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Electrodes' Configuration Influences the Agreement Between Surface EMG and B-Mode Ultrasound Detection of Motor Unit Fasciculation

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This work involved human subjects or animals in its research. Approval of all ethical and experimental procedures and protocols was granted by the Regional Ethics Committee (Commissione di Vigilanza, Servizio Sanitario Nazionale-Regione Piemonte-ASL 1-Torino, Italy), and performed in line with the Declaration of Helsinki.

ABSTRACT Muscle fasciculations, resulting from the spontaneous activation of motor neurons, may be associated with neurological disorders, and are often assessed with intramuscular electromyography (EMG). Recently, however, both ultrasound (US) imaging and multichannel surface EMG have been shown to be more sensitive to fasciculations. In this study we combined these two techniques to compare their detection sensitivity to fasciculations occurring in different muscle regions and to investigate the effect of EMG electrodes' configuration on their agreement. Monopolar surface EMGs were collected from medial gastrocnemius and soleus with an array of 32 electrodes (10 mm Inter-Electrode Distance, IED) simultaneously with b-mode US images detected alongside either proximal, central or distal electrodes groups. Fasciculation potentials (FP) were identified from single differential EMGs with 10 mm (SD1), 20 mm (SD2) and 30 mm (SD3) IEDs, and fasciculation events (FE) from US image sequences. The number, location, and size of FEs and FPs in 10 healthy participants were analyzed. Overall, the two techniques showed similar sensitivities to muscle fasciculations. US was equally sensitive to FE occurring in the proximal and distal calf regions, while the number of FP revealed by EMG increased significantly with the IED and was larger distally, where the depth of FE decreased. The agreement between the two techniques was relatively low, with a percentage of fasciculation classified as common ranging from 22% for the smallest IED to 68% for the largest IED. The relevant number of events uniquely detected by the two techniques is discussed in terms of different spatial sensitivities of EMG and US, which suggest that a combination of US-EMG is likely to maximise the sensitivity to muscle fasciculations.

INDEX TERMS Fasciculation, motor unit, electromyography, HD-EMG, ultrasonic imaging, amyotrophic lateral sclerosis (ALS), motor neuron disease.

I. INTRODUCTION

A motor unit comprises the *a*-motoneuron located in the spinal cord, its axon and the family of muscle fibers it innervates [1]. Excitation of the *a*-motoneuron and hence associated muscle fibers, results in the translation of chemical energy to mechanical force and changes in muscle shape, which during voluntary contraction often leads to movement of the skeleton. Motor units can also demonstrate spontaneous, intermittent, involuntary activity, termed fasciculation, whereby muscle fiber excitation translates to small, localized muscle tissue displacements or twitches. Fasciculations may therefore be assessed in terms of muscle excitation or movement. Fasciculation occurs in healthy

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individuals, however in clinical practice fasciculation in muscles across multiple body regions is deemed a sign of on-going denervation, indicative of devastating neurodegenerative diseases, such as Motor Neurone Disease for which there is currently no treatment [2]. For instance, fasciculations represent an early pathophysiological hallmark of amyotrophic lateral sclerosis often preceding the onset of muscle weakness [2], [3]. Being able to increase the sensitivity to fasciculation occurrence and therefore the ability to follow its progression may have a relevant impact on the diagnostic process.

In clinical practice and experimental studies, muscle excitation is commonly recorded using electromyography (EMG) while the resulting mechanical tissue displacement can be captured using ultrasound imaging. While these two techniques have traditionally been used independently, there is growing interest in the potential value of combining them to, for example, improve sensitivity of neurodegenerative disease diagnosis [4] or increase understanding of neuro-mechanical aspects of skeletal muscle function [5]–[8]. Each technique however captures different elements of the motor unit activation, and each has different limitations in terms of the size of the muscle region from which information may be extracted. For example, surface EMG is traditionally thought to be limited to detecting activity in the most superficial portion of muscle with limited possibility for studying deeper muscles. Although application of multi-channel electrode arrays provide possibility to alter inter-electrode distances (IEDs) and influence the depth from which activation may be detected [9]. On the other hand, ultrasound can image multiple muscle layers but captures only a short (typically ∼50 - 60 mm [10]), thin, cross-sectional slice through the muscle volume. As such, while both techniques can detect motor unit fasciculation, the degree to which both electrophysiological and mechanical information can be extracted from the same fasciculation event may be influenced by the inter-relationship between the detection volume of each system.

Previous work has shown that both ultrasound and multi-channel surface EMG are more sensitive detectors of fasciculation than intramuscular EMG in some muscles [11], [12]. However, the literature is lacking systematic comparisons between sensitivity to fasciculations of surface EMG and ultrasound. Our preliminary work suggested low rate of agreement between the two (<75%), tested in calf muscles of healthy individuals [13]. However, this work was based on a small number of participants ($N = 5$) and, to our knowledge, no previous work takes advantage of the recent technical advances in both fields. For example, our novel computational analysis of ultrasound images can robustly and objectively detect occurrence of fasciculation [14], [15], providing a measure of the muscle region in which the fasciculation event occurred. Similarly, we have shown the use of grids of electrodes may increase the sensitivity of surface EMGs to excitation taking place at deep muscle regions [9]. It is therefore timely to evaluate whether combining US and grids of electrodes could maximize the potential for identification of muscle fasciculations.

In this study we build on our preliminary work, with two main goals: i) to assess whether both techniques are equally sensitive to detecting the same muscle fasciculations, based on the number of matched fasciculations observed concurrently in surface EMG and US images; ii) to investigate whether the sensitivity to matched potentials increases when surface EMGs are detected for greater IEDs. Owing to the limited, depth view of surface electrodes and the limited proximo-distal field of US view, anticipating the number of expected matched fasciculations would be tentative at best. However, given the number of motor units represented in the surface EMGs increases with the IED [9], [16], we hypothesize the number of fasciculations identified in the surface EMGs increases with IED and so the number of EMG-US matched fasciculations. Our results are expected to advance our knowledge on the detection of fasciculations events, likely opening new fronts for the study of fasciculation and motor unit characteristics.

