Surface EMG crosstalk quantified at the motor unit population level for muscles of the hand, thigh, and calf

Carina M Germer[1,2], Dario Farina[3], Leonardo A Elias[1,4], Stefano Nuccio[5], François Hug[6,7,8] and Alessandro Del Vecchio[9]

[1] Neural Engineering Research Laboratory, Center for Biomedical Engineering, University of Campinas, Brazil
[2] Department of Bioengineering, Federal University of Pernambuco, Recife, Brazil
[3] Department of Bioengineering, Faculty of Engineering, Imperial College London, United Kingdom
[4] Department of Electronics and Biomedical Engineering, School of Electrical and Computer Engineering, University of Campinas, Brazil
[5] Department of Movement, Human and Health Sciences, University of Rome "Foro Italico", Rome, Italy
[6] Laboratory "Movement, Interactions, Performance" (MIP), Nantes University, F-44000 Nantes, France
[7] Institut Universitaire de France (IUF), Paris, France
[8] School of Biomedical Sciences, The University of Queensland, Brisbane, Australia.
[9] Department of Artificial Intelligence in Biomedical Engineering, Faculty of Engineering, Friedrich-Alexander University, Erlangen-Nuremberg, Germany

Corresponding Author: Prof. Dr. Alessandro Del Vecchio
E-mail: alessandro.del.vecchio@fau.de
Address: Department of Artificial Intelligence in Biomedical Engineering, Faculty of Engineering, Friedrich-Alexander University, Erlangen-Nuremberg, 91052, Germany

Received xxxxxx
Accepted for publication xxxxxx
Published xxxxxx

Abstract

Crosstalk is an important source of error in interpreting surface electromyography (EMG) signals. Here, we aimed at characterizing crosstalk for three groups of synergistic muscles by the identification of individual motor unit action potentials. Moreover, we explored whether spatial filtering (single and double differential) of the EMG signals influences the level of crosstalk. Three experiments were conducted. Participants (total twenty-five) performed isometric contractions at 10% of the maximal voluntary contraction (MVC) with digit muscles and knee extensors, and at 30% MVC with plantar flexors. High-density surface EMG signals were recorded and decomposed into motor unit spike trains. For each muscle, we quantified the crosstalk induced to neighboring muscles and the level of contamination by the nearby muscle activity. We also estimated the influence of crosstalk on the EMG power spectrum and intermuscular correlation. Most motor units (80%) generated significant crosstalk signals to neighboring muscle EMG in monopolar recording mode, but this proportion decreased with spatial filtering (50% and 42% for single and double differential, respectively). Crosstalk induced overestimations of intermuscular correlation and has a small effect on the EMG power spectrum, which indicates that crosstalk is not reduced with high-pass temporal filtering. Conversely, spatial filtering reduced the crosstalk magnitude and the overestimations of intermuscular correlation, confirming to be an effective and simple technique to reduce crosstalk. This paper presents a new method for the identification and quantification of crosstalk at the motor unit level and clarifies the influence of crosstalk on EMG interpretation for muscles with different anatomy.

Keywords: Electromyography, Signal contamination, Motor unit, High-density sEMG, Spike triggered averaging

New & Noteworthy

We proposed a new method for the identification and quantification of crosstalk at the motor unit level. We show that surface EMG crosstalk can lead to physiological misinterpretations of EMG signals such as overestimations in the muscle activity and intermuscular correlation. Crosstalk had little influence on the EMG power spectrum which indicates that conventional temporal filtering cannot minimize crosstalk. Spatial filter (single and double differential) effectively reduces but not abolish crosstalk.

1. Introduction

Surface electromyography (EMG) is a widely used tool for extracting information concerning the neuromuscular system. Because of its simplicity, EMG usage ranges from neurophysiological research to clinical applications and myoelectric control of assistive devices. An important source of error in EMG interpretation is crosstalk, i.e., the signal recorded over a muscle that is generated by another nearby muscle [1–4]. Crosstalk may cause an overestimation of the activity level of a muscle [5] and bias coherence analysis [6].

It is well known that crosstalk is influenced by anatomical features. For example, muscle fiber length and subcutaneous tissue thickness [7–9] are important influencing factors since they affect the spatial distribution of electrical activity from the sources to the recording electrodes. Typically, long fibers generate relatively large propagating components whereas short fibers generate action potentials dominated by non-propagating component. However, the propagating component decays faster with distance [7], so that the non-propagating component is the main determinant of crosstalk [2]. Therefore, the level of crosstalk greatly varies across muscles. Previous studies identified crosstalk on a limited number of muscle groups of the lower and upper limbs [2,5,8,10–13], however, no study has systematically characterized crosstalk in different muscles.

Typically, the voluntary contraction of a muscle also involves coactivation of other muscles, which introduces an important challenge to the identification and quantification of crosstalk. Classical approaches for crosstalk quantification involved selective activation of a target muscle, such as by using electrical stimulation [1,8] or functional isolation [14,15], while measuring the activity on a nearby quiescent muscle. These methods cannot ensure selective activation of the target muscle and a natural condition paradigm of voluntary activation of motor units. Due to inadequate methods, an exact quantification of crosstalk and its effects on the interpretation of the EMG signals is still lacking [3,16].

Estimation of EMG crosstalk may be feasible with the acquisition of high-density surface EMG (HD sEMG) [6,11]. Once the EMG signals are decomposed into motor unit discharge times, the crosstalk from individual motor units can be estimated by spike-triggered averaging (STA) the EMG signals recorded from multiple muscles [6]. This approach allows the identification of crosstalk even in the presence of coactivation of several muscles in natural conditions of force generation.

The aim of this study is to quantify the level of crosstalk at the motor unit level for muscles of different groups and with different anatomy (size and architecture). We present a methodology to assess the contribution of individual motor units to crosstalk and to determine how crosstalk affects the physiological interpretation of surface EMG signals. Moreover, we evaluated the effects of spatial filtering (single [SD] and double differential [DD]) for reducing crosstalk. SD and DD selectively attenuate common signal components from consecutive electrodes and therefore are expected to reduce crosstalk.

2. Methodology

Three independent studies were conducted for the hand, knee extensor, and plantarflexor muscles. Volunteers with no history of neurological impairments gave their informed consent before the experiments. Procedures were in accordance with the *Declaration of Helsinki* and were approved by the local ethics committee (Imperial College Research Ethics Committee, N 18IC4685; The University of Queensland Ethics Committee, 2013001448; Institutional Review Board of the University of Rome "Foro Italico", N 2018/07).

2.1 Recording of high-density surface EMG

HD sEMG signals were recorded with a multichannel amplifier (OT Bioelettronica Quattrocento; bandwidth: 10-500 Hz; resolution: 16 bits; sampling rate: 2048 Hz) in monopolar mode.

2.2 Experimental Protocols

2.2.1 Hand muscles

For the hand muscles, the experimental protocol is described in detail in [6]. Briefly, eight participants (26 ± 2 years, seven males) were asked to simultaneously perform steady isometric index finger abduction and thumb flexion at 10% of the maximal voluntary contraction (MVC). The MVC for each digit was defined as the maximum value in two attempts for maximum effort task (performed independently), separated by 1 min of rest. The steady contraction task of 60-s duration was performed twice. Participants received a visual feedback of the force as a cursor in which the x-axis and y-axis were controlled by the thumb and index finger, respectively. The force exerted by each digit was measured with a three-axis force transducer (Nano25, ATI Industrial Automation), digitized (2048 Hz, USB-6225, National Instruments), and filtered (15

Hz low-pass cutoff frequency). Two 13 x 5 flexible grids of electrodes (4-mm interelectrode distance, ELSCH064NM4, OT Bioelettronica) were placed over the first dorsal interosseous (FDI) and thenar muscles (Figure 1A). The matrix placement on the thenar muscle targeted the flexor pollicis brevis and abductor pollicis brevis.

2.2.2 Knee extensors

Eight participants (27.6 ± 2.4 years, all males) were asked to perform knee extension steady contractions. First, the MVC was defined as the peak value in three attempts of maximum effort task, separated with 1 min of rest. Subsequently, participants performed two 60-s visually guided steady contractions at 10 %MVC. Participants received a visual feedback of the force with a constant visual gain. The knee angle was set at 45° of flexion and the knee extensor force was measured bilaterally using a Kin-Com dynamometer (KinCom, Denver, USA), sampled at 2048 Hz, and low-pass filtered with a cut-off frequency of 15 Hz (4th order, zero-lag, Butterworth filter).

Two 13 x 5 flexible grids of electrodes (8-mm interelectrode distance; ELSCH064NM2, OT Biolettronica) were oriented and attached over the vastus lateralis (VL) and vastus medialis (VM) muscle bellies following the procedures of a previous study [17] (Figure 1A). Force and HD sEMG data were concurrently collected through the software OT BioLab (OT Bioelettronica, Turin, Italy).

2.2.3 Plantarflexors (triceps surae muscles)

The experimental procedure is described in [18]. Nine participants (31 ± 9 years, all males) were asked to perform submaximal isometric plantarflexion contractions. They laid prone on a custom-made dynamometer equipped with a torque sensor (TRE-50K, Dacell, Korea). Their knee was fully extended, and their ankle angle was set to 10° of plantarflexion (0° being the foot perpendicular to the shank). Participants first performed three maximal isometric contractions for 3 to 5 s with 2 min rest in between. The maximal value obtained from a moving average window of 250 ms was considered as the MVC. Then, participants performed four contractions at 30 %MVC, which involved a 5-s ramp-up, a 30-s plateau and a 5-s ramp down phase. The contractions were separated by 120 s of rest. Participants received a visual feedback of the target contraction intensity and the torque output.

Two 13 x 5 flexible grids of electrodes (8-mm interelectrode distance, ELSCH064NM2, OT Bioelettronica) were placed over the gastrocnemius medialis (GM) and the gastrocnemius lateralis muscle (GL) and aligned with the main fascicle direction, as determined using B-mode ultrasound (Aixplorer, Supersonic Imagine, France). In addition, an 8×4 flexible grid of electrodes (10-mm interelectrode distance GR10MM0804, SpesMedica, Battipaglia, Italy) was placed on the medial part of the soleus (SOL), below the myotendinous junction of the GM muscle.

2.3 Data Analysis

2.3.1 HD surface EMG signals

The multi-channel EMG signals were visually inspected, and the channels with low signal-to-noise ratio were removed from the analysis (i.e., signals with power line interference and visible spurious activity). EMG signals were low-pass filtered at 500 Hz (fourth-order Butterworth) offline. The initial and final segments for force stabilization at the target level were discarded. For the hand muscles and knee extensors, we discarded the initial 9.5 s and 7 s, respectively. Due to the late recruitment of GL, initial 12.5 s (5-s ramp up and 7.5-s during plateau) were removed. Therefore, for each participant, we analyzed two trials of 50 s for FDI-thenar and VL-VM, and four trials of 12.5 s for GL-GM-SOL. Two or three groups of five neighboring electrodes were used to estimate SD and DD EMG signals [9], respectively (Figure 1A).

2.3.2 EMG decomposition

HD sEMG was decomposed into motor unit spike trains with a blind source separation algorithm [19]. The motor unit spike trains were visually inspected and corrected by experienced examiners, according to the guidelines described [20]. Motor units with high interspike variability (i.e., mean coefficient of variation above 40%) were discarded since they are typically associated with intermittent activities. We also checked if the identified motor units were associated to the right muscle. For this, we compared the amplitude and the spatial distribution of the motor unit action potential in the HD sEMG grid between the neighboring muscles. In monopolar recording, the amplitude is greater for the electrodes close to the innervation zone. Consequently, the grid over the motor unit has a heterogeneous spatial distribution, unlike crosstalk, which is characterized by signals with small amplitude and homogeneous spatial distribution.

