Spatial variation of compound muscle action potentials across human gastrocnemius medialis

Vieira TM, Botter A, Minetto MA, Hodson-Tole EF. Spatial variation of compound muscle action potentials across human gastrocnemius medialis. J Neurophysiol 114: 1617–1627, 2015. First published July 8, 2015; doi:10.1152/jn.00221.2015.—The massed action potential (M wave) elicited through nerve stimulation underpins a wide range of physiological and mechanical understanding of skeletal muscle structure and function. Although systematic approaches have evaluated the effect of different factors on M waves, the effect of the location and distribution of activated fibers within the muscle remains unknown. By detecting M waves from the medial gastrocnemius (MG) of 12 participants with a grid of 128 electrodes, we investigated whether different populations of muscle units have different spatial organization within MG. If populations of muscle units occupy discrete MG regions, current pulses of progressively greater intensities applied to the MG nerve branch would be expected to lead to local changes in M-wave amplitudes. Electrical pulses were therefore delivered at 2 pps, with the current pulse amplitude increased every 10 stimuli to elicit different degrees of muscle activation. The localization of MG response to increases in current intensity was determined from the spatial distribution of M-wave amplitude. Key results revealed that increases in M-wave amplitude were detected somewhat locally, by 10–50% of the 128 electrodes. Most importantly, the electrodes detecting greatest increases in M-wave amplitude were localized at different regions in the grid, with a tendency for greater stimulation intensities to elicit M waves in the more distal MG region. The presented results indicate that M waves recorded locally may not provide a representative MG response, with major implications for the estimation of, e.g., the maximal stimulation levels, the number of motor units, and the onset and normalization in H-reflex studies.

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wirth et al. 2010]. Stimulation at an intensity that produces a defined percentage $M_{\text{max}}$ can facilitate recruitment of a consistent population of muscle units between different trials, although a degree of alternation does occur because of fluctuations in axon thresholds (Bostock et al. 1998; Burke et al. 2001). Increasing stimulation intensity leads to an increase in M-wave (Keenan et al. 2006) and joint torque (Vieira et al. 2013) amplitude, which is assumed to reflect an increase in the number of activated motor units. As such, M waves are also used in the study of neuromuscular fatigue (Botter et al. 2009a; Merletti et al. 1994) and reflex responses [e.g., Hoffman’s reflex (Maffulli et al. 2001)] and to normalize myoelectric signals recorded during voluntary activation (Carolan and Caffarelli 1992). Eliciting and recording M-wave responses in skeletal muscles therefore underpin a wide range of physiological and mechanical understanding of skeletal muscle structure and function.

The characteristics of M waves detected by surface electrodes are known to be influenced by a number of factors. For instance, signal waveform can be influenced by 1) the conduction velocity of action potentials along the muscle fiber sarcomlemma (Merletti et al. 1999), with faster conduction leading to potentials with shorter durations; 2) the variability in the times at which action potentials reach the neuromuscular junctions and the shape of the intracellular action potential (Keenan et al. 2006); and 3) the amount of subcutaneous fat tissue (Doheny et al. 2010) and the distance between the motor unit muscle fibers and recording electrodes (Bromberg and Spiegelberg 1997); both decrease the amplitude of surface potentials.

In addition to those noted above, a further factor that could influence M-wave properties, but has not yet been well explored, is muscle fiber architecture (geometric arrangement of fibers in the muscle belly). The location and distribution of activated muscle fibers within the muscle belly could influence the spatial distribution of the resulting M waves over the surface of the muscle. This is likely to be particularly true in muscles with a pennate fascicle architecture, where action potentials travel from deep to superficial regions of the muscle and surface electrodes are most likely to record potentials from fibers close to their location (Mesin et al. 2011). If motor unit fibers are evenly distributed throughout the muscle belly this factor may not be as critical. However, it has recently been suggested that in human gastrocnemius medialis (MG) muscle the fibers of units activated during standing occupy relatively localized regions along the longitudinal axis of the muscle.
[~40 mm (Vieira et al. 2011)]. In such cases, M-wave properties may be significantly influenced by the location of the recording electrode over the muscle. As the majority of studies of M-wave properties utilize single electrodes placed over the muscle of interest, in a standardized location, it is possible that they do not capture 1) M-wave characteristics that are representative of all motor unit properties present within the muscle or 2) a true \( M_{\text{max}} \) for the muscle. Indeed, in human MG muscle it has previously been shown that low-level stimulation of the tibial nerve produces M waves localized in the proximal muscle region, which are not represented in distal muscle regions (Hodson-Tole et al. 2012).