II. METHODS

We carried-out an experimental study to compare, in healthy subjects, the detection sensitivity of EMG and US to fasciculations occurring in different muscle regions and to investigate the effect of EMG electrodes' configuration on their agreement. To this end we performed simultaneous acquisition of EMG signals and US images from calf muscles during rest.

A. SUBJECTS

Ten participants (age 29 ± 6 years; height 173 ± 8 cm; body mass: 69 ± 5 kg, seven males and three females), with no self-reported history of neurological or musculoskeletal impairment or disease were recruited. Experimental procedures conformed with the latest revision of the Declaration of Helsinki and were approved by the Regional Ethics Committee (Commissione di Vigilanza, Servizio Sanitario Nazionale-Regione Piemonte- ASL 1-Torino, Italy). Informed consent was obtained from all participants after providing detailed explanation of the study procedures.

B. PROTOCOL

1) EXPERIMENTAL PROCEDURE

Participants laid prone on a padded bed, with the knee fully extended and the ankle in neutral position. The participant was asked to relax and to keep the same position during the entire experiment. Surface EMG and US images were recorded simultaneously during rest from four muscle regions along the proximo-distal axis of the leg (Fig. 1). While the EMG electrodes were positioned to cover the whole calf, because of its limited length, the US probe covered roughly one quarter of the calf. For this reason, four trials were necessary for the concurrent collection of surface EMG and US images from different calf

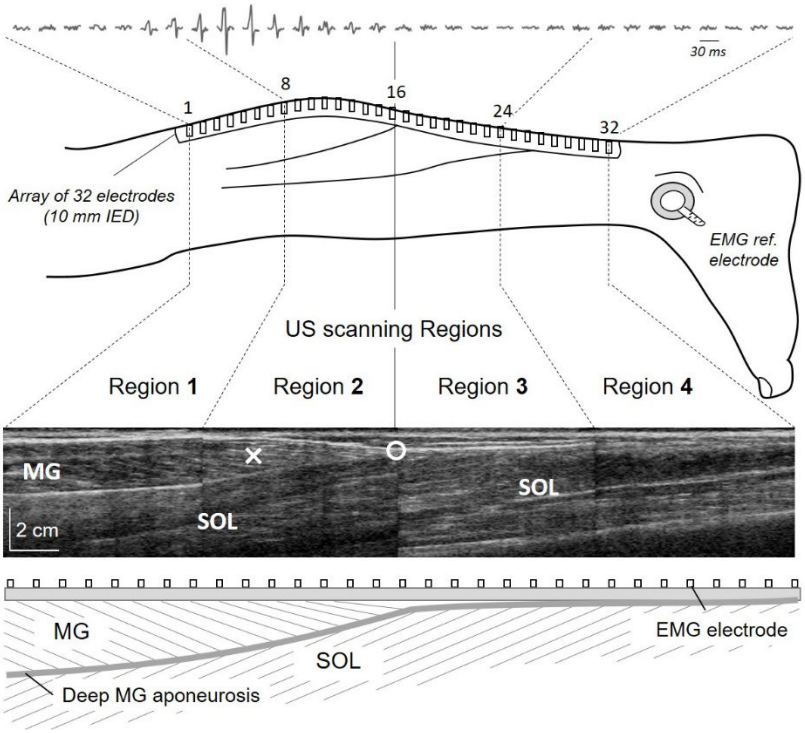

FIGURE 1. Electrode positioning over the posterior leg. Ultrasound (US) videos (80 fps) were detected simultaneously with EMGs from four adjacent regions along the electrode array composed by two linear arrays of 16 electrodes with 10 mm inter-electrode distance (IED). Lower panels: composite image of four longitudinal US scans and schematic representation of calf muscles' architecture. The white circle in the US image indicates the gastrocnemius myo-tendon junction. An example of fasciculation potential detected in single differential configuration (10 mm IED) is reported in the upper part of the figure. The location of the corresponding fasciculation event identified in US images is indicated with a white cross. MG: medial gastrocnemius; SOL: soleus.

regions. More specifically, four trials, lasting 60 s each, were applied with the muscle at rest and the US probe positioned at four consecutive, parasagittal calf regions, one at a time.

2) SURFACE EMG RECORDINGS

Surface EMGs were detected from the medial gastrocnemius (MG) and the distal part of soleus using two linear arrays of 16 electrodes each (LISiN, Politecnico di Torino, Torino, Italy) (Fig. 1). Both arrays were secured serially to the skin using a single, bi-adhesive pad. This single pad ensured the same inter-electrode distance (IED = 10mm) across the two arrays. The 32 Ag-AgCl electrodes (10 mm^2 area) were aligned parallel to the muscle longitudinal axis, with the most proximal electrode being positioned 20 mm medially from the junction between the two gastrocnemius heads. Care was taken to ensure the 16th electrode covered the MG myo-tendon junction (Fig.1). Both the junction between gastrocnemius heads and the muscle myo-tendon junction were identified with ultrasound scanning (7 MHz, Echoblaster 128, Telemed Ltd., Vilnius, Lithuania) and marked on the skin with a felt-tip pen (see supplementary material on [17]). Surface EMG signals were detected in monopolar derivation (referenced to a remote reference on the medial malleolus, Fig. 1), amplified, band-pass filtered (3 dB bandwidth, 10–500 Hz), sampled at 2048 Hz and A/D converted with 12 bits resolution (multichannel surface EMG amplifier, EMG-USB2, OT Bioelettronica, Torino, Italy) [18]. An external trigger pulse signaling the start and the end of the US acquisition was acquired synchronously with EMG signals.