2.3.3 Crosstalk features in each muscle

For all identified motor units of each muscle, we characterized the extent of crosstalk that contaminated the EMG of the neighboring muscle(s) (i.e., muscles within the same group). The motor unit action potential (MUAP) and the crosstalk MUAP (*Cross* MUAP) from each motor unit were extracted by spike-triggered averaging (STA) the multi-channel EMG signals (Figure 1B). For example, motor unit discharge times identified from the HD EMG grids over the GM muscle were used to extract the two-dimensional action potential waveform for GM (MUAP), GL and SOL (*Cross* MUAPs). This procedure was iterated for all motor units and muscles and allowed us to quantify the amount of crosstalk for each motor unit. For each muscle, we pooled all extracted motor units and estimated the proportion of motor units that induced significant crosstalk (as defined in 2.3.4). We also assessed the association between *Cross* MUAP and MUAP amplitudes by linear regression and computed a crosstalk index as the peak-to-peak amplitude ratio (*Cross* MUAP/MUAP x 100%). The linear regression and the crosstalk index were estimated for motor units that showed significant *Cross* MUAP.

2.3.4 Method to assess crosstalk significance

We applied statistical analysis to define a level of significance for crosstalk. For each motor unit, we estimated the *Cross* MUAP in the neighboring EMG (Figure 1B). The *Cross* MUAP was considered significant if its amplitude was greater than the noise. The baseline noise value was estimated by applying STA on the EMG signal but using random triggers. For each motor unit, two hundred random sequences of triggers were obtained by bootstrapping (random sampling with replacement) the interspike intervals, and each sequence had the same number of triggers as the number of motor unit discharges. Therefore, the discharge properties (i.e., mean, and standard deviation) of the random triggers were similar to the original motor unit, but the discharge time instants were randomly allocated. The *Cross* MUAP was considered significant if its peak-to-peak amplitude exceeded the 95th percentile (one-tailed test at the 0.05 level) of the amplitude distribution of the two hundred resampled versions (Figure 1B).

2.3.5 Influence of crosstalk on the surface EMG

The influence of crosstalk on the global EMG signals was assessed for each individual muscle in time and frequency domains. For each muscle, we reconstructed the interference pattern from the decomposed motor units (*Synthetic* EMG), the crosstalk from each neighboring muscle (*Cross* EMG), and the EMG after crosstalk removal (*Clean* EMG). The *Synthetic* EMG was assessed by summing all motor unit action potential trains (Figure 1C), which were estimated by convolving the MUAP obtained by STA on the EMG signal with the motor unit discharge times. Similarly, the *Cross* EMG from each neighboring muscle was reconstructed with the cross MUAP extracted by triggering the EMG signal with the discharge times from the neighboring motor units (Figure 1C). Lastly, *Clean* EMG was obtained by subtracting the neighboring muscle(s) *Cross* EMG from the original EMG. Note that the crosstalk from other active synergistic muscles were not included in the analysis (e.g., the knee extensors vastus intermedius and rectus femoris were not measured and, thus, were not considered for the *Clean* EMG calculation).

For each muscle, the total crosstalk magnitude that originated from the measured neighboring muscles was estimated as the sum of all neighboring muscle *Cross* EMG RMS normalized to its EMG RMS. Moreover, the EMG signal was compared to the *Synthetic* and C*ross* EMG using the Pearson's correlation coefficient (ρ). These measures roughly indicate how much of the EMG signal can be attributed to the activity of the decomposed motor units and crosstalk, respectively. The critical value for an α = 0.05 of a large sample data was estimated by: $r_{crit} = 1.645/\sqrt{n-2+1.645^2}$, where *n* is the sample size [21].

The influence of crosstalk on the frequency content of the EMG signal was assessed by comparing the EMG and *Clean* EMG power spectrum. The power spectrum was estimated by Welch's averaged periodogram with non-overlapping Hanning window of 1 s duration. First, the EMG signals were full wave rectified and detrended. The median frequency was estimated for each participant and we computed the average power spectrum for all participants for visualization.

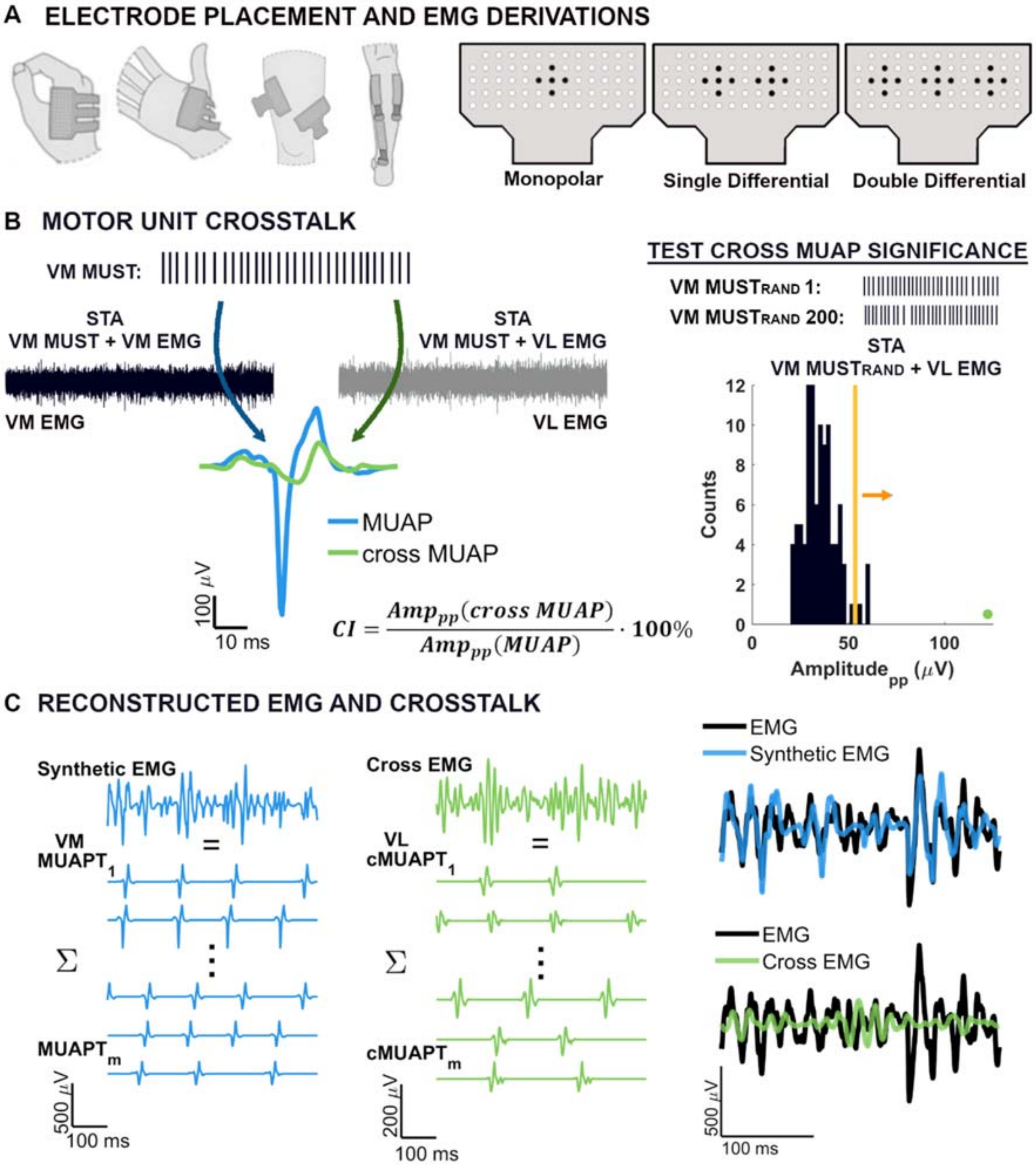


Figure 1. (A) Schematic representation of high-density surface EMG grids placement and electrode selection used to estimate the monopolar, single differential and double differential derivations for the EMG signal. (B) Analysis of individual motor unit crosstalk. A representative motor unit spike train (MUST) from vastus medialis muscle (VM) was used to trigger the VM EMG by spike-triggered averaging (STA). The crosstalk from the motor unit into the vastus lateralis (VL) EMG was also estimated by STA. The significance of the motor unit crosstalk was tested by comparing the selected cross MUAP amplitude to the amplitude obtained from triggering two hundred shuffling versions of the VM MUST (MUSTrand) on the VL EMG signal. The cross MUAP was considered significant if its amplitude was above the 95th percentile of the amplitude distribution for the random estimations. The crosstalk index (CI) for individual motor units with significant crosstalk was estimated by the ratio between the peak-to-peak amplitude of the Cross MUAP and MUAP. (C) Representative reconstruction of the VM Synthetic EMG computed by summing all decomposed VM motor unit action potential trains (MUAPT). The representative crosstalk from VL into VM EMG (Cross EMG) was estimated as the summation of the crosstalk (cMUAPT) from all decomposed VL motor units in the VM EMG (note the amplified scale of the Cross EMG). The Synthetic EMG and Cross EMG (or Clean EMG, see Methods) were compared with the original EMG.

2.3.6 Crosstalk influence on intermuscular correlation

We quantified the effect of crosstalk on the cross-correlation analysis between muscles by comparing the |ρ| between EMG envelopes when considering either the original or *Clean* EMG. The EMG envelope was computed by low-pass filtering (cut-off at 8 Hz, second-order Butterworth) the full-wave rectified EMG. This analysis is particularly relevant for studies of muscle synergy [6,22].

2.3.7 Statistical Analysis

Statistical analysis was performed using SPSS (IBM, version 23). Assumption for normality distribution was tested by the Shapiro-Wilk test.

Linear regression analysis tested the correlation between *Cross* MUAP and MUAP amplitude for all extracted motor units with significant crosstalk (see section 2.3.4). In the case of significant correlation, analysis of covariance (ANCOVA) tested the effect of *EMG Recording Mode* (monopolar, SD and DD) on the regression slopes. The effect of *EMG Recording Mode* was also tested in the population mean with an analysis of variance (ANOVA) in the cases of weak or no correlation.

The crosstalk index was compared in a two-way ANOVA for *Muscle Pairs* (FDI to thenar, thenar to FDI, VL to VM, VM to VL, GL to GM, GL to SOL, GM to GL, GM to SOL, SOL to GL and SOL to GM) and *EMG Recording Mode*.

The relative contribution of crosstalk to the EMG signal (|ρ| between EMG and *Cross* EMG) was evaluated with a mixed two-way ANOVA with the repeated factor being the *EMG Recording Mode* and *Muscle Pairs* as independent factor. Similarly, a mixed two-way ANOVA compared the EMG and *Synthetic* EMG |ρ| for the different *Muscles* (FDI, thenar, VL, VM, GL, GM, SOL) and *EMG Recording Mode* as repeated factor. A mixed two-way ANOVA compared the EMG RMS and relative *Cross* EMG RMS for the *EMG Recording Mode* for the muscles in the hand, thigh and calf separately.

A two-way repeated measure ANOVA estimated the effect of *EMG Recording Mode* and *EMG Crosstalk Type* (EMG and *Clean* EMG) on the median frequency of the EMG power spectrum and |ρ| between EMG envelopes in a multivariate design for individual *Muscles* or *Simple Muscles Pairs* (FDI-thenar, VL-VM, GL-GM, GL-SOL, GM-SOL), respectively. Pairwise comparison was conducted with Bonferroni's *post hoc* test, and a 95% significance level was adopted. Data are reported as mean values ± 95% confidence interval.

3. Results

In total, 739 motor units were decomposed (FDI: 196, thenar: 68, VL: 115, VM: 75, GL: 96, GM: 200, SOL: 79), with an average number of motor units per subject per contraction of 12.25 ± 2.08 for the FDI, 4.25 ± 0.98 for the thenar, 7.19 ± 3.00 for the VL, 4.69 ± 1.61 for the VM, 9.00 ± 3.58 for the GL, 20.30 ± 4.72 for the GM, and 7.44 ± 2.70 for the SOL.

