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RESEARCH ARTICLE

Control of Movement

MRCP as a biomarker of motor action with varying degree of central and peripheral contribution as defined by ultrasound imaging

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Abstract

Motor imagination is an alternative rehabilitation strategy for people who cannot execute real movements. However, it is still a matter of debate to which degree it involves activation of deeper muscle structures, which cannot be detected by surface electromyography (SEMG). Sixteen able-bodied participants performed cue based isometric ankle plantar flexion (active movement) followed by active relaxation under four conditions: executed movements with two levels of muscle contraction (fully executed and attempted movements, EM and AM) and motor imagination with and without detectable muscle twitches (IT and I). The most prominent peaks and distinctive phases of movement-related cortical potential (MRCP) were compared between conditions. Ultrasound imaging (USI) and SEMG were used to detect movements. IT showed spatially distinctive significant differences compared to both I and AM during active movement preparation and reafferentation phase; further widespread differences were found between IT and AM during active movement execution and posteriorly during preparation for active relaxation. EM and AM showed the largest differences frontally during active movement planning and posteriorly during execution of active relaxation. Movement preparation positivity P1 showed a significant difference in amplitude between IT and AM but not between IT and I. USI can detect subliminal movements (twitches) better than SEMG. MRCP is a biomarker sensitive to different levels of muscle contraction and relaxation. IT is a motor condition distinguishable from both I and AM. EEG biomarkers of movements could be used to identify pathological conditions, that manifest themselves during either active contraction or active relaxation.

NEW & NOTEWORTHY Ultrasound imaging can detect subtle muscle movements (twitches) that are not detectable with electromyography. Almost a quarter of trials of imagined movements in able-bodied people are accompanied by twitches. Analysis of movement-related cortical potential showed that motor imagination with twitches is a condition distinguishable from motor imagination without twitches and from motor attempts.

EEG; EMG; MRCP; motor imagery; ultrasound imaging

INTRODUCTION

Different types of overt and covert motor actions are defined by distinctive levels of activation of corticospinal tract and proprioceptive afferent feedback, related to motor planning/execution and evaluation of a real or simulated actions (1). The afferent contribution of a motor action is typically assessed by measuring the accompanied muscular activity by surface electromyography (SEMG). However, SEMG cannot provide information about the activity of deeper muscular structures and cannot always detect subliminal muscle activity, that is, muscle twitches, which may accompany covert motor actions, such as motor imagery (MI).

In recent years, the popularity of ultrasound imaging (USI) of muscular activity has increased due to the availability of portable and inexpensive ultrasound devices (2, 3) and real-time USI has been used to teach patients to control the activity of the deep muscles that might be hard to consciously control in isolation from other muscles (4). Although USI is frequently interpreted by specialists, an automated method of USI analysis enables unbiased quantitative analysis, similar to the analysis of SEMG (5).

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Although USI typically has a much lower temporal resolution than SEMG, it can provide information about the activation of deeper muscle structures and can detect subliminal movