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

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## 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

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## 47 **New & Noteworthy**

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

## 54 **1. Introduction**

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

61 It is well known that crosstalk is influenced by anatomical features. For example, muscle fiber  
62 length and subcutaneous tissue thickness [7–9] are important influencing factors since they affect  
63 the spatial distribution of electrical activity from the sources to the recording electrodes.  
64 Typically, long fibers generate relatively large propagating components whereas short fibers  
65 generate action potentials dominated by non-propagating component. However, the propagating  
66 component decays faster with distance [7], so that the non-propagating component is the main  
67 determinant of crosstalk [2]. Therefore, the level of crosstalk greatly varies across muscles.  
68 Previous studies identified crosstalk on a limited number of muscle groups of the lower and upper

69 limbs [2,5,8,10–13], however, no study has systematically characterized crosstalk in different  
70 muscles.

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

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

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

## 92 2. Methodology

93 Three independent studies were conducted for the hand, knee extensor, and plantarflexor  
94 muscles. Volunteers with no history of neurological impairments gave their informed consent  
95 before the experiments. Procedures were in accordance with the *Declaration of Helsinki* and were  
96 approved by the local ethics committee (Imperial College Research Ethics Committee, N  
97 18IC4685; The University of Queensland Ethics Committee, 2013001448; Institutional Review  
98 Board of the University of Rome “Foro Italico”, N 2018/07).

### 99 2.1 Recording of high-density surface EMG

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

### 103 2.2 Experimental Protocols

#### 104 2.2.1 Hand muscles

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

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

### 118 2.2.2 Knee extensors

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

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

### 132 2.2.3 Plantarflexors (*triceps surae muscles*)

133 The experimental procedure is described in [18]. Nine participants ( $31 \pm 9$  years, all males)  
134 were asked to perform submaximal isometric plantarflexion contractions. They laid prone on a  
135 custom-made dynamometer equipped with a torque sensor (TRE-50K, Dacell, Korea). Their  
136 knee was fully extended, and their ankle angle was set to  $10^\circ$  of plantarflexion ( $0^\circ$  being the foot

137 perpendicular to the shank). Participants first performed three maximal isometric contractions  
138 for 3 to 5 s with 2 min rest in between. The maximal value obtained from a moving average  
139 window of 250 ms was considered as the MVC. Then, participants performed four contractions  
140 at 30 %MVC, which involved a 5-s ramp-up, a 30-s plateau and a 5-s ramp down phase. The  
141 contractions were separated by 120 s of rest. Participants received a visual feedback of the target  
142 contraction intensity and the torque output.

143 Two 13 x 5 flexible grids of electrodes (8-mm interelectrode distance, ELSCH064NM2, OT  
144 Bioelettronica) were placed over the gastrocnemius medialis (GM) and the gastrocnemius  
145 lateralis muscle (GL) and aligned with the main fascicle direction, as determined using B-mode  
146 ultrasound (Aixplorer, Supersonic Imagine, France). In addition, an 8x4 flexible grid of  
147 electrodes (10-mm interelectrode distance GR10MM0804, SpesMedica, Battipaglia, Italy) was  
148 placed on the medial part of the soleus (SOL), below the myotendinous junction of the GM  
149 muscle.

## 150 2.3 Data Analysis

### 151 2.3.1 HD surface EMG signals

152 The multi-channel EMG signals were visually inspected, and the channels with low signal-to-  
153 noise ratio were removed from the analysis (i.e., signals with power line interference and visible  
154 spurious activity). EMG signals were low-pass filtered at 500 Hz (fourth-order Butterworth)  
155 offline. The initial and final segments for force stabilization at the target level were discarded.  
156 For the hand muscles and knee extensors, we discarded the initial 9.5 s and 7 s, respectively. Due  
157 to the late recruitment of GL, initial 12.5 s (5-s ramp up and 7.5-s during plateau) were removed.  
158 Therefore, for each participant, we analyzed two trials of 50 s for FDI-thenar and VL-VM, and

159 four trials of 12.5 s for GL-GM-SOL. Two or three groups of five neighboring electrodes were  
160 used to estimate SD and DD EMG signals [9], respectively (Figure 1A).

### 161 2.3.2 EMG decomposition

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

### 173 2.3.3 Crosstalk features in each muscle

174 For all identified motor units of each muscle, we characterized the extent of crosstalk that  
175 contaminated the EMG of the neighboring muscle(s) (i.e., muscles within the same group). The  
176 motor unit action potential (MUAP) and the crosstalk MUAP (*Cross* MUAP) from each motor  
177 unit were extracted by spike-triggered averaging (STA) the multi-channel EMG signals (Figure  
178 1B). For example, motor unit discharge times identified from the HD EMG grids over the GM  
179 muscle were used to extract the two-dimensional action potential waveform for GM (MUAP),  
180 GL and SOL (*Cross* MUAPs). This procedure was iterated for all motor units and muscles and  
181 allowed us to quantify the amount of crosstalk for each motor unit. For each muscle, we pooled

182 all extracted motor units and estimated the proportion of motor units that induced significant  
183 crosstalk (as defined in 2.3.4). We also assessed the association between *Cross* MUAP and  
184 MUAP amplitudes by linear regression and computed a crosstalk index as the peak-to-peak  
185 amplitude ratio ( $Cross\ MUAP/MUAP \times 100\%$ ). The linear regression and the crosstalk index  
186 were estimated for motor units that showed significant *Cross* MUAP.

#### 187 *2.3.4 Method to assess crosstalk significance*

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

#### 199 *2.3.5 Influence of crosstalk on the surface EMG*

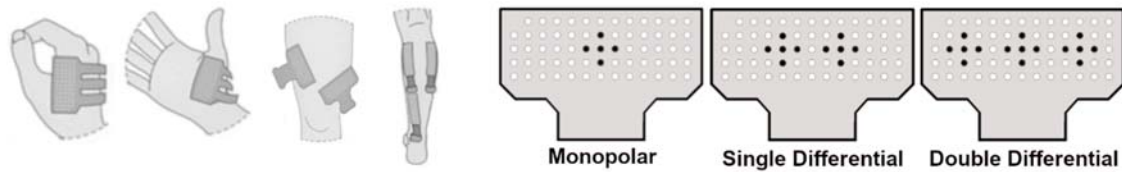
200 The influence of crosstalk on the global EMG signals was assessed for each individual muscle  
201 in time and frequency domains. For each muscle, we reconstructed the interference pattern from  
202 the decomposed motor units (*Synthetic* EMG), the crosstalk from each neighboring muscle  
203 (*Cross* EMG), and the EMG after crosstalk removal (*Clean* EMG). The *Synthetic* EMG was  
204 assessed by summing all motor unit action potential trains (Figure 1C), which were estimated by

205 convolving the MUAP obtained by STA on the EMG signal with the motor unit discharge times.  
206 Similarly, the *Cross* EMG from each neighboring muscle was reconstructed with the cross  
207 MUAP extracted by triggering the EMG signal with the discharge times from the neighboring  
208 motor units (Figure 1C). Lastly, *Clean* EMG was obtained by subtracting the neighboring  
209 muscle(s) *Cross* EMG from the original EMG. Note that the crosstalk from other active  
210 synergistic muscles were not included in the analysis (e.g., the knee extensors vastus intermedius  
211 and rectus femoris were not measured and, thus, were not considered for the *Clean* EMG  
212 calculation).

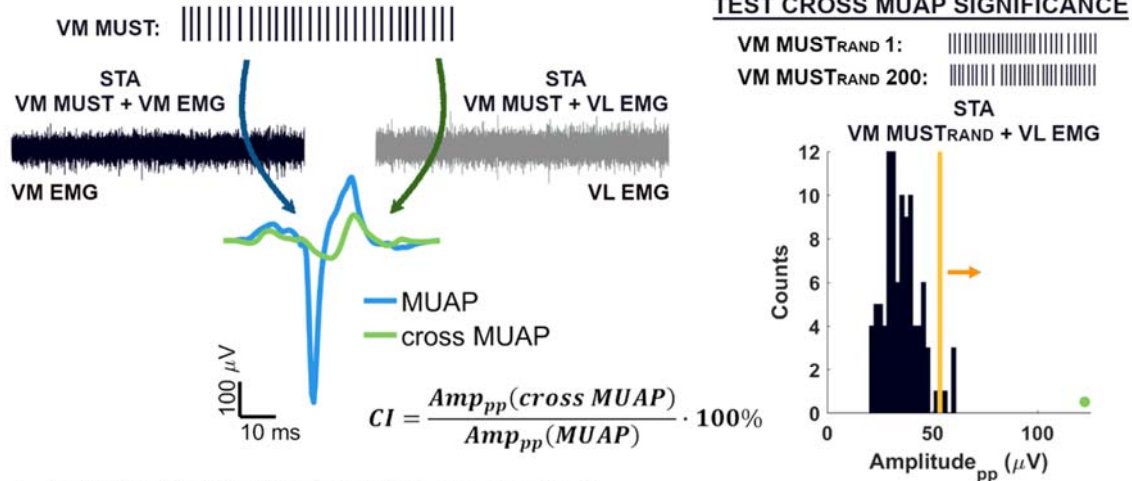
213 For each muscle, the total crosstalk magnitude that originated from the measured neighboring  
214 muscles was estimated as the sum of all neighboring muscle *Cross* EMG RMS normalized to its  
215 EMG RMS. Moreover, the EMG signal was compared to the *Synthetic* and *Cross* EMG using  
216 the Pearson's correlation coefficient ( $\rho$ ). These measures roughly indicate how much of the EMG  
217 signal can be attributed to the activity of the decomposed motor units and crosstalk, respectively.  
218 The critical value for an  $\alpha = 0.05$  of a large sample data was estimated by:  $r_{crit} =$   
219  $1.645/\sqrt{n - 2 + 1.645^2}$ , where  $n$  is the sample size [21].

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

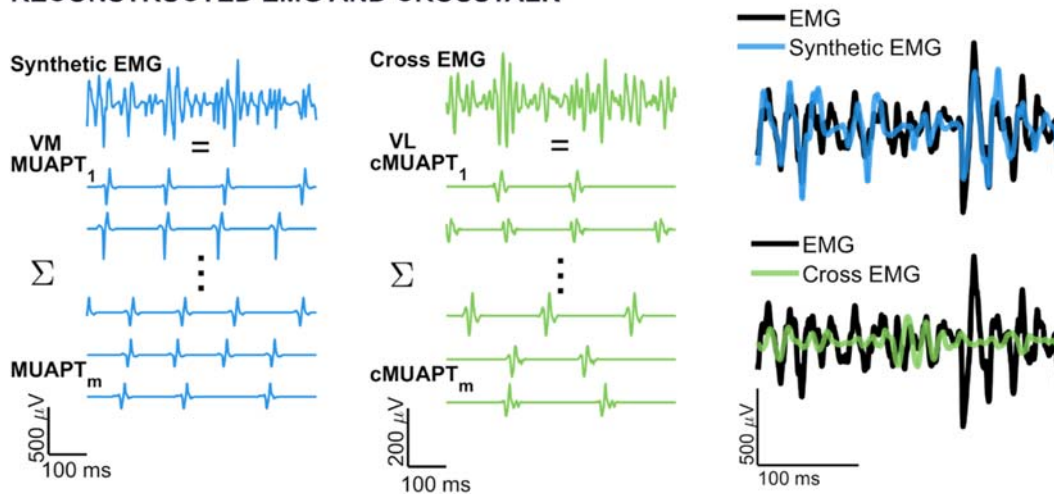
## A ELECTRODE PLACEMENT AND EMG DERIVATIONS



## B MOTOR UNIT CROSSTALK



## C RECONSTRUCTED EMG AND CROSSTALK



226

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

### 240 2.3.6 Crosstalk influence on intermuscular correlation

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

### 246 2.3.7 Statistical Analysis

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

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

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

258 The relative contribution of crosstalk to the EMG signal ( $|\rho|$  between EMG and *Cross* EMG)  
259 was evaluated with a mixed two-way ANOVA with the repeated factor being the *EMG Recording*  
260 *Mode* and *Muscle Pairs* as independent factor. Similarly, a mixed two-way ANOVA compared  
261 the EMG and *Synthetic* EMG  $|\rho|$  for the different *Muscles* (FDI, thenar, VL, VM, GL, GM, SOL)

262 and *EMG Recording Mode* as repeated factor. A mixed two-way ANOVA compared the EMG  
263 RMS and relative *Cross* EMG RMS for the *EMG Recording Mode* for the muscles in the hand,  
264 thigh and calf separately.

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

### 271 3. Results

272 In total, 739 motor units were decomposed (FDI: 196, thenar: 68, VL: 115, VM: 75, GL: 96,  
273 GM: 200, SOL: 79), with an average number of motor units per subject per contraction of  $12.25$   
274  $\pm 2.08$  for the FDI,  $4.25 \pm 0.98$  for the thenar,  $7.19 \pm 3.00$  for the VL,  $4.69 \pm 1.61$  for the VM,  
275  $9.00 \pm 3.58$  for the GL,  $20.30 \pm 4.72$  for the GM, and  $7.44 \pm 2.70$  for the SOL.

#### 276 3.1 Crosstalk features in different muscles

277 We evaluated the significance of crosstalk for all the identified motor units (Figure 1B). Only  
278 signals with amplitude greater than a critical value (95<sup>th</sup> percentile EMG amplitude estimated  
279 from random motor unit spike trains) were considered as *Cross* MUAPs. Notably, most motor  
280 units induced crosstalk on the EMG of nearby muscle for the monopolar recording mode  
281 (79.73%, pooled motor units from all muscles), but SD and DD showed a smaller proportion  
282 (50.42% and 41.69%, respectively) (Figure 2A).