3.1 Crosstalk features in different muscles

We evaluated the significance of crosstalk for all the identified motor units (Figure 1B). Only signals with amplitude greater than a critical value (95th percentile EMG amplitude estimated from random motor unit spike trains) were considered as *Cross* MUAPs. Notably, most motor units induced crosstalk on the EMG of nearby muscle for the monopolar recording mode (79.73%, pooled motor units from all muscles), but SD and DD showed a smaller proportion (50.42% and 41.69%, respectively) (Figure 2A).

The amplitude values for the motor unit crosstalk (*Cross* MUAP) and original MUAP are shown in Figure 2B. The *Cross* MUAP was significantly correlated to the MUAP amplitude for most pair of muscles in monopolar (Table 1), indicating that motor units with higher amplitudes tend to induce more crosstalk. The slope for the regressions is small (Table 1), and the highest slopes were found for VL to VM EMG (0.264) and GL to SOL EMG (0.244). Therefore, although the MUAP amplitude ranged from 30 μV to 1900 μV, the crosstalk amplitude was mostly confined within a smaller range, from 20 μV to 300 μV (Figure 2B). For SD and DD, the correlation was not significant (Table 1) in most cases, and the crosstalk amplitude was confined within the 20 μV to 100 μV range.

Spatial filter significantly decreased the amplitude of crosstalk for most muscle pairs, except for VM to VL EMG (Table 1). Noteworthy, although spatial filter decreased the *Cross* MUAP amplitude for most muscles, signals from the neighboring muscle were still present in the global EMG signal (see next section).

The relation between *Cross* MUAP and MUAP amplitude was also assessed by estimating the crosstalk index (Figure 2C). A small proportion of motor units (total of 6%) showed a crosstalk index greater than 100%, indicating a larger *Cross* MUAP than MUAP amplitude. This is an artefact of the methodological procedure due to the selection of the channels in the middle of the HD sEMG grid (Figure 1A) regardless of the motor unit innervation zone and amplitude distribution map. Of note, all motor units were addressed to the right muscle (see Section 2.3.2). The crosstalk index reduced with spatial filtering for some muscles ($p < 0.001$ for the interaction: *Muscles Pairs* and *EMG Recording Mode*). SD reduced the crosstalk index only for the calf muscles ($p < 0.001$, for GL to SOL EMG and GM to both GL and SOL EMG) and DD reduced the crosstalk index for motor units from FDI to thenar ($p = 0.016$), VM to VL ($p = 0.011$), GL to SOL ($p < 0.001$) and GM to both GL and SOL EMG ($p < 0.001$). These results agree with the significant reduction in the *Cross* MUAP amplitude with SD and DD for some muscles (Figure 2B).

A greater index of crosstalk was found for thenar in FDI EMG compared to FDI in thenar EMG ($p < 0.001$ for monopolar and DD) and GM compared to GL ($p < 0.001$ for monopolar and SD). It is important to point out that these results should be interpreted carefully. One might wrongly extrapolate that thenar muscles induce more crosstalk than FDI. However, the crosstalk index is a measure of relative amplitude (*Cross* MUAP/MUAP), and an asymmetry might emerge from differences in the amplitude of the MUAP rather than *Cross* MUAP.

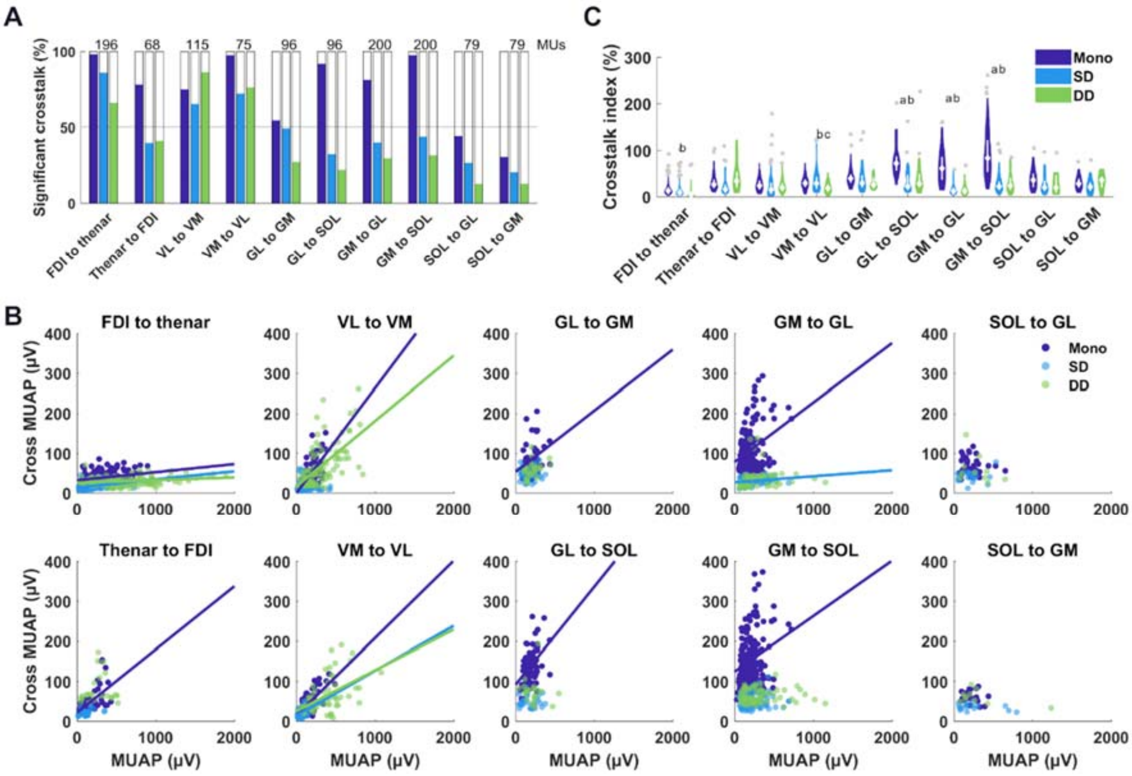


Figure 2. (A) Proportion of motor units with significant crosstalk for the EMG in monopolar (Mono), single differential (SD) and double differential (DD) modes. Numbers above bars indicate the number of motor units decomposed for each muscle. First muscle in the legend is the targeted (control) and the second muscle is where the crosstalk was estimated. (B) Peak-to-peak amplitude of individual motor unit crosstalk (Cross MUAP) relative to the motor unit action potential (MUAP). Significant linear regressions are shown as continuous lines. Only motor units with significant cross MUAP are shown. (C) Motor unit crosstalk index (CI). Significant differences for EMG Muscle and Recording Mode ($p < 0.05$) are demarked by letters (a: Mono vs SD, b: Mono vs DD, c: SD vs DD). Grey dots indicate outliers.

3.2 Crosstalk effect on the EMG

In order to quantify the effect of crosstalk on the interference EMG, we reconstructed the crosstalk from the neighboring muscle(s) (*Cross* EMG, Figure 1C) and we estimated the *Clean* EMG by removing the *Cross* EMG(s) from the recorded signal. First, we evaluated whether the algebraic summation of all motor unit crosstalk is a good estimation of the total muscle crosstalk. For this, we evaluated the contribution of the decomposed motor units (*Synthetic* EMG, from the summation of MUAPs, Figure 1C) to the acquired EMG signal. In the following subsections we describe the results for each of these analyses.

3.2.1 Contribution of the decomposed motor units to the global interference EMG signal

We synthetized the EMG from the spike trains of the decomposed motor units, as shown in Figure 1C. The *Synthetic* EMG signal corresponds to a crosstalk-free version of the EMG signal with contribution only from the decomposed motor units. Therefore, the background activity of the undecomposed action potentials and noise are removed. The *Synthetic* EMG was significantly correlated to the acquired EMG signal ($r_{crit} = 0.005$, Figure 3A). The correlation was moderate ($|\rho|$ between 0.5 and 0.7) for most muscles, but weaker ($|\rho|$ between 0.2 and 0.4) for GL and SOL. The weak correlation could be attributed to few motor units decomposed and/or high background noise (including crosstalk). In fact, high levels of GM crosstalk were observed on the GL and SOL EMG acquired in monopolar mode (see next section). SD increased the $|\rho|$ for VM ($p = 0.002$), GL and GM ($p < 0.001$), but not for the hand muscles ($p > 0.999$), VL ($p = 0.559$) and SOL ($p = 0.062$). A greater effect was found for DD, with significant increase in the $|\rho|$ for the thigh ($p < 0.001$) and calf ($p < 0.001$) muscles. In general, differentiating the EMG signals increases the correlation and the representativeness of motor unit activity.

3.2.2 Contribution of the crosstalk to the global interference EMG signal

We found significant correlations between the EMG and *Cross* EMG (Figure 3B). The relative contribution of crosstalk to the EMG signal depended on the muscle and *EMG Recording Mode* (significant interaction, $p < 0.001$). Thenar EMG was more affected by the FDI activity than the opposite way ($p = 0.052$, $p < 0.001$, $p = 0.002$, for EMG in monopolar, SD, and DD, respectively). The two knee extensor muscles showed similar amounts of crosstalk ($p > 0.276$). In the triceps surae muscle group, GM EMG was equally affected by GL and SOL crosstalk ($p = 0.999$, for all *EMG Recording Mode*), but a greater contribution from GM crosstalk was found in GL EMG ($p < 0.007$, for all *EMG Recording Mode*) and SOL EMG in DD ($p = 0.157$, $p = 0.032$, $p = 0.026$, for EMG in monopolar, SD, and DD, respectively).

Spatial filtering reduced the crosstalk contribution on the EMG signal but not for all muscles ($p < 0.001$). SD decreased the $|\rho|$ for GL ($p < 0.001$ from GM crosstalk) and SOL ($p < 0.001$, from GL and GM crosstalk), and DD significantly decreased the $|\rho|$ for thenar ($p < 0.001$), VL ($p = 0.040$), GL ($p < 0.001$ for GM crosstalk) and SOL ($p < 0.001$ for crosstalk from GL and GM). Reduction on the $|\rho|$ with spatial filter was non-significant for FDI ($p > 0.313$, for SD and DD), VM ($p = 0.999$, for both SD and DD) and GM ($p > 0.090$ for SD and DD for crosstalk from GL and SOL).

Interestingly, GL EMG in monopolar was equally correlated to the GL *Synthetic* EMG (Figure 3A) and GM crosstalk (*Cross* EMG, GL_{GM} Figure 3B). Although this result may be partly influenced by the small number of identified motor units, it suggests that a significant part of the GL EMG signal originated from GM crosstalk during the task evaluated in the present study. Also, spatial filter decreased the crosstalk influence as demonstrated by a consistent increase in the correlation to the GL *Synthetic* EMG and a decrease in the correlation to GM crosstalk with SD and DD.

3.2.3 Relation between crosstalk and EMG amplitude

The asymmetric contribution of crosstalk to the EMG signal (ρ between EMG and *Cross* EMG) described for FDI-thenar might be explained by the differences in EMG amplitudes. Albeit each digit muscle contracted at 10 %MVC, a higher EMG amplitude was found for the FDI (Table 2, $p < 0.015$, for monopolar and DD) and, consequently, the crosstalk was more pronounced in the thenar EMG ($p < 0.001$, for all *EMG Recording Modes*). On average, crosstalk amplitude corresponded to approximately 23% of the thenar EMG signal (but higher for monopolar, $p < 0.001$) and only 8% for FDI (Figure 3C, $p > 0.05$ for multiple comparisons for *EMG Recording Mode*). The asymmetry was not found for the knee extensors, where both muscles contributed equally to the contraction task at 10 %MVC ($p = 0.918$) and similar relative crosstalk amplitude was found for VL and VM (13% and 16%, respectively; $p = 0.208$). Conversely, all triceps surae muscles contributed to the plantarflexion contraction at 30 %MVC, however, we found different EMG RMS ($p = 0.036$) between the muscles. A higher amplitude was detected in SOL compared to GL ($p = 0.032$), but not compared to GM ($p = 0.545$). Relative crosstalk amplitude was approximately 27% for GL (higher for monopolar, $p < 0.001$), 15% for GM and 29% for SOL (higher for monopolar, $p < 0.001$). The relative crosstalk amplitude corresponded to 41% and 44% for the GL and SOL EMG in monopolar (Figure 3C). Therefore, when neighboring muscles present different EMG amplitudes, the muscle with the smallest EMG RMS is likely the one most affected by crosstalk.

