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Influence of the number of trials on evoked motor cortical activity in EEG recordings

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Influence of the number of trials on evoked motor cortical activity in EEG recordings

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Keywords: electroencephalography (EEG), motor-related activity, event-related desynchronization/synchronization (ERD/ERS), LORETA

Abstract

Objective. Improvements in electroencephalography enable the study of the localization of active brain regions during motor tasks. Movement-related cortical potentials (MRCPs), and event-related desynchronization (ERD) and synchronization are the main motor-related cortical phenomena/neural correlates observed when a movement is elicited. When assessing neurological diseases, averaging techniques are commonly applied to characterize motor related processes better. In this case, a large number of trials is required to obtain a motor potential that is representative enough of the subject's condition. This study aimed to assess the effect of a limited number of trials on motor-related activity corresponding to different upper limb movements (elbow flexion/extension, pronation/supination and hand open/close). **Approach.** An open dataset consisting on 15 healthy subjects was used for the analysis. A Monte Carlo simulation approach was applied to analyse, in a robust way, different typical time- and frequency-domain features, topography, and low-resolution electromagnetic tomography. **Main results.** Grand average potentials, and topographic and tomographic maps showed few differences when using fewer trials, but shifts in the localization of motor-related activity were found for several individuals. MRCP and beta ERD features were more robust to a limited number of trials, yielding differences lower than 20% for cases with 50 trials or more. Strong correlations between features were obtained for subsets above 50 trials. However, the inter-subject variability increased as the number of trials decreased. The elbow flexion/extension movement showed a more robust performance for a limited number of trials, both in population and in individual-based analysis. **Significance.** Our findings suggested that 50 trials can be an appropriate number to obtain stable motor-related features in terms of differences in the averaged motor features, correlation, and changes in topography and tomography.

1. Introduction

Every voluntary movement is induced by complex neurologic mechanisms of the brain (Kandel *et al* 2014). The analysis of the cortical processes associated with the execution of movements through the electroencephalogram has been widely studied for several decades (Gilden *et al* 1966, Colebatch 2007, Chen *et al* 2018). In fact, electroencephalography

(EEG) activity already provides information during the movement planning period, such as the location of the target, the trajectory of the limb or the configuration of the arm position (Rao 2013, Luck 2014). In this way, neural activity reflects the movement intention independently of whether the physical movement can take place or not. The brain areas that are active during motor control change according to the characteristics of

the movement performed by the subject (Yeom *et al* 2020).

Improvements on the EEG recordings enable the broad study of the localization of active brain regions during motor tasks. For instance, movement-related cortical potentials (MRCPs), either self-initiated such as the Bereitschaftspotential or stimulus-related, occur before the movement (Colebatch 2007, Chen *et al* 2018, Nann *et al* 2019). Although the comparison between the self-initiated and the stimulus-related motor potentials is debatable, there is a clear similarity because both MRCPs are slow potentials containing elements related to stimulus anticipation and motor readiness (Schurger *et al* 2021). MRCPs sources have been attributed to the supplementary motor area, the primary motor area, and the premotor cortex (Rektor *et al* 2003, Colebatch 2007). Moreover, voluntary movements result also in an event-related desynchronization (ERD) prior to movement-onset in the sensorimotor alpha- μ (8–13 Hz) and beta (14–30 Hz) rhythms, and that continues for around 500 ms after the movement-onset (Heinrichs-Graham *et al* 2014, Qiu *et al* 2016). Next, an event-related synchronization (ERS) is presented right after the end of the motor execution (Pfurtscheller and Lopes da Silva 1999).

One of the first concerns to be decided in motor-activity study protocols is about how many trials are necessary to elicit a stable, consistent and representative average motor potential. The selection of the number of trials is a trade-off between improving the potential characterization and optimizing time resources. In the literature, the number of the trials is very variable depending on the task, the subject condition and the analysed motor potential and features. Although the number of trials varied from 20 to 1000 trials for tasks such as grasping an object and extending the fingers, respectively (Gerloff *et al* 2006, Jeon *et al* 2011), most works usually selected around 50–100 trials (Dean *et al* 2012, Bizovičar *et al* 2014, Visani *et al* 2019). However, to the best of our knowledge, there are no studies in the literature that have investigated the impact of the number of trials on the EEG recordings related to voluntary motor activity.

Collecting EEG data from a large number of trials requires signal recording protocols that can make tasks tedious and tiresome for the subjects. In long acquisition protocols, a significant cognitive effort is required to concentrate on the different stimuli that can cause fatigue, frustration and loss of attention. These factors can distort the analysis by affecting signal features, signal-to-noise ratio, repeatability and replicability. For example, EEG analyses during motor imagery have shown changes due to fatigue in spectral power especially below 12 Hz (Boksem *et al* 2005, Talukdar *et al* 2019, Jacquet *et al* 2021). Moreover, participants less motivated to respond to the stimulus have a detrimental impact on the MRCP amplitude (Linssen *et al* 2011). To

avoid this, it is important to limit the number of trials recorded as much as possible, provided that the averaged results obtained are sound and significant.

Prior works in the literature have shown that recommendations on the number of trials needed to obtain a stable ERP rely on several factors such as the nature of the ERP component, external noise, attention, background neural activity and sample size (Marco-Pallares *et al* 2011, Thigpen *et al* 2017, Biabani *et al* 2018). In general, their guidelines state that only a few trials are required to obtain a stable grand-average ERP component. However, these studies have ignored the subsequent statistical analyses in order to determine the number of trials necessary to detect between-group or condition effects; therefore, these fairly small values may lead to underestimating the number of trials required to get statistically significant outcomes (Boudewyn *et al* 2018). In any case, the effect of the number of trials on the movement related cortical potentials has not yet been analysed.

The main aim of this paper was to assess the effect of a limited number of trials on potentials related to motor activity. In particular, MRCP and ERD/ERS corresponding to different self-initiated upper limb movements (elbow flexion/extension, forearm pronation/supination and hand close/open) were obtained by using a S1–S2 paradigm. The effect of decreasing the number of trials was assessed considering different approaches commonly used in the literature: time- and frequency-domain features of the averaged ERPs, scalp topography activity, and low-resolution electromagnetic tomography (LORETA) (Dushanova *et al* 2009, Ibáñez *et al* 2014, Peng *et al* 2015, Li *et al* 2018).