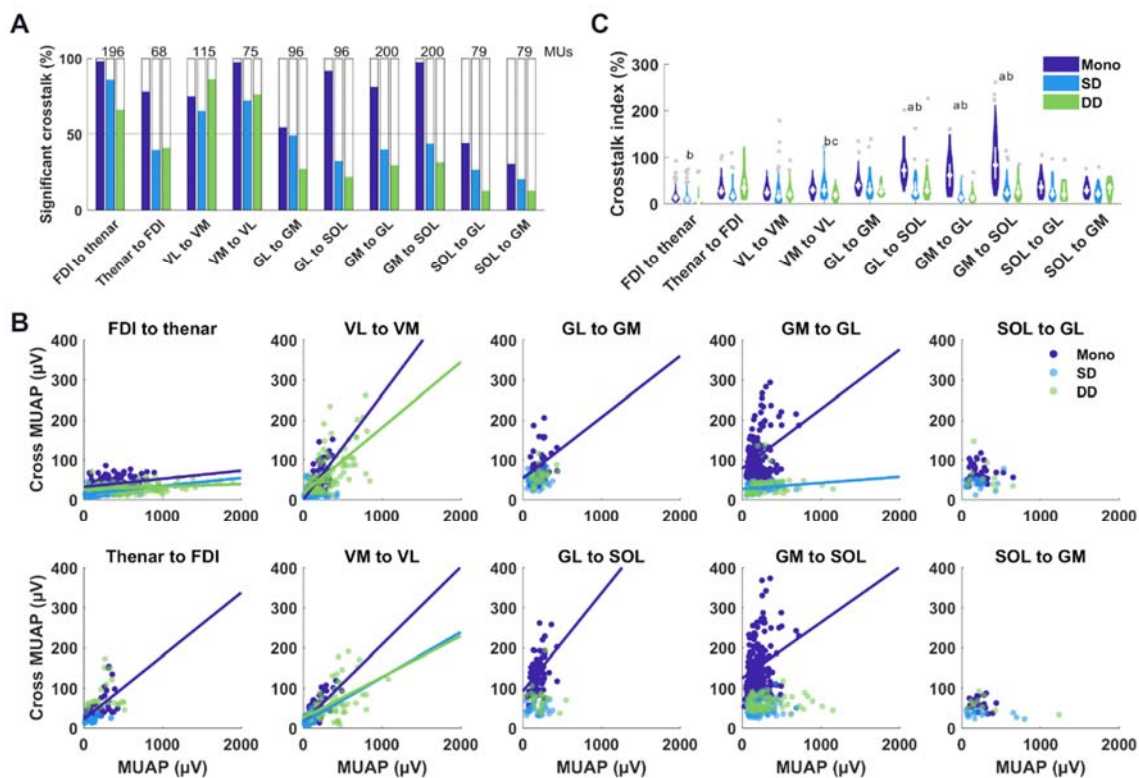
283 The amplitude values for the motor unit crosstalk (*Cross* MUAP) and original MUAP are  
284 shown in Figure 2B. The *Cross* MUAP was significantly correlated to the MUAP amplitude for  
285 most pair of muscles in monopolar (Table 1), indicating that motor units with higher amplitudes  
286 tend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

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

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

307 significant reduction in the *Cross* MUAP amplitude with SD and DD for some muscles (Figure  
308 2B).

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



315

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

323

## 324 3.2 Crosstalk effect on the EMG

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

### 332 3.2.1 Contribution of the decomposed motor units to the global interference EMG 333 signal

334 We synthesized the EMG from the spike trains of the decomposed motor units, as shown in  
335 Figure 1C. The *Synthetic* EMG signal corresponds to a crosstalk-free version of the EMG signal  
336 with contribution only from the decomposed motor units. Therefore, the background activity of  
337 the undecomposed action potentials and noise are removed. The *Synthetic* EMG was significantly  
338 correlated to the acquired EMG signal ( $r_{crit} = 0.005$ , Figure 3A). The correlation was moderate  
339 ( $|\rho|$  between 0.5 and 0.7) for most muscles, but weaker ( $|\rho|$  between 0.2 and 0.4) for GL and SOL.  
340 The weak correlation could be attributed to few motor units decomposed and/or high background  
341 noise (including crosstalk). In fact, high levels of GM crosstalk were observed on the GL and  
342 SOL EMG acquired in monopolar mode (see next section). SD increased the  $|\rho|$  for VM ( $p =$   
343 0.002), GL and GM ( $p < 0.001$ ), but not for the hand muscles ( $p > 0.999$ ), VL ( $p = 0.559$ ) and  
344 SOL ( $p = 0.062$ ). A greater effect was found for DD, with significant increase in the  $|\rho|$  for the  
345 thigh ( $p < 0.001$ ) and calf ( $p < 0.001$ ) muscles. In general, differentiating the EMG signals  
346 increases the correlation and the representativeness of motor unit activity.

## 347 3.2.2 Contribution of the crosstalk to the global interference EMG signal

348 We found significant correlations between the EMG and *Cross* EMG (Figure 3B). The relative  
349 contribution of crosstalk to the EMG signal depended on the muscle and *EMG Recording Mode*  
350 (significant interaction,  $p < 0.001$ ). Thenar EMG was more affected by the FDI activity than the  
351 opposite way ( $p = 0.052$ ,  $p < 0.001$ ,  $p = 0.002$ , for EMG in monopolar, SD, and DD, respectively).  
352 The two knee extensor muscles showed similar amounts of crosstalk ( $p > 0.276$ ). In the triceps  
353 surae muscle group, GM EMG was equally affected by GL and SOL crosstalk ( $p = 0.999$ , for all  
354 *EMG Recording Mode*), but a greater contribution from GM crosstalk was found in GL EMG ( $p$   
355  $< 0.007$ , for all *EMG Recording Mode*) and SOL EMG in DD ( $p = 0.157$ ,  $p = 0.032$ ,  $p = 0.026$ ,  
356 for EMG in monopolar, SD, and DD, respectively).

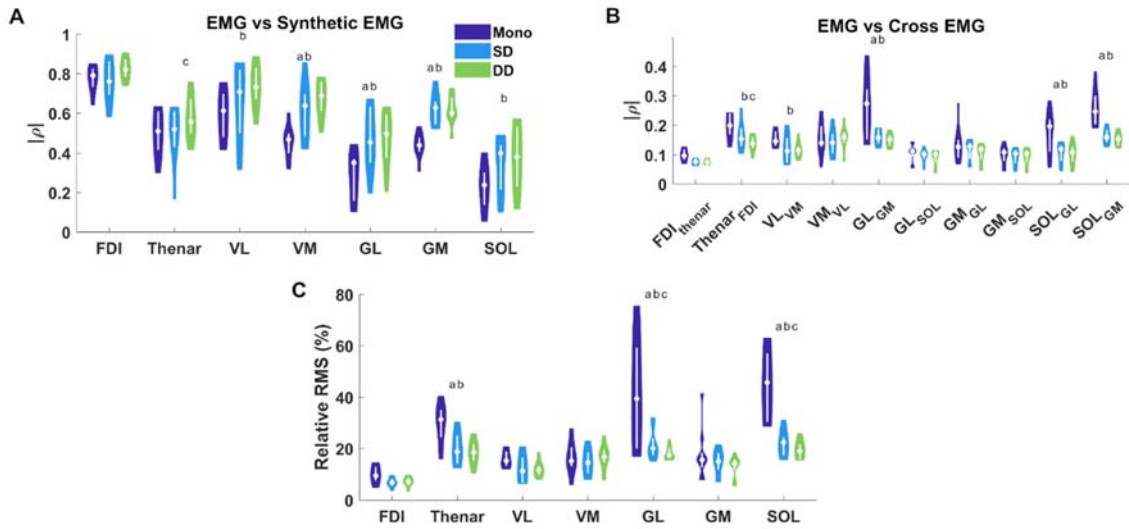
357 Spatial filtering reduced the crosstalk contribution on the EMG signal but not for all muscles  
358 ( $p < 0.001$ ). SD decreased the  $|\rho|$  for GL ( $p < 0.001$  from GM crosstalk) and SOL ( $p < 0.001$ ,  
359 from GL and GM crosstalk), and DD significantly decreased the  $|\rho|$  for thenar ( $p < 0.001$ ), VL ( $p$   
360  $= 0.040$ ), GL ( $p < 0.001$  for GM crosstalk) and SOL ( $p < 0.001$  for crosstalk from GL and GM).  
361 Reduction on the  $|\rho|$  with spatial filter was non-significant for FDI ( $p > 0.313$ , for SD and DD),  
362 VM ( $p = 0.999$ , for both SD and DD) and GM ( $p > 0.090$  for SD and DD for crosstalk from GL  
363 and SOL).

364 Interestingly, GL EMG in monopolar was equally correlated to the GL *Synthetic* EMG (Figure  
365 3A) and GM crosstalk (*Cross* EMG,  $GL_{GM}$  Figure 3B). Although this result may be partly  
366 influenced by the small number of identified motor units, it suggests that a significant part of the  
367 GL EMG signal originated from GM crosstalk during the task evaluated in the present study.  
368 Also, spatial filter decreased the crosstalk influence as demonstrated by a consistent increase in

369 the correlation to the GL *Synthetic* EMG and a decrease in the correlation to GM crosstalk with  
370 SD and DD.

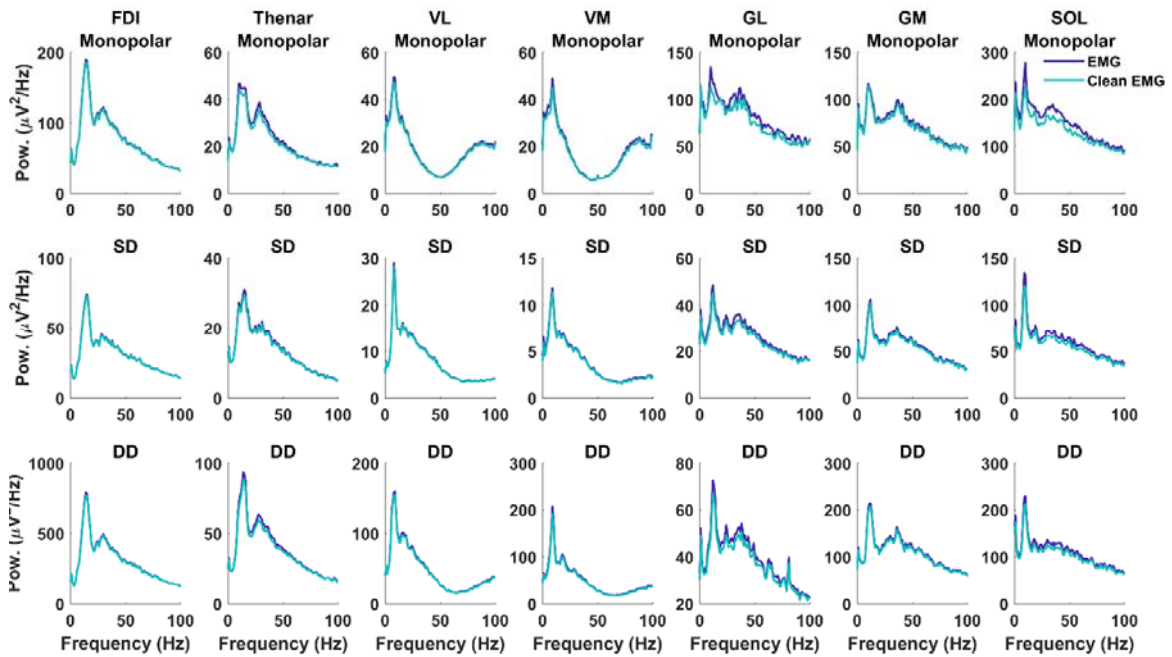
### 371 3.2.3 Relation between crosstalk and EMG amplitude