3) ULTRASOUND RECORDINGS

Ultrasound B-mode images (where pixel gray-level intensity represents amplitude of returned echo signal) were acquired with the EchoBlaster 128 device (Telemed Ltd., Vilnius, Lithuania) equipped with a linear-array transducer (LV7.5/60/128Z-2) with variable frequency 5–8 MHz (set to 7 MHz to analyze skeletal muscle). The ultrasound probe was aligned parallel to the proximal-distal axis of the leg and positioned just medially to the electrode array. The transverse distance between the electrodes and the US probe was approximately 20 mm. Gain, depth and focus depth were defined, for each participant, to optimize image quality, to provide the clearest view of muscle fascicles and the aponeuroses. These system-setting parameters were then kept constant in all trials. The ultrasound images were recorded

at ∼80 frames/s [19] and transferred to a workstation for analysis.

C. DATA ANALYSIS

1) IDENTIFICATION OF FASCICULATION POTENTIALS FROM EMG

Data analysis was performed in Matlab (R2016b, The MathWorks Inc., MA, USA). Monopolar EMGs were band-pass filtered in the 20-400 Hz frequency band (4th-order zero-phase Butterworth filter). The identification of fasciculation potentials (FPs) from EMGs was performed for single-differential (SD) signals with different IEDs. Briefly, the algebraic difference between monopolar EMGs detected with the 32-electrode array was computed offline for IEDs of 10 mm (SD1), 20 mm (SD2) and 30 mm (SD3). FP onsets were identified using an amplitude threshold set at three standard deviations of the background noise. The threshold was defined for each EMG channel over the whole trial and for each of the three bipolar montages. All EMGs were visually inspected by two expert operators (A.B. and T.V.) to verify the correctness of the automatically-identified FP onsets and to exclude spurious FPs due to noise or artifact-related threshold crossing. The location of the FP was defined as the skin region covered by the channels detecting above-threshold potentials and the EMG amplitudes provided by these channels were used to compute the barycenter of the FP. The absolute and the relative (over 60 s) number of FPs as well as their location were considered to assess differences between detection montages and association with fasciculation events observed in ultrasound images.

2) IDENTIFICATION OF FASCICULATION EVENTS FROM US

Fasciculation events (FEs) were detected in the ultrasound image sequences using a computational method based on foreground detection using a Gaussian mixture model [14], [20]. This model was applied using the approach described in Bibbings *et al.* [14] for the gastrocnemius muscle, with three Gaussian distributions used with learning rate set to 0.5. As fasciculation events are typically spatially localized within the muscle, the algorithm identified clusters of foreground pixels for each frame. Clusters were classified as foreground objects whenever their size was greater than 20% of the image size (682 × 563 pixels), to prevent image noise (e.g. salt-pepper speckle) being wrongly identified as potential FE [14]. Foreground objects in subsequent frames were FE candidates; these objects were defined as FE when appearing in at least for four consecutive frames (i.e. ∼50 ms). Finally, the size and the centroid location of the FEs were computed using connective components, extracting the convex hull of a mask derived from all frames of that event. Computationally identified fasciculation events were cross-checked with a visual analysis in terms of: twitch onset, duration, centroid location, and area of the displaced muscle tissue. The visual analysis of US videos was independently performed by two operators (E.H.T. and A.B.). The high inter-rater agreement was verified through Kappa index (always >0.97) computed on the FE occurrences over 1-s time windows. As for the fasciculations assessed in the EMGs, FEs were assessed in terms of absolute and relative number as FEs per minute. In addition, benefiting from the 2D identification of FEs in the US images, the coordinate of the centroid of FEs in the depth direction was quantified and considered as a predictor for matches between FPs and FEs, as detailed below, with the image-specific time-stamp information used to ensure appropriate alignment between modalities [19]. FEs were classified as belonging to MG or SOL and deeper muscles motor units based on the location of the twitch region. If the movement involved the aponeurosis between muscles or was seen across a region spanning both muscles, the FE location was classified as uncertain (MG ap in figure 2).

3) ANALYSIS COMBINING BOTH EMG AND US DETECTION TECHNIQUES

The fasciculation potential (FP, EMG) and event (FE, US) onsets and locations were used to identify whether pairs detected in EMG *and* US could be attributed to the same muscle fasciculation, i.e. whether the pair defined by the i-th fasciculation event (FE_i) and the j-th fasciculation potential (FP_j) was indeed associated with the mechanical and electrical representation of the same physiological happening. To this end, we defined the following criteria: (a) the location of FP_j must be included in the region scanned by the US probe and (b) the time lag between FP_j and the detection of FE_i must be smaller than 150 ms. If both criteria were met, FEi and FPj were classified as matched. It should be noted that 150 ms (criterion b) is longer than the rise time of the mechanical twitch associated with the excitation of the calf muscles [5], [21]. The 150 ms figure is therefore a conservative selection for the period within which a mechanical twitch in the US image should be observed in response to FPs observed in the EMGs. For each EMG electrode configuration, we reported the number of matches as percentage with respect to the number of FE identified by US.

The subset of fasciculations identified by both EMG and US (matches) was analyzed to study the association between electrical and mechanical/anatomical properties of fasciculations. Specifically, for the three EMG detection configurations, we analyzed the depth of FEs both matching and not matching the FPs. Finally, for occurrences of fasciculation matches in EMG and US, we computed the correlation between the features of the FE (size, proximal-distal location, depth) and those of the FP (amplitude, spatial dimension, barycenter).