We therefore aimed to address the question: Are changes in M-wave amplitude, resulting from increased stimulation intensity, represented equally over different regions of MG? If populations of muscle units activated by a given stimulation increment occupy discrete, localized regions within MG, current pulses applied to the MG nerve branch would be expected to lead to local changes in M-wave amplitudes. Changes in the intensity of stimulation pulses would consequently evoke MG responses in different muscle regions. Alternatively, if the fibers of MG motor units are widely distributed through the muscle belly, increases in stimulation intensity would lead to equal changes in M-wave amplitude over the surface of the MG muscle. The presented results have both methodological and physiological implications, such as 1) informing principles for placement of electrodes over MG, 2) calculation of MUNE/MUNIX values and normalization in H-reflex studies, 3) understanding of different motor unit properties in MG, and 4) development of functional electrical stimulation protocols.

MATERIALS AND METHODS

Participants. Twelve participants took part in the study (1 woman, 11 men; range values: age 23–26 yr, height 165–188 cm, body mass 58–85 kg). All participants reported that they were free from musculoskeletal dysfunction and pathology and provided written informed consent. Experimental procedures conformed to the Declaration of Helsinki and were approved by the Institutional Ethical Committee.

Data collection. Participants were seated on a customized chair, with the hip joint slightly flexed, the knee fully extended, and the ankle at 90°. The right foot was positioned onto a piezoelectric force plate, positioned vertically in front of the participant (see Fig. 1 in Gallina et al. 2011). Reaction forces were measured by highly sensitive sensors (~0.9 mV/N; 9286AA Kistler, Zurich, Switzerland) and digitized with a 12-bit A/D converter, which provided an approximate resolution of ~2.7 N/bit.

Monopolar surface EMGs were recorded from MG of the right leg with 128 circular electrodes (8-mm diameter) arranged in a 16 × 8 grid with 10-mm center-to-center interelectrode distance. Ultrasound imaging (7.5-MHz linear array transducer, MyLab25, Esaote, Genoa, Italy) was used to ensure that electrodes were placed within the MG boundaries and to facilitate placement over fascicles under the superficial aponeurosis, whose pennation angle prevented action potential propagation being detected along the electrode array (for example, see Fig. 1 in Hodson-Tole et al. 2012) and hence ensured that each electrode recorded signals from different populations of muscle units along the proximal-distal muscle axis. Before the electrodes were positioned, the skin was shaved and carefully cleansed with alcohol. For each individual, the amplification factor (100-1,000) was set to maximize the signal-to-noise ratio while avoiding saturation (10- to 750-Hz EMG-USB amplifier, OT Bioeletronica, Turin, Italy). During each trial, EMGs and trigger pulses, each signaling the delivery of a stimulation pulse, were recorded synchronously at 2,048 samples/s (12-bit A/D converter, 5-V dynamic range, EMG-USB amplifier, OT Bioeletronica).