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



390

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



396

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

400

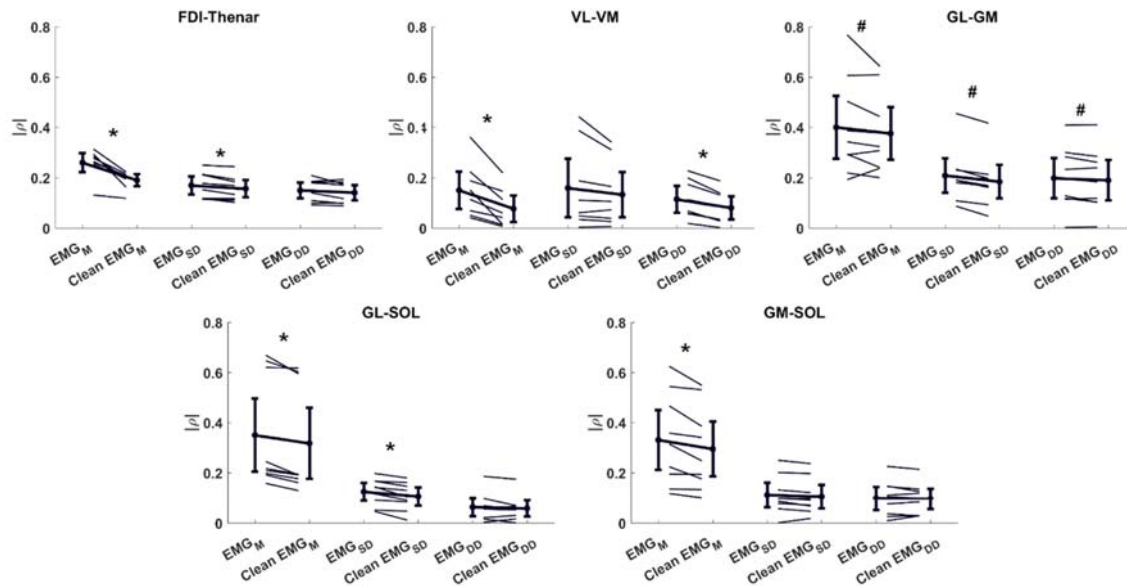
401

### 402 3.2.4 Effect of crosstalk on the EMG power spectrum

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

### 410 3.3 Influence of crosstalk on the intermuscular correlation

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



422

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

428

#### 429 4. Discussion

430 We evaluated crosstalk in the surface EMG for two muscles of the hand, and two groups of  
 431 synergistic muscles of the thigh and calf. We identified and quantified the level of crosstalk of  
 432 each muscle and the effect of each muscle in contaminating neighboring muscle activities. Our  
 433 results showed that all muscles were contaminated by crosstalk, but the magnitude of crosstalk  
 434 differed among muscles. We also found an asymmetric level of crosstalk between the muscles in  
 435 the hand and calf, but not in the thigh. This asymmetry was likely related to the difference in the  
 436 EMG amplitude. Moreover, crosstalk caused an overestimation of intermuscular correlation  
 437 between EMG envelopes for all muscle pairs. We also showed that spatial filtering effectively  
 438 reduced crosstalk effects in the time-domain metrics, especially the DD recording mode,  
 439 confirming it as a convenient method to minimize crosstalk. A summary of the effects of crosstalk  
 440 on the EMG metrics and on the influence of *EMG Recording Mode* is presented in Table 2. These

441 results add to the knowledge of crosstalk identification in different muscles and elucidate some  
442 of its confounding effects. In the following sections we present a discussion on each of these  
443 findings.

#### 444 *4.1 Crosstalk features in different muscle groups*

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

463 fiber length and orientation relative to the detection system [25] also determine the magnitude of  
464 the non-propagating components and might corroborate the asymmetric level of crosstalk found.

465 Albeit nearly 50% more motor units were identified in VL than VM, the two knee extensor  
466 muscles induced similar levels of crosstalk, which is consistent with previous studies [8,26].  
467 Noteworthy, the majority of the identified motor units from each muscle (more than 76%) was  
468 detected in EMG of the other muscle, even though VL and VM are separated by the rectus  
469 femoris and the vastus intermedius muscles. At a shared contraction level of 10 %MVC, both  
470 muscles had similar EMG amplitudes and the relative crosstalk corresponded to 14% of the EMG  
471 RMS. VL has a larger physiological cross-section area than VM [27,28], yet they have similar  
472 architecture (muscle thickness, fascicle angle and fiber length) at the distal region of the thigh  
473 [27,29], where the electrodes were placed. Therefore, similarities in the level of crosstalk might  
474 reflect similarities in the characteristics of the sources and activity levels.

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

487 activation [34] or muscle compartmentalization [32,35] could also have contributed to the  
488 asymmetric crosstalk features between the heads of the triceps surae.

#### 489 *4.2 Influence of crosstalk on the surface EMG*

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

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

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

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

526 In the frequency domain, crosstalk removal significantly increased the median frequency of  
527 the EMG power spectrum for all evaluated muscles, but the shift was small (maximum 4 Hz).  
528 Our findings are in agreement with previous results from the FDI and the abductor pollicis brevis  
529 muscles in which the power spectra were almost identical before and after crosstalk removal [10].  
530 Notwithstanding, the power spectrum for the non-rectified EMG was more robust to crosstalk,  
531 showing an even smaller but significant shift in the median frequency (about 0.3 Hz, data not  
532 shown). Furthermore, the removal of crosstalk slightly reduced the relative area of the rectified  
533 EMG power spectrum in the alpha and beta bands (data not shown; see Figure 4). These findings

534 could be attributed to the high-frequency content of crosstalk from the non-propagating signal  
535 [8,38], but also indicate that crosstalk also carries low-frequency components that might be  
536 related to the volume conduction effect. In sum, crosstalk induced a small, but significant shift  
537 on the median frequency of the EMG power spectrum, but the effect unlikely leads to signal  
538 misinterpretations. From the results, we may suggest that crosstalk mainly influences time  
539 domain analysis, especially metrics of muscle activity (amplitude). Importantly, however, the  
540 results on EMG power spectra clearly indicate that high-pass filtering of EMG signals is not  
541 effective in reducing crosstalk due to the end-of-fiber effect, as also concluded previously on a  
542 theoretical basis [2,8,16,39].

#### 543 *4.3 Effect of spatial filters on EMG crosstalk*

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

557 attenuates crosstalk to a lesser extent [41]. Overall, it is particularly difficult to predict the effect  
558 of any spatial filter on the non-propagating signal [9], yet, our results confirm that DD is a  
559 convenient method to reduce crosstalk for the targeted muscle pairs.

#### 560 4.4 Methods to quantify crosstalk

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

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

## 580 4.5 Limitations

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

604 study to investigate in-depth aspects of the motor unit action potential and its crosstalk. For  
605 instance, we did not aim at separating propagating and non-propagating components and their  
606 role in crosstalk. Also, we did not evaluate the effect of the volume conductor on the spatial  
607 distribution of the motor unit action potentials. These detailed biophysical analyses will be the  
608 subject of future studies.

## 609 5. Conclusion

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

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720

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

734 Figure 2. (A) Proportion of motor units with significant crosstalk for the EMG in monopolar (Mono), single differential (SD) and  
735 double differential (DD) modes. Numbers above bars indicate the number of motor units decomposed for each muscle. First muscle  
736 in the legend is the targeted (control) and the second muscle is where the crosstalk was estimated. (B) Peak-to-peak amplitude of  
737 individual motor unit crosstalk (Cross MUAP) relative to the motor unit action potential (MUAP). Significant linear regressions are  
738 shown as continuous lines. Only motor units with significant cross MUAP are shown. (C) Motor unit crosstalk index (CI). Significant  
739 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).  
740 Grey dots indicate outliers.

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

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

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

754

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

		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 <sup>2</sup>	0.077	0.285	0.385	0.440	0.076	0.108	0.098	0.062	-0.021	-0.015
	p	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.026</b>	<b>0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	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 <sup>2</sup>	0.136	0.075	0.031	0.305	-0.022	-0.033	0.057	0.001	0.020	0.084
	p	<b>&lt;0.001</b>	<b>0.090</b>	<b>0.071</b>	<b>&lt;0.001</b>	<b>0.872</b>	<b>0.841</b>	<b>0.018</b>	<b>0.309</b>	<b>0.250</b>	<b>0.146</b>
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 <sup>2</sup>	0.042	-0.029	0.392	0.224	0.103	-0.040	-0.015	-0.016	0.028	0.212
	p	<b>0.011</b>	<b>0.579</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.060</b>	<b>0.635</b>	<b>0.699</b>	<b>0.990</b>	<b>0.295</b>	<b>0.102</b>
Homogeneity of regression slopes test / ANOVA	F	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	<b>0.005</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.051</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	$\eta^2$	0.022	0.214	0.230	0.034	0.260	0.467	0.463	0.505	0.250	0.465
	M vs SD	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	M vs DD	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.764	>0.999
SD vs DD	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>		0.430	0.106	>0.999	0.431	0.091	<b>&lt;0.001</b>	

759

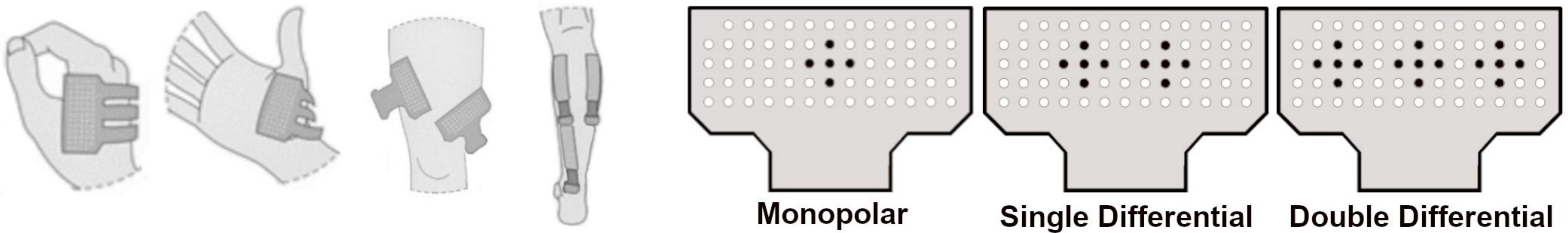
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761 Table 2. Summary of the results from the analysis of the influence of crosstalk on the EMG signal, and the evaluation of the efficiency  
 762 of single (SD) and double differential (DD) spatial filtering in reducing crosstalk.

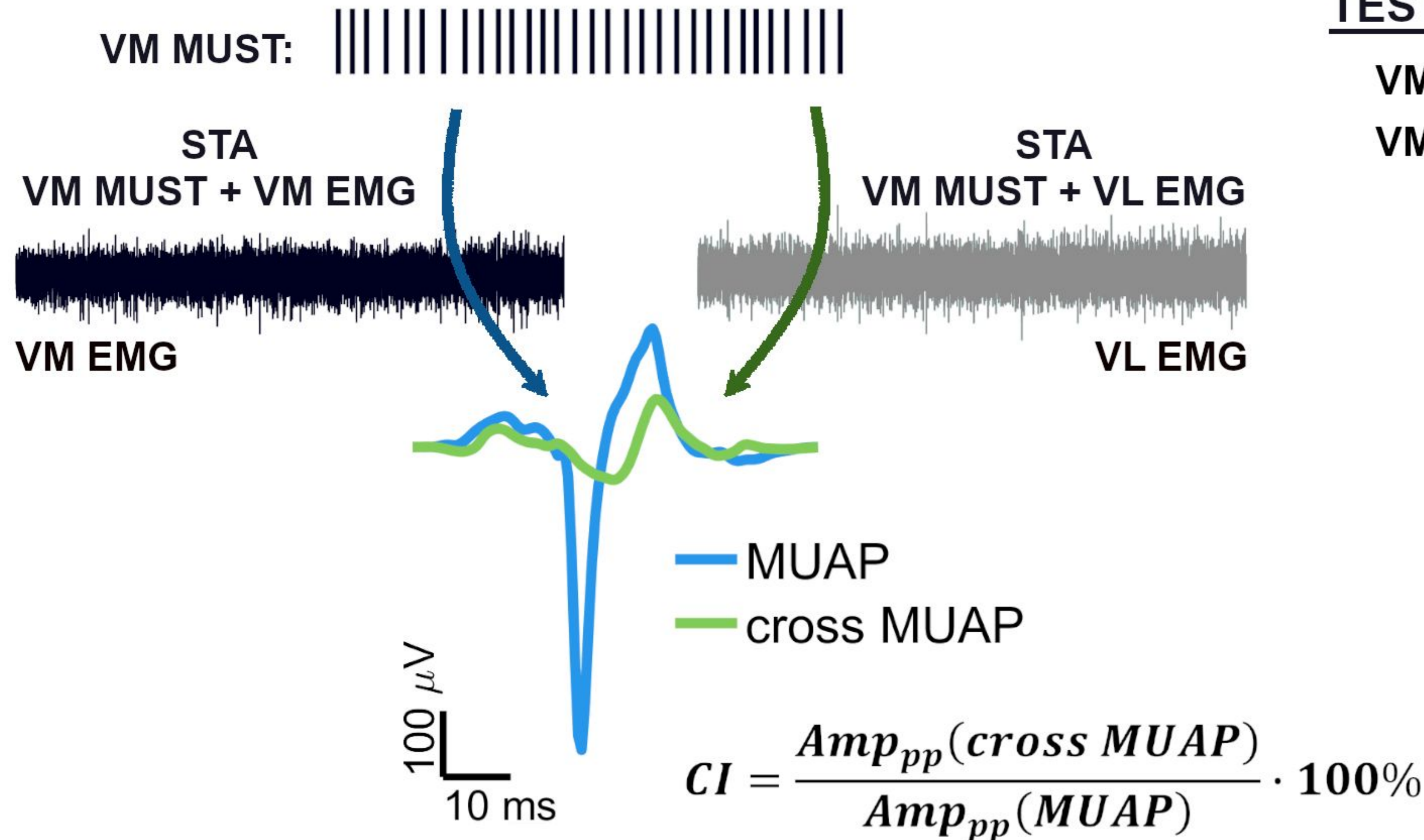
	FDI-thenar	VL-VM	GL-GM-SOL	Conclusion
<b>Surface EMG metrics:</b>	<ul style="list-style-type: none"> <li>- Crosstalk was more pronounced in thenar EMG signal.</li> <li>- Spatial filter reduced proportion of significant crosstalk.</li> <li>- Spatial filter reduced Cross MUAP amplitude from FDI but increased from thenar motor units.</li> <li>- Spatial filter reduced the relative contribution of crosstalk for thenar EMG signal.</li> <li>- Crosstalk slightly reduced the median frequency of the FDI and thenar EMG power spectrum.</li> <li>- Spatial filter did not reduce the influence of crosstalk on the EMG power spectrum.</li> </ul>	<ul style="list-style-type: none"> <li>- Similar amount of crosstalk between muscles.</li> <li>- Spatial filter reduced proportion of significant crosstalk.</li> <li>- Spatial filter slightly reduced Cross MUAP amplitude.</li> <li>- Spatial filter did not reduce the relative contribution of crosstalk (except SD for VL EMG).</li> <li>- Crosstalk slightly reduced the median frequency of the EMG power spectrum.</li> <li>- Spatial filter did not reduce the influence of crosstalk on the EMG power spectrum.</li> </ul>	<ul style="list-style-type: none"> <li>- Greater crosstalk from GM.</li> <li>- Spatial filter reduced proportion of significant crosstalk.</li> <li>- Spatial filter reduced Cross MUAP amplitude, except for SOL motor units.</li> <li>- Spatial filter reduced the relative contribution of crosstalk to the EMG signal for GL and SOL.</li> <li>- Crosstalk reduced the median frequency of the EMG power spectrum.</li> <li>- Spatial filter did not reduce the influence of crosstalk on the EMG power spectrum.</li> </ul>	<ul style="list-style-type: none"> <li>- 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).</li> <li>- Frequency domain: Crosstalk slightly increased the EMG median frequency for all muscles, and spatial filter did not reduce this effect.</li> </ul>
<b>Intermuscular correlation:</b>	<ul style="list-style-type: none"> <li>- Crosstalk led to an overestimation of cross-correlation between the two signals.</li> <li>- Spatial filter was effective in partially (SD) or totally (DD) reduce the effect.</li> </ul>	<ul style="list-style-type: none"> <li>- Crosstalk led to an overestimation of cross-correlation between the two signals.</li> <li>- Spatial filter was effective in partially (SD) reduce the effect.</li> </ul>	<ul style="list-style-type: none"> <li>- Crosstalk overestimated the cross-correlation between muscle pairs.</li> <li>- Except for GL-GM, spatial filter was effective in partially (SD) or totally (SD, DD) reduce the effect.</li> </ul>	<ul style="list-style-type: none"> <li>- Crosstalk biased correlation estimates, and spatial filtering partially or totally reduce this effect.</li> </ul>