2. Methods

2.1. Subjects and experimental design

An open dataset was used for the analysis (Ofner *et al* 2017). Fifteen non-disabled volunteers (nine women) aged between 22 and 40 years (mean: 27.0 years and standard deviation 5.0 years) participated in the study. All the subjects, but one, were right-handed. All participants signed a written informed consent.

Subjects sat on a chair with their right arm fully supported by an exoskeleton. The experimental session was divided into ten runs to reduce the fatigue effect. A S1–S2 contingent negative variation (CNV) paradigm was conducted to record motor anticipation. Participants watched a computer screen where cues were displayed. At the beginning of a trial, a beep sound and a warning cross appeared on the screen (S1) to remind the participant to pay attention to the task. Two seconds after S1, subjects were instructed to respond to a picture presented on the screen with the imperative movement task (S2). Six different sustained upper limb movement types were required: elbow flexion, elbow extension, supination, pronation, hand close and hand open. Finally, volunteers

moved back to the initial position: lower arm extended to 120° in a neutral position and hand half open. A break with a random duration between 2 and 3 s was applied between trials. At the end of the experimental session, 60 trials were acquired for each movement. Movements were grouped into three categories with respect to their joint movements: (a) elbow flexion/extension, (b) forearm pronation/supination, and (c) hand close/open; resulting in a total of 120 trials for each category.

2.2. Data acquisition

EEG data was recorded from 61 active electrodes according to the international 5/10 system and referenced to the right mastoid. Additionally, three electrooculography (EOG) channels were recorded as auxiliary signals for ocular artefact reduction. EEG and EOG signals were analogically band-pass filtered between 0.01 and 200 Hz and recorded with a sampling frequency of 512 Hz by means of four 16-channel amplifiers (g.tec medical engineering GmbH, Austria). Additionally, data from accelerometers in the exoskeleton and grip pressure sensors in a Data Glove (5DT, USA) were acquired to monitor physical movement.

2.3. Data analysis

Firstly, raw EEG and EOG data were digitally filtered using a band-pass filter (0.3–100 Hz). Secondly, an ocular reduction automatic procedure based on blind source separation was applied to raw EEG signals. In particular, the second order blind identification algorithm was used to decompose EEG and EOG data into source components (Belouchrani *et al* 1997). The automatic identification of the ocular-related sources was based on frequency and scalp topography features of the obtained components (Romero *et al* 2008). After reconstructing the EEG excluding the ocular components, signals were segmented into ERP trials from -0.5 s before to 7 s after the S1 stimulus. Artefactual EEG channels were automatically rejected by means of outlier detection based on trial amplitude and kurtosis. For each subject and movement category, trials with less than 75% of artefact-free channels were also rejected. Finally, EEG was re-referenced to the common average (Platz *et al* 2000) considering the excluded channels.

2.4. Movement detection

Movement onsets and offsets were obtained from the exoskeleton and glove sensors, taking the derivative of the signal of the best sensor according to each movement. As these signals showed noisy upward or downward steps depending on the type of movement, the derivative was thresholded to obtain a rough approximation of the desired landmarks. These values were subsequently refined by looking for the first local minimum or maximum appearing just before the step, which was taken as the onset or offset time.

2.5. Monte Carlo simulation

The sets of trials contributing to the average motor ERP were selected via a Monte Carlo approach. Specifically, 1000 random simulations were performed for each movement category; in each one a random 10% of trials were removed successively until only the 20% of the trials remained. In this way, each Monte Carlo simulation included random trial sets for each movement category with a number of trials ranging from 90% (108 trials) to 20% (24 trials), in 10% decreases.

2.6. Motor related cortical potential (MRCP)

The CNV potential has two components: an early wave related to the warning stimulus (S1) and a late component related to the imperative stimulus (S2). This second targeted wave has been associated with motor preparation and response readiness (Kononowicz and Penney 2016). MRCPs have been commonly characterized by the maximal amplitude and the onset of the wave (Chen *et al* 2018, Nann *et al* 2019). In our study, EEG signals were band-pass filtered between 0.3 and 10 Hz (4th-order Butterworth) before averaging windows from -2.5 to 2.5 s with respect to movement-onset. For each subject and movement class, MRCP onset latency was computed, for each subject, as the last zero crossing of the Cz channel before the rise of the late MRCP component. Peak amplitude of the MRCP was calculated as the average of Cz in a window from -100 to 100 ms centred on the maximum peak detected in the 500 ms before the movement onset.

2.7. Event-related desynchronization and synchronization (ERD/ERS)

ERD and ERS are defined as percentage power decreases or increases, respectively, in relation to a reference period. They are considered to indicate activation and subsequent recovery of the motor cortex during the planning, execution and completion of a voluntary movement. The most common studied rhythms are mu and beta.

The calculation of ERD and ERS followed the classical methodology using mu band from 8 to 13 Hz and beta band from 14 to 30 Hz (Pfurtscheller and Lopes da Silva 1999). Whereas ERD was estimated by averaging trials in a window from -2.5 to 5.5 s with respect to the movement onset, ERS used a window from -5 to 3 s with respect to the movement offset. Both ERD and ERS were obtained as the percentage change with respect to the reference window (1.5 – 1 s before the movement onset).

Minimum and maximum ERD/ERS amplitudes are commonly used to characterize the sensorimotor response (Heida *et al* 2014, Aoh *et al* 2019, Visani *et al* 2019). Minimum ERD was calculated as the average ERD in a window of 200 ms around the movement onset (we will refer to this feature as ERD from now on). Recovery ERS was the difference between

the maximum ERS (average in a window of 200 ms around the ERS peak) and the average ERS just before the movement offset, also in a window of 200 ms (we will refer to this feature as ERS from now on).

Whereas the MRCP peak was mainly located at Cz, mu and beta ERD showed a more spread distribution consisting of bilateral central foci, which were stronger on the contralateral hemisphere of the movement (McFarland *et al* 2000). That is why different regions of interest (ROIs) were considered for mu and beta, considering the channels showing higher ERD consistently. The ROI in the mu band comprised the channels CPP3h, CCP3h, CP1, CP3 and P3; and in the beta band included the channels CCP3h, CP3, CP1 and CCP1h.