M waves were elicited with monopolar electrical stimulation as fully described in Vieira et al. (2013); in monopolar stimulation, the cathode electrode is positioned nearby whereas the anode is positioned away from the targeted nerve or motor point (cf. Fig. 1A in Vieira et al. 2013). Briefly, the anode (80 × 50 mm) was fixed above the patella. A pen electrode (small cathode: 10-mm² surface; Globus Italia, Codognè, Italy) was used to deliver biphasic square-wave current pulses (200-μs duration; Digitimer DS7, Digitimer, Welwyn Garden City, UK) while the experimenter searched for the location on the tibial nerve branch that 1) exclusively innervated MG (Wolf and Kim 1997) and 2) was the mostexciting stimulation site, defined as the point where a muscle twitch was evoked with the smallest current amplitude. Once this location was identified, an adhesive pregelled stimulation electrode (10 × 10 mm; Spes Medica, Battaglia, Italy) was placed over the identified location.

The amplitude of the current pulses was varied to elicit responses from different MG muscle units (Fig. 1). Pulses were delivered for 50 s at a rate of 2 pps. The first stimulation level corresponded to the

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**Fig. 1. Staircase stimulation protocol.** A schematic illustration of the stimulation protocol as well as of the procedure for the calculation of incremental M waves is shown. Stimulation intensity increased equally from zero to the current intensity \( I_{\text{max}} \), after which no changes in the amplitude of gastrocnemius twitches were observed. For each stimulation level, 10 stimuli were applied at a rate of 2 pps. Incremental M waves were calculated from M waves averaged over these 10 stimuli, as shown at bottom. Note that the amplitude of incremental M waves is large only when an increase in stimulation intensity leads to marked changes in the amplitude/shape of M waves.
smallest current for which the recorded peak ankle plantar flexion torque was significantly higher than the background noise level. Every 10 stimuli (5 s), stimulation amplitude was increased by a fixed amount. The highest stimulation level matched the current intensity at which the greatest peaks in ankle torque were recorded without discomfort. Ankle torque rather than M-wave amplitude was used to define maximum stimulation level because, as hypothesized in this study, the amplitude of M waves detected locally could lead to erroneous indications of the maximal stimulation intensity for the MG muscle.

Data analysis. The spatial distribution of electrically evoked responses in MG was quantified through the amplitude distribution of incremental M waves. When stimulation intensity is increased, a new population of muscle units may be activated if stimulation intensity exceeds the activation threshold of their motor axons. The characteristics of any newly stimulated units are clearly represented by the algebraic difference between a pair of M waves elicited by different stimulation intensities: the incremental M wave (Botter et al 2009b). By progressively increasing the stimulation intensity, as schematically illustrated in Fig. 1 and outlined below, we therefore considered the incremental M waves to quantify the response of different regions of MG to the stimulation pulses.

Incremental M waves were calculated separately for each of the 128 electrodes and each of the 10 consecutive stimuli delivered at each stimulation level. First, each group of 10 M waves, elicited at a given stimulation level, were averaged (20-ms epochs). The incremental M wave was then taken as the difference between average M waves for consecutive stimulation levels (Fig. 1), separately for each of the 128 electrodes.

Localized changes in the response of MG to the increases in the intensity of stimulation pulses were specifically calculated through the centroid of the amplitude distribution of incremental M waves. Initially, the root mean square (RMS) amplitude of incremental M waves was computed separately for each electrode and stimulation level, providing a total of 1,152 values per participant (128 electrodes × 9 increments of stimulation level). After evaluation of the presence of action potential propagation between adjacent electrodes, only electrodes located on skin regions over the superficial aponeurosis, where the fascicle pennation angle ensured sampling from different groups of MG fibers, were used for further analysis (Vieira et al 2011). As the ultrasound imaging revealed different MG lengths between participants, the number of rows of electrodes considered varied between them, from 8 to 11 rows. After that, for each stimulation level the electrodes detecting incremental M waves with greatest RMS amplitude were identified with an automated and validated segmentation technique (Vieira et al 2010); these electrodes were termed segmented electrodes (Fig. 2A). Finally, the centroid of the resulting RMS distribution was calculated as the weighted average of the coordinates of segmented electrodes, separately across rows and columns (Fig. 2A). The centroid coordinates across the rows and the columns of electrodes indicate where the mean of the RMS amplitude distribution is located along the muscle proximo-distal and mediolateral axes, respectively.