763

# A ELECTRODE PLACEMENT AND EMG DERIVATIONS



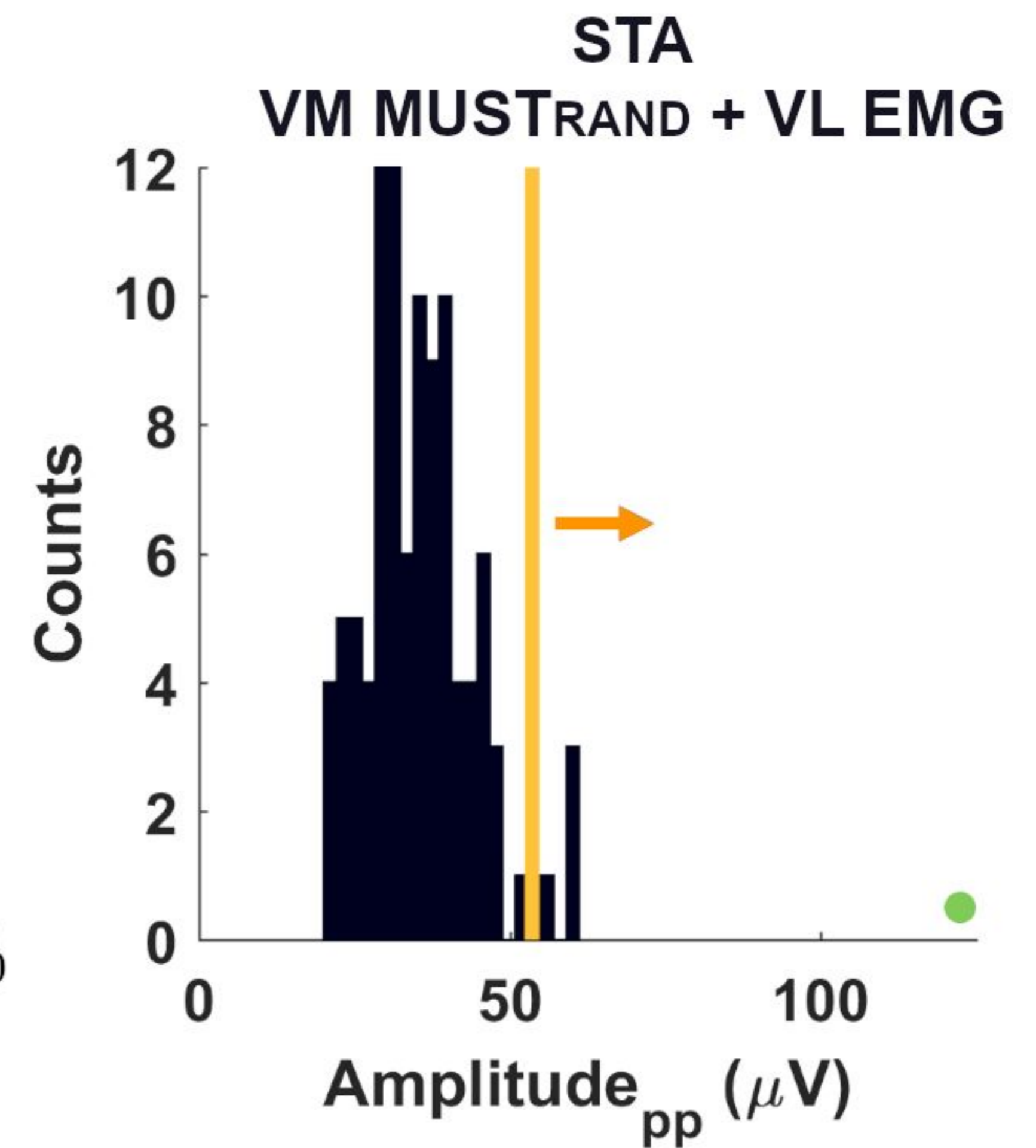
# B MOTOR UNIT CROSSTALK



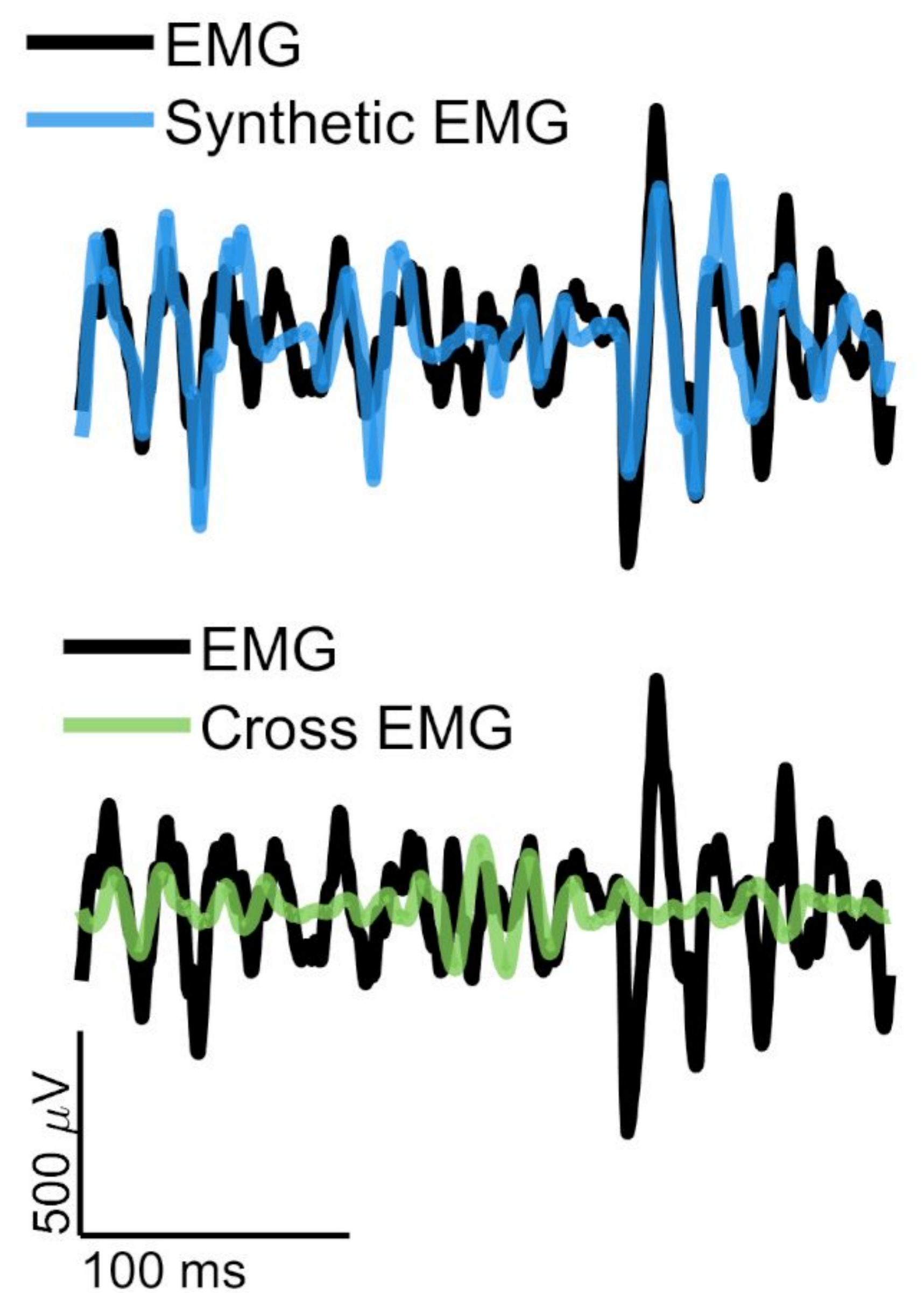
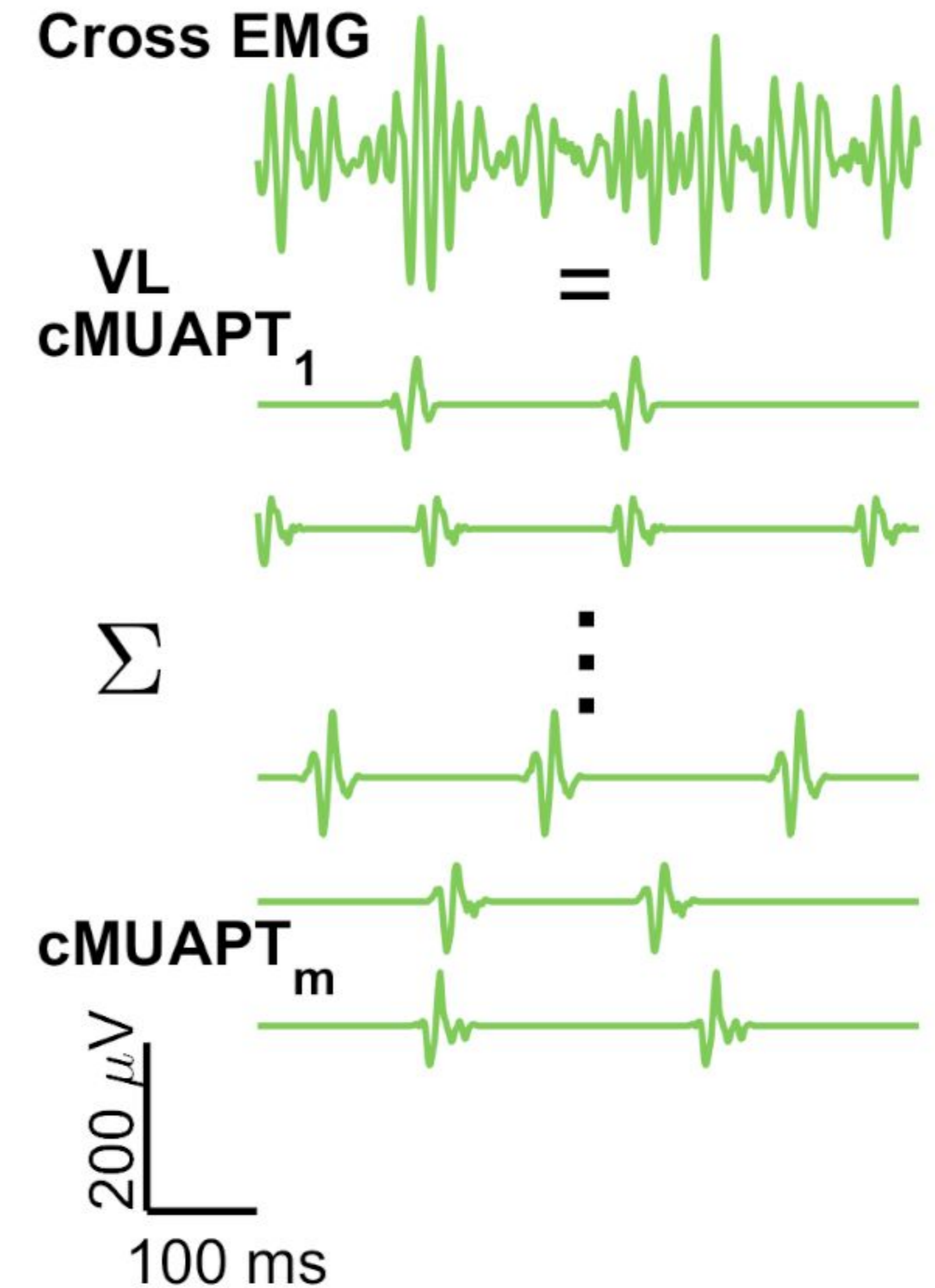
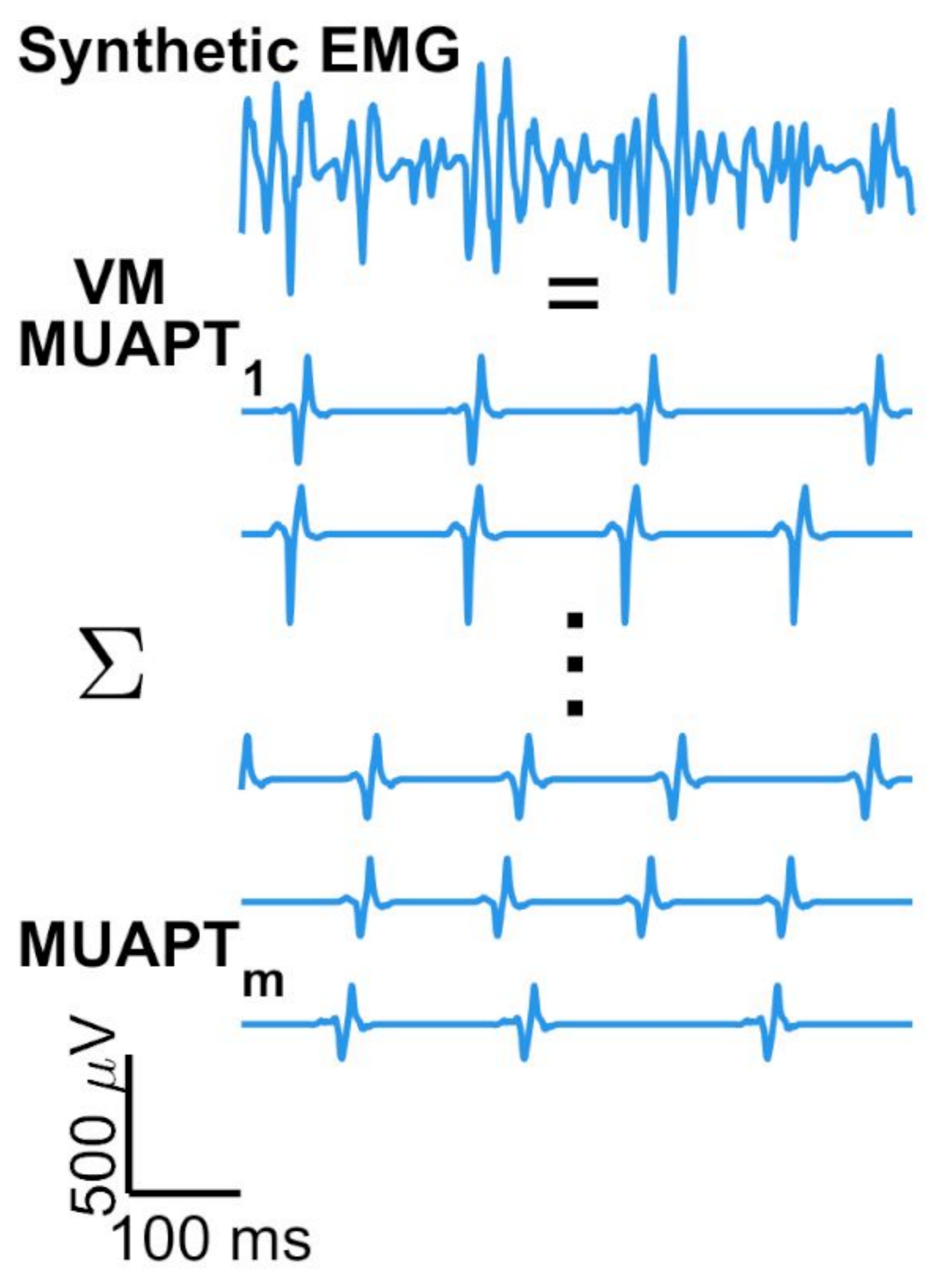
# TEST CROSS MUAP SIGNIFICANCE

