



# Contraction level, but not force direction or wrist position, affects the spatial distribution of motor unit recruitment in the biceps brachii muscle

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## Abstract

**Purpose** Different motor units (MUs) in the biceps brachii (BB) muscle have been shown to be preferentially recruited during either elbow flexion or supination. Whether these different units reside within different regions is an open issue. In this study, we tested whether MUs recruited during submaximal isometric tasks of elbow flexion and supination for two contraction levels and with the wrist fixed at two different angles are spatially localized in different BB portions.

**Methods** The MUs' firing instants were extracted by decomposing high-density surface electromyograms (EMG), detected from the BB muscle of 12 subjects with a grid of electrodes (4 rows along the BB longitudinal axis, 16 columns medio-laterally). The firing instants were then used to trigger and average single-differential EMGs. The average rectified value was computed separately for each signal and the maximal value along each column in the grid was retained. The center of mass, defined as the weighted mean of the maximal, average rectified value across columns, was then considered to assess the medio-lateral changes in the MU surface representation between conditions.

**Results** Contraction level, but neither wrist position nor force direction (flexion vs. supination), affected the spatial distribution of BB MUs. In particular, higher forces were associated with the recruitment of BB MUs whose action potentials were represented more medially.

**Conclusion** Although the action potentials of BB MUs were represented locally across the muscle medio-lateral region, discrimination between elbow flexion or supination seems unlikely from the surface representation of MUs action potentials.

**Keywords** High-density EMG · Surface EMG · Motor units decomposition · Motor control

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## Abbreviations

ANOVA	Analysis of variance
ARV	Averaged and rectified value
BB	Biceps brachii
CoM	Center of mass position
EMG	Electromyography
MUs	Motor units
MVC	Maximum voluntary contractions

## Introduction

It is well established in the literature that skeletal muscles are innervated by hundreds of motor units (MUs) (Brown 1972; Daube 1995; McComas et al. 1971; Stein and Yang 1990). Even though there seems to be a size rule governing the recruitment of MUs within a pool (Henneman et al. 1965), different studies on human subjects observed

that MUs in the same upper limb muscle may be activated selectively (Herrmann and Flanders 1998; Riek and Bawa 1992; ter Romeny et al. 1984). Moreover, the selective activation of different muscle sub-portions (Brown et al. 2007; Holtermann et al. 2009; Pappas et al. 2002) further suggests that MUs may be recruited according to functional demands imposed by the motor task and, thus, according to their location within the muscle. While the selective recruitment of MUs in different locations may be well appreciated in muscles with broad attachment, the selective activation of MUs in muscles attached via strap-like tendons is still elusive.

Specifically concerning the biceps brachii (BB) muscle, conflicting views appear to exist on the task-related spatialization of MUs. With intramuscular electrodes, ter Haar Romeny and collaborators (1984) observed that MUs in the most lateral side of the muscle were recruited during elbow flexion, while units in the medial part of the BB long head were preferentially recruited during supination. These authors tested their hypothesis with wire EMG electrodes inserted laterally and medially with respect to the centerline of the muscle head. On the contrary, Herrmann and Flanders showed that MUs detected in similar locations with intramuscular electrodes could be recruited during different tasks, suggesting a lack of task-related spatialization on the BB. Although intramuscular electrodes provide a genuine representation of action potentials of single MUs (Merletti and Farina 2009), their pick-up volume is markedly small (Lowery et al. 2006). The detection of action potentials of different MUs with selective EMG recordings, taken from two locations within BB in different tasks, may not be sufficient to test the hypothesis of regional recruitment of MUs. In fact, the two detection sites could either intersect the boundaries of MU territories centered in distinct locations or be located at the same relative position within the territories. Only with the sampling of MUs from multiple BB locations it would be possible to test for the hypothesis of task-related spatialization of MUs.