4) STATISTICAL ANALYSIS

Normality was assessed with the Shapiro-Wilk test. FE depths and the depth of matched and non-matched FP-FE occurrences were normally distributed and were therefore analyzed with one-way ANOVA (factor: ''region''). In this case data from all subjects was merged into one dataset. The effect

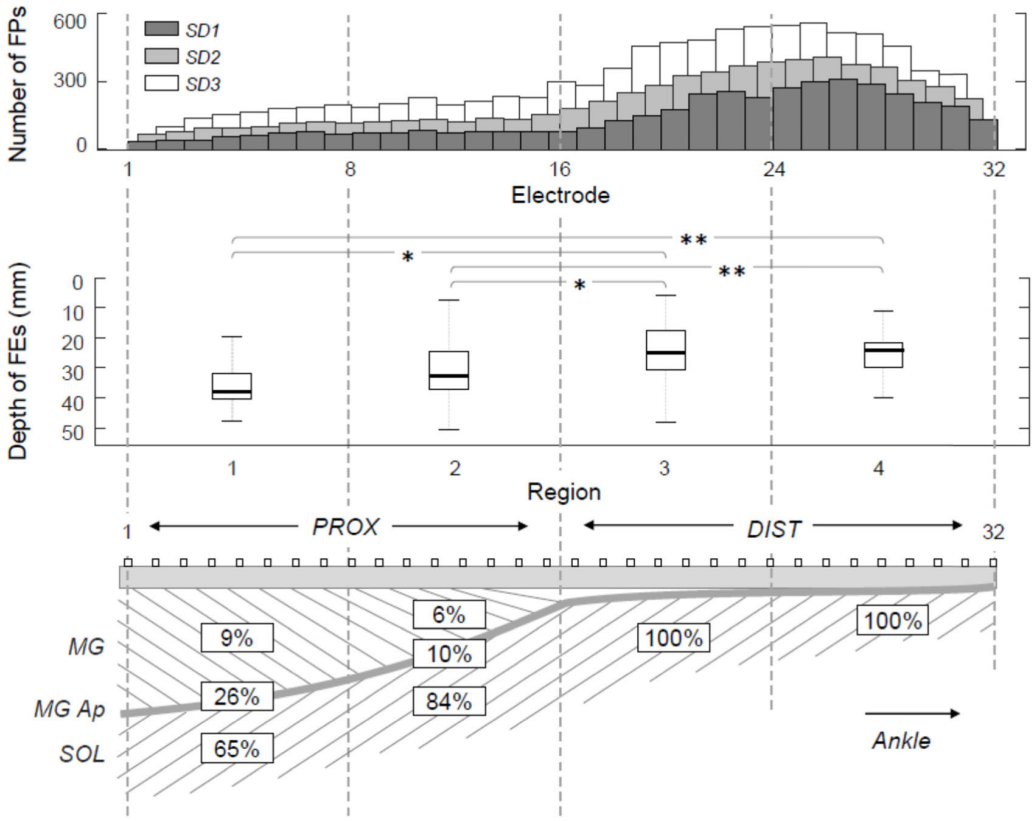

FIGURE 2. Upper panel: distribution of fasciculation potentials (FPs) across the channels of the three electrodes' configurations for all participants (SD1, SD2 and SD3 indicate single differential EMGs with 10, 20 and 30 mm inter-electrode distance respectively). Mid panel: depth of the identified fasciculation events (FEs) in the four scanning regions (*p<0.05 **p<0.001). The spatial relationship between scanning regions, EMG electrodes and anatomical structures of underlying muscles is reported in the lower panel, along with the percentage of FE classified as belonging to MG or SOL and deeper muscles motor units. Percentages in correspondence of the MG aponeurosis (MG Ap) refer to event non uniquely attributable to MG or SOL.

of IED on the depth of matched and non-matched FPs-FEs was analyzed with a two-way ANOVA (factors: IED, Match). Post-hoc pairwise assessments were conducted whenever a main effect was verified. Non-parametric tests were used for the number of fasciculation and the number of matches, as their distribution was not Gaussian. Specifically, Friedman ANOVA was adopted to evaluate the effect of detection system and detection region on the number of detected fasciculation and matches. In cases where Friedman ANOVA revealed an additive effect, Wilcoxon signed rank test (pairwise comparisons) and Mann-Whitney U test (comparison between independent samples) were used for all relevant variable combinations. All post-hoc analyses were corrected according to Bonferroni. The Pearson's correlation coefficient was computed on the merged dataset across subjects to determine the association between electrical and mechanical/anatomical properties of fasciculations (the variable list is reported in the previous section). The threshold for statistical significance was set at $p < 0.05$. Tests were performed with Statistica 7.0 (Stat Soft. Inc, Tulsa, OK, USA). All results are reported as median [first quartile – third quartile].

III. RESULTS

A total number of 998 FPs in SD3 (750 in SD2 and 484 in SD1) and 377 FEs were detected in the ten participants enrolled in this study. The distribution of FP locations along the skin for each of the three detection systems is reported in the upper panel of Fig 2. FEs were significantly deeper in the most proximal scanning region and progressively became more superficial as the scanning region moved distally (mid panel Fig 2). The relative distribution of FE across muscles showed that for all the scanning regions the majority of events occurred in SOL (lower panel Fig 2).

Figure 3a shows the number of fasciculations per minute (fpm) identified by the three EMG detection configurations (SD1, SD2, SD3) and by US. Significantly more fasciculations were observed in SD3 (12.9 [10.1 – 17.9] fpm) compared to both SD1 (4.1 [1.8 – 6.7] fpm, p=0.0012) and SD2 (9.63 [5.8 – 12.0] fpm, p=0.012). No statistically significant differences were observed between SD3 and US (10.4 [3.2 – 14.6] fpm). The comparison between the number of fasciculation detected in the proximal and distal calf regions (Figure 3b) showed that the US sensitivity to

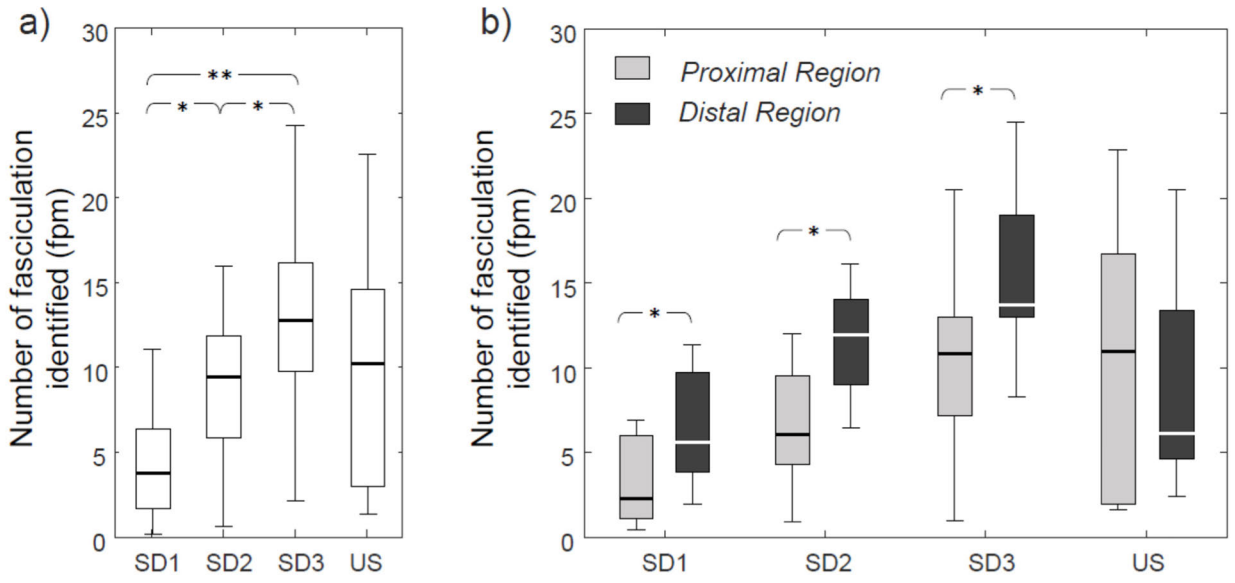

FIGURE 3. Comparison between the number of fasciculation per minute identified from the same muscle portion by the three EMG configurations (SD1, SD2, SD3) and by US imaging considering the entire posterior leg (a) and the proximal and distal regions separately (b).

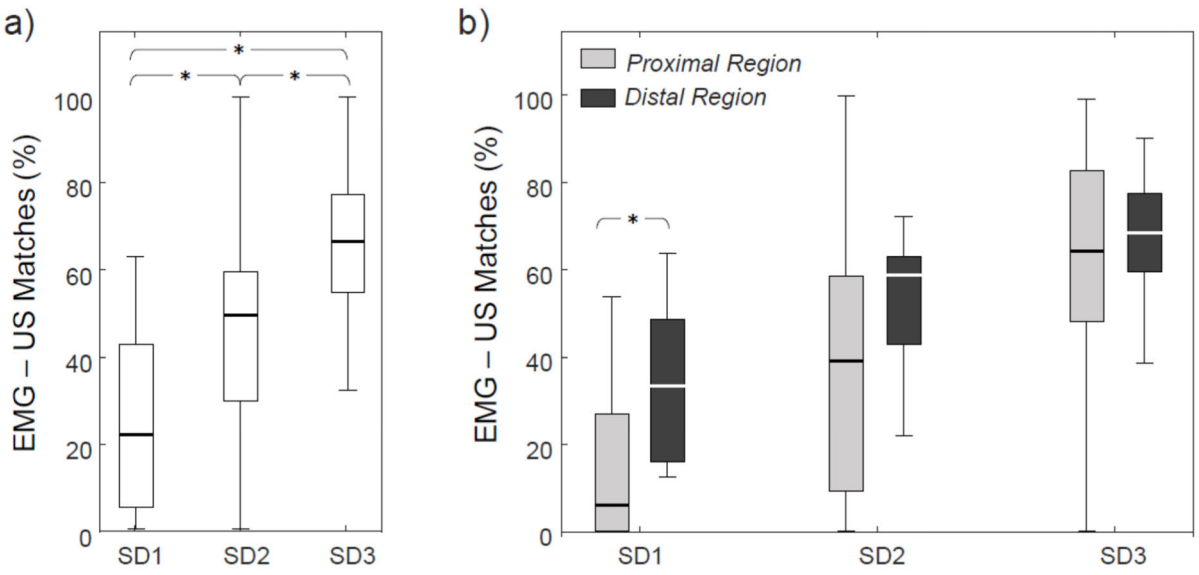

FIGURE 4. Comparison between the number of matches between fasciculation potentials and events (EMG-US matches) for the three EMG configurations (SD1, SD2, SD3) considering the entire posterior leg (a) and the proximal and distal regions separately (b).

fasciculation did not depend on the detection region. In contrast, the number of fasciculation detected by EMGs was region dependent, with significantly greater occurrences observed distally for all electrodes' configurations.

Although the number of fasciculation detected by EMG and US was comparable, only a small fraction of the identified events was classified as common and therefore attributable to the activation of the same motor unit. The percentage of FEs that were identified in EMG (matches) ranged from 22% to 68% (median values) and increased significantly with greater distance between EMG electrodes (i.e., from SD1 to SD3), as shown in Figure 4a. The results reported in figure 4b show that the median number of matches is higher distally, although the statistical significance was observed for only SD1.

The subset of FEs matching FPs in EMG were clustered in the superficial muscle region, as shown by the average depth of FEs detected and not detected in EMG (Figure 5, significant main effect of Match, $p < 0.001$ for both proximal and distal regions). By increasing the IED (from SD1 to SD3),

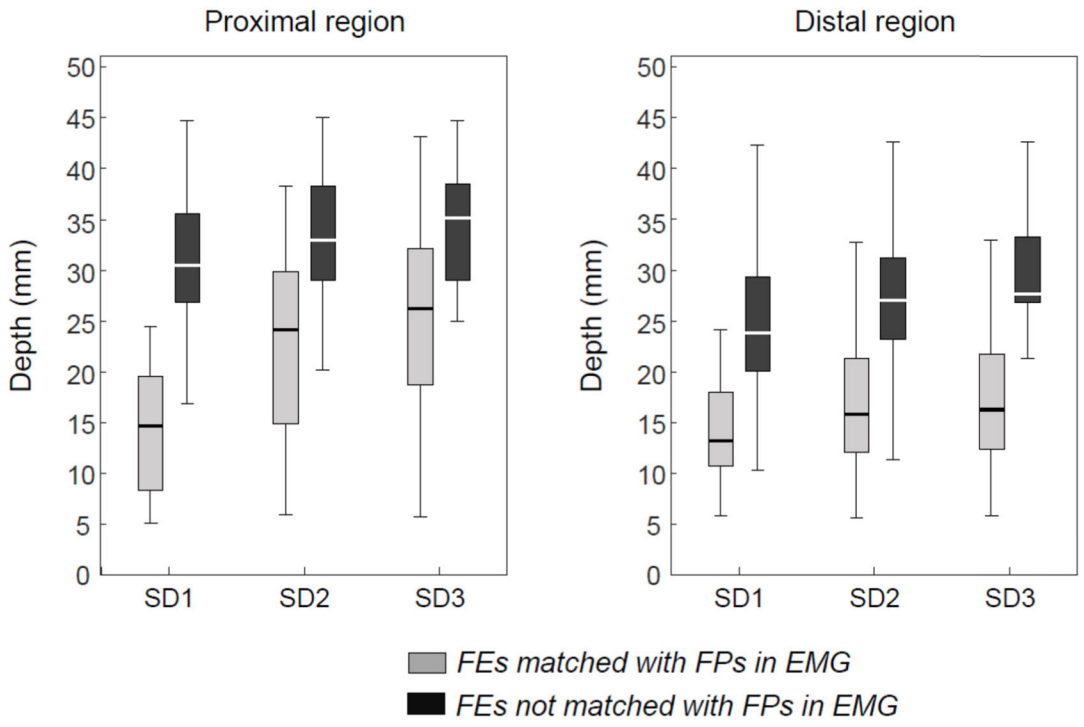

FIGURE 5. Depth of fasciculation events (FEs) matched (grey boxes) and not matched (black boxes) with fasciculation potentials (FPs) in EMG. Data is provided for the three electrodes' configurations (SD1, SD2, SD3) in the proximal (a) and distal (b) muscle regions. A significant main effect of match ($p < 0.001$) was found for both proximal and distal regions.

the average depth of matched fasciculations increased. This trend was clear in the proximal region (significant interaction IED x Match $p < 0.05$), whereas in the distal region it was not statistically significant.

The analysis of the association between mechanical and electrical characteristics of the fasciculations showed a significant correlation between the barycenter of the FP and the proximal-distal coordinate of the centroid of the twitch region ($r = 0.86$, $p < 0.001$). All other combinations of variables (amplitude and location for FPs and depth and area of the twitching region for FE) did not show significant correlation.

IV. DISCUSSION

The possibility of non-invasively assessing muscle excitation and movement has attracted an increasing interest for the study of fasciculation in the last ten years [13], [15], [4]. As compared to the standard electrophysiological approach based on intramuscular detections, both surface EMGs and b-mode US imaging have proved to increase the detection sensitivity to fasciculations [11], [12]. In this study we characterized the fasciculation potentials and events detected simultaneously by multichannel EMG and US recordings. When EMG and US are recorded from the same muscle region, they proved to be similarly sensitive to muscle fasciculations, however the agreement between the two techniques in detecting the same fasciculation depends on the characteristics of the EMG detection system and on the anatomy of the investigated muscles.

Before delving into the discussion of the study results, few important considerations on the characteristics of the US and EMG detections are required. US and EMG measure respectively the mechanical and the electrical responses of excited muscle fibers. US is sensitive to movements taking place in muscle sections lying in the plane of the US probe. One dimension of this plane is defined by the size of the probe while the other (depth) depends on the ultrasound wave properties. In this study, the probe was 60-mm long and the depth was set to 50 mm to sample both MG and SOL in all participants. Surface EMG is predominantly sensitive to electrical events occurring closer to the skin surface. The contribution of deeper electrical sources can be attenuated to a lesser extent however when surface EMGs are detected with greater IEDs [9], [16]. In this study EMGs were detected with a linear array of electrodes covering longitudinally the medial gastrocnemius muscle and the distal portion of soleus. This electrode arrangement allowed us to map where FPs occurred in the calf and thus to study the extent to which the number and location of FPs, and the agreement with FEs detected by US, depend on the detection sensitivity (inter-electrode distance) of single differential channels. Another important consideration concerns the classification of FE and FP. In this study we counted and localized the occurrences of FPs and FEs but did not classify them. Indeed, while the classification of FPs based on their spatiotemporal characteristics is feasible, a higher temporal resolution of US recordings would be required to robustly complete a classification of FEs.

Therefore, we do not provide information about the number of sources generating the observed fasciculations.

A. RELATIONSHIP BETWEEN THE SPATIAL DISTRIBUTION OF FE AND FP

We found that FPs distribute unevenly along the longitudinal axis of the posterior leg (Fig 2). Specifically, a larger proportion of FPs was observed in the distal half of the electrode array, and therefore distal to the medial gastrocnemius myo-tendon junction for all participants. This result, observed for the three IEDs, can be explained by the location of fibers of active motor units in relation to the surface electrodes. The analysis of the dimensions and centroids of the twitch areas detected in US showed that FEs were predominantly associated with the activation of motor units located in the SOL (Fig 2 mid and lower panels). In the proximal region, SOL is covered by the MG that progressively tapers to the myo-tendon junction, after which the distance between SOL and EMG electrodes is determined by the subcutaneous adipose tissue thickness (Fig 1). Assuming most of the fasciculations took place in SOL, as corroborated by the results shown in Fig.2, we therefore propose the larger number of FP detected distally to be a direct consequence of the reduced source-electrode distance in that region [22]. This consideration has important implications for the interpretation of results concerning the sensitivity of US and EMG to fasciculations in different muscle regions, as reported in the following sections.

B. NUMBER OF FASCICULATION DETECTED BY EMG AND US

In virtue of a larger area covered by the detection system, multichannel EMG is potentially more sensitive to fasciculations than US. Indeed, when the entire array of electrodes is considered the number of fasciculation detected by EMG is larger than US for all IEDs (see first paragraph of Results section). This factor represents however an obvious bias towards EMG which would not allow a generalization of the results. To ensure a like-with-like comparison between techniques, only FPs detected by EMG electrodes covering the same area of the US probe were analyzed.

The number of fasciculation identified by EMG and US from the same muscle region is similar; about ten per minute for the healthy individuals studied. However, Fig 3a shows that the EMG sensitivity to FPs depends on the inter-electrode distance. This observation is in line with our initial hypothesis and can be explained by the increased detection volume associated with the larger distance between differential EMG electrodes. For 20 and 30 mm IED the EMG sensitivity to fasciculations was comparable to that of US, while it was smaller for 10 mm IED. With respect to sensitivity in the proximo-distal direction, Figure 3b shows that fasciculations in the distal calf region are more likely to be observed in EMGs than in US. Two factors presumably explain the different proximo-distal sensitivity of EMGs to fasciculations. First, when compared to MG, a greater number of fasciculations were observed in SOL. Second, the distance between SOL and the skin is smaller in the distal region, where MG is not present. Accordingly, while US is equally sensitive to superficial and deep sources in its scanning plane, EMG detection filters deep sources resulting in a lower number of FP detected proximally. In this context, the greatest IED provided an EMG sensitivity to fasciculation comparable to that of US (see the comparison between SD3 and US proximal in Fig3b).

C. MATCHES AND CORRELATIONS BETWEEN FP AND FE

The number of matches between FP and FE increased with IED, which is likely a direct consequence of the increased sensitivity to deep fasciculations provided by larger IEDs. This interpretation is corroborated by two additional observations. Firstly, for the most selective configuration (SD1) the number of matches observed proximally are significantly lower than distally (Fig 4b), where fasciculations are more superficial (Fig 2). Secondly, as for the number of matches, the depth of matched FEs increases with the IED (FEs detected in EMG, Fig 5). This behavior is clear in the proximal region and less evident distally. Although these results suggest a clear association between EMG detection volume and number of matches, it is worth noting that about 40% (median) of the fasciculations are unmatched even for the largest IED (Fig 4b), despite this the sensitivity of SD2 and SD3 is similar or slightly higher than US (Fig 3a). This result may be explained by the different spatial sensitivities of EMG and US: a superficial, hemispherical volume for EMG and a proximo-distal section of the tissue for US (Fig 6). Indeed, excited fibers outside the scanning plane may not generate a detectable movement in the US image although their action potential may cross the detection volume of EMG (FE#1 Fig 6a). The probability for this to happen depends on the medio-lateral dimension of motor unit territories, on the fibers' curvature across the parasagittal plane, and on the limb circumference in the considered region. For these reasons, we believe missing FEs is more likely to occur in the proximal portion of the SOL (see fig 2) [22], [23]. On the other hand, a fasciculation taking place below the US probe in the deeper muscle region (FE#3 Fig 6a) can be captured by US but may not exceed the noise threshold in EMG signals, even when detected with the 30-mm IED [9]. In this respect, it is important to note that the detectability of a FP depends on the minimum distance between the electrodes and the propagating potential. For in-depth pennate muscles the minimum distance is determined by the superficial aponeurosis, where action potentials extinguish (e.g. FE#2 in fig 6). Therefore, in these muscles, surface electrodes may detect FPs associated with FEs taking place outside their detection volume (FE#2 Fig 6a and b). This possibility appears more likely proximally, where both imaged muscle (MG and SOL) have in-depth pennate architecture. Distally, FEs may arise from deeper muscle with different architectures (e.g. flexor hallucis longus), with their associated FPs not reaching the detection volume of electrodes (FE#3 in fig 6). The same