2.8. Low-resolution electromagnetic tomography (LORETA) analysis

Cortical sources related to the generation of the MRCP were estimated using standardized LORETA (sLORETA) (Pascual-Marqui 2002), a tool that computes a 3D intracerebral current density distribution from the voltage values acquired at the scalp. The final solution space, restricted to the cortical grey matter and hippocampus, consists of 6239 voxels with a spatial resolution of 0.125 cm³. MRCP brain electrical sources were determined by averaging sLORETA images in the interval between -100 and 100 ms around the MRCP peak.

2.9. Statistical analysis

For each Monte Carlo simulation, Pearson's correlation coefficient was calculated between the motor-related features extracted for the whole set and a lower subset of trials. The probability of obtaining a statistically significant difference in each motor feature (100% vs a lower number of trials) was estimated by simulating 1000 different trial combinations (number of significant results obtained divided by 1000). Statistical differences on topographic distributions of MRCP and ERD/ERS features, as a function of the number of trials used for the ERP averaging, were analysed by paired *t*-tests at each electrode. The false discovery rate (FDR) method was used for controlling Type I error of multiple comparisons (Benjamini and Hochberg 1995). In addition, statistical differences between sLORETA images were assessed using paired *t*-tests computed for log-transformed current density values at each voxel. To correct for multiple comparisons, a non-parametric test based on the theory of randomization and permutation was applied (Holmes *et al* 1996).

3. Results

A total of 2.2 ± 1.0 (mean \pm standard deviation) independent components related to ocular

contamination were rejected by the automatic artefact reduction procedure. On average, 1.8 ± 1.7 artefactual trials (1.5% of all trials) for flexion/extension, 11.7 ± 3.9 (9.8%) for pronation/supination and 5.8 ± 2.6 trials (4.9%) for hand open/close were excluded from the averaging.

3.1. Population-based analysis

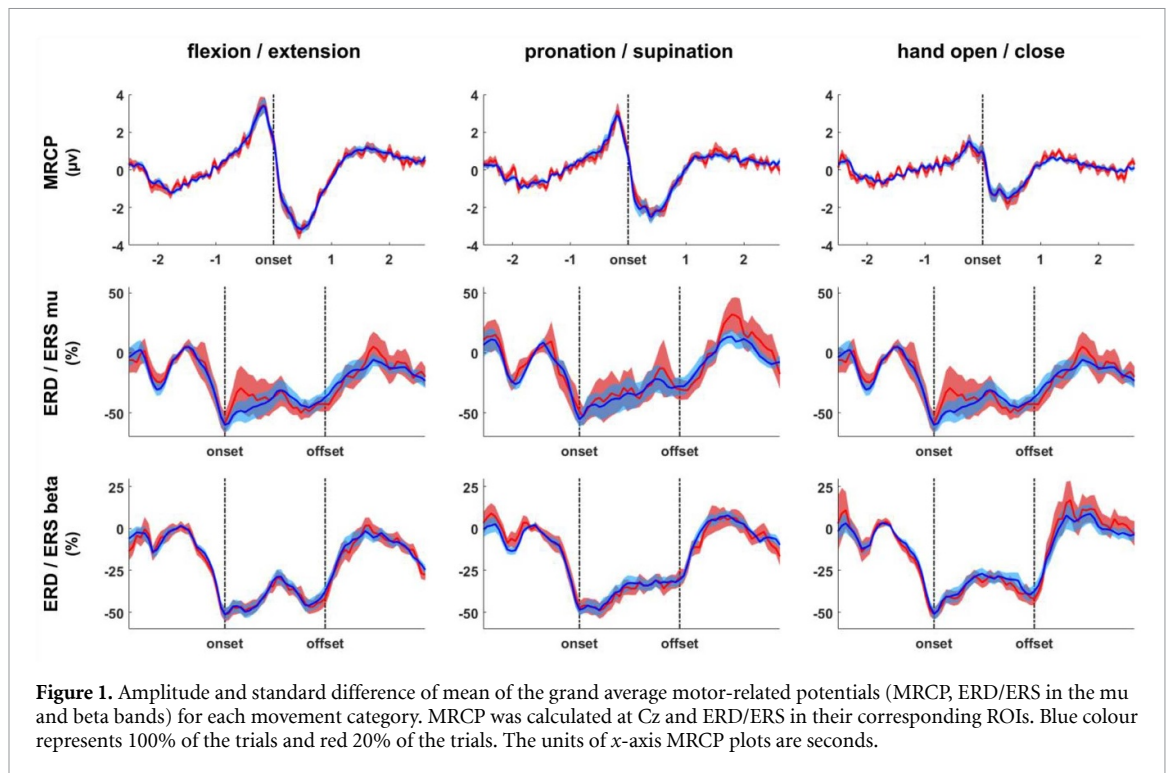
Movement onset-locked averages of motor-related potentials (MRCP and ERD/ERS calculated at Cz electrode and corresponding ROIs, respectively) for one Monte Carlo trial-set simulation (the one in which the chronological order of the trials is respected) are shown in figure 1 as a representative case (similar behaviours were obtained for all simulations). Motor potentials for 100% and 20% of the trials are shown (mean and standard error of mean). ERD/ERS curves were obtained by averaging the time-normalized movement onset- and movement offset-locked trials. A time-normalization method, derived from Aeschbach and Borbély (1993), was applied to compensate for inter-subject differences in the duration of sustained movement. The procedure was based on dividing pre-movement and post-movement periods into 100 equal parts, and then averaging normalized trials within and across subjects. Grand-average motor-related potentials were very similar for all the different cases with a lower number of trials. However, as the number of trials decreased, inter-subject variability increased.

Moreover, the percentage of absolute differences were calculated for features obtained from a lower number of trials compared to these obtained from all trials, considering all the Monte Carlo simulations. Table 1 shows the average and standard deviation of these differences for all features and all movements.

As expected, averaged differences increased with decreasing numbers of trials. Except for MRCP latency, positive and negative differences would have cancelled themselves to some extent had absolute value not been applied. Differences for MRCP and ERD features were lower than 20% for trial percentages above 40%, but they clearly increased when the number of trials was reduced to 20%. Interestingly, the sensorimotor beta rhythm seemed more robust to the reduction in the number of trials than the mu rhythm, and ERS-related features were more sensitive to this change.