In this study, we investigated whether action potentials of MUs recruited during elbow flexion and supination are predominantly located at different medio-lateral BB regions. Differently from previous similar studies on BB, we used grids of electrodes to sample surface electromyograms (EMGs) across BB. This methodology has been shown to be remarkably selective (Vieira et al. 2017). Combined with a validated decomposition algorithm (Holobar et al. 2011), our grid of electrodes provides the topography of action potentials of single MUs. If BB MUs are functionally segregated according to the location of their fibers, we would expect to observe action potentials in different BB transverse locations during different force tasks.

## Methods

### Subjects

Twelve subjects provided written informed consent before participating in the study (11 males and 1 female; age range 24–44 years; height 162–187 cm; body mass 60–94 kg). All participants did not report any known musculoskeletal or neurological dysfunction and pathology prior to and during the experiments. Experimental procedures conformed to the Declaration of Helsinki and were approved by the Regional Ethical Committee (Commissione di Vigilanza, Servizio Sanitario Nazionale-Regione Piemonte-ASL 1-Torino Italy, Prot. No. 0010610).

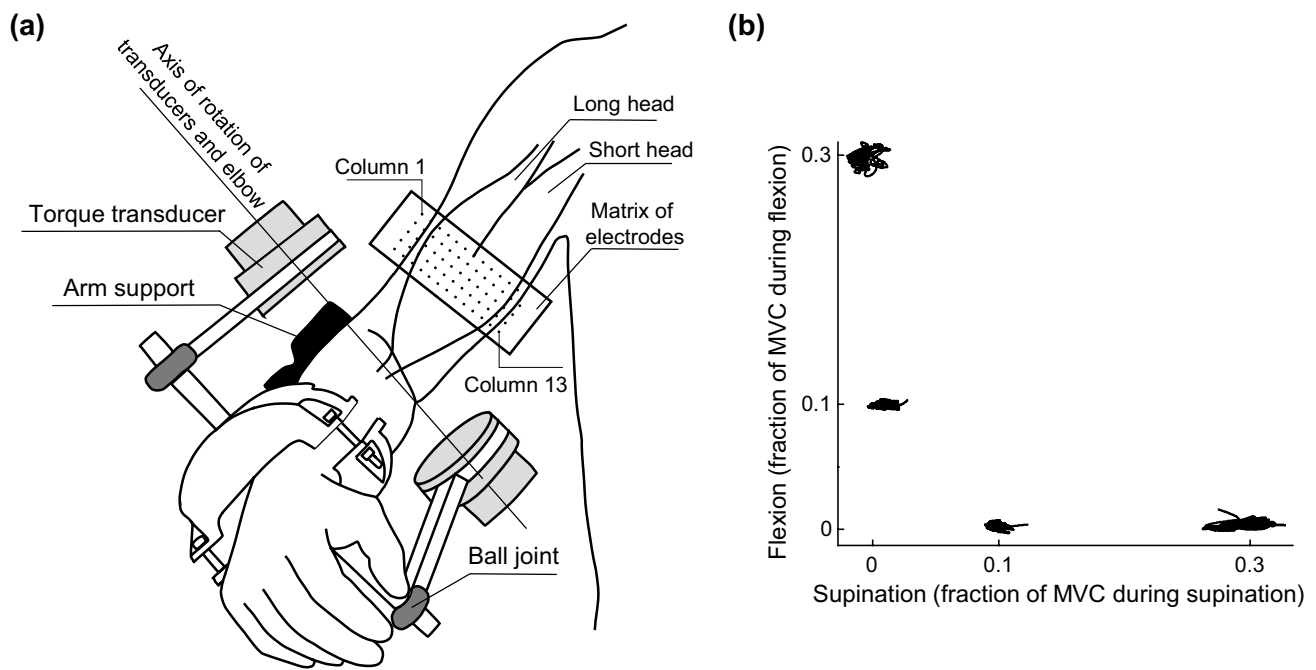
### Experimental protocol

Subjects were asked to sit in a chair with their right arm abducted at 45° and the forearm flexed to 90°. The elbow was laid on a support with its axis of rotation aligned coaxially with the axes of rotation of two torque transducers (model TR11, CCT Transducers, Torino, Italy). The wrist was secured to a custom-made ring whose oval internal side was cased with foam to reduce discomfort and prevent wrist deflections (Fig. 1a). The ring was designed to fix the forearm at 90° (neutral position) or supinated at 45° (full supination: 0°).