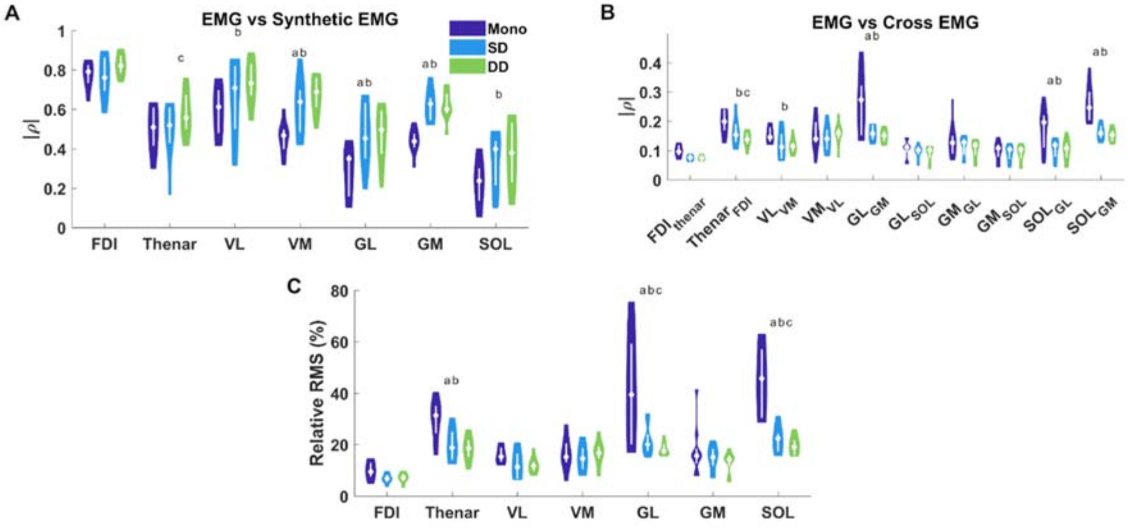


Figure 3. Absolute Pearson's correlation coefficient (|ρ|) between the muscle's EMG and the global activity from its motor units (Synthetic EMG) (A), or the crosstalk from another muscle (Cross EMG, subscribed in the x-axis label) (B) Correlations were estimated for the EMG in monopolar (Mono), single differential (SD) and double differential (DD) recording modes. (C) Root mean square (RMS) for the Cross EMG relative to EMG in percent. Letters indicate significant differences between EMG Recording Mode ($p < 0.05$, a: Mono vs SD, b: Mono vs DD and c: SD vs DD).

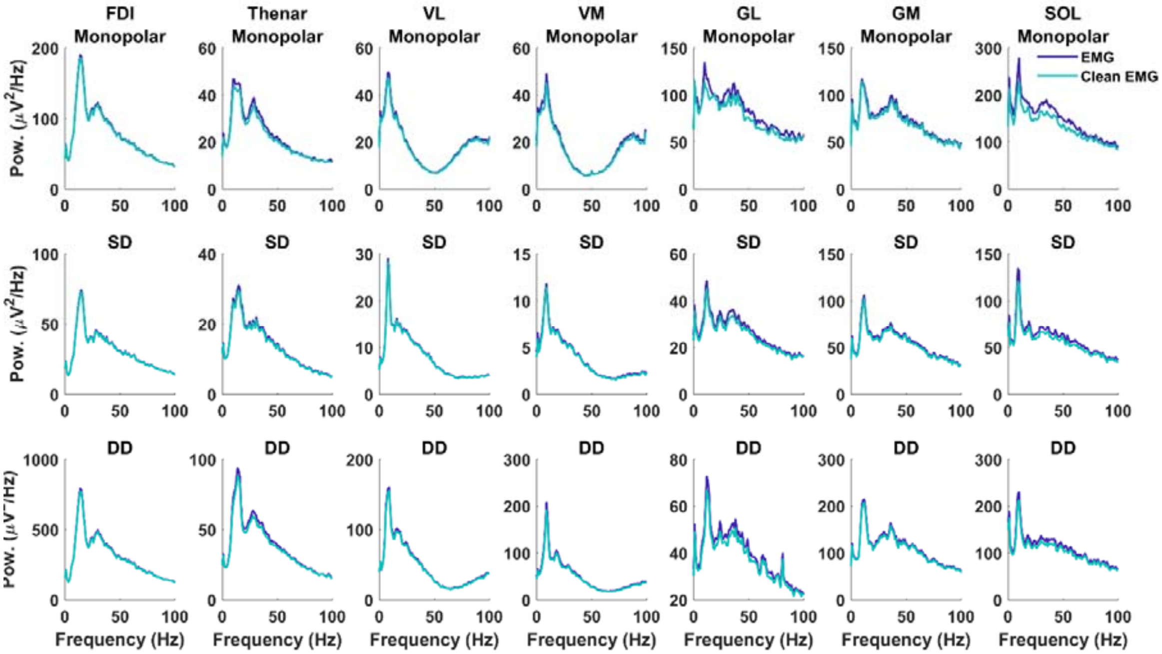


Figure *4.* Average power spectrum for different muscle EMGs in monopolar, single differential (SD) and double differential (DD) recording modes, evaluated on the original rectified EMG and after crosstalk removal (Clean EMG). Note differences in the y-axis scale.

3.2.4 Effect of crosstalk on the EMG power spectrum

The averaged power spectra for the original and *Clean* EMG are presented in Figure 4. The removal of crosstalk slightly increased ($p < 0.003$, for all *Muscles*) the median frequency of the rectified EMG power spectrum regardless of *EMG Recording Mode* ($p > 0.057$ for the interaction Table 2). Therefore, crosstalk has an effect of slightly shifting the EMG power spectrum to lower frequencies. Nevertheless, the shift was small. The maximal shift of frequency was approximately 4 Hz (FDI: 0.62 Hz, thenar: 1.76 Hz, VL: 0.32 Hz, VM: 0.53 Hz, GL: 3.94 Hz, GM: 2.17 Hz, SOL: 4.25 Hz).

3.3 Influence of crosstalk on the intermuscular correlation

The $|\rho|$ between original EMG signals was significantly higher than the $|\rho|$ between *Clean* EMG signals (Figure 5), indicating that crosstalk increased the intermuscular correlation. A significant interaction between *EMG Recording Mode* and *EMG Crosstalk Type* (EMG and *Clean* EMG) was found for most muscle pairs ($p < 0.026$), except for GL-GM ($p = 0.416$). For GL-GM, reduction of the $|\rho|$ with crosstalk removal was irrespective of *EMG Recording Mode* ($p = 0.008$). Conversely, a significant difference was found for the EMG in monopolar for all other muscle pairs ($p < 0.010$) compared to SD and DD. Thus, spatial filters effectively reduced or removed the crosstalk effect for most pairs of muscles. For SD, there was a significant effect of crosstalk on the correlation between FDI-thenar ($p = 0.007$) and GL-SOL ($p = 0.003$), but not for VL-VM ($p = 0.112$) and GM-SOL ($p = 0.098$). On the other hand, DD completely removed the crosstalk effect for most muscle pairs ($p > 0.119$), except for VL-VM ($p < 0.001$).

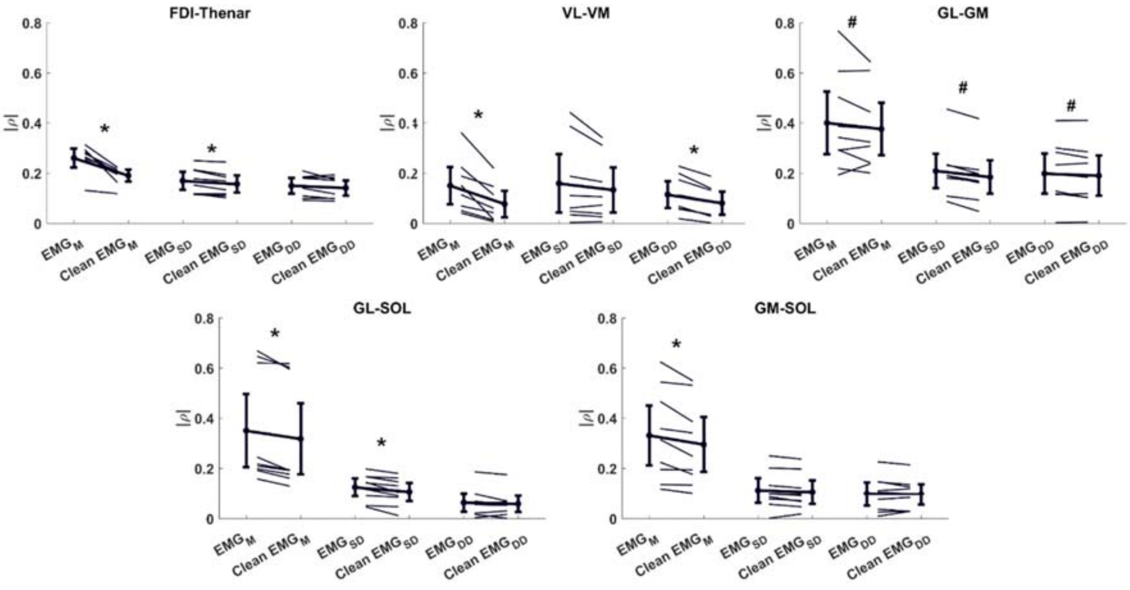


Figure 5. Absolute Pearson's correlation (|ρ|) between pairs of muscle EMGs in monopolar (M), single differential (SD) and double differential (DD) recording modes, evaluated on the recorded signal (EMG) and after crosstalk removal (Clean EMG). Error bars represent the 95% confidence interval (n = 8 for hand and thigh, n = 9 for calf muscles). Hash and asterisk indicate significant differences for EMG and Clean EMG (p < 0.05) for main effect (no significant interaction) and simple main effect (significant interaction), respectively.

4. Discussion

We evaluated crosstalk in the surface EMG for two muscles of the hand, and two groups of synergistic muscles of the thigh and calf. We identified and quantified the level of crosstalk of each muscle and the effect of each muscle in contaminating neighboring muscle activities. Our results showed that all muscles were contaminated by crosstalk, but the magnitude of crosstalk differed among muscles. We also found an asymmetric level of crosstalk between the muscles in the hand and calf, but not in the thigh. This asymmetry was likely related to the difference in the EMG amplitude. Moreover, crosstalk caused an overestimation of intermuscular correlation between EMG envelopes for all muscle pairs. We also showed that spatial filtering effectively reduced crosstalk effects in the time-domain metrics, especially the DD recording mode, confirming it as a convenient method to minimize crosstalk. A summary of the effects of crosstalk on the EMG metrics and on the influence of *EMG Recording Mode* is presented in Table 2. These results add to the knowledge of crosstalk identification in different muscles and elucidate some of its confounding effects. In the following sections we present a discussion on each of these findings.