For each Monte Carlo simulation, Pearson's correlation coefficients (and 95% confidence intervals) were obtained between features from averaged motor potentials. In addition, paired *t*-tests were performed comparing features from all trials and each reduced percentage. The probability of obtaining a significant difference was estimated from the number of significant *t*-tests among all the Monte Carlo simulations.

Figure 2(a) shows the average correlation coefficients for all simulations as a function of the numbers



of trials. Strong correlations (higher than 0.75) were obtained for all trial percentages above 40%. As expected, mean correlation values decreased and the confidence interval increased as the number of trials was reduced. Figure 2(b) shows the probability of obtaining a significant difference in features as a function of the number of trials averaged. Although probabilities increased as the number of trials was reduced, it was not a significant trend because the subject variability increased at the same time, thus reducing statistical power. However, MRCP latency was a particular case where the probability to obtain a significant change was very high because latencies were shortened for most subjects as the number of trials decreased.

The effect of the reduction of the number of trials on the topographical distribution of MRCP and ERD/ERS features was also studied. Figure 3 shows an illustrative example of the maps obtained for one simulation using the 100%, 60% and 20% of the trials, respecting the chronological order of the appearance of the trials in the recordings. Three maps are presented for each feature, movement and percentage of trials: average potential, within-subject variability (standard deviation) and percentage difference. All features and all movement categories exhibited similar maps when reducing the number of trials. Considering all features and movements, no statistically significant differences were found when comparing maps obtained with all the trials to those corresponding to a lower number of trials. Because of the multiple comparisons involved, an FDR correction was applied. The standard deviation clearly increased as the number of trials was lower, especially in regions

not related to motor activity. This effect was more noticeable in the error maps, which showed some within-subject average differences (with respect to all trials) higher than 50% for the lowest number of trials.

The effect of trial reduction on the sources of motor-related cortical potentials was assessed by LORETA (sLORETA). Figure 4 shows, as an example, the source solutions of the MRCP peak obtained for one Monte Carlo simulation (first chronologically trials) as a function of the number of trials. Within-subject average activations showed similar current density patterns when reducing the number of trials: for all cases its maximum values were located at the Brodmann area 6 (medial frontal gyrus) for elbow flexion/extension; at the Brodmann area 7 (postcentral gyrus) for forearm pronation/supination; and at the Brodmann area 4 (precentral gyrus) for hand open/close. However, source locations obtained from fewer trials appeared to be less accurate or precise, as indicated by the standard deviation, also included in the figure to assess inter-subject variability. To make the comparison between movements easier, the colour scale of standard deviation images was set to the 90% of the maximum value obtained when using 60% of the trials in each category.

The number of high standard deviation regions increased and spread as the number of trials was reduced. For each movement, the Holmes non-parametric correction was performed for all voxels between the all-trials case and the lower-trials cases for correcting the effect of multiple comparisons. Statistical differences were found for percentages

Table 1. Grand average and standard deviation for all subjects of absolute differences (%) calculated between 80%, 60%, 40% and 20% of the trials and the 100% of the different motor features calculated for each movement category. All the Monte Carlo simulations were considered for average calculation. Minimum and maximum absolute differences values were also depicted.

Feature	80% Avg. Diff. \pm STD	60% Avg. Diff. \pm STD	40% Avg. Diff. \pm STD	20% Avg. Diff. \pm STD
Flexion/extension				
MRCP peak (min max)	4,7 \pm 6,7 [2,0 18,0]	6,9 \pm 7,8 [3,3 21,7]	9,3 \pm 9,4 [4,9 25,6]	14,1 \pm 12,4 [8,6 31,0]
MRCP lat. (min max)	4,2 \pm 6,7 [0,9 12,3]	7,4 \pm 9,7 [2,0 18,3]	11,2 \pm 12,0 [3,9 22,9]	20,0 \pm 16,6 [7,7 32,0]
ERD beta (min max)	2,6 \pm 2,3 [0,8 7,3]	4,3 \pm 3,8 [1,3 12,3]	6,5 \pm 5,7 [1,9 18,1]	11,0 \pm 9,6 [3,4 30,1]
ERD mu (min max)	6,3 \pm 8,8 [0,4 43,2]	8,9 \pm 9,9 [0,7 48,7]	11,9 \pm 11,8 [1,1 51,3]	17,3 \pm 16,0 [2,1 48,8]
ERS beta (min max)	9,4 \pm 9,4 [3,6 31,3]	14,9 \pm 14,0 [5,3 50,7]	20,5 \pm 17,5 [7,4 55,7]	30,0 \pm 22,7 [13,0 48,6]
ERS mu (min max)	15,4 \pm 15,8 [5,6 47,9]	21,1 \pm 17,0 [9,8 48,7]	27,9 \pm 19,9 [15,1 50,3]	37,4 \pm 24,0 [26,8 51,4]
Pronation/supination				
MRCP peak (min max)	6,0 \pm 6,8 [2,4 20,3]	9,3 \pm 9,4 [4,0 25,4]	13,3 \pm 12,6 [5,9 31,4]	20,0 \pm 16,9 [9,0 38,9]
MRCP lat. (min max)	6,4 \pm 8,9 [0,8 24,0]	10,6 \pm 12,3 [1,5 32,7]	15,9 \pm 15,7 [2,3 38,2]	25,0 \pm 19,7 [6,0 50,6]
ERD beta (min max)	3,7 \pm 3,6 [1,4 12,8]	6,3 \pm 6,0 [2,5 22,0]	9,5 \pm 8,8 [3,7 30,8]	15,6 \pm 13,3 [5,7 38,4]
ERD mu (min max)	7,9 \pm 10,3 [0,6 37,8]	11,3 \pm 12,1 [1,1 72,4]	15,3 \pm 13,8 [1,7 49,0]	22,0 \pm 18,5 [3,5 51,1]
ERS beta (min max)	9,3 \pm 8,0 [5,2 19,9]	15,2 \pm 12,6 [8,8 30,4]	22,6 \pm 17,9 [13,7 39,1]	33,5 \pm 23,2 [24,1 46,8]
ERS mu (min max)	16,7 \pm 15,6 [5,9 37,2]	24,6 \pm 20,3 [9,9 43,7]	32,0 \pm 23,0 [14,9 47,5]	40,1 \pm 25,5 [27,3 51,2]
Hand open/close				
MRCP peak (min–max)	9,1 \pm 7,1 [3,6 45,9]	13,8 \pm 10,9 [6,0 54,1]	17,8 \pm 13,7 [8,7 48,6]	25,9 \pm 18,0 [14,6 65,9]
MRCP lat. (min max)	5,9 \pm 9,1 [1,4 18,1]	10,1 \pm 13,4 [2,8 24,7]	15,2 \pm 17,0 [5,1 39,1]	24,5 \pm 21,4 [10,5 53,8]
ERD beta (min max)	3,1 \pm 2,7 [0,7 6,7]	5,1 \pm 4,5 [1,2 11,1]	7,8 \pm 6,9 [1,7 16,7]	13,3 \pm 11,7 [3,1 25,7]
ERD mu (min max)	6,0 \pm 7,4 [0,6 18,9]	9,9 \pm 12,0 [0,9 29,1]	14,3 \pm 16,3 [1,4 40,5]	21,1 \pm 20,9 [2,5 46,9]
ERS beta (min max)	7,3 \pm 6,2 [3,8 16,0]	11,1 \pm 9,1 [5,9 26,7]	16,0 \pm 13,1 [8,6 37,8]	25,4 \pm 19,2 [15,2 39,5]
ERS mu (min max)	12,4 \pm 10,4 [6,2 25,0]	20,1 \pm 16,0 [10,3 37,4]	28,7 \pm 21,2 [15,8 44,4]	39,1 \pm 25,5 [25,4 47,4]