Experiments started after a familiarization session, in which subjects were provided with visual feedback of elbow flexion torque and forearm supination torque and were asked to maintain it predominantly in either direction. First, the supination and flexion torques produced during maximum voluntary contractions (MVC) were calculated as the maximum value across two repetitions. Three minutes intervals were provided between MVCs. Subjects were asked to either isometrically flex their elbow or supinate their forearm at 10% and 30% of their maximum voluntary torque (Fig. 1b). Each direction and torque level were tested twice, separately for the forearm in neutral position or supinated at 45°. A total of 16 trials were applied (2 wrist positions × 2 torque levels × 2 force direction × 2 repetitions), in random order and with 15 s interval in between.

### Torque and EMG measurements

Elbow torque was measured with a customized torque brace, with two torque sensors measuring flexion torque from each brace at the wrist level (cf. ball joints in Fig. 1a). Torque data were amplified with a general-purpose amplification device (Forza, OTBioelettronica, Turin, Italy). If the subject flexed his elbow without any pronosupination,



**Fig. 1** Setup. **a** Experimental setup. **b** Torque profiles produced during elbow flexion and forearm supination at 10% and 30% MVC, with the wrist at neutral position

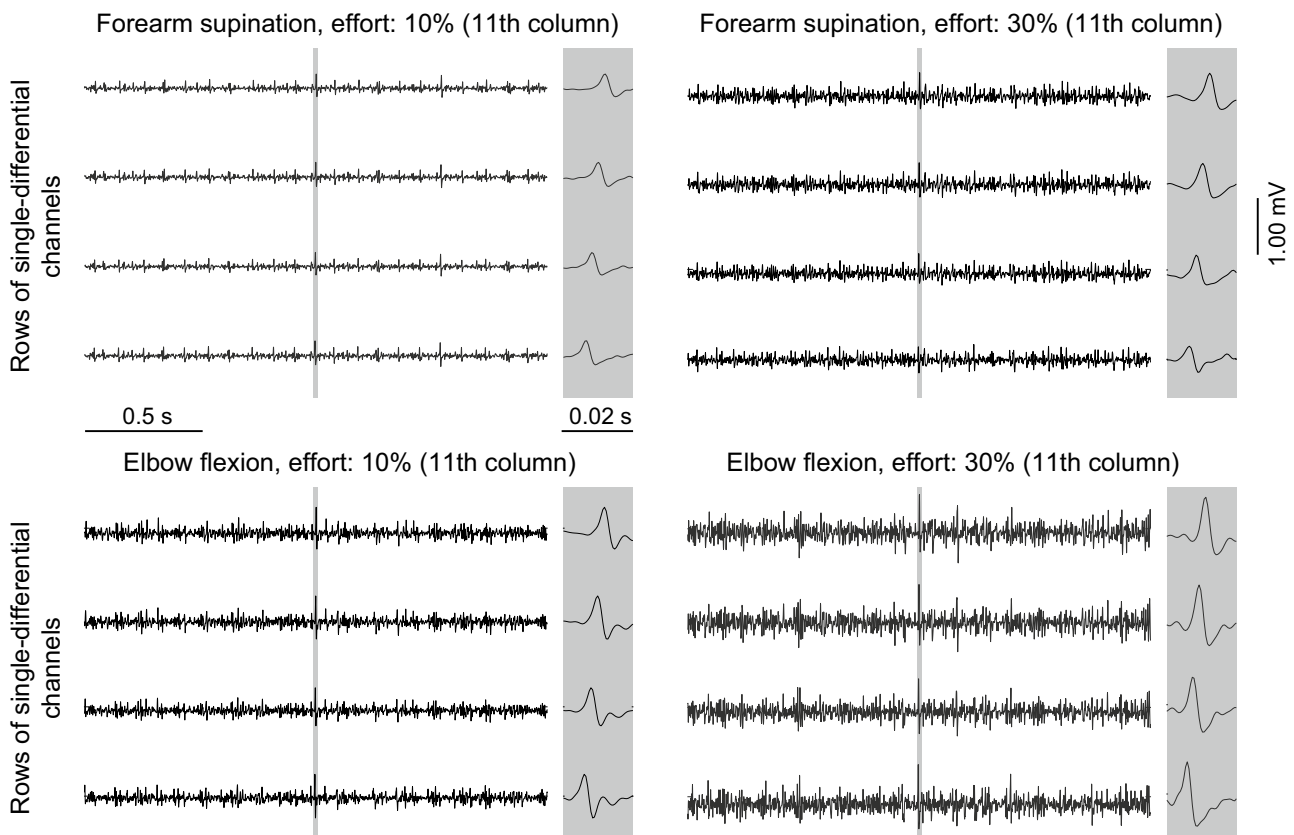
both transducers would measure a torque of the same magnitude and sign. In contrast, if the subject supinated his forearm, the two transducers would measure torque values equal in magnitude but with opposite signs. Therefore, flexion and supination torques were respectively calculated as the sum and difference of the torques measured by each sensor. Visual feedback of elbow flexion and forearm supination torques was given to the subject as the displacement of a virtual circular cursor in a Cartesian plane (zero: the subject was not exerting any torque with his arm,  $x$ -axis: forearm supination torque,  $y$ -axis: elbow flexion torque; Fig. 1b), displayed by a monitor placed in front of the subject. The feedback was updated every 0.25 s.