Statistics. Nonparametric statistics were used to assess localized changes in the RMS distribution of the incremental M waves. To ensure that no assumptions on the linearity of these data were made, the Spearman correlation coefficient was calculated for scatterplots created with the stimulation increment (numbered 1–9) in the x-axis and the centroid coordinates in the y-axis. Correlation coefficients were evaluated separately for each subject, as consecutive stimulation increments were potentially not comparable between participants. Moreover, as we could not anticipate a predominant direction for the shifts in centroid position along either the muscle longitudinal or transverse axis, the significance of the Spearman correlation coefficient was tested bilaterally, i.e., we tested whether it was significantly greater or smaller than zero.

Not all stimulation increments were considered for the calculation and testing of correlation coefficients. As illustrated for a single participant in Fig. 3, only stimulation increments leading to a significant increase in the M-wave RMS amplitudes were considered for analysis. Including instances corresponding to marginal changes in the M-wave amplitudes would possibly lead to misleading indications on the region where muscle response was evoked, as these occurrences would be associated with the stimulation of a very few, or no, additional muscle units. RMS values were calculated for M waves from each individual participant and then averaged over the segmented electrodes to produce a single RMS value for each stimulation pulse, level, and participant. To determine which stimulation increments to include in the correlation coefficients, Tukey honestly significant difference (HSD) post hoc analysis was considered for the paired comparison of M-wave RMS amplitude between consecutive stimulation levels.

RESULTS

General description. None of the participants complained about discomfort on the staircase stimulation protocol considered in this study (Fig. 1). The maximal current intensity applied varied from 56 mA to 90 mA, whereas the current leading to the first observable MG twitches ranged from 12 mA to 32 mA across the 12 participants. Not all stimulation increments led to significant increases in the amplitude of M waves, and there was no relationship between the increment number and significant increases in M-wave amplitude. Nevertheless, the amplitude of incremental M waves was consistently positively correlated to increments in peak plantar flexion torque for all subjects tested (Fig. 4; Pearson R > 0.57; P < 0.11); for every 1-μV increase in the RMS amplitude of incremental M waves, the plantar flexion peak torque increased from 0.012 Nm to 0.069 Nm. The number of electrodes detecting significant changes in M-wave amplitude was relatively small (interquartile interval: ~10%-50% of electrodes in the grid). Most importantly, as revealed by the incremental M waves and as illustrated in the following sections, these electrodes were located over different MG regions.

Incremental M-wave properties. Increases in the stimulation intensity evoked localized MG responses, which were well represented in the RMS amplitude of the incremental M waves. Figure 2A shows an example of the distribution of RMS amplitude calculated for M waves elicited for two consecutive current intensities (Fig. 2A, top) and the corresponding incremental M waves (Fig. 2A, bottom) from one participant. For the smallest stimulation intensity shown, 21 mA, high-RMS amplitude values were observed for M waves detected by the most proximal row and central columns of electrodes. By increasing stimulation intensity to 28 mA, increases in RMS amplitude were observed, with M waves detected over a relatively larger region that extended toward the distal and lateral electrodes. The region leading to greatest changes in the RMS amplitude of M waves, when stimulation intensity increased from 21 mA to 28 mA, was evident in the RMS amplitude of incremental M waves. This region was automatically identified with the segmentation technique, and its location in the grid is well represented by the centroid coordinates (cf. Fig. 2A, bottom).