lower than 40% of the trials. For reduced percentages of trials (20% and 30%), the number of statistical suprathreshold voxels did not reach the 2% of the total number of sLORETA solution space (6239 voxels). Furthermore, suprathreshold voxels for 20% and 30% of trials did not define a compact region, but they were rather scattered throughout different regions of the brain.

3.2. Individual-based analysis

Percentage absolute differences in motor features between considering the whole or a reduced number of trials showed a high variability between subjects independently of the number of trials, i.e. there were

subjects whose differences were very high or reasonably low in spite of considering a reduced number of trials (see minimum and maximum values in table 1).

As a general rule, subjects did not present substantial variations on topographic maps when reducing the number of trials, but in some individual cases scalp distributions changed for MRCP peak and ERD features after reducing the number of trials. Particularly, this happened to a greater extent in the forearm pronation/supination and hand open/close movements and for ERD and ERS activity (see examples in figure 5).

Regarding the individual source tomographies, there were not substantial differences when reducing

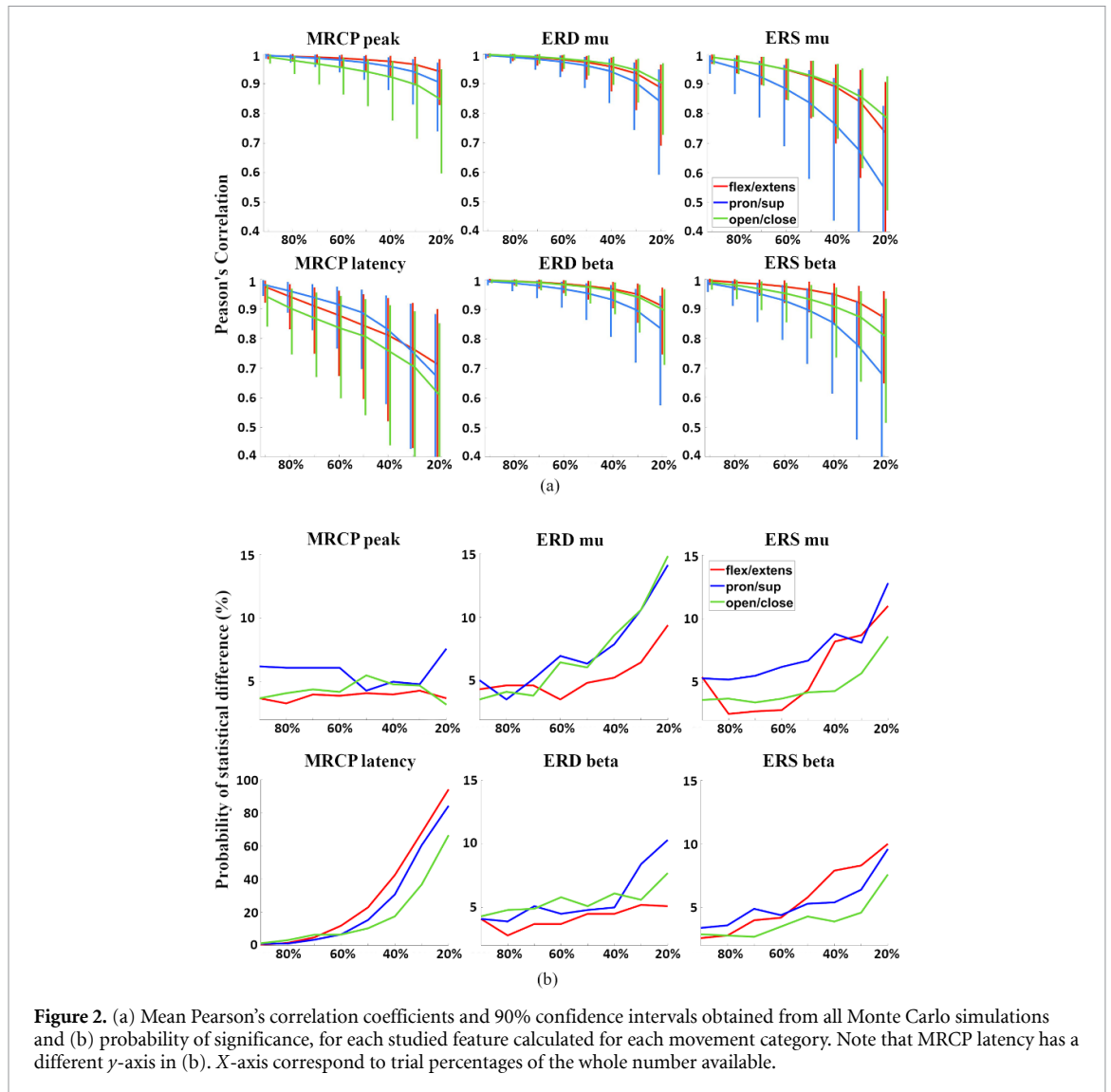


Figure 2. (a) Mean Pearson's correlation coefficients and 90% confidence intervals obtained from all Monte Carlo simulations and (b) probability of significance, for each studied feature calculated for each movement category. Note that MRCP latency has a different y-axis in (b). X-axis correspond to trial percentages of the whole number available.

the number of trials, analogously with topography analysis, except in some subjects during pronation/supination and hand open/close.