Monopolar surface EMG signals were acquired with a matrix of 64 electrodes (13 columns  $\times$  5 rows, with one missing electrode; inter-electrode distance 8 mm) from BB (see Fig. 1a). Columns were aligned parallel to the muscle fibers, with the seventh column placed along the junction between the BB heads. The central row was placed where the arm circumference was greatest, allowing for the visualization of at least one innervation zone for each BB head. EMGs were amplified (gain between 500 and 5000) and sampled synchronously with torque data at 2048 Hz (12 bit A/D resolution; EMG-USB2, OTBioelettronica, Turin, Italy). A custom-made script was written in Matlab<sup>®</sup> (MathWorks, Natick, MA) for data acquisition and for the real-time display of elbow torque.

## Data analysis

Single-differential EMGs between pairs of adjacent electrodes along each column of the matrix were computed from the monopolar signals and inspected for quality control. High-quality signals were defined based on the visual identification of propagation of action potentials in all consecutive rows of the grid (cf. Fig. 2). Channels presenting contact problems or power line interference were linearly interpolated with data collected from the closest channels of the same column. After ensuring the high quality of all differential EMGs, monopolar signals were filtered (2<sup>nd</sup>-order Butterworth filtered, between 15 and 350 Hz) and decomposed (Holobar et al. 2011), separately for each trial. After decomposition, the firing instants of identified MUs were used to trigger and average single-differential EMGs over 30 ms epochs, providing the surface representation of single motor unit action potential (Farina et al. 2002).

The presence of common MUs during the different tasks of flexion and supination was assessed by the comparison of their action potentials. First, the channels providing action potentials with peak amplitude greater than 70% of the maximal peak value across the grid were identified and termed active channels (Gallina and Vieira 2015). The two sets of action potentials for each pair of motor units were aligned in time by maximizing their cross-correlation function. The mean square difference was computed between the two sets



**Fig. 2** Example of data collected from subject 1 (11th column of the EMG matrix) during four different trials exerted with the wrist rotated in a neutral position: forearm supination at 10% MVC (up-

left), forearm supination at 30% MVC (up-right), elbow flexion at 10% MVC (down-left), elbow flexion at 30% MVC (down-right)

of time-aligned action potentials, averaged across channels and then normalized with respect to the mean energy of the two sets of action potentials. Finally, pairs of MUs leading to action potentials with a mean square difference smaller than 5% were considered common (Farina et al. 2008).