4.1 Crosstalk features in different muscle groups

Recordings of myoelectric activity of index abduction and thumb flexion are contaminated by crosstalk, which is in agreement with previous studies [6,10]. Nevertheless, we also showed that the crosstalk features vary across muscles. The activity from FDI is more likely to induce crosstalk on thenar, as measured by the proportion of motor units with significant crosstalk on the other muscle (82% for FDI vs. 50% for thenar), and higher level of contamination to thenar EMG (23% vs. 8%). Our data indicate that the level of contamination is related to the activity level (e.g. global EMG amplitude). Even though participants performed simultaneous contractions at the same relative force (10 %MVC, independently), the myoelectric activity recorded over the thenar muscle was smaller than the FDI in terms of EMG RMS. Consequently, the level of crosstalk contamination over the thenar muscle was greater. It is important to note that index finger abduction is mainly due to activation of the FDI, whereas thumb flexion at the metacarpophalangeal and interphalangeal joint requires coactivation of the abductor pollicis brevis, flexor pollicis brevis and flexor pollicis longus. Hence, a dispersion of the active region for thumb flexion could have contributed to the lower activity (or smaller MUAPs) detected. Moreover, although FDI has less motor units than abductor pollicis brevis (120 vs. 170, respectively [23,24]), FDI has a higher innervation ratio (340 vs. 106 [23,24]). Consequently, the composite action potentials generated by FDI motor units, and thus the capacity to induce crosstalk, should be larger than the abductor pollicis brevis. These and other features, such as fiber length and orientation relative to the detection system [25] also determine the magnitude of the non-propagating components and might corroborate the asymmetric level of crosstalk found.

Albeit nearly 50% more motor units were identified in VL than VM, the two knee extensor muscles induced similar levels of crosstalk, which is consistent with previous studies [8,26]. Noteworthy, the majority of the identified motor units from each muscle (more than 76%) was detected in EMG of the other muscle, even though VL and VM are separated by the rectus femoris and the vastus intermedius muscles. At a shared contraction level of 10 %MVC, both muscles had similar EMG amplitudes and the relative crosstalk corresponded to 14% of the EMG RMS. VL has a larger physiological cross-section area than VM [27,28], yet they have similar architecture (muscle thickness, fascicle angle and fiber length) at the distal region of the thigh [27,29], where the electrodes were placed. Therefore, similarities in the level of crosstalk might reflect similarities in the characteristics of the sources and activity levels.

We found a smaller proportion of motor units with significant crosstalk between calf muscles (approximately 46%, 54% and 25% for GL, GM and SOL, respectively). Notwithstanding, crosstalk was still present and significantly influenced the EMG metrics. Even though all muscles contributed to the plantarflexion task, a smaller myoelectric activity was recorded in GL than GM and SOL, which is in agreement with another study [18]. Consequently, GL contamination was more evident. The lower myoelectric activity over the GL can be attributed to a higher motor unit recruitment threshold compared to SOL and GM [30] and considerably smaller muscle structure (i.e., physiological cross-section area [27,31]). Also, we found particularly interesting that most of SOL crosstalk in GL and GM was below the noise threshold. This can be due to the distance between the source and the detection point (e.g., region of activation [32], and source amplitude). In fact, SOL contains 80% of slow twitch fibers, which are associated to small motor unit size, compared to only 57% for gastrocnemius [33]. Moreover, regionalization of motor unit activation [34] or muscle compartmentalization [32,35] could also have contributed to the asymmetric crosstalk features between the heads of the triceps surae.

4.2 Influence of crosstalk on the surface EMG

Crosstalk was present in all EMG signals from the evaluated muscles, and the relative crosstalk amplitude (RMS) ranged from 8% to 44% of the EMG signal in monopolar mode. These levels of crosstalk are relevant and might lead to misinterpretation of the EMG signal. The efficacy of surface EMG to measure the activity level of a muscle has been questioned before [36]. For instance, surface EMG suggested an activation of the rectus femoris during gait whereas intramuscular EMG did not detect activity for this muscle [5]. A similar result was found for the sternocleidomastoid muscle during progressive inspiratory task, where surface EMG mainly registered crosstalk from other muscles [37].

In addition to a crosstalk influence on the activity level measured on the EMG signal, crosstalk caused overestimation on the cross-correlation between the EMG amplitude from pairs of muscles. Intermuscular correlation is commonly used to address muscle synergy and, thus, should be interpreted cautiously [6,22]. It is known that the crosstalk signal does not resemble the source signal due to changes in the MUAP shape with distance [2], which is why cross-correlation is not recommended to estimate the amount of crosstalk [2,7]. Nonetheless, we evaluated the cross-correlation between EMG envelopes, which is minimally influenced by the individual action potential waveform shapes. Regardless of changes in the shape of the detected signals (as illustrated in Figure 1B), crosstalk contributed to the amplitude modulation (envelope) of the EMG and overestimated the estimation of intermuscular correlation. An important consideration is that crosstalk is mainly determined by the extinction of the action potentials (end-of-fiber effects), which represent the non-propagating component of the source [2].

Therefore, we may suggest that EMG will be correlated if the end-of-fiber effect is detected over the source and the contaminated nearby muscle. This can be minimized by electrode placement away from the musculotendinous junction [9] but is unavoidable for some muscles due to the pennation angle of the fibers. This is why crosstalk is a hindrance to EMG measurements. The orientation of the fibers oblique to the skin predominates the end-of-fiber effect. The amplitude of the non-propagating component also depends on the electrode location, fat thickness, and fiber depth [2,8]. Such factors could determine the effect of crosstalk on the cross-correlation analysis and could explain the different results between muscle pairs.

Moreover, our data suggest that individual motor unit crosstalk amplitude is confined within the range 20 μV to 300 μV regardless of muscle and motor unit action potential amplitude. Therefore, it is intuitive to expect that cross-correlation and other metrics will be less affected by crosstalk if the background activity is higher and the signal-to-noise ratio is lower. This is because crosstalk has a low power relative to the EMG signals from the target muscle. A lower relative power of crosstalk is expected when more motor units in the targeted muscle are recruited (since the target muscle would produce EMG with greater power), thus reducing the relative influence of crosstalk.

In the frequency domain, crosstalk removal significantly increased the median frequency of the EMG power spectrum for all evaluated muscles, but the shift was small (maximum 4 Hz). Our findings are in agreement with previous results from the FDI and the abductor pollicis brevis muscles in which the power spectra were almost identical before and after crosstalk removal [10]. Notwithstanding, the power spectrum for the non-rectified EMG was more robust to crosstalk, showing an even smaller but significant shift in the median frequency (about 0.3 Hz, data not shown). Furthermore, the removal of crosstalk slightly reduced the relative area of the rectified EMG power spectrum in the alpha and beta bands (data not shown; see Figure 4). These findings could be attributed to the high-frequency content of crosstalk from the non-propagating signal [8,38], but also indicate that crosstalk also carries low-frequency components that might be related to the volume conduction effect. In sum, crosstalk induced a small, but significant shift on the median frequency of the EMG power spectrum, but the effect unlikely leads to signal misinterpretations. From the results, we may suggest that crosstalk mainly influences time domain analysis, especially metrics of muscle activity (amplitude). Importantly, however, the results on EMG power spectra clearly indicate that high-pass filtering of EMG signals is not effective in reducing crosstalk due to the end-of-fiber effect, as also concluded previously on a theoretical basis [2,8,16,39].

4.3 Effect of spatial filters on EMG crosstalk

Spatial filters reduced the amplitude of the *Cross* MUAP and the proportion of motor units that were identified as crosstalk. Consequently, the relative contribution of crosstalk to the recorded EMG signal decreased with the use of SD and DD with respect to monopolar mode. These results corroborate previous suggestions for reducing crosstalk [1,8,40,41]. DD is recommended due to its high selectivity and rejection of common signal components [1], particularly important to filter the non-propagating components that determine crosstalk. Spatial filters also minimized the overestimations of the intermuscular correlation for most muscle pairs, and DD effectively abolished this overestimation for the FDI-thenar, GL-SOL, and GM-SOL. It should be pointed out that DD is sensitive to interelectrode distance, orientation relative to the fiber, and distance to the end plate [9]. These factors might explain why DD was less effective in minimizing the crosstalk between VL and VM. For the thigh muscles, the two HD sEMG grids were almost orthogonal to each other. Consequently, the double differentiation direction was nearly perpendicular to the source of the crosstalk (fibers from the neighboring muscle) and, thus, attenuates crosstalk to a lesser extent [41]. Overall, it is particularly difficult to predict the effect of any spatial filter on the non-propagating signal [9], yet, our results confirm that DD is a convenient method to reduce crosstalk for the targeted muscle pairs.

4.4 Methods to quantify crosstalk

We presented different methods to quantify crosstalk with the use of individual motor units decomposed from the HD sEMG. The potential of a muscle in contaminating the EMG signal of another muscle was measured by the proportion of motor units with significant crosstalk. To our knowledge, this is the first time that crosstalk significance is evaluated on a statistical basis for single motor units. Moreover, with the reconstructed crosstalk signal (*Cross* EMG), we could quantify the level of contamination and the influence of crosstalk on the EMG signal interpretation. The latter has been addressed before [10], however, the crosstalk signal in the referred paper was blindly estimated by source separation without direct validation. Conversely, in the present study, *Cross* EMG was reconstructed from the trains of action potentials of decomposed motor units.

The commonly used method to quantify crosstalk based on the ratio between peak-to-peak amplitude of the *Cross MUAP* and MUAP (the index of crosstalk) can lead to erroneous interpretation. Here we showed that the index of crosstalk is not indicative of the amount of crosstalk and cannot be used to compare crosstalk among muscles. For instance, one can wrongly suggest that GL induces more crosstalk to GM EMG based on the index of crosstalk when, in fact, nearly 40% of the signal recorded over GL is GM crosstalk. The reason for the GL crosstalk index being greater than GM is the smaller MUAP amplitude. Therefore, it is important to remember that this value cannot disentangle the absolute proportion of crosstalk from different muscles.

4.5 Limitations

Some methodological aspects require consideration. First, the crosstalk estimation depends on the number of identified motor units. Although we interpret our results as representative for each muscle, the *Cross* EMG might be underestimated. Moreover, the method only accounted for the crosstalk from the motor units within the electrodes' pick-up volume and did not consider different regions of activation and their crosstalk. For instance, the soleus muscle comprises four compartments [32], but in this study, we only recorded the activity over the medial-posterior compartment. Furthermore, due to task-specificity, the medial-posterior compartment could be less active during the plantar-flexion task than the not-recorded compartments. Second, our participants' sample was predominantly male (only one female), which imposes a limitation on the generalizability of the study. Anatomical differences between sexes, such as subcutaneous tissue thickness, may influence crosstalk and could lead to slightly different outcomes. We believe the main findings of the study are sufficiently general, but further evaluations would need to specifically consider sex-related differences. Third, due to the simultaneous contraction, motor units across muscles could have synchronized activity, as reported for FDI-thenar [6] and VL-VM [42] muscles, but less in the calf muscles [18]. Short-term synchronization biases the STA-derived motor unit action potentials amplitude and width [43], and could potentially affect the crosstalk estimates in this study. Synchronization also biases the correlation analysis. Therefore, it is possible that the metric of crosstalk quantification based on the correlation between EMG and *Cross* EMG can be overestimated. Yet, we consider that the effect would be negligible due to differences in the *Cross* MUAP and MUAP shapes. Regarding the intermuscular correlation, it is important to stress that we did not propose cross-correlation between EMG envelopes as a metric to quantify crosstalk. Rather, we showed that this commonly used metric, which is a measure of synchronized activity, is affected by crosstalk. Finally, it was not the purpose of the study to investigate in-depth aspects of the motor unit action potential and its crosstalk. For instance, we did not aim at separating propagating and non-propagating components and their role in crosstalk. Also, we did not evaluate the effect of the volume conductor on the spatial distribution of the motor unit action potentials. These detailed biophysical analyses will be the subject of future studies.

5. Conclusion

Crosstalk contaminated the surface EMG for all the evaluated muscle pairs. The contamination level depended on the EMG amplitude of the targeted muscle. Crosstalk overestimated muscle activity and intermuscular correlation analysis. The influence of crosstalk on the EMG power spectrum was significant, but small, and indicates that conventional temporal filtering of EMG signals does not minimize crosstalk. Conversely, spatial filter (SD and DD) is an effective and simple technique to reduce crosstalk.

Acknowledgements

CMG was the recipient of a postdoctoral scholarship from Instituto Eldorado (Campinas, Brazil). LAE is supported by CNPq (Brazilian NSF, 312442/2017-3), and received a research grant from FAPESP (The Sao Paulo Research Foundation, 2017/22191-3). FH is supported by a fellowship from the *Institut Universitaire de France* (IUF) and received support from the French national research agency (ANR-19-CE17-002-01, COMMODE project).

References

[1] De Luca C J and Merletti R 1988 Surface myoelectric signal cross-talk among muscles of the leg *Electroencephalogr. Clin. Neurophysiol.* **69** 568–75

[2] Farina D, Merletti R, Indino B and Graven-Nielsen T 2004 Surface EMG Crosstalk Evaluated from Experimental Recordings and Simulated Signals: Reflections on Crosstalk Interpretation, Quantification and Reduction *Methods Inf. Med.* **43** 30–5

[3] Talib I, Sundaraj K, Lam C K, Hussain J and Ali M A 2019 A review on crosstalk in myographic signals *Eur. J. Appl. Physiol.* **119** 9–28

[4] Enoka R M and Duchateau J 2015 Inappropriate interpretation of surface EMG signals and muscle fiber characteristics impedes understanding of the control of neuromuscular function *J. Appl. Physiol.* **119** 1516–8

[5] Barr K M, Miller A L and Chapin K B 2010 Surface electromyography does not accurately reflect rectus femoris activity during gait: Impact of speed and crouch on vasti-to-rectus crosstalk *Gait Posture* **32** 363–8

[6] Del Vecchio A, Germer C M, Elias L A, Fu Q, Fine J, Santello M and Farina D 2019 The human central nervous system transmits common synaptic inputs to distinct motor neuron pools during non-synergistic digit actions *J. Physiol.* **597** 5935–48

[7] Lowery M M, Stoykov N S and Kuiken T A 2003 A simulation study to examine the use of cross-correlation as an estimate of surface EMG cross talk *J. Appl. Physiol.* **94** 1324–34

[8] Farina D, Merletti R, Indino B, Nazzaro M and Pozzo M 2002 Surface EMG crosstalk between knee extensor muscles: Experimental and model results *Muscle Nerve* **26** 681–95

[9] Farina D, Cescon C and Merletti R 2002 Influence of anatomical, physical, and detection-system parameters on surface EMG. *Biol. Cybern.* **86** 445–56

[10] Kilner J M, Baker S N and Lemon R N 2001 A novel algorithm to remove electrical cross-talk between surface EMG recordings and its application to the measurement of short-term synchronisation in humans *J. Physiol.* **583** 919–30

[11] Vieira T M, Botter A, Muceli S and Farina D 2017 Specificity of surface EMG recordings for gastrocnemius during upright standing *Sci. Rep.* **7** 1–11

[12] Raiteri B J, Cresswell A G and Lichtwark G A 2015 Ultrasound reveals negligible cocontraction during isometric plantar flexion and dorsiflexion despite the presence of antagonist electromyographic activity *J. Appl. Physiol.* **118** 1193–9

[13] Selvanayagam V S, Riek S and Carroll T J 2012 A systematic method to quantify the presence of cross-talk in stimulus-evoked EMG responses: Implications for TMS studies *J. Appl. Physiol.* **112** 259–65

[14] Staudenmann D and Taube W 2015 Brachialis muscle activity can be assessed with surface electromyography *J. Electromyogr. Kinesiol.* **25** 199–204

[15] Mezzarane R A and Kohn A F 2009 A method to estimate EMG crosstalk between two muscles based on the silent period following an H-reflex *Med. Eng. Phys.* **31** 1331–6

[16] Farina D, Merletti R and Enoka R M 2014 The extraction of neural strategies from the surface EMG: an update *J. Appl. Physiol.* **117** 1215–30

[17] Nuccio S, Del Vecchio A, Casolo A, Labanca L, Rocchi J E, Felici F, Macaluso A, Mariani P P, Falla D, Farina D and Sbriccoli P 2020 Muscle fiber conduction velocity in the vastus lateralis and medialis muscles of soccer players after ACL reconstruction *Scand. J. Med. Sci. Sport.* **30** 1976–84

[18] Hug F, Del Vecchio A, Avrillon S, Farina D and Tucker K 2021 Muscles from the same muscle group do not necessarily share common drive: evidence from the human triceps surae *J. Appl. Physiol.* **130** 342–54

[19] Holobar A and Zazula D 2007 Multichannel blind source separation using convolution Kernel compensation *IEEE Trans. Signal Process.* **55** 4487–96

[20] Del Vecchio A, Holobar A, Falla D, Felici F, Enoka R M and Farina D 2020 Tutorial: Analysis of motor unit discharge characteristics from high-density surface EMG signals *J. Electromyogr. Kinesiol.* **53** 102426

[21] McKean H E 1969 Statistical Tables *Technometrics* **11** 841–2

[22] Laine C M and Valero-Cuevas F J 2017 Intermuscular Coherence Reflects Functional Coordination *J. Neurophysiol.* jn.00204.2017

[23] Santo Neto H, Meciano Filho J, Passini R and Marques M J 2004 Number and Size of Motor Units in Thenar Muscles *Clin. Anat.* **17** 308–11

[24] Feinstein B, Lindegard B, Nyman E and Wohlfard G 1955 Morphologic studies of motor units in normal human muscles *Acta Anat.* 127–42

[25] Kerkhof F D, van Leeuwen T and Vereecke E E 2018 The digital human forearm and hand *J. Anat.* **233** 557–66

[26] Winter D A, Fuglevand A J and Archer S E 1994 Crosstalk in surface electromyography: Theoretical and practical estimates *J. Electromyogr. Kinesiol.* **4** 15–26

[27] Ward S R, Eng C M, Smallwood L H and Lieber R L 2009 Are current measurements of lower extremity muscle architecture accurate? *Clin. Orthop. Relat. Res.* **467** 1074–82

[28] Hug F, Goupille C, Baum D, Raiteri B J, Hodges P W and Tucker K 2015 Nature of the coupling between neural drive and force-generating capacity in the human quadriceps muscle *Proc. R. Soc. B Biol. Sci.* **282**

[29] Blazevich A J, Gill N D and Zhou S 2006 Intra- and intermuscular variation in human quadriceps femoris architecture assessed in vivo *J. Anat.* **209** 289–310

[30] Héroux M E, Dakin C J, Luu B L, Inglis J T and Blouin J 2014 Absence of lateral gastrocnemius activity and differential motor unit behavior in soleus and medial gastrocnemius during standing balance *J. Appl. Physiol.* **116** 140–8

[31] Fukunaga T, Roy R R, Shellock F G, Hodgson J A, Day M K, Lee P L, Kwong-Fu H and Edgerton V R 1992 Physiological cross-sectional area of human leg muscles based on magnetic resonance imaging *J. Orthop. Res.* **10** 926–34

[32] Bolsterlee B, Finni T, D'Souza A, Eguchi J, Clarke E C and Herbert R D 2018 Three-dimensional architecture of the whole human soleus muscle in vivo *PeerJ* **2018** 1–22

[33] Gollnick P D, Sjödin B, Karlsson J, Jansson E and Saltin B 1974 Human soleus muscle: A comparison of fiber composition and enzyme activities with other leg muscles *Pflügers Arch. Eur. J. Physiol.* **348** 247–55

[34] Vieira T M M, Loram I D, Muceli S, Merletti R and Farina D 2011 Postural activation of the human medial gastrocnemius muscle: are the muscle units spatially localised ? *J. Physiol.* **2** 431–43

[35] Olewnik Ł, Zielinska N, Paulsen F, Podgórski M, Haładaj R, Karauda P and Polguj M 2020 A proposal for a new classification of soleus muscle morphology *Ann. Anat.* **232**

[36] Hug F 2011 Can muscle coordination be precisely studied by surface electromyography? *J. Electromyogr. Kinesiol.* **21** 1–12

[37] Chiti L, Biondi G, Morelot-Panzini C, Raux M, Similowski T and Hug F 2008 Scalene muscle activity during progressive inspiratory loading under pressure support ventilation in normal humans *Respir. Physiol. Neurobiol.* **164** 441–8

[38] Dimitrova N A, Dimitrov G V. and Nikitin O A 2002 Neither high-pass filtering nor mathematical differentiation of the EMG signals can considerably reduce cross-talk *J. Electromyogr. Kinesiol.* **12** 235–46

[39] Farina D, Merletti R and Enoka R M 2004 The extraction of neural strategies from the surface EMG *J. Appl. Physiol.* **96** 1486–95

[40] Koh T J and Grabiner M D 1993 Evaluation of methods to minimize cross talk in surface electromyography *J. Biomech.* **26** 151–7

[41] van Vugt J P and van Dijk J G 2001 A convenient method to reduce crosstalk in surface EMG. *Clin. Neurophysiol.* **112** 583–92

[42] Laine C M, Martinez-Valdes E, Falla D, Mayer F and Farina D 2015 Motor Neuron Pools of Synergistic Thigh Muscles Share Most of Their Synaptic Input *J. Neurosci.* **35** 12207–16

[43] Westad C and Westgaard R H 2005 The influence of contraction amplitude and firing history on spike-triggered averaged trapezius motor unit potentials *J. Physiol.* **562** 965–75

Figure 1. (A) Schematic representation of high-density surface EMG grids placement and electrode selection used to estimate the monopolar, single differential and double differential derivations for the EMG signal. (B) Analysis of individual motor unit crosstalk. A representative motor unit spike train (MUST) from vastus medialis muscle (VM) was used to trigger the VM EMG by spike-triggered averaging (STA). The crosstalk from the motor unit into the vastus lateralis (VL) EMG was also estimated by STA. The significance of the motor unit crosstalk was tested by comparing the selected cross MUAP amplitude to the amplitude obtained from triggering two hundred shuffling versions of the VM MUST (MUSTrand) on the VL EMG signal. The cross MUAP was considered significant if its amplitude was above the 95th percentile of the amplitude distribution for the random estimations. The crosstalk index (CI) for individual motor units with significant crosstalk was estimated by the ratio between the peak-to-peak amplitude of the Cross MUAP and MUAP. (C) Representative reconstruction of the VM Synthetic EMG computed by summing all decomposed VM motor unit action potential trains (MUAPT). The representative crosstalk from VL into VM EMG (Cross EMG) was estimated as the summation of the crosstalk (cMUAPT) from all decomposed VL motor units in the VM EMG (note the amplified scale of the Cross EMG). The Synthetic EMG and Cross EMG (or Clean EMG, see Methods) were compared with the original EMG.

Figure 2. (A) Proportion of motor units with significant crosstalk for the EMG in monopolar (Mono), single differential (SD) and double differential (DD) modes. Numbers above bars indicate the number of motor units decomposed for each muscle. First muscle in the legend is the targeted (control) and the second muscle is where the crosstalk was estimated. (B) Peak-to-peak amplitude of individual motor unit crosstalk (Cross MUAP) relative to the motor unit action potential (MUAP). Significant linear regressions are shown as continuous lines. Only motor units with significant cross MUAP are shown. (C) Motor unit crosstalk index (CI). Significant differences for EMG Muscle and Recording Mode ($p < 0.05$) are demarked by letters (a: Mono vs SD, b: Mono vs DD, c: SD vs DD). Grey dots indicate outliers.

Figure 3. Absolute Pearson's correlation coefficient ($|\rho|$) between the muscle's EMG and the global activity from its motor units (Synthetic EMG) (A), or the crosstalk from another muscle (Cross EMG, subscribed in the x-axis label) (B) Correlations were estimated for the EMG in monopolar (Mono), single differential (SD) and double differential (DD) recording modes. (C) Root mean square (RMS) for the Cross EMG relative to EMG in percent. Letters indicate significant differences between EMG Recording Mode ($p < 0.05$, a: Mono vs SD, b: Mono vs DD and c: SD vs DD).

Figure 4. Average power spectrum for different muscle EMGs in monopolar, single differential (SD) and double differential (DD) recording modes, evaluated on the original rectified EMGs and after crosstalk removal (Clean EMG). Note differences in the y-axis scale.

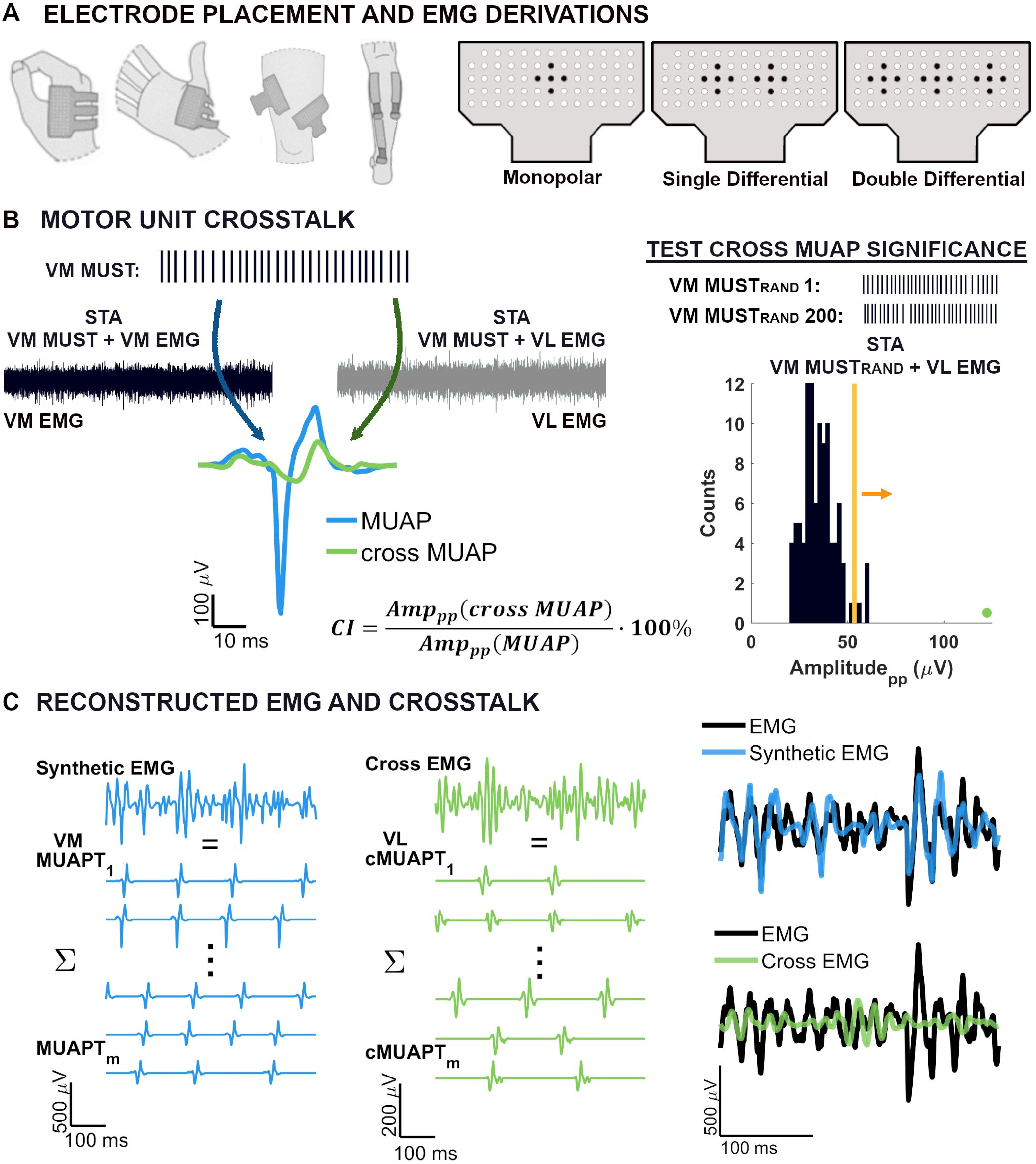
Figure 5. Absolute Pearson's correlation ($|\rho|$) between pairs of muscle EMGs in monopolar (M), single differential (SD) and double differential (DD) recording modes, evaluated on the recorded signal (EMG) and after crosstalk removal (Clean EMG). Error bars represent the 95% confidence interval ($n = 8$ for hand and thigh, $n = 9$ for calf muscles). Hash and asterisk indicate significant differences for EMG and Clean EMG ($p < 0.05$) for main effect (no significant interaction) and simple main effect (significant interaction), respectively.

Table 1. Linear regression analysis for Cross MUAP with MUAP amplitude for monopolar, single differential (SD) and double differential (DD) recording modes. Statistics from the homogeneity of regression slopes test (ANCOVA) for the cases with a clear correlation between Cross MUAP and MUAP amplitude and ANOVA for the cases with weak or no correlation. Bold numbers indicate significance ($p < 0.05$). Effect sizes are reported as the partial eta-squared (η^2).

		FDI to Thenar	Thenar to FDI	VL to VM	VM to VL	GL to GM	GL to SOL	GM to GL	GM to SOL	SOL to GL	SOL to GM
Monopolar	**N**	192	53	86	73	52	88	162	195	35	24
	Slope	0.020	0.158	0.264	0.194	0.153	0.244	0.150	0.139	-0.016	-0.026
	Offset	31.777	21.275	-0.629	14.036	53.024	91.496	77.876	124.000	72.441	66.686
	aR^2	0.077	0.285	0.385	0.440	0.076	0.108	0.098	0.062	-0.021	-0.015
	p	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.026**	**0.001**	**<0.001**	**<0.001**	0.584	0.423
SD	**N**	129	25	75	54	47	31	80	88	21	16
	Slope	0.020	0.031	-0.026	0.113	0.004	-0.006	0.015	0.014	0.027	-0.013
	Offset	13.705	17.902	19.769	13.412	48.176	49.327	26.73	48.958	35.053	38.606
	aR^2	0.136	0.075	0.031	0.305	-0.022	-0.033	0.057	0.001	0.020	0.084
	p	**<0.001**	0.090	0.071	**<0.001**	0.872	0.841	**0.018**	0.309	0.250	0.146
DD	**N**	168	27	99	57	26	21	59	63	10	10
	Slope	0.001	0.028	0.164	0.102	0.085	-0.030	0.006	<0.001	-0.072	-0.028
	Offset	25.681	70.025	16.188	25.041	40.754	80.850	35.272	63.777	80.973	69.326
	aR^2	0.042	-0.029	0.392	0.224	0.103	-0.040	-0.015	-0.016	0.028	0.212
	p	**0.011**	0.579	**<0.001**	**<0.001**	0.060	0.635	0.699	0.990	0.295	0.102
Homogeneity of regression slopes test / ANOVA		F(2,483)	F(2,102)	F(2,181)	F(2,178)	F(2,122)	F(2,137)	F(2,298)	F(2,343)	F(2,63)	F(2,47)
	F	5.454	27.457	38.399	3.024	21.466	59.963	128.304	174.707	10.493	20.388
	p	**0.005**	**<0.001**	**<0.001**	0.051	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	η^2	0.022	0.214	0.230	0.034	0.260	0.467	0.463	0.505	0.250	0.465
	M vs SD	**<0.001**	**<0.001**	**<0.001**		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	M vs DD	**<0.001**	**<0.001**	**0,001**		**0.001**	**<0.001**	**<0.001**	**<0.001**	0.764	>0.999
	SD vs DD	**<0.001**	**<0.001**	**<0.001**		0.430	0.106	>0.999	0.431	0.091	**<0.001**

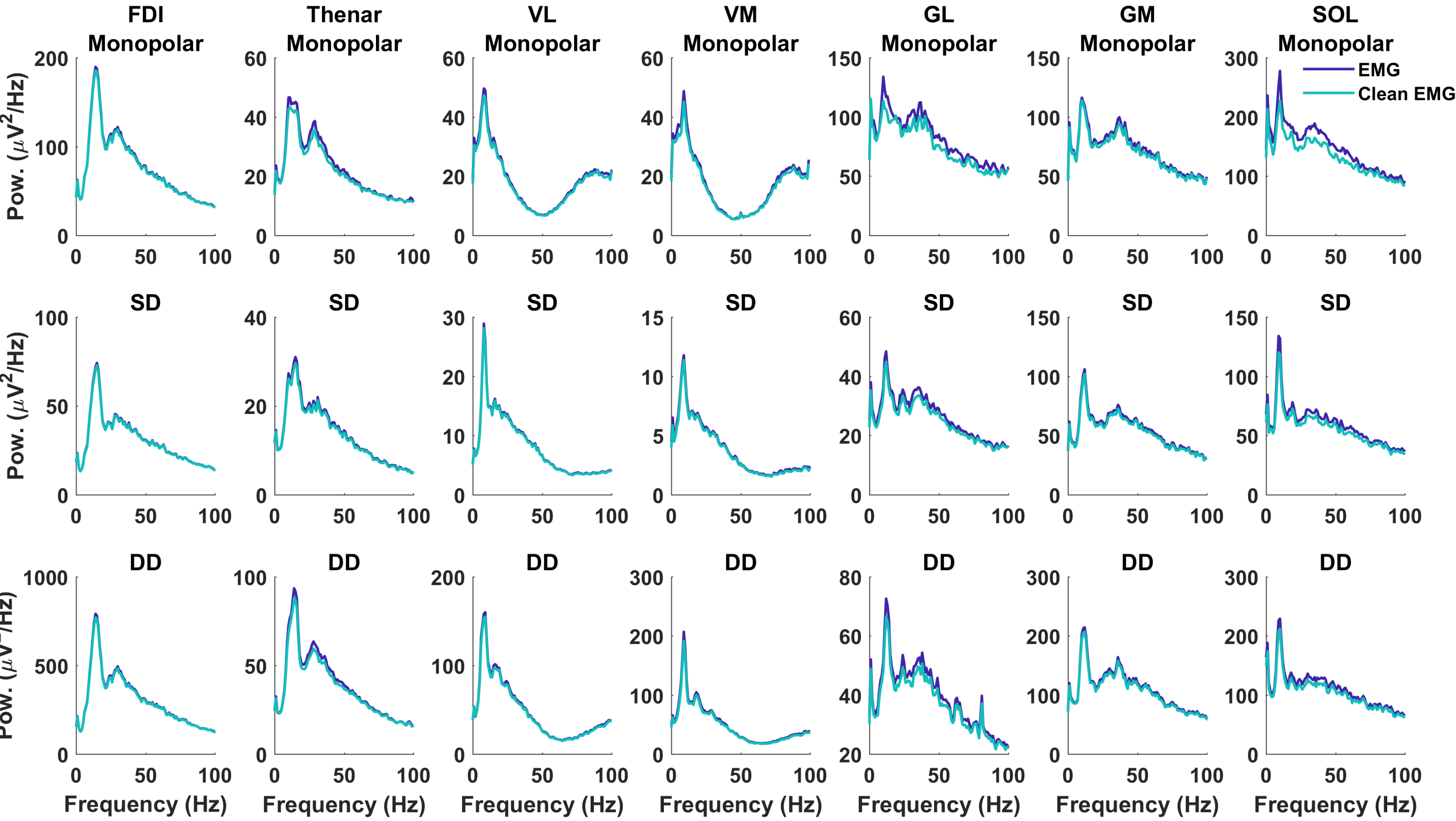
Table 2. Summary of the results from the analysis of the influence of crosstalk on the EMG signal, and the evaluation of the efficiency of single (SD) and double differential (DD) spatial filtering in reducing crosstalk.

	FDI-thenar	**VL-VM**	**GL-GM-SOL**	**Conclusion**
Surface EMG metrics:	- Crosstalk was more pronounced in thenar EMG signal. - Spatial filter reduced proportion of significant crosstalk. - Spatial filter reduced *Cross* MUAP amplitude from FDI but increased from thenar motor units. - Spatial filter reduced the relative contribution of crosstalk for thenar EMG signal. - Crosstalk slightly reduced the median frequency of the FDI and thenar EMG power spectrum. - Spatial filter did not reduce the influence of crosstalk on the EMG power spectrum.	- Similar amount of crosstalk between muscles. - Spatial filter reduced proportion of significant crosstalk. - Spatial filter slightly reduced *Cross* MUAP amplitude. - Spatial filter did not reduce the relative contribution of crosstalk (except SD for VL EMG). - Crosstalk slightly reduced the median frequency of the EMG power spectrum. - Spatial filter did not reduce the influence of crosstalk on the EMG power spectrum.	- Greater crosstalk from GM. - Spatial filter reduced proportion of significant crosstalk. - Spatial filter reduced *Cross* MUAP amplitude, except for SOL motor units. - Spatial filter reduced the relative contribution of crosstalk to the EMG signal for GL and SOL. - Crosstalk reduced the median frequency of the EMG power spectrum. - Spatial filter did not reduce the influence of crosstalk on the EMG power spectrum.	- Time domain: All EMG signals were contaminated by crosstalk. Spatial filters reduced the proportion of crosstalk and *Cross* MUAP amplitude for most muscles (except SOL) and reduced the relative contribution of crosstalk to the EMG for some muscles (thenar, GL and SOL). - Frequency domain: Crosstalk slightly increased the EMG median frequency for all muscles, and spatial filter did not reduce this effect.
Intermuscular correlation:	- Crosstalk led to an overestimation of cross-correlation between the two signals. - Spatial filter was effective in partially (SD) or totally (DD) reduce the effect.	- Crosstalk led to an overestimation of cross-correlation between the two signals. - Spatial filter was effective in partially (SD) reduce the effect.	- Crosstalk overestimated the cross-correlation between muscle pairs. - Except for GL-GM, spatial filter was effective in partially (SD) or totally (SD, DD) reduce the effect.	- Crosstalk biased correlation estimates, and spatial filtering partially or totally reduce this effect.


A ELECTRODE PLACEMENT AND EMG DERIVATIONS
Monopolar
Single Differential
Double Differential
B MOTOR UNIT CROSSTALK
VM MUST:
STA
VM MUST + VM EMG
STA
VM MUST + VL EMG
VM EMG
VL EMG
MUAP
cross MUAP
100 μV
10 ms
$CI = \frac{Amp_{pp}(cross\ MUAP)}{Amp_{pp}(MUAP)} \cdot 100\%$
TEST CROSS MUAP SIGNIFICANCE
VM MUSTRAND 1:
VM MUSTRAND 200:
STA
VM MUSTRAND + VL EMG
Counts
12
10
8
6
4
2
0
0
50
100
Amplitude_{pp} (μV)
C RECONSTRUCTED EMG AND CROSSTALK
Synthetic EMG
=
VM
$MUAPT_1$
Σ
$MUAPT_m$
500 μV
100 ms
Cross EMG
=
VL
$cMUAPT_1$
Σ
$cMUAPT_m$
200 μV
100 ms
EMG
Synthetic EMG
EMG
Cross EMG
500 μV
100 ms


A
Significant crosstalk (%)
100
50
0
196
68
115
75
96
96
200
200
79
79
MUs
FDI to thenar
Thenar to FDI
VL to VM
VM to VL
GL to GM
GL to SOL
GM to GL
GM to SOL
SOL to GL
SOL to GM
C
Crosstalk index (%)
300
200
100
0
Mono
SD
DD
b
bc
ab
ab
ab
FDI to thenar
Thenar to FDI
VL to VM
VM to VL
GL to GM
GL to SOL
GM to GL
GM to SOL
SOL to GL
SOL to GM
B
FDI to thenar
VL to VM
GL to GM
GM to GL
SOL to GL
Mono
SD
DD
Thenar to FDI
VM to VL
GL to SOL
GM to SOL
SOL to GM
Cross MUAP (µV)
400
300
200
100
0
0
1000
2000
MUAP (µV)


A
EMG vs Synthetic EMG
Mono
SD
DD
|ρ|
1
0.8
0.6
0.4
0.2
0
c
b
a b
a b
a b
b
FDI
Thenar
VL
VM
GL
GM
SOL
B
EMG vs Cross EMG
|ρ|
0.4
0.3
0.2
0.1
0
b c
b
a b
a b
a b
FDI thenar
Thenar FDI
VL VM
VM VL
GL GM
GL SOL
GM GL
GM SOL
SOL GL
SOL GM
C
Relative RMS (%)
80
60
40
20
0
a b
a b c
a b c
FDI
Thenar
VL
VM
GL
GM
SOL


FDI
Monopolar
Thenar
Monopolar
VL
Monopolar
VM
Monopolar
GL
Monopolar
GM
Monopolar
SOL
Monopolar
EMG
Clean EMG
SD
SD
SD
SD
SD
SD
SD
DD
DD
DD
DD
DD
DD
DD
Pow. (μV^2/Hz)
Pow. (μV^2/Hz)
Pow. (μV^2/Hz)
0
50
100
Frequency (Hz)
Frequency (Hz)
Frequency (Hz)
Frequency (Hz)
Frequency (Hz)
Frequency (Hz)
Frequency (Hz)


FDI-Thenar
VL-VM
GL-GM
GL-SOL
GM-SOL
|ρ|
0
0.2
0.4
0.6
0.8
EMG_M
Clean EMG_M
EMG_SD
Clean EMG_SD
EMG_DD
Clean EMG_DD
*
#

Table 1. Linear regression analysis for Cross MUAP with MUAP amplitude for monopolar, single differential (SD) and double differential (DD) recording modes. Statistics from the homogeneity of regression slopes test (ANCOVA) for the cases with a clear correlation between Cross MUAP and MUAP amplitude and ANOVA for the cases with weak or no correlation. Bold numbers indicate significance ($p < 0.05$). Effect sizes are reported as the partial eta-squared (η^2).

		FDI to Thenar	**Thenar to FDI**	**VL to VM**	**VM to VL**	**GL to GM**	**GL to SOL**	**GM to GL**	**GM to SOL**	**SOL to GL**	**SOL to GM**
Monopolar	**N**	192	53	86	73	52	88	162	195	35	24
	Slope	0.020	0.158	0.264	0.194	0.153	0.244	0.150	0.139	-0.016	-0.026
	Offset	31.777	21.275	-0.629	14.036	53.024	91.496	77.876	124.000	72.441	66.686
	aR^2	0.077	0.285	0.385	0.440	0.076	0.108	0.098	0.062	-0.021	-0.015
	p	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.026**	**0.001**	**<0.001**	**<0.001**	0.584	0.423
SD	**N**	129	25	75	54	47	31	80	88	21	16
	Slope	0.020	0.031	-0.026	0.113	0.004	-0.006	0.015	0.014	0.027	-0.013
	Offset	13.705	17.902	19.769	13.412	48.176	49.327	26.73	48.958	35.053	38.606
	aR^2	0.136	0.075	0.031	0.305	-0.022	-0.033	0.057	0.001	0.020	0.084
	p	**<0.001**	0.090	0.071	**<0.001**	0.872	0.841	**0.018**	0.309	0.250	0.146
DD	**N**	168	27	99	57	26	21	59	63	10	10
	Slope	0.001	0.028	0.164	0.102	0.085	-0.030	0.006	<0.001	-0.072	-0.028
	Offset	25.681	70.025	16.188	25.041	40.754	80.850	35.272	63.777	80.973	69.326
	aR^2	0.042	-0.029	0.392	0.224	0.103	-0.040	-0.015	-0.016	0.028	0.212
	p	**0.011**	0.579	**<0.001**	**<0.001**	0.060	0.635	0.699	0.990	0.295	0.102
Homogeneity of regression slopes test / ANOVA		$F(2,483)$	$F(2,102)$	$F(2,181)$	$F(2,178)$	$F(2,122)$	$F(2,137)$	$F(2,298)$	$F(2,343)$	$F(2,63)$	$F(2,47)$
	F	5.454	27.457	38.399	3.024	21.466	59.963	128.304	174.707	10.493	20.388
	p	**0.005**	**<0.001**	**<0.001**	0.051	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	η^2	0.022	0.214	0.230	0.034	0.260	0.467	0.463	0.505	0.250	0.465
	M vs SD	**<0.001**	**<0.001**	**<0.001**		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	M vs DD	**<0.001**	**<0.001**	**0,001**		**0.001**	**<0.001**	**<0.001**	**<0.001**	0.764	>0.999
	SD vs DD	**<0.001**	**<0.001**	**<0.001**		0.430	0.106	>0.999	0.431	0.091	**<0.001**

Table 1. Summary of the results from the analysis of the influence of crosstalk on the EMG signal, and the evaluation of the efficiency of single (SD) and double differential (DD) spatial filtering in reducing crosstalk.

	FDI-thenar	**VL-VM**	**GL-GM-SOL**	**Conclusion**
Surface EMG metrics:	- Crosstalk was more pronounced in thenar EMG signal. - Spatial filter reduced proportion of significant crosstalk. - Spatial filter reduced *Cross* MUAP amplitude from FDI but increased from thenar motor units. - Spatial filter reduced the relative contribution of crosstalk for thenar EMG signal. - Crosstalk slightly reduced the median frequency of the FDI and thenar EMG power spectrum. - Spatial filter did not reduce the influence of crosstalk on the EMG power spectrum.	- Similar amount of crosstalk between muscles. - Spatial filter reduced proportion of significant crosstalk. - Spatial filter slightly reduced *Cross* MUAP amplitude. - Spatial filter did not reduce the relative contribution of crosstalk (except SD for VL EMG). - Crosstalk slightly reduced the median frequency of the EMG power spectrum. - Spatial filter did not reduce the influence of crosstalk on the EMG power spectrum.	- Greater crosstalk from GM. - Spatial filter reduced proportion of significant crosstalk. - Spatial filter reduced *Cross* MUAP amplitude, except for SOL motor units. - Spatial filter reduced the relative contribution of crosstalk to the EMG signal for GL and SOL. - Crosstalk reduced the median frequency of the EMG power spectrum. - Spatial filter did not reduce the influence of crosstalk on the EMG power spectrum.	- Time domain: All EMG signals were contaminated by crosstalk. Spatial filters reduced the proportion of crosstalk and *Cross* MUAP amplitude for most muscles (except SOL) and reduced the relative contribution of crosstalk to the EMG for some muscles (thenar, GL and SOL). - Frequency domain: Crosstalk slightly increased the EMG median frequency for all muscles, and spatial filter did not reduce this effect.
Intermuscular correlation:	- Crosstalk led to an overestimation of cross-correlation between the two signals. - Spatial filter was effective in partially (SD) or totally (DD) reduce the effect.	- Crosstalk led to an overestimation of cross-correlation between the two signals. - Spatial filter was effective in partially (SD) reduce the effect.	- Crosstalk overestimated the cross-correlation between muscle pairs. - Except for GL-GM, spatial filter was effective in partially (SD) or totally (SD, DD) reduce the effect.	- Crosstalk biased correlation estimates, and spatial filtering partially or totally reduce this effect.