Figure 6 depicts sLORETA images as an example of these cases. This subject exhibited the two extreme situations that arose when reducing the number of trials: either no effect in source localization (see flexion/extension) or high deviation and irregularities in source localization (see movements of pronation/supination and hand open/close). Related to the latter case, localizations of MRCP activity obtained for the 20% case shifted markedly to occipital or temporal brain regions.

4. Discussion

The characterization of EEG motor activity is well established as a valuable clinical tool to assess the degree of motor impairment related to neurological diseases such as Parkinson's (Dushanova *et al* 2009), stroke (Monge-Pereira *et al* 2017), myoclonus (Visani *et al* 2019), spinocerebellar ataxia (Aoh *et al*

2019), and amyotrophic lateral sclerosis (Bizovičar *et al* 2014) among others. EEG can also be a useful tool to assess the effectiveness of rehabilitation and drug therapies in an objective fashion (Ibáñez *et al* 2014). While brain computer interface systems focus on the detection/prediction of movement execution/intention based on single trial processing (Cecotti and Ries 2017), other applications often rely on averaging techniques to better characterize motor related potentials, improving signal to noise ratio (Pfurtscheller and Lopes da Silva 1999). In this case, a large number of trials is required to obtain a motor potential that is representative enough of the patient status.

The aim of this paper was to assess the effect of the number of trials on the MRCP and ERD/ERS potentials related to different upper limb movements. EEG MRCPs are commonly used to represent and interpret motor activity (Pfurtscheller and Lopes da Silva 1999, Colebatch 2007) as well as to characterize and understand the pathogenesis of motor impairment in specific neurological diseases (Dushanova

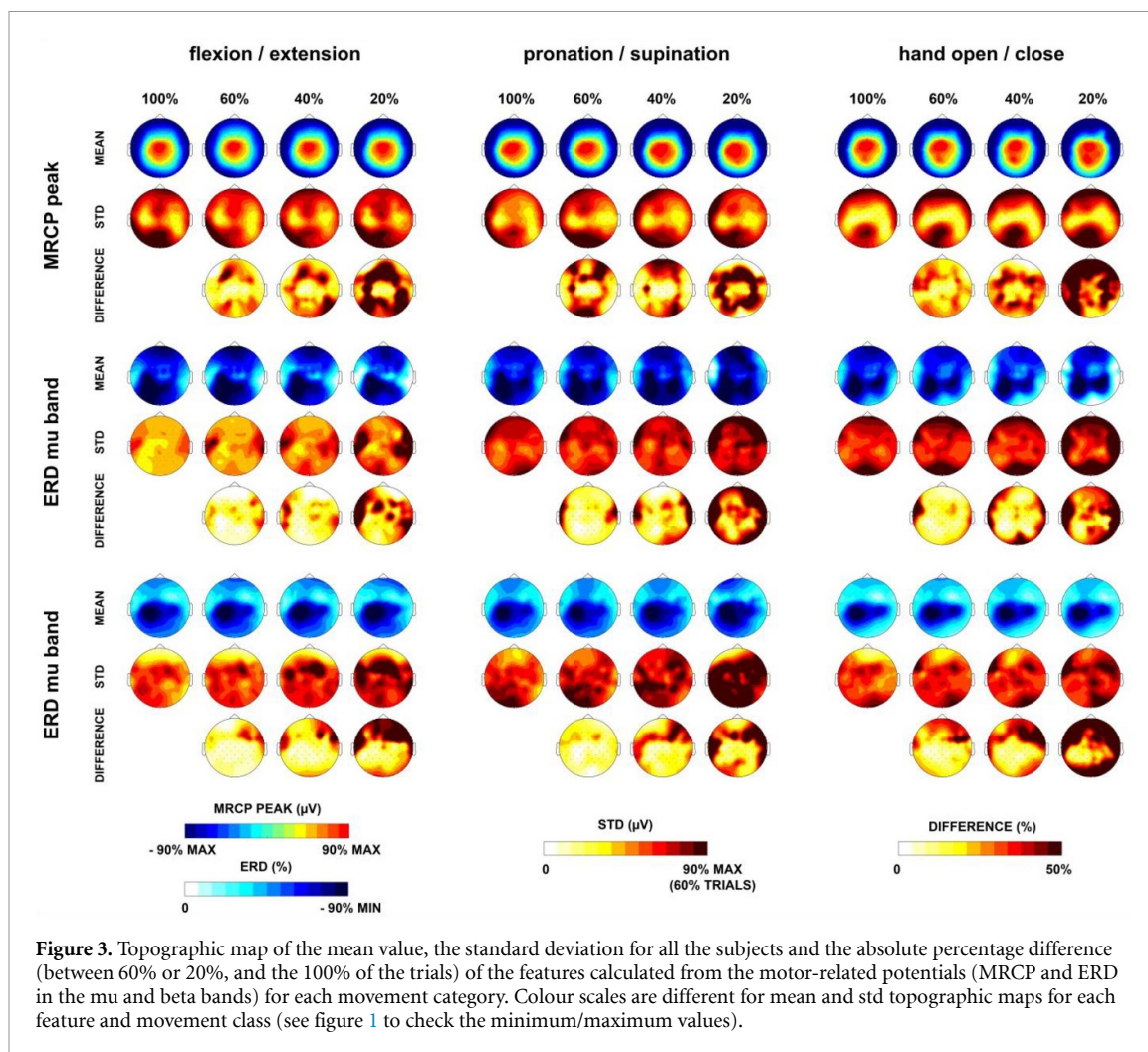


Figure 3. Topographic map of the mean value, the standard deviation for all the subjects and the absolute percentage difference (between 60% or 20%, and the 100% of the trials) of the features calculated from the motor-related potentials (MRCP and ERD in the mu and beta bands) for each movement category. Colour scales are different for mean and std topographic maps for each feature and movement class (see figure 1 to check the minimum/maximum values).

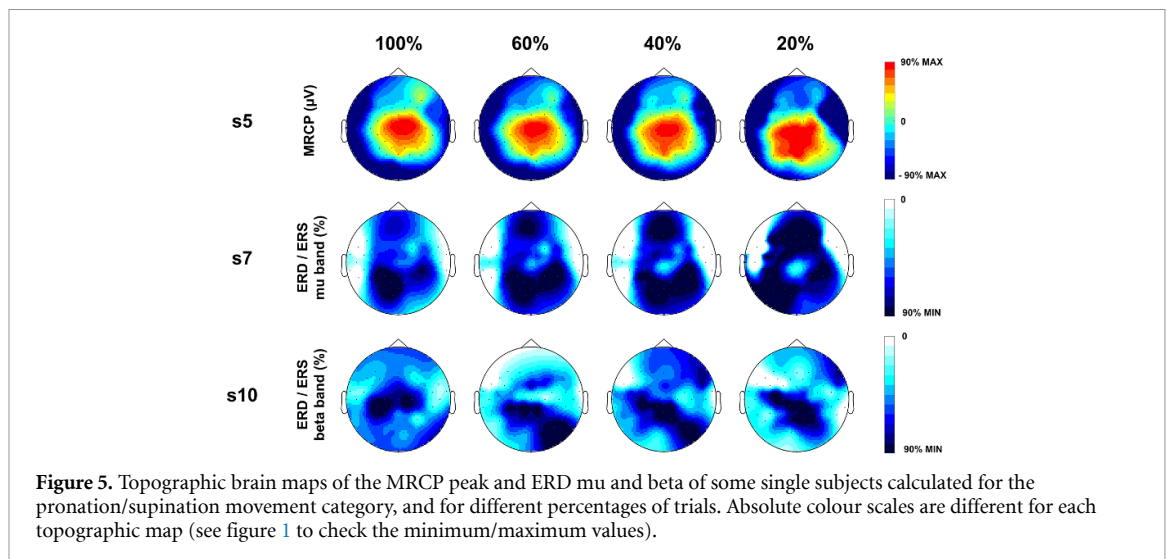
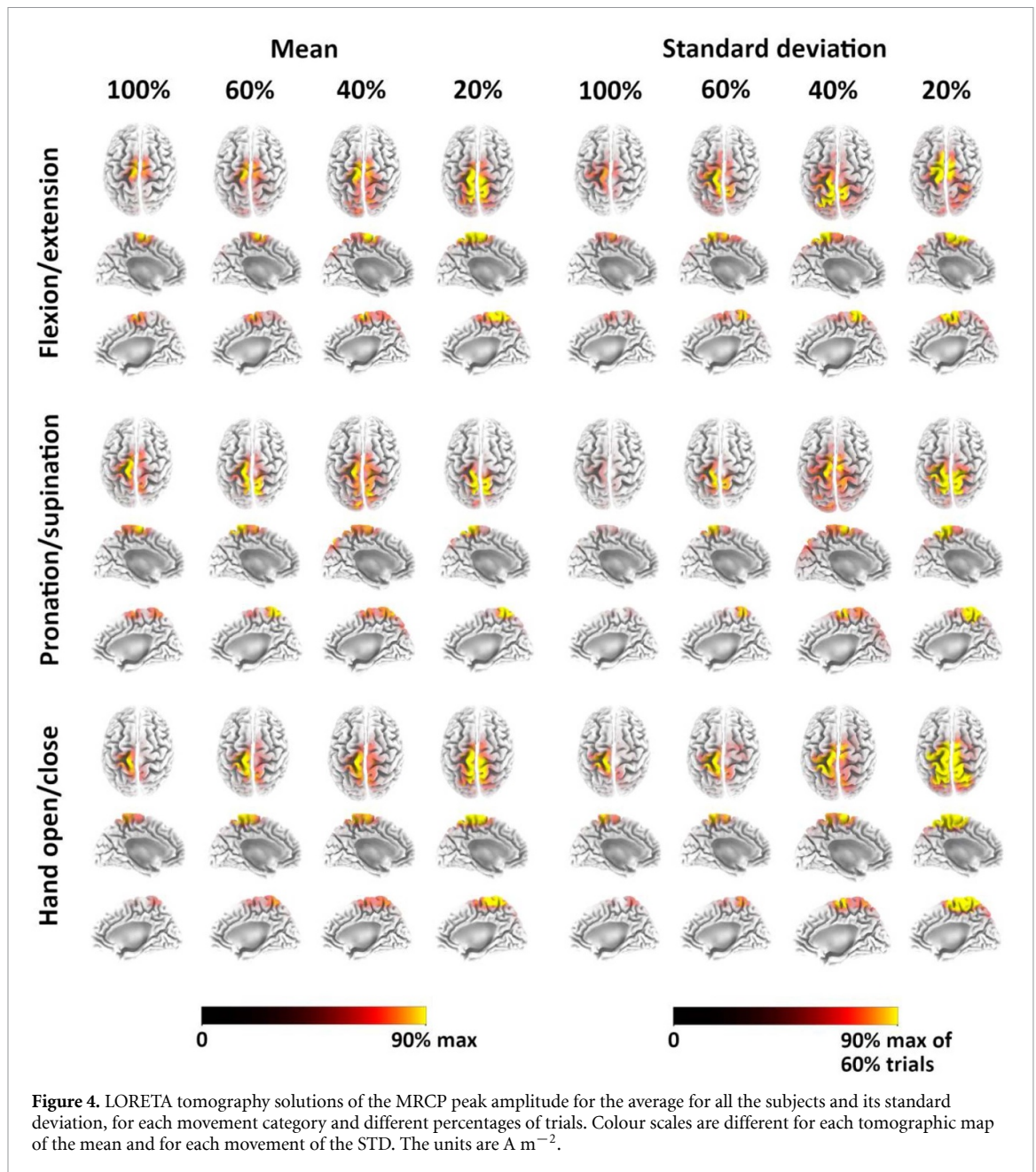
et al 2009, Bizovičar et al 2014, Monge-Pereira et al 2017, Aoh et al 2019, Visani et al 2019). EEG MRCPs also assess the improvement or prognosis after some rehabilitation therapies (Ibáñez *et al* 2014, Bartur *et al* 2017, Cassidy *et al* 2021, Hakiki *et al* 2021).

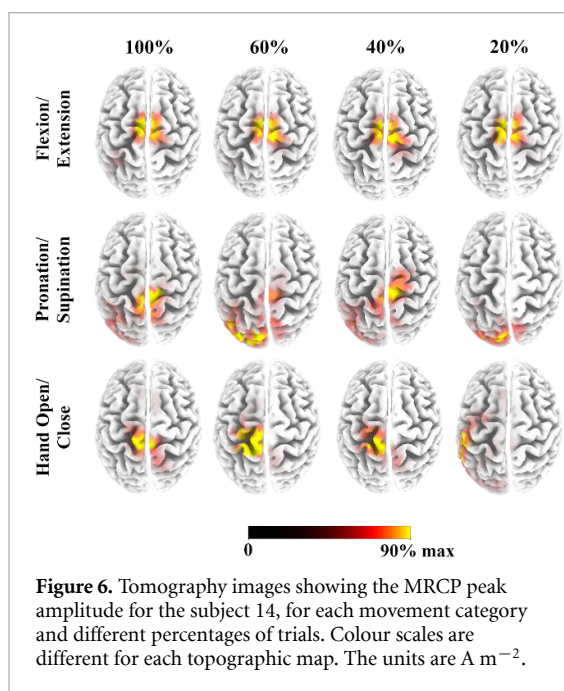
Our analyses show that the impact of the number of trials on the average MRCP and mu and beta ERD/ERS time courses was scarce, since the average motor potentials mostly overlapped for the different percentages of trials studied (see figure 1). Although the amplitude of these potentials hardly varied when the number of trials was lower, the subject variability noticeably increased. A 1000 trial-set Monte Carlo simulations were applied to assess a robust and consistent effect.

We observed strong correlations (>0.75) between the motor features obtained using all the trials and percentages of trials higher than 40% (around 50 trials). Moreover, we obtained low probabilities ($<10\%$) of statistical difference for 40% of trials and higher and all motor features, despite MRCP latency presented a greater sensitivity to the decrease in the number of trials. Regarding MRCP correlation, the hand open/close generated a worse

impact on correlation when reducing the number of trials. Although the ERD/ERS correlations were higher, the forearm pronation/supination movement was more sensitive to a limited number of trials than the other movements. In general, the elbow flexion/extension movements were more robust when reducing the number of trials both, in population and in individual-based analysis, for all the features studied.

As expected, the averaged absolute differences increased as the number of trials decreased for all the motor features analysed (see table 1). Although motor potential averages did not change when limiting the number of trials, there was an obvious absolute difference suggesting a compensation performance between subjects. These results were confirmed by the topographic analysis: similar average MRCP and ERD/ERS scalp patterns for reducing number of trials but increases of the within-subject variability and the absolute differences, especially in the regions not related to motor activity (see figure 3). Although these changes in non-motor channels do not affect the feature extraction, they influence the solution of the source localization of motor activity.





According to this, within-subject averages of sLORETA images corresponding to the MRCP peak showed a slight improvement of its localization with increasing percentage of trials, especially in terms of dispersion. That is, the results for all trials presented a more compact source localization of the MRCP peak than for 20% of the trials (see figure 4). Along the same lines, this behaviour was more evident in the standard deviation images, evidencing increased variability with lower percentages of trials. However, no statistical differences were found between tomographies corresponding to all trials and lower percentages down to 40%.

For all the movement categories and all the different numbers of trials analysed, the maximum sLORETA activations were located at the brain regions associated with movement planning and execution, spatial guidance of movement and the visuo-motor coordination (Brodman 2006). As it was mentioned above, within-subject average topographic and tomographic images for different numbers of trials remained virtually unaltered (see figures 3 and 4). Additionally, individual analysis did not show substantial topographic and tomographic differences when reducing the number of trials, especially for MRCP peak. However, we found several cases with shifts of the localization of the source (see figures 5 and 6).

In summary, our overall findings are in accordance with the literature on different ERP components: the lower the number of trials, the greater the variability (Goldsworthy *et al* 2016). The effect of the number of trials had a small impact, in terms of population, on the analysis of averaged features and maps. However, the variability of the results was relevant, being more pronounced when decreasing the number of trials.

High variability with lower number of trials is present in all the approaches considered in our study: motor-related time-course potentials, features, topographies and tomographies. In studies relying on the comparison of two or more groups (pathology vs controls, pre-rehabilitation vs post-rehabilitation, etc), high variability decreases statistical power. Our population analysis demonstrates that, although increasing the number of trials improves the consistency of the motor potentials, 50 trials can be an adequate number to obtain reliable motor-related features in terms of differences, correlation, topography and tomography. This is a recommended general number of trials considering globally all the analysed motor features and movements, but if the study is focused on some specific motor feature, such as amplitude ERD, reliable results can be obtained with more reduced number of trials (around 25). Fifty trials are a reasonable and moderate number in MRCPs, especially compared to the number of trials recommended for obtaining a stable ERP component in other studies: around ten trials for P300, 15 for event-related negativity, 35 for transcranial magnetic stimulation evoked potential, and 45 trials for lateralized readiness potential. In our study, the analysis was extended to demonstrate statistically significant differences in topographical and tomographical locations of motor activity. In any case, increasing the number of trials could be of great interest as long as the time effort were affordable assuming that this increase would not lead to fatigue or lack of attention to the task performed (Boudewyn *et al* 2018).

It is important to point out that our findings were obtained for young and healthy participants during upper limb movement execution. Thus, more studies are needed to assess the effect in other populations (elderly or coordination and movement pathologies such as stroke or ataxia), other movement types (such as lower limb movements), the impact of the sample size on motor potentials, and the outcomes where the physical movement was not possible, such as in motor imagery studies.

Data availability statement


The data that support the findings of this study are openly available at the following URL/DOI: <https://doi.org/10.1371/journal.pone.0182578>.

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