Before processing the amplitude distribution of single MU action potentials, decomposition results were inspected for spurious units. Visual analysis was conducted on all the extracted signals and non-physiological potentials (i.e., non-propagating potentials) were discarded. An example of a retained MU is shown in Fig. 3a.

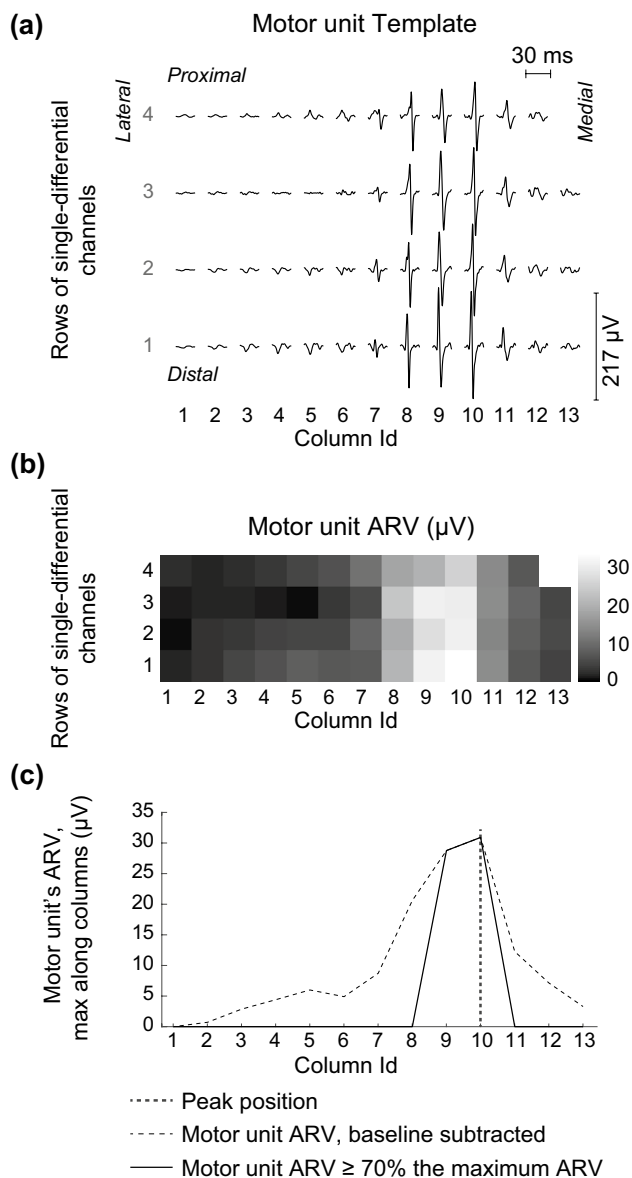
For each retained MU, the averaged and rectified value (ARV) was calculated across 30 ms epoch centered on individual action potentials (Fig. 3b). The maximum ARV value was calculated along each column in the grid and the smallest ARV value across columns was subtracted from all other ARV values, providing the transverse, surface distribution of MU action potential amplitude (Fig. 3c). The number of columns whose ARV values were higher than 70% of the maximum (Vieira et al. 2010), multiplied by the inter-electrode distance (i.e., 8 mm), provided an indication on the size of the muscle region from which action potentials were mostly represented. If it was greater than half of the

superficial surface of BB, approximated as 35% of the largest arm circumference during rest, its spatialization could thus be not ascertained from the ARV distribution. We therefore decided to discard those units, as we were unsure whether they indeed had large medio-lateral or deep, spatially localized territories (Roeleveld et al. 1997); both conditions would lead to such highly diffused ARV distributions. For the remaining units, the ARV values lower than 70% of the maximum were set to zero.