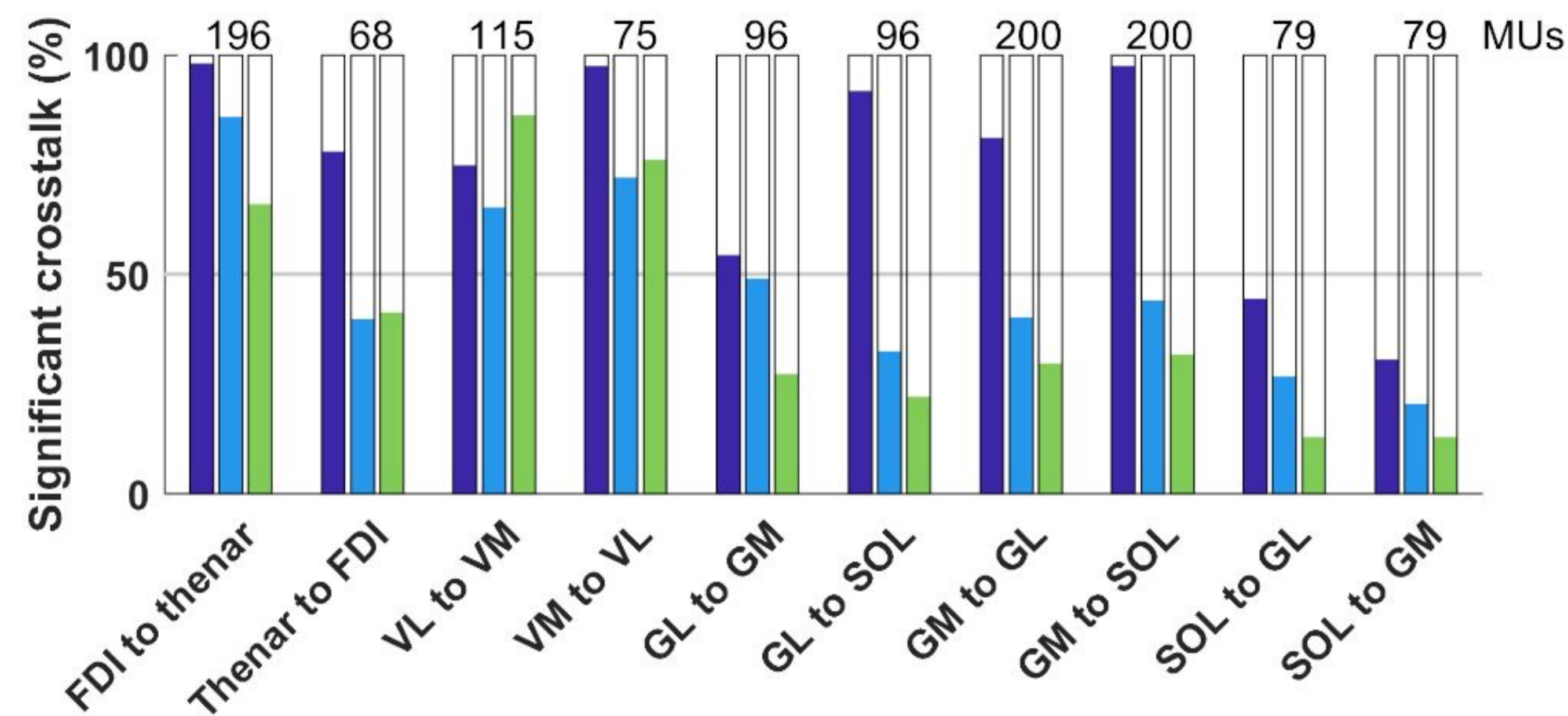
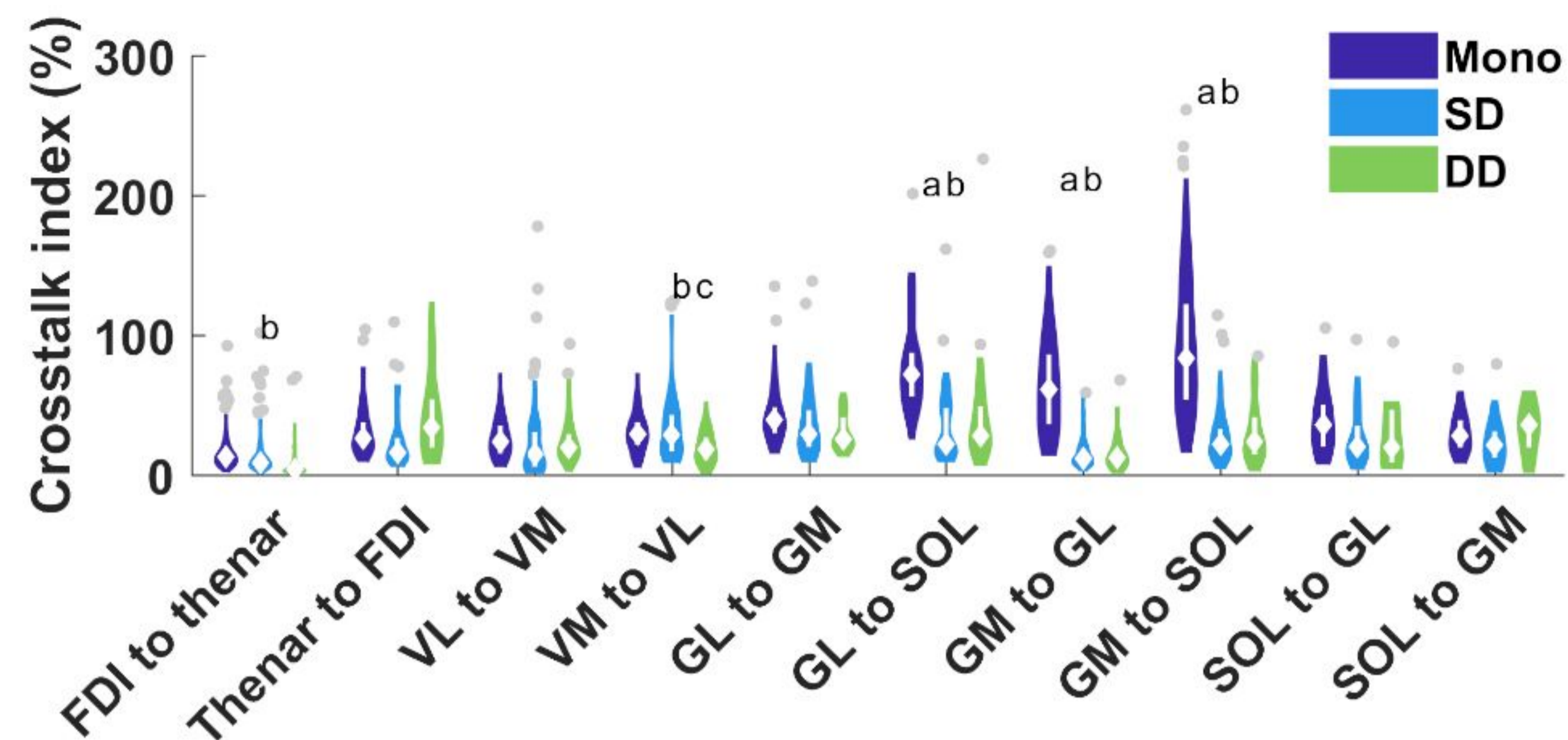
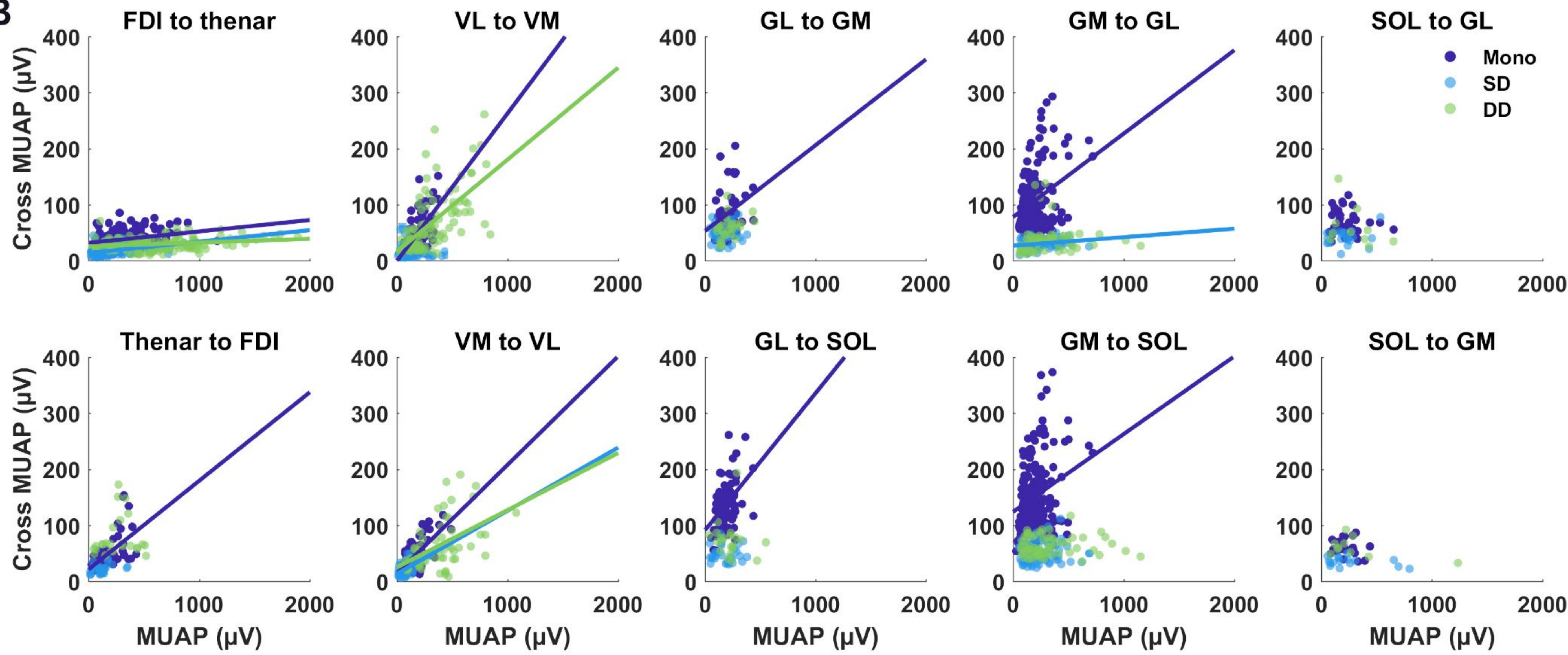
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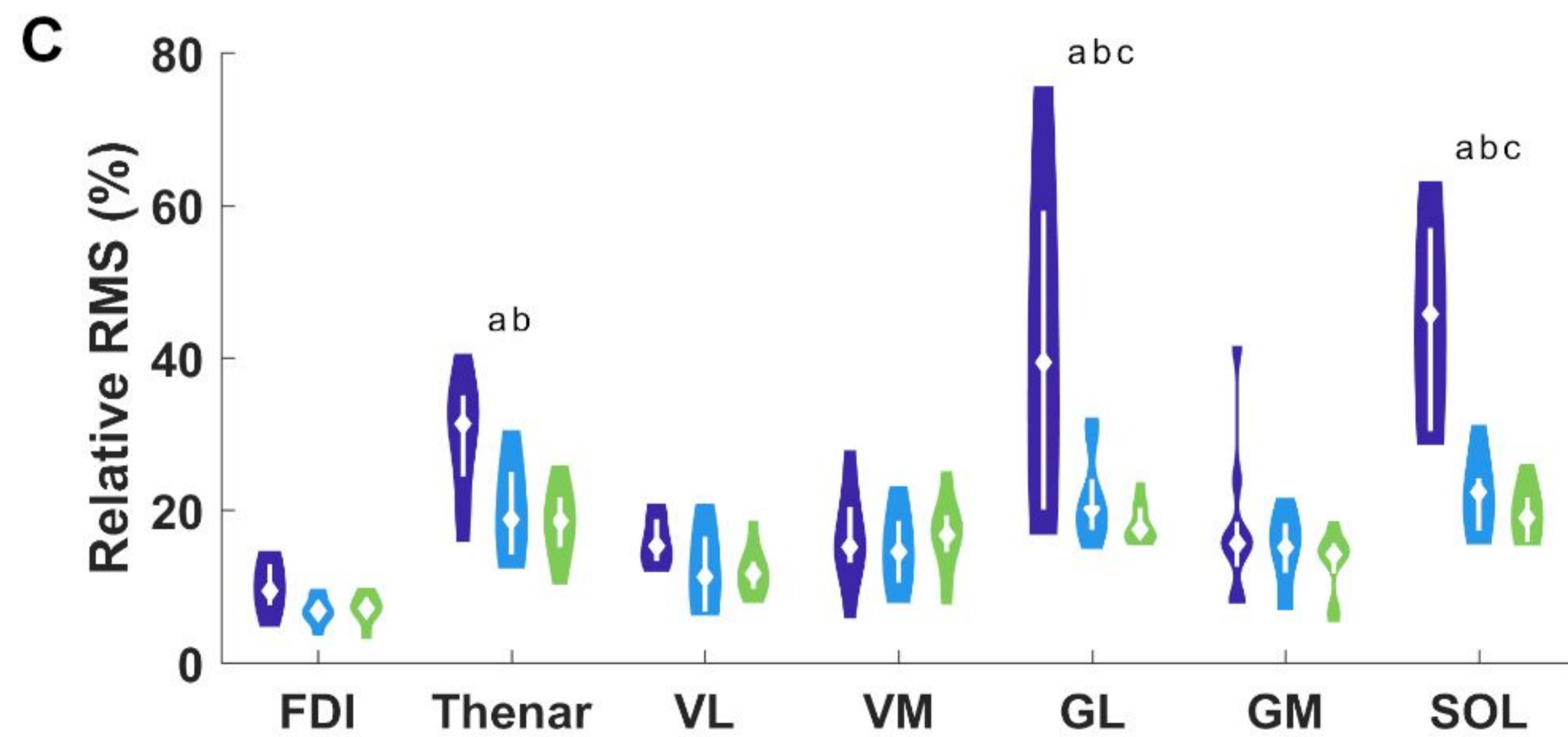
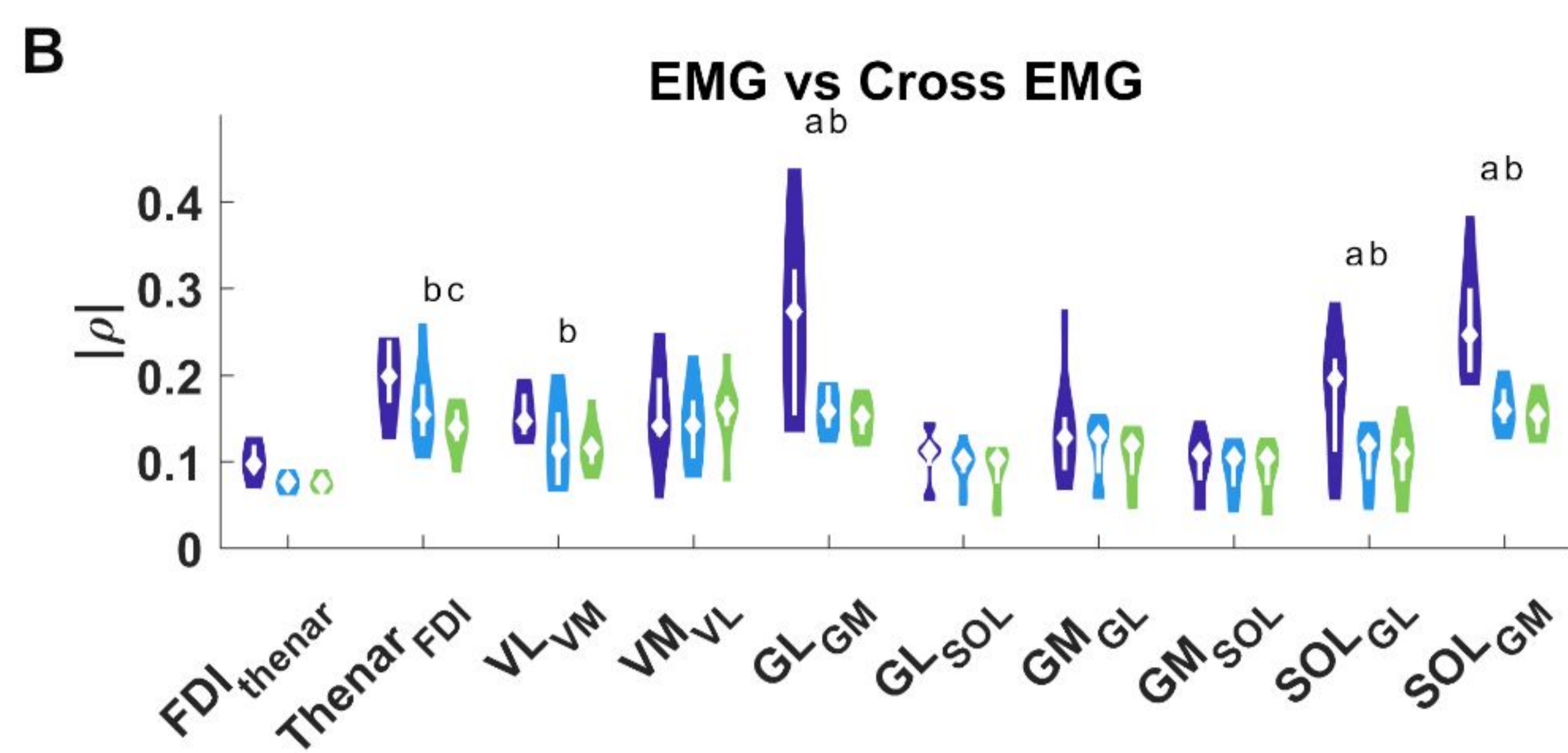
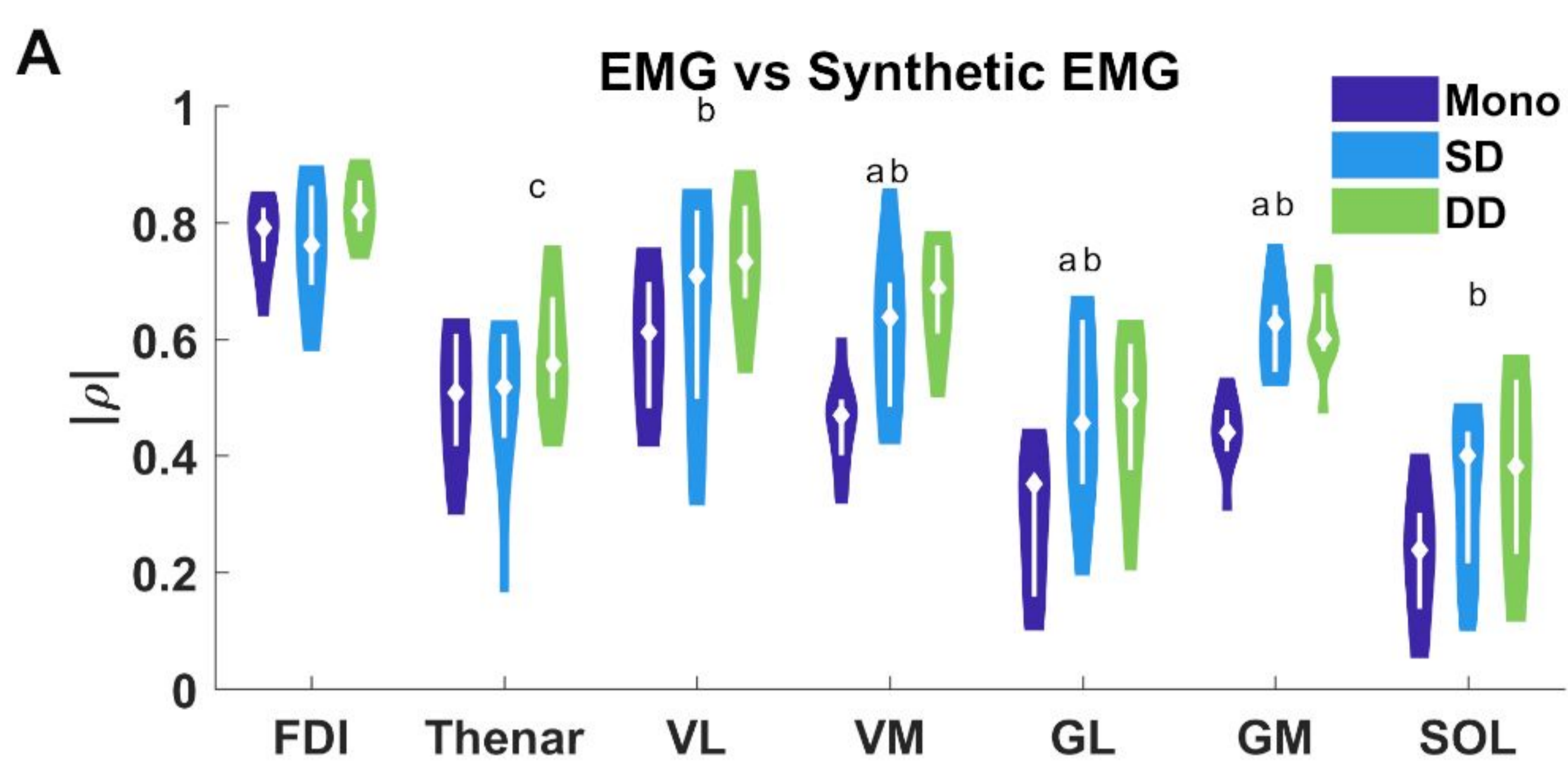
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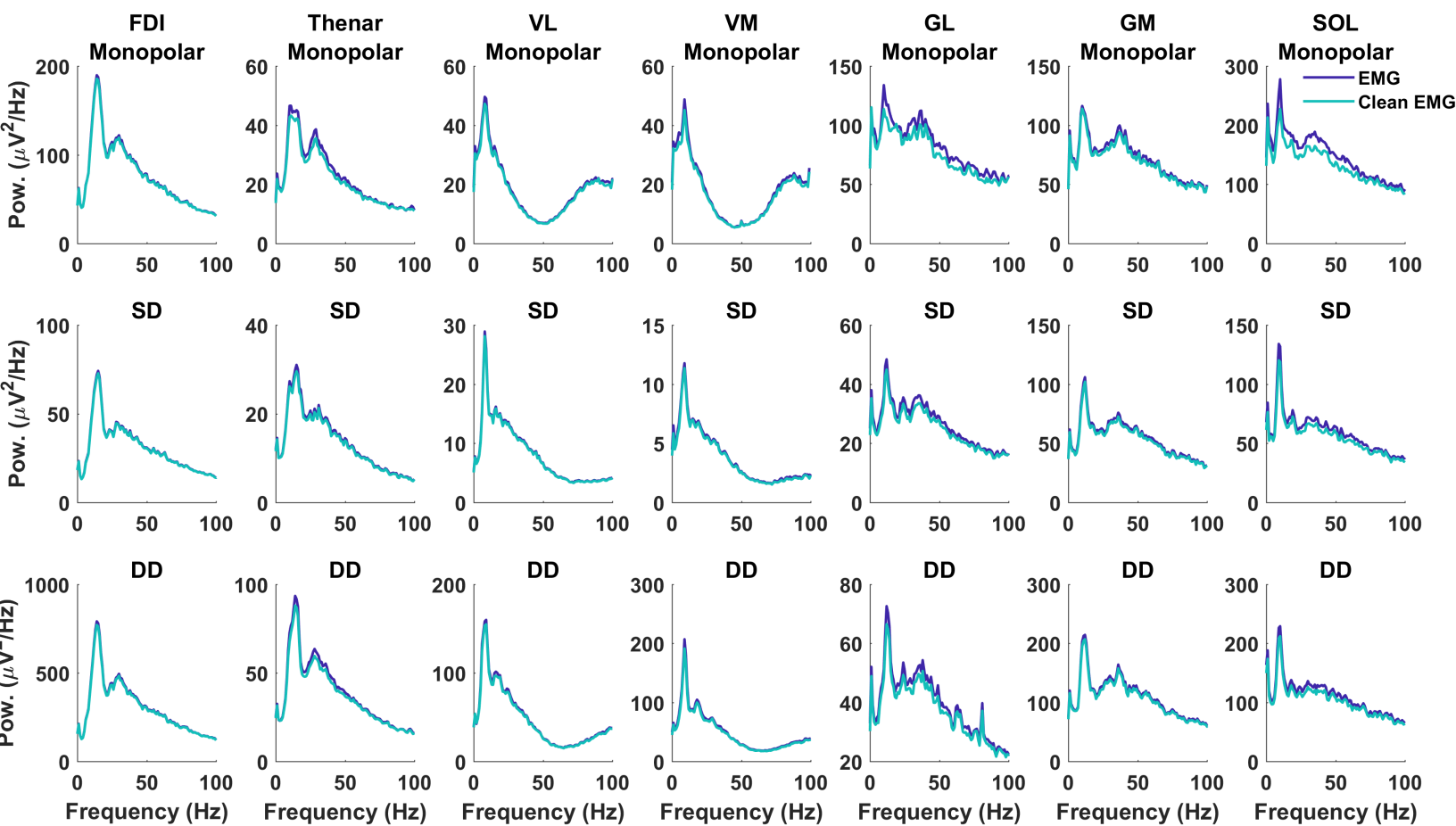