The location of the greatest incremental M waves in the grid of electrodes changed markedly with the progressive increase...
Fig. 2. Incremental M waves and their local representation. A: root mean square (RMS) values computed from M waves obtained for a single participant. M waves were averaged over 10 consecutive stimuli (top), separately for the third (21 mA) and fourth (28 mA) stimulation steps (cf. Fig. 1) and for each of the 64 electrodes (8 rows × 8 columns) positioned over the muscle superficial aponeurosis. The RMS amplitude of incremental M waves is shown at bottom. Electrodes providing incremental M waves with greatest amplitudes are represented with small circles, whereas the coordinate of their barycenter along rows and columns is represented with a large crossed circle. B: RMS amplitude of incremental M waves obtained for the 9 progressive increases in stimulation intensity. Changes in the stimulation intensity leading to each of the 9 increments are indicated at top of each plot. Ranges in bold above array plots denote significant difference in the RMS amplitude of M waves with the increase in stimulation intensity (cf. Fig. 3).

in stimulation intensity. For the participant whose data is shown in Fig. 2B, the number of electrodes detecting greatest RMS amplitude ranged from 2 to 40 of 64 electrodes across all stimulation levels. As revealed by the centroid position, these electrodes were located in different regions during the different stimulation levels. In the example shown in Fig. 2B, the centroid of the RMS distribution shifted predominantly distally and laterally when stimulation intensity increased, from 7 mA through to 35 mA and then again for 63 mA to 70 mA. For each of these five increments the RMS amplitude of raw M waves increased significantly (Tukey HSD post hoc; \( P < 0.001; n = 100; 10 \) stimuli × 10 stimulation steps). For the four stimulation increments, from 35 mA through to 63 mA, the centroid position changed in the opposite direction, moving proximally and toward the medial aspect of the muscle. These increments, however, led to marginal increases in the amplitude of raw M waves (Tukey HSD post hoc; \( P > 0.4 \)).

Proximo-distal changes in distribution of incremental M-wave amplitudes. The centroid of the RMS amplitude distribution shifted predominantly toward the distal muscle region with increasing stimulation level. Ten of the twelve participants tested showed a positive correlation coefficient, with the centroid coordinate moving from proximal to distal locations between stimulation levels (Fig. 5). Five occurrences were statistically strongly significant, with the total shift in the centroid position amounting to ~40 mm in the distal direction (subjects 2, 4, 6, 7, and 9; Spearman \( \rho > 0.85, P < 0.01, n = 9 \) stimulation increments). Three instances revealed a moderately high correlation coefficient (subjects 1, 5, and 11; \( \rho > 0.58, P < 0.16 \), with the centroid position moving distally by 30 mm on average from the smallest to the largest stimulation level. The other two participants showed negligible correlation coefficients (subjects 8 and 10; \( \rho \leq 0.15, P \geq 0.72 \)). In some cases (e.g., subjects 1, 5, 8, and 11) the strength of the correlation was influenced by a single data point. For example,
in subject 8 the centroid position changed markedly, from a distal to proximal location, for the first stimulation increment and then from the proximal to more distal locations as stimulation level increased. Negative correlation coefficients were obtained in two participants (subject 3: $p = -0.58$ and subject 12: $p = -0.10$), although the centroid position changed by ~20 mm in the proximal direction.

**Medio-lateral changes in distribution of incremental M-wave amplitudes.** There was not a predominant, medio-lateral direction along which the centroid coordinate of the incremental M waves moved in response to increasing stimulation level. From the Spearman analysis a positive correlation related to a lateral movement of the centroid coordinate, with a negative correlation relating to movement medially. A positive correlation was found in five participants (subjects 1, 3, 4, 10, and 12; Fig. 6) and a negative correlation in the other seven. Strong, statistically significant correlation coefficients were observed for four participants: centroid moved significantly in the lateral direction for subjects 1 and 4 (i.e., positive correlation coefficients; $p > 0.86$) and in the medial direction for subjects 7 and 9 (Fig. 6; $p < -0.98$, $P < 0.05$ for the 4 cases). Five participants showed moderate negative correlation coefficients (subjects 2, 5, 6, 8, and 11; $p < -0.55$, $P < 0.14$), whereas a modest positive correlation was observed for subject 12 ($p = 0.80$, $P = 0.11$). For all occurrences of moderately and strongly significant correlation coefficients, the centroid medio-lateral position changed by at least 30 mm as the stimulation intensity increased (Fig. 6). As for the proximo-distal direction, a negligible association between centroid position and stimulation increments was observed in the medio-lateral direction for subject 10. Negligible correlation was also observed for subject 3 despite a marked change in the centroid position with the first and last stimulation increments.

**DISCUSSION**

Local responses in MG evoked by changes in stimulation level. Recording and quantifying M-wave characteristics form an important part of many investigations of neuromuscular function, although it is known that several factors can influence the signal waveform. Here we investigated the influence of MG pinnate fascicle geometry on M waves sampled from different muscle locations. Specifically, we wished to identify whether changes in M-wave amplitude resulting from increased stimulation intensity were represented equally over different regions of MG. It was found that increases in stimulation intensity did lead to different representations of M waves across the electrode grid, with the location of newly recruited units (identified through the incremental M wave) changing between stimulation levels.

The incremental M-wave calculation provides a means of quantifying changes in M-wave amplitude between stimulation levels and hence identifying recruitment of new muscle units. If the fibers of newly recruited units were widely distributed through the muscle volume, it would be expected that changes in incremental M-wave amplitudes would be widely distributed across the electrode channels. Instead, the results show localized changes in incremental M-wave amplitude (Fig. 2), making it possible to segment the electrode grid into regions of high and low amplitude values. Representation of M waves in localized regions of MG has previously been observed (Hodson-Tole et al. 2012). We interpret such a pattern as evidence...
that the fibers of newly activated motor units were localized within the muscle volume. This is in agreement with recent work suggesting that fibers of motor units in MG, activated during postural control, span small regions (~40 mm) along the proximo-distal axis (Vieira et al. 2011). Coupled with the proximal-distal variation in fascicle geometric properties (Narici et al. 1996) and aponeurosis displacement during contractions (Kubo et al. 2002), spatial organization of muscle fiber types in MG may represent potential for optimization of fiber mechanical properties or other physiological factors such as blood flow determined by intramuscular pressure distribution (van Leeuwen and Spoor 1992), although further work is required to fully understand whether any such advantages exist.

The interpretation of localized populations of muscle units elicited for different stimulation intensities is not in disagreement with recent findings. Héroux and colleagues (2015) have recently evaluated the largest longitudinal distance within MG where intramuscular electrodes could detect action potentials from individual motor units. These authors observed that the longitudinal distance between action potential recordings from single motor units may range from small (<4 cm) to large (up to 18 cm) values. Even though these figures possibly indicate the region along the MG longitudinal axis confining fibers of individual motor units, the distribution of fibers within such a region remains an open issue. In a previous article we reported that the probability of finding fibers of individual MG motor units outside a region corresponding to 4 cm is low (Vieira et al. 2011). Our previous and present findings do not exclude the possibility of fibers of individual units being located at regions longer than 4 cm. Following our current and previous results, and considering animal data (Bodine et al. 1988; Burke and Tsairis 1973), we suggest rather that the MG fibers tend to be grouped within the muscle. In consideration of the results presented here, and regardless of the different interpretations for the electrophysiological data reported in the literature, M waves elicited for different stimulation intensities are not detected equally from different skin regions (Figs. 2, 3, 5, and 6). Increases in the amplitude of M waves posit the basis for the determination of motor unit number estimation, as increases in M-wave amplitude are indicative of newly elicited populations of motor units. Here we show that M-wave amplitude increases differently in different MG locations.

Fig. 4. Incremental muscle activation and response. Changes in the peak plantar flexion torque and the RMS amplitude of incremental M waves are shown for each of the 12 subjects tested. The Pearson correlation coefficient, its P value, and the slope of regression (dashed lines) fitted to individual data are shown.
The finding of localized changes in the incremental M-wave amplitude across the electrode grid has some significant methodological and physiological implications related to the identification of M-wave characteristics and the muscle unit organization in human MG. The following sections discuss these implications in more detail.

**Methodological implications of localized MG M-wave responses.** The pennate arrangement of MG fascicles leads to myoelectric activity from different fibers being represented in different channels along the length of the electrode array. The majority of studies involving collection of myoelectric signals utilize single, closely spaced electrodes placed over the muscle belly, often in accordance with SENIAM guidelines (Hermens et al. 2000). The results presented here, however, suggest that reliance on a single recording location may lead to inconsistencies in the recorded data between individual participants. For example, different pairs of electrodes in the grid provided equal estimates of the M-wave amplitude for subject 8 (Fig. 7A). For subject 2, however, M waves detected proximally and distally exhibited very different amplitude profiles. In this participant, the amplitude of M waves detected proximally increased progressively, reaching a plateau for stimulation levels \( \geq 60\% \) of the maximum, whereas the amplitude of distally detected M waves showed marked increases only for the greatest stimulation levels (Fig. 7B). Moreover, regardless of the stimulation level M waves were generally greater in the more proximal MG regions. These results have marked consequences, for example, in using the amplitude of M waves detected in a single region of the muscle to define \( M_{\text{max}} \) and hence the maximal stimulation level required for a given study.
Similar implications are evident in studies focusing on the timing and magnitude of reflex responses (Henry et al. 1998) as well as in surveys relying on $M_{\text{max}}$ to normalize EMGs (Carolan and Cafarelli 1992). As such, the location of electrode placement over the muscle is critical in studies aiming to quantify and characterize the M-wave properties (Bromberg and Spiegelberg 1997).

A simple potential method of overcoming such problems could be provided by using multiple electrodes to record M-wave characteristics. Within our experimental protocol it was possible to record from 128 electrodes. Such a large number is, however, unlikely to be required in the majority of cases, although from our results it is not possible to anticipate the simplest electrode configuration that would provide enough information for measurement of M-wave properties in human MG. Ideally, the pickup volume of surface electrodes should include all fibers of the target, MG muscle, and of no other muscles (e.g., soleus). For an isotropic medium, previous accounts suggest that the pickup volume of bipolar electrodes includes fibers located within one length of the center-to-center distance between the two surface electrodes (Lynn et al. 1978). Because of the large physiological cross-sectional area of MG (Narici et al. 1996), the MG volume sampled by a single pair of electrodes is small and, according to our results (Figs. 2–7), unrepresentative of the whole MG volume. Increasing the distance between bipolar electrodes would be the most convenient and simplest solution for sampling from a relatively larger MG volume, with care to not sample from other (e.g., soleus) muscles (De Luca et al. 2012). Indeed, preliminary results from intramuscular and surface EMGs collected during

Fig. 6. Location of incremental M waves along the muscle medio-lateral axis. The RMS amplitude of mean M waves (black circles) and the medio-lateral coordinate of the barycenter (cf. Fig. 2) of incremental M waves are shown for each of the 12 subjects tested. Gray circles indicate the barycenter coordinates obtained in correspondence to changes in stimulation intensity leading to a statistically significant increase in the RMS amplitude of mean M waves (cf. Fig. 3). Spearman $\rho$ and its $P$ value are indicated within each plot. Regression lines are shown so as to provide a qualitative indication of how much the location of incremental M waves (i.e., in their barycenter) changed with increases in stimulation intensity. Only gray circles were considered for the correlation and regression analyses.
standing suggest that action potentials of individual motor units in the soleus muscle contribute marginally to surface EMGs sampled from the MG muscle with at most 5-cm interelectrode distance (Vieira et al. 2014). In view of such a large interelectrode distance, and considering the average MG length (\( \approx 27 \) cm; Narici et al. 1996), two or three consecutive pairs of surface electrodes could provide a representative, proximo-distal view of MG activity (Fig. 7). However, at the moment, the two or three bipolar electrodes with 5-cm interelectrode distance should be regarded as a speculative indication more than a recommendation. The appropriate distance between bipolar electrodes, maximizing the representation of the target muscle activity in the surface EMGs without leading to cross talk, may change according to interindividual differences in, for example, subcutaneous adipose tissue and muscle thickness. While compelling evidence on the relationship between pickup volume of surface electrodes and the representation of activity within the whole MG volume, but no other muscles, is not available, our results suggest that electrically evoked MG responses should be sampled from different proximo-distal and medio-lateral muscle regions.

Physiological implications of localized MG M-wave responses. As previously discussed, the incremental M-wave calculation enabled identification of regions where new muscle units were recruited with increasing stimulation level. Segmentation of the resulting spatial maps into regions of high RMS amplitude was possible, indicating that discrete localized changes in M-wave amplitude occurred. The interesting phenomenon reported in Figs. 5 and 6 could therefore be indicative of some form of spatial arrangement of either 1) nerve fibers in the motor nerve at the site of stimulation or 2) muscle fibers of motor units in MG.

Topological arrangement in the nervous system has previously been reported and is seen as a common feature of many muscles that are described as compartmentalized on the basis of anatomical (e.g., partitioned by intramuscular tendon) or neuromuscular (e.g., number of innervating primary nerve branches) features (Bennett et al. 1989; Bennett and Ho 1988; Weeks and English 1985, 1987). Human MG is, however, composed of a single neuromuscular compartment supplied by a single primary nerve branch and contains no internal tendinous divisions (Wolf and Kim 1997), so spatial arrangement of nerve fibers to human MG does not fit with current literature. Our findings could therefore suggest that topological organization in the nervous system may not be limited to compartmentalized muscles and warrants further investigation.

Spatial arrangement of muscle fiber properties within skeletal muscles, even with single neuromuscular compartments, has been discussed previously (for review see Kernell 1998), with important implications for sampling sites for muscle biopsies and intramuscular EMG recording. Coupled with proximo-distal variation in fascicle geometric properties (Na-
rici et al. 1996) and aponeurosis displacement during contrac-
tions (Kubo et al. 2002), spatial organization of muscle fiber
types in MG may represent potential for optimization of fiber
mechanical properties or other physiological factors such as
blood flow determined by intramuscular pressure distribution
(van Leeuwen and Spoor 1992). Further work is, however,
required to determine both the potential extent of spatial
organization of muscle and/or nerve fibers in MG and any
functional implications. Such information would be valuable
for the development of novel neural interfaces to, for example,
integrate with prosthetic devices.

Potential limitations. The results presented here should be
considered in light of some limiting factors. For example, the
experimental protocol used did not lead to maximal stimulation
of MG, and as such we cannot describe characteristics of M\textsubscript{max}
across different regions of MG. Our protocol was adopted after
pilot work revealed the difficulty in achieving M\textsubscript{max} across all
electrodes of the grid. Moreover, the stimulation amplitudes
required to attempt to achieve M\textsubscript{max} were not well tolerated by
participants and activation of synergistic muscles was appar-
ent. The difficulty in achieving M\textsubscript{max} across all electrode
channels was surprising and highlights the influence of elec-
trode position in protocols in which determining M\textsubscript{max} is the
goal.

A second consideration is the size of our electrode grid
relative to the actual size of the muscle across participants. We
used a 16 × 8 grid, with a 10-mm interelectrode distance. MG
muscle belly length has been reported to be 251 ± 21 mm
(Barber et al. 2009) in a study group of similar heights (mean
muscle belly length has been reported to be 251
units, and the onset and amplitude of reflex responses.
of, e.g., the maximal stimulation levels, the number of motor
unit fibers may not provide a representative response to
results indicate that EMGs recorded locally from the gastroc-
nemius muscle may not provide a representative response to
increases in the recorded M-wave amplitudes (e.g., Figs. 5 and
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No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: T.M.V., A.B., M.A.M., and E.F.H.-T. conception and
design of research; T.M.V., A.B., and M.A.M. performed experiments; T.M.V.
and A.B. analyzed data; T.M.V., A.B., M.A.M., and E.F.H.-T. interpreted
results of experiments; T.M.V., A.B., and E.F.H.-T. prepared figures; T.M.V.,
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