The peak position  $p$  was identified for each unit as the grid column at which the maximum ARV occurred. The center of the amplitude distribution for single action potentials, that is, the location across the muscle where MU action potentials are more clearly represented, was quantified as the center of mass position (CoM)  $\bar{c}$  of amplitude distribution determined separately for each subject, task, effort level, and wrist angle:

$$\bar{c} = \frac{\sum_j A_j \cdot p_j}{\sum_j A_j}, \quad (1)$$

where  $A_j$  is the maximum ARV of the  $j$ -th column, subtracted by the minimum ARV across all channels, and  $p_j$  is



**Fig. 3** Procedure for the determination of the CoM of one motor unit. **a** Example of motor unit recruited during the flexion task (10% MVC, wrist in neutral position) by subject 1 (row channels from 1 to 13 move from lateral to medial, while column channels from 1 to 4 move from distal to proximal). **b** ARV of each channel. **c** Maximum ARV calculated along each column (dotted line) and after setting to zero the values lower than 70% of the maximum ARV (continuous line). The position of the peak is indicated with a dotted thick line

the column index. The CoM was then averaged across MUs, separately for each subject, task, wrist position and effort level, and considered for analysis.

### Statistical analysis

A Lilliefors test was conducted on the data collected during each combination of groups of independent variables

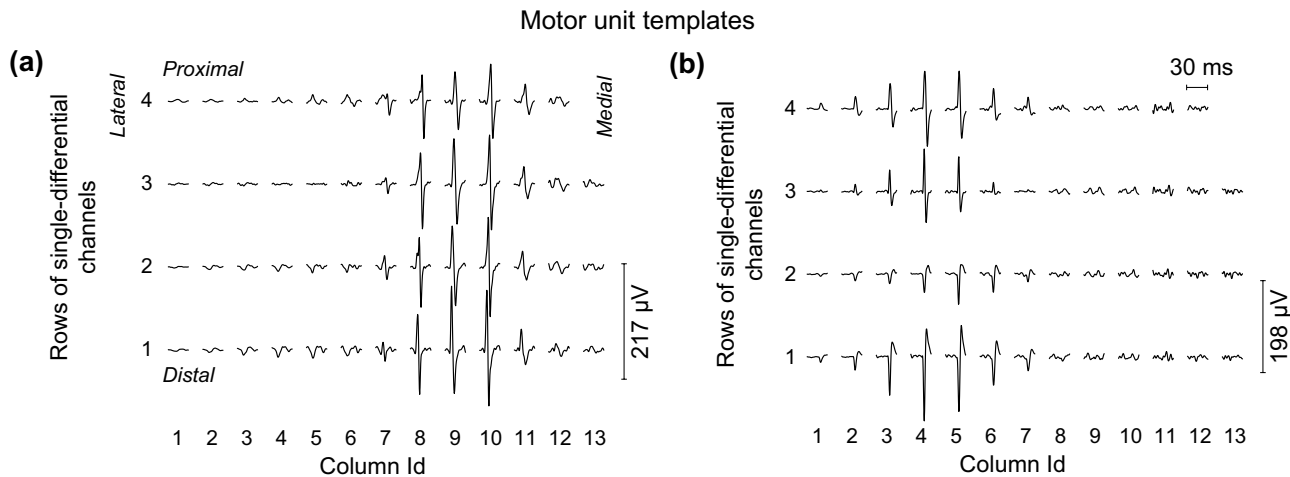
(wrist angle, effort level, task, and repetition) to test for whether the data distribution was Gaussian. The Levene's test was computed for each combination of groups of independent variables, from their group means, to test for the homogeneity of variances. After ensuring data, Gaussianity ( $p > 0.077$  in all cases) and homoscedasticity ( $p = 0.986$ ), a four-way ANOVA (Matlab<sup>®</sup> function `anovan`) was performed to identify the effect of four factors on the center of mass of motor unit action potentials. The factors were: the supination angle (two levels: neutral and supinated at  $45^\circ$ ), task (two levels: elbow flexion and forearm supination), repetition (two levels: first and second repetition) and effort level (two levels: 10% and 30% the maximum voluntary torque). A Bonferroni correction was applied for pairwise comparisons, whenever a main or interaction effect was observed.