# C RECONSTRUCTED EMG AND CROSSTALK

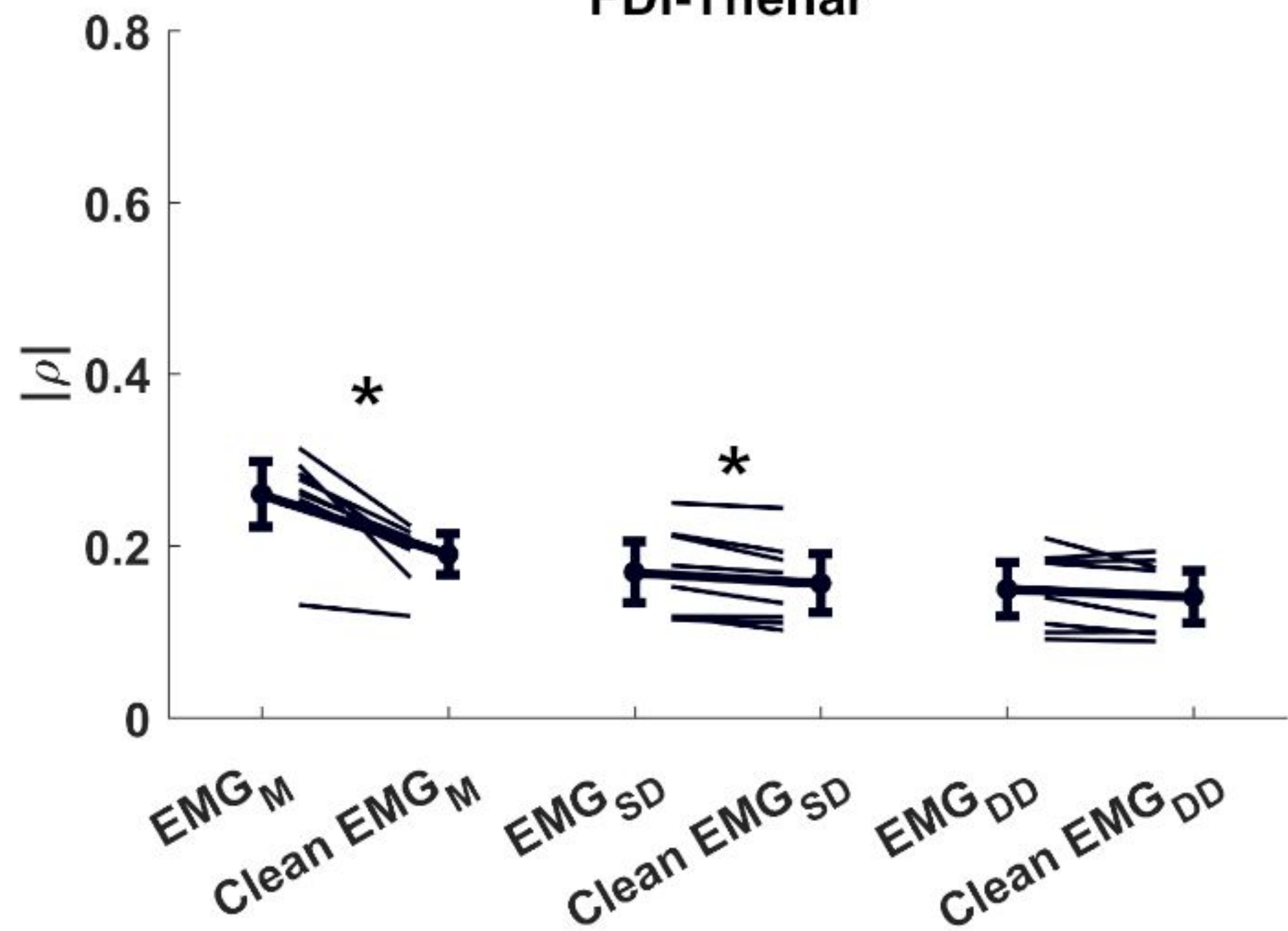


**A****C****B**

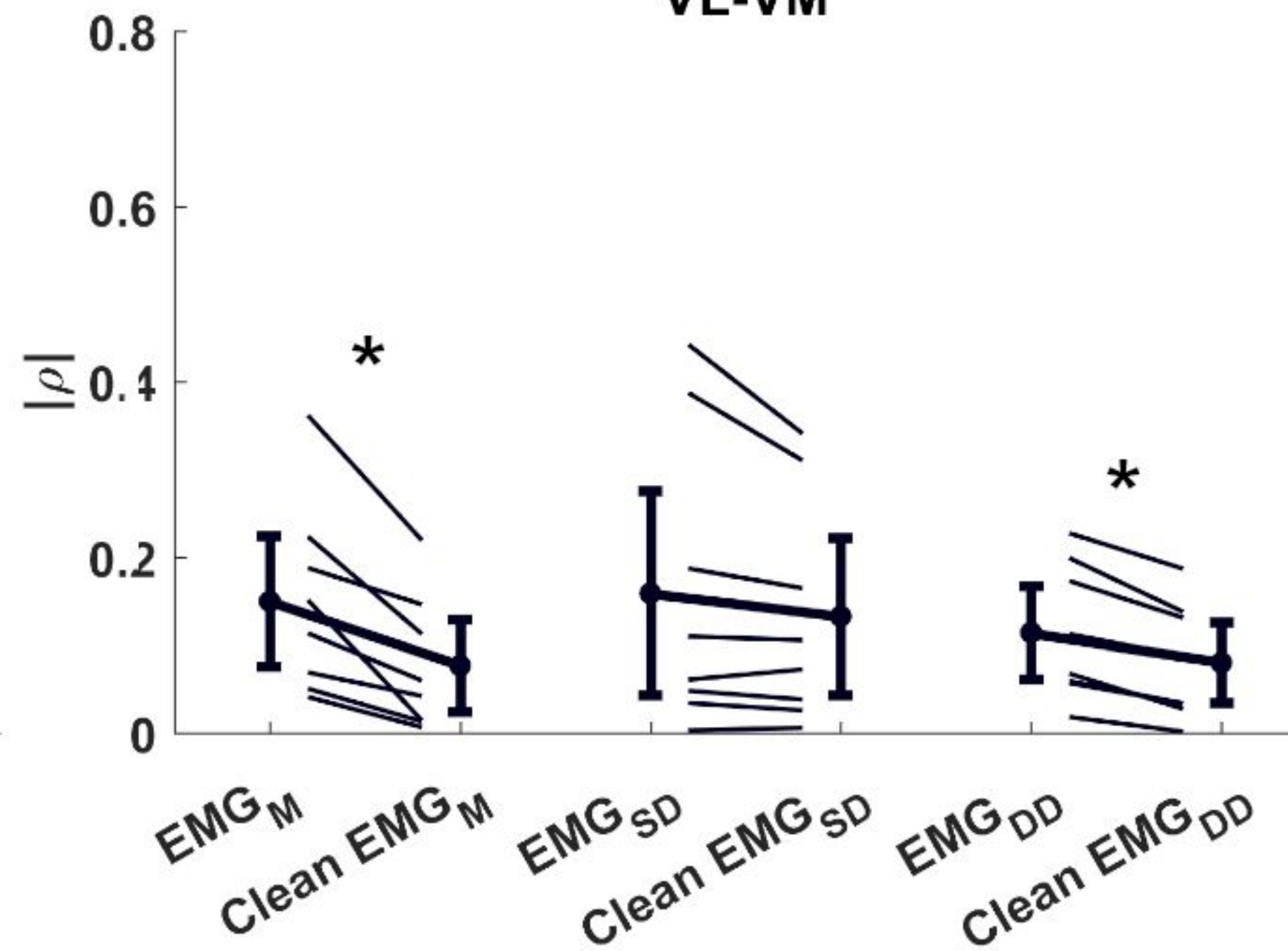




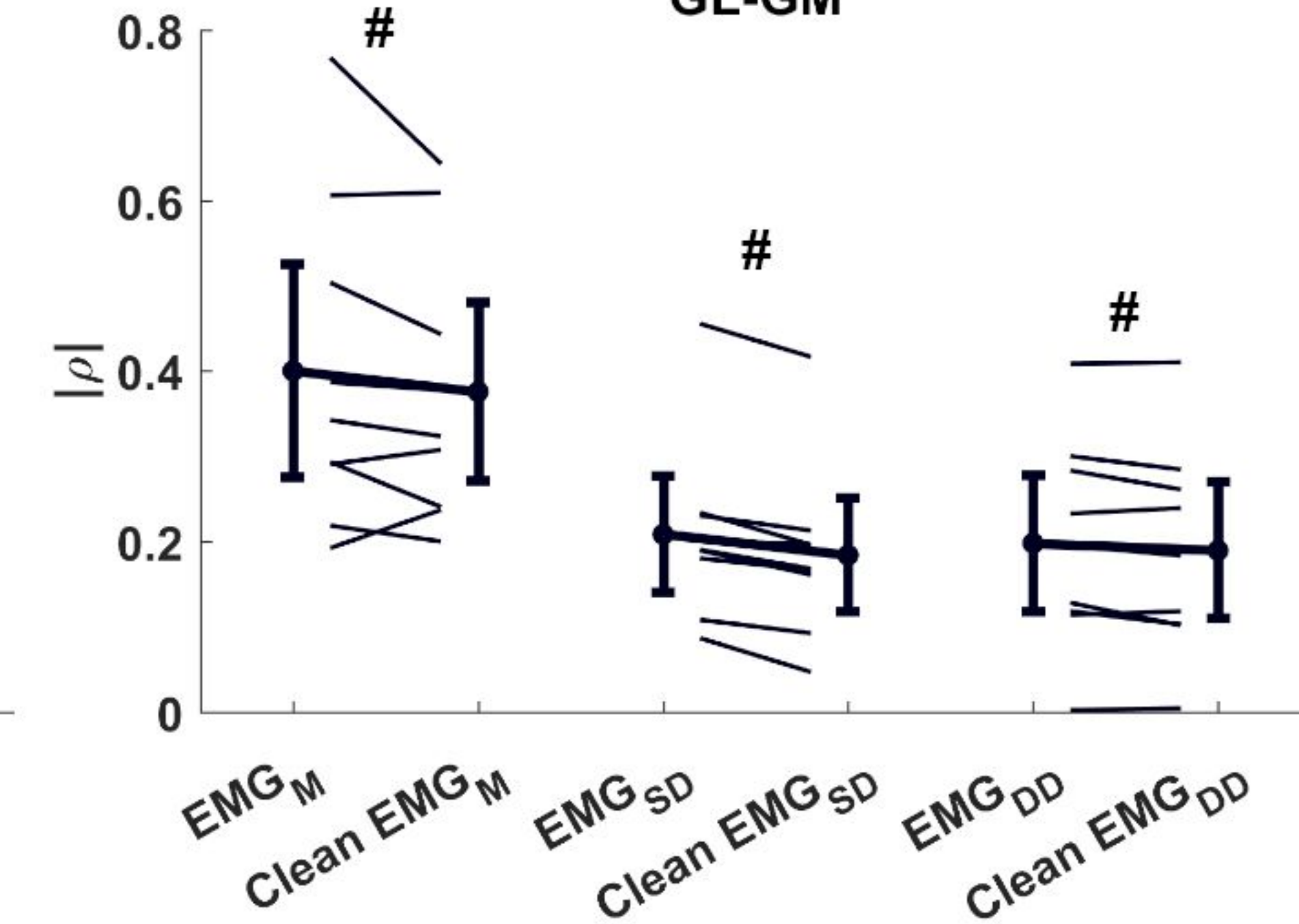
FDI-Thenar



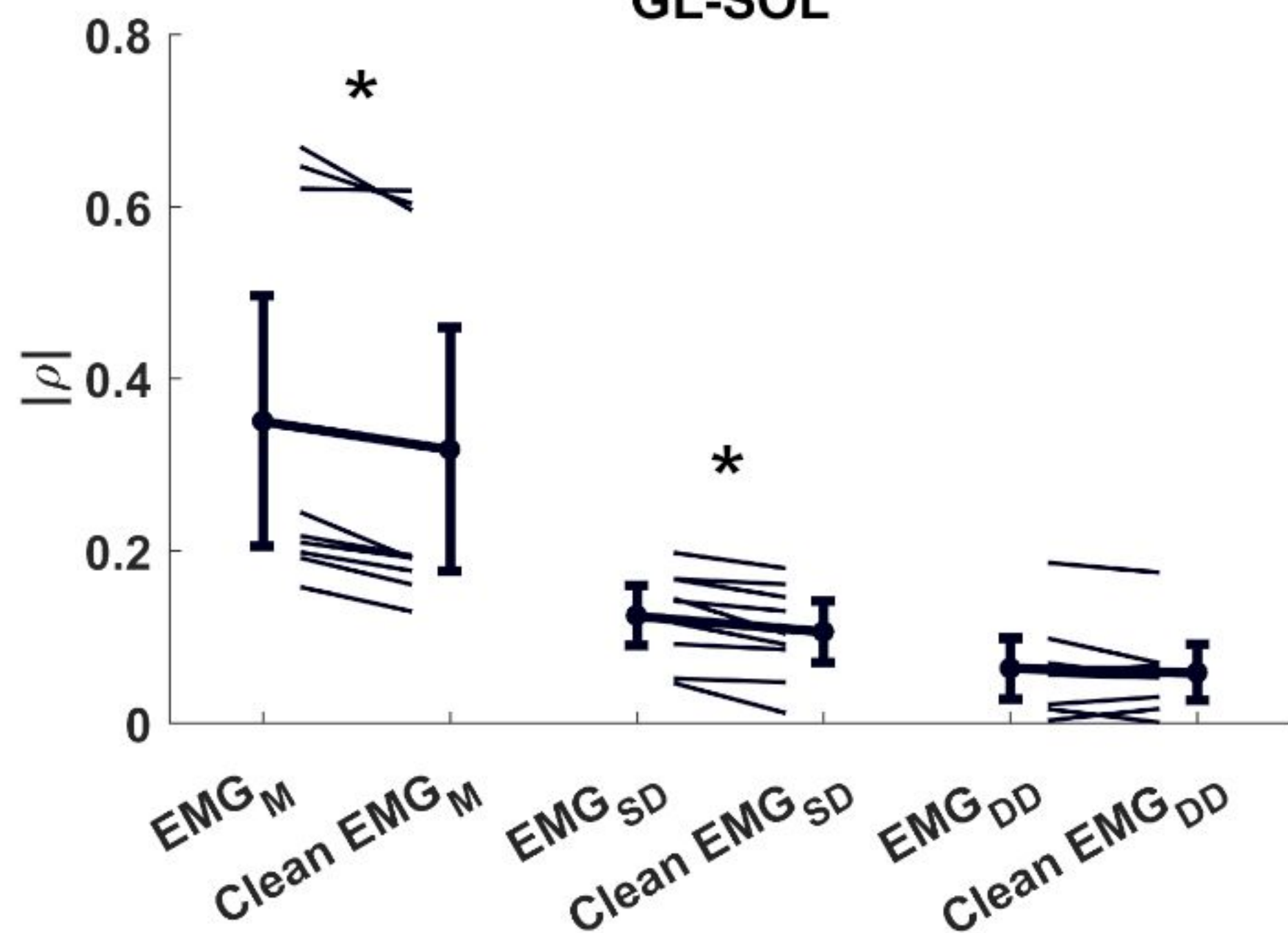
VL-VM



GL-GM



GL-SOL



GM-SOL

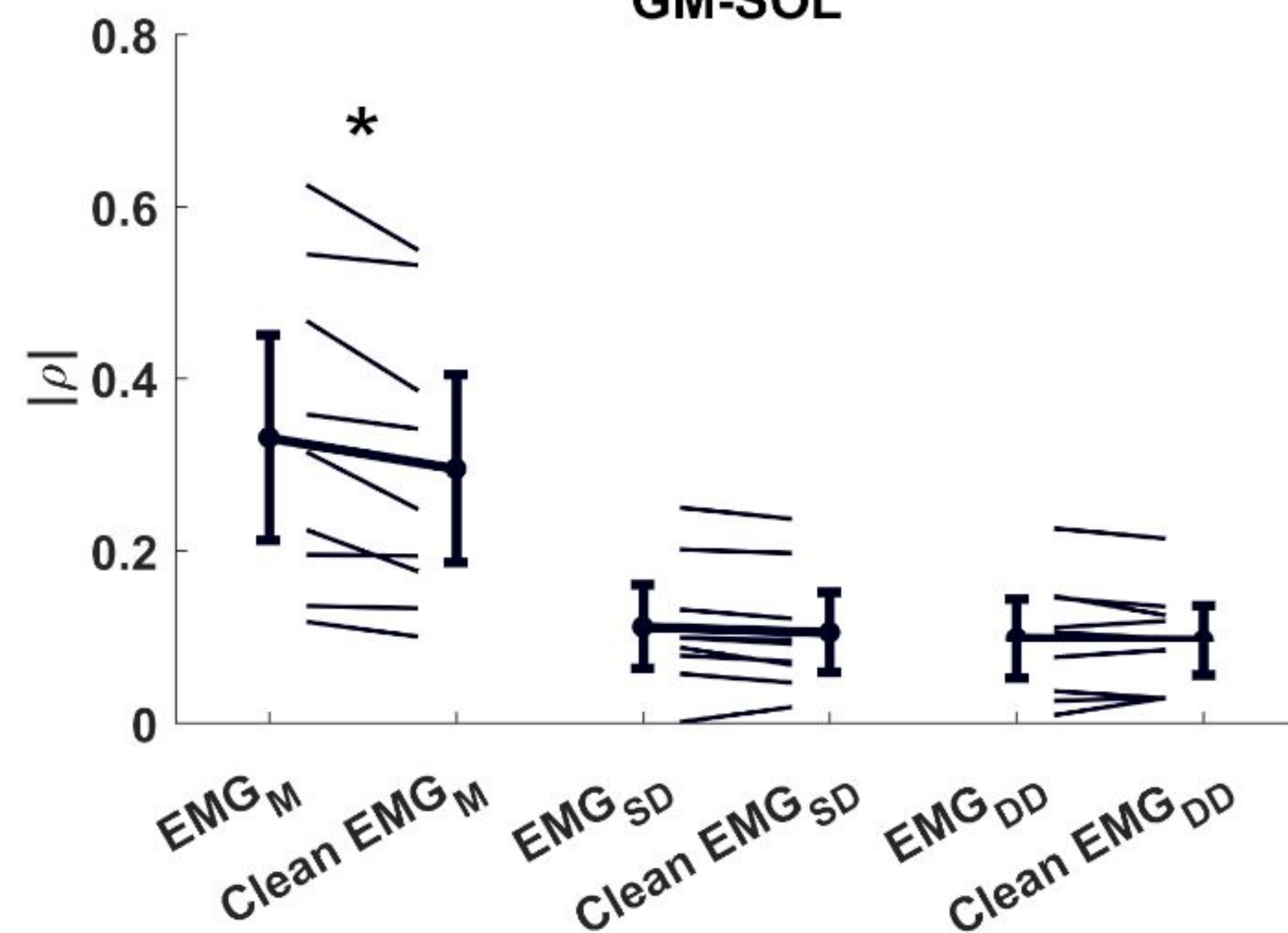


Table 1. Linear regression analysis for Cross MUAP with MUAP amplitude for monopolar, single differential (SD) and double differential (DD) recording modes. Statistics from the homogeneity of regression slopes test (ANCOVA) for the cases with a clear correlation between Cross MUAP and MUAP amplitude 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 ( $\eta^2$ ).

		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
<b>Monopolar</b>	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 <sup>2</sup>	0.077	0.285	0.385	0.440	0.076	0.108	0.098	0.062	-0.021	-0.015
	p	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.026</b>	<b>0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.584	0.423
<b>SD</b>	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 <sup>2</sup>	0.136	0.075	0.031	0.305	-0.022	-0.033	0.057	0.001	0.020	0.084
	p	<b>&lt;0.001</b>	0.090	0.071	<b>&lt;0.001</b>	0.872	0.841	<b>0.018</b>	0.309	0.250	0.146
<b>DD</b>	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 <sup>2</sup>	0.042	-0.029	0.392	0.224	0.103	-0.040	-0.015	-0.016	0.028	0.212
	p	<b>0.011</b>	0.579	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.060	0.635	0.699	0.990	0.295	0.102
<b>Homogeneity of regression slopes test / ANOVA</b>		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	<b>0.005</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.051	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	$\eta^2$	0.022	0.214	0.230	0.034	0.260	0.467	0.463	0.505	0.250	0.465
	M vs SD	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	SD vs DD	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.764	>0.999

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.

	<b>FDI-thenar</b>	<b>VL-VM</b>	<b>GL-GM-SOL</b>	<b>Conclusion</b>
<b>Surface EMG metrics:</b>	<ul style="list-style-type: none"> <li>- Crosstalk was more pronounced in thenar EMG signal.</li> <li>- Spatial filter reduced proportion of significant crosstalk.</li> <li>- Spatial filter reduced <i>Cross</i> MUAP amplitude from FDI but increased from thenar motor units.</li> <li>- Spatial filter reduced the relative contribution of crosstalk for thenar EMG signal.</li> <li>- Crosstalk slightly reduced the median frequency of the FDI and thenar EMG power spectrum.