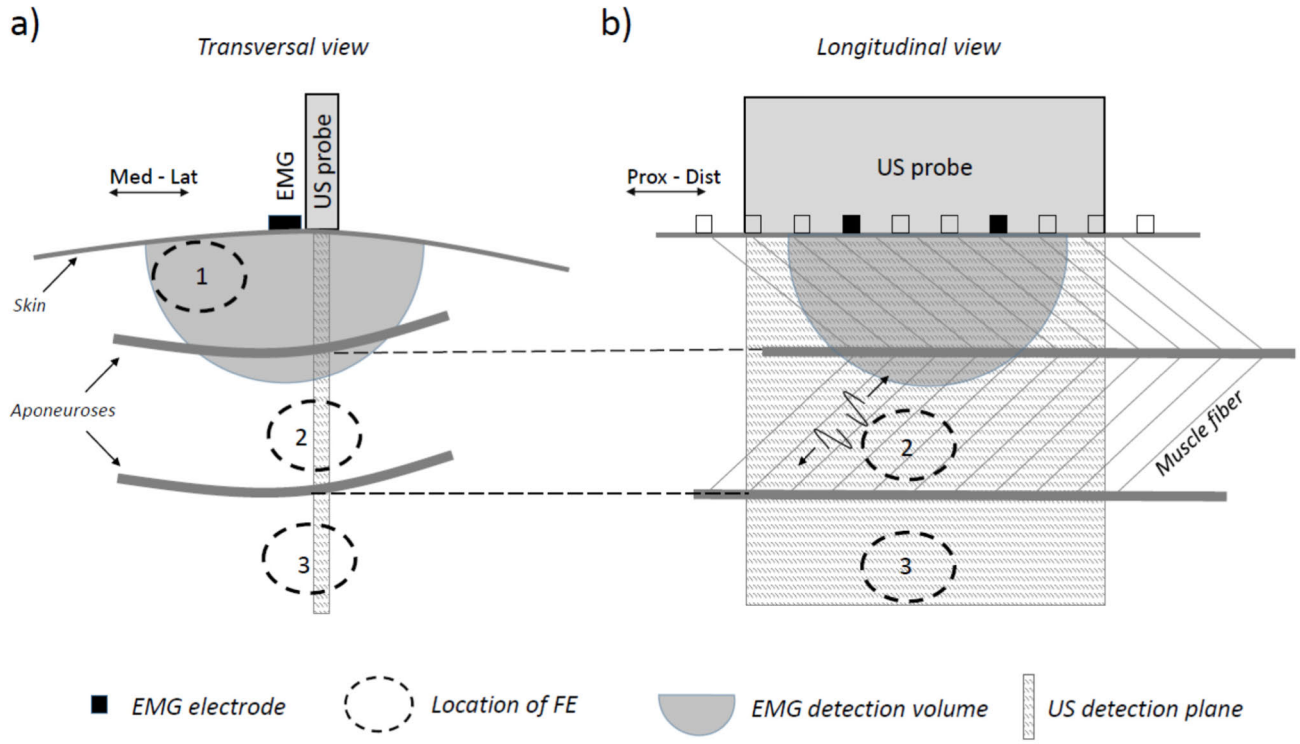

FIGURE 6. Schematic representation of the transverse (a) and longitudinal (b) muscle sections showing how the differences between EMG and US detection regions may determine mismatches between EMG and US fasciculation detection. The grey-shaded semicircle is the detection volume of EMG electrodes and the patterned rectangle is the imaging plane of US. The area identified by the dashed line represents the muscle region where a fasciculation generates an active movement detectable in US. Three cases are illustrated. Fasciculation event #1 (FE#1) is not detected in US but the associated fasciculation potential (FP) is included in the EMG detection volume. In FE#3, a FE is identified but the correspondent FP is too deep to be detected in EMG. The movement associated with the fasciculation for FE#2 is outside the detection volume of EMG but, owing to the in-depth pinnation of the calf muscles, the FP propagates towards the muscle superficial aponeurosis and therefore enters in the detection volume of EMG electrodes (see propagating potential in panel b).

IED may therefore detect more, and deeper matches proximally than distally, as shown in Fig 5. Although we cannot provide direct evidence supporting these interpretations of our results, theoretical and experimental evidence seem to suggest this explanation, indicating an avenue for future research [9], [16], [22], [24].

The differences between EMG and US detection volumes may also explain another unexpected result. In fact, although the association between the motor unit anatomical properties (size and depth) and the correspondent motor unit action potential amplitude is well established, in our study neither the size of the twitch area nor its depth was correlated to the amplitude of the FPs. A possible confounding factor we believe may be responsible for the lack of correlation between FE and FP properties is that the measured area of a FE depends on how the US imaging plane crosses the twitching volume. The same area may correspond to a large volume intersected by the imaging plane peripherally or to a small volume intersected centrally. Additionally, the muscle fiber architecture (e.g. length and orientation) coupled with the base-material muscle properties (e.g. stiffness) and current state (e.g. background, tonic activity) are likely to influence how the motor unit excitation is encoded through the muscle tissue displacements. Further investigations, coupling 3D US scans and bi-dimensional arrays of EMG electrodes, alongside muscle simulations, may help to quantify the effect of deep and misaligned sources on the agreement between EMG- and US-based fasciculation detection and variable estimation.

D. CLINICAL IMPLICATIONS

While the fasciculation frequency found here is similar to that previously reported for healthy adults [25], we were surprised by the fraction that were unique to either EMG or US detection. This should therefore also be a consideration for implementation of these approaches as a clinical tool for identification of on-going denervation. Specifically, it seems that rather than focusing on one technique, a combination of US-EMG is the most comprehensive means of detecting fasciculation. Although muscle atrophy, which will occur as neurodegeneration progresses, may reduce the fraction of unique events identified in each modality the ability to assess, comprehensively, muscles in the early stages of disease (i.e. larger, less affected) is likely of value for early detection of disease. Equally, changes in the fraction of unique events within the muscles may be a useful indicator of combined changes in muscle size/shape and motor unit pool characteristics particularly given fasciculation frequency has been

suggested a sensitive indicator of longitudinal change [3]. Future work should therefore include evaluation of patients living with neurodegenerative diseases, such as Motor Neurone Disease. Additionally, due to the requirement to evaluate muscles across different body regions for diagnosis of Motor Neurone Disease [2], work should evaluate differences in fasciculation detection rates in different muscle groups (e.g. upper limb, paraspinal region) particularly where fascicle architectures different to the muscles studied here exist.

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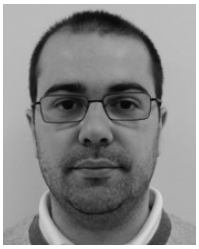

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