### Results

A total of 1194 MUs were identified during elbow flexion [ $N = 618$  units, median (interquartile interval) across subjects 50 (20)] and supination [ $N = 576$  units; 46 (18)], most of which ( $91.3 \pm 6.3\%$ , mean  $\pm$  std across subjects) were not recruited during both the tasks of elbow flexion and forearm supination. Of these, respectively, for elbow flexion and forearm supination, the amplitude of action potentials of 8 and 7 units was distributed roughly across the whole medio-lateral columns. After discarding these units, 1179 [94 (31), median (interquartile interval) across subjects] units were retained for analysis. MUs were identified for both effort levels. The total number of identified units was similar during low (586) and high (593) contraction levels.

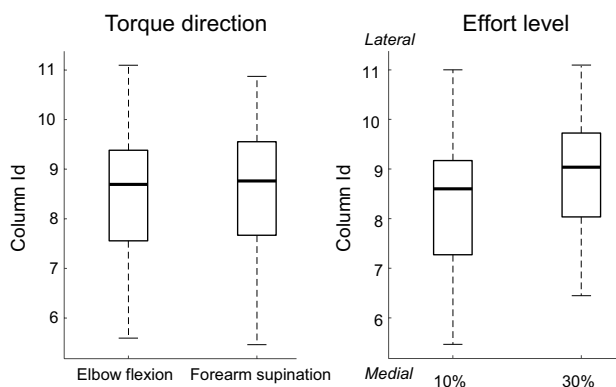
Action potentials of MUs were observed in different transverse regions. While the amplitude distribution of action potentials of 68% units ( $N = 803$ ) was centered from column 7 to 13 (Fig. 4a), the action potentials of 32% of units ( $N = 376$ ) were represented from columns 1 to 7 (Fig. 4b).

Group results revealed differences in the distribution of CoM values. These differences were observed between effort levels (main effect;  $F = 8.05$ ,  $p = 0.005$ ,  $N = 16$ , 2 effort levels  $\times$  2 forearm rotation  $\times$  2 torque directions  $\times$  2 repetitions, 12 subjects, 4 discarded outlier), though not for the other factors tested. Specifically, no main effect for torque direction, forearm rotation, and repetition and no interaction effect were observed (ANOVA;  $p > 0.543$  for all cases). Post hoc analyses revealed that regardless of the forearm rotation, torque direction, and repetition considered, the CoM shifted laterally as contraction intensity increased ( $p = 0.006$ , see Fig. 5).



**Fig. 4** Examples of MUs extracted from subject 1 during the flexion task (10% MVC) with the wrist in a neutral position. **a** Example of an accepted MU whose activity is mostly located in the proximal part of the BB muscle (row channels from 1 to 13 move from lateral to

medial, while column channels from 1 to 4 move from distal to proximal). **b** Example of an accepted MU whose activity is mostly located in the distal part of the BB muscle



**Fig. 5** MUs' CoM positions during different torque directions (left) and effort levels (right)

## Discussion

Surface EMGs were recorded from both heads of the human BB to address the question of whether MUs recruited during the elbow flexion and supination are represented in different muscle regions. The surface representation of BB MUs was estimated by spike-triggered averaging surface EMGs recorded with a matrix of electrodes. Our results revealed that: (1) most MUs were represented in a few, consecutive columns of electrodes and (2) contraction level but not forearm rotation and torque direction significantly affected the surface distribution of MU action potentials.

## Regionalization of biceps brachii motor units

Different motor units were recruited during different tasks. This observation is consistent with previous studies (Herrmann and Flanders 1998; Riek and Bawa 1992; ter Romeny et al. 1984) that identified task-specific recruitment of MUs of upper limb muscles. However, the different units, recruited during different tasks, were not identified to be spatially localized in different portions of the BB during different tasks. This observation is in line with the study performed by Herrmann and Flanders (1998), who demonstrated that MUs with closely located territories may have different directional tuning. Our results, however, differ from those reported by ter Haar Romeny and collaborators (1984), who observed a preferential recruitment of units in the most lateral and medial regions of BB long head during elbow flexion and forearm supination, respectively. This discrepancy could be due to the different portions of the BB muscle investigated. While ter Haar Romeny and collaborators used wire EMG electrodes, which assess a genuine representation of deep motor units, from 2.0 to 3.5 cm within BB, but with a markedly small pick-up, in this study we investigate motor units spatialized across the whole BB surface. Moreover, ter Haar Romeny and collaborators assessed exclusively the BB long head. In contrast, we extracted units from EMGs sampled from both BB heads and observed that they were centered mostly in the medial BB region (columns from 7 to 13; ~68% of all units) which likely corresponds to the BB short head. If there is any functional organization of MUs within BB, according to our results this is likely to apply to deep rather than to superficial MUs, as suggested by studies performed with MRI (Pappas et al. 2002).

Unexpectedly, a different spatial distribution of MUs was observed between different contraction levels. In particular, a shift toward the medial BB portion was observed for higher effort levels regardless of the wrist position considered. Assuming that units recruited at 30% contraction level are larger than those recruited at 10%, as often observed for isometric, well-controlled tasks (Gydikov and Kosarov 1974; Henneman et al. 1965; Moritani and Muro 1987), these results possibly suggest a spatialization of superficial units according to their recruitment order, i.e., with their size. Indirect evidence on the regionalization of MUs in relation to recruitment order has been reported for other muscles (Ansved et al. 1991; Vieira et al. 2015). Further experiments, based for example on the recruitment threshold of MUs identified during force-varying contractions, will be necessary to test this possibility.

### Anatomical and physiological implications

While our findings support the hypothesis of task-specific MUs in the BB, when performing a task, they show that the central nervous system does not recruit MUs distributed on a specific superficial sub-portion of the muscle, but recruits MUs represented across the whole medio-lateral BB region. Therefore, the logic for the MUs recruitment is not merely topographical and the task-specific recruitment of MUs is not a consequence of the different mechanical actions generated by the fibers composing each MU, possibly acting along two different directions. Consequently, other hypotheses should be investigated. For example, our findings might be explained by a shared synaptic input among MUs of different muscles (Laine et al. 2015).

We even identified that the MUs recruited during tasks performed with different levels of effort were identified in different sub-portions of the BB. This finding gives novel anatomical insights into the organization of the MUs, and suggests a topographic separation of MUs based on their characteristics.

### Practical implications

Our findings suggest that during a task that involves the BB, the nervous system recruits MUs represented locally across the whole medio-lateral BB region. For this reason, the use of more surface EMG electrodes, collected from different BB regions, does not allow discriminating between elbow flexion and forearm supination. Notwithstanding the high number of detection points on BB, applications focused on the use of EMGs, e.g., to control prosthetic arms (Roche et al. 2014; Uellendahl 2017), would require sampling of EMGs from additional muscles. An additional implication of our results is with regard to electrode positioning. Even though there was not a preferential surface representation

for individual BB MUs with force direction and forearm rotation, most of them were represented locally on the surface (Fig. 4). This local, superficial representation of MUs suggests that an optimal location for centering bipolar electrodes on BB could not be identified.

In conclusion, the action potentials of the majority (99%) of the 1194 MUs identified were represented locally on the skin. Contraction level but not wrist position and force direction (flexion vs. supination) affected the spatial distribution of the recruited BB MUs. Greater force demands were associated with the recruitment of BB MUs with action potentials represented more medially.

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**Author contributions** DB, TMV, MG, and AB conceived and designed the research. DB conducted the experiments. DB, TMV, MG, and AB analyzed the data. DB, TMV, MG, AB, AdA, and LG wrote the manuscript. All authors read and approved the manuscript.

### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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