</li> <li>- Spatial filter did not reduce the influence of crosstalk on the EMG power spectrum.</li> </ul>	<ul style="list-style-type: none"> <li>- Similar amount of crosstalk between muscles.</li> <li>- Spatial filter reduced proportion of significant crosstalk.</li> <li>- Spatial filter slightly reduced <i>Cross</i> MUAP amplitude.</li> <li>- Spatial filter did not reduce the relative contribution of crosstalk (except SD for VL EMG).</li> <li>- Crosstalk slightly reduced the median frequency of the EMG power spectrum.</li> <li>- Spatial filter did not reduce the influence of crosstalk on the EMG power spectrum.</li> </ul>	<ul style="list-style-type: none"> <li>- Greater crosstalk from GM.</li> <li>- Spatial filter reduced proportion of significant crosstalk.</li> <li>- Spatial filter reduced <i>Cross</i> MUAP amplitude, except for SOL motor units.</li> <li>- Spatial filter reduced the relative contribution of crosstalk to the EMG signal for GL and SOL.</li> <li>- Crosstalk reduced the median frequency of the EMG power spectrum.</li> <li>- Spatial filter did not reduce the influence of crosstalk on the EMG power spectrum.</li> </ul>	<ul style="list-style-type: none"> <li>- Time domain: All EMG signals were contaminated by crosstalk. Spatial filters reduced the proportion of crosstalk and <i>Cross</i> MUAP amplitude for most muscles (except SOL) and reduced the relative contribution of crosstalk to the EMG for some muscles (thenar, GL and SOL).</li> <li>- Frequency domain: Crosstalk slightly increased the EMG median frequency for all muscles, and spatial filter did not reduce this effect.</li> </ul>
<b>Intermuscular correlation:</b>	<ul style="list-style-type: none"> <li>- Crosstalk led to an overestimation of cross-correlation between the two signals.</li> <li>- Spatial filter was effective in partially (SD) or totally (DD) reduce the effect.</li> </ul>	<ul style="list-style-type: none"> <li>- Crosstalk led to an overestimation of cross-correlation between the two signals.</li> <li>- Spatial filter was effective in partially (SD) reduce the effect.</li> </ul>	<ul style="list-style-type: none"> <li>- Crosstalk overestimated the cross-correlation between muscle pairs.</li> <li>- Except for GL-GM, spatial filter was effective in partially (SD) or totally (SD, DD) reduce the effect.</li> </ul>	<ul style="list-style-type: none"> <li>- Crosstalk biased correlation estimates, and spatial filtering partially or totally reduce this effect.</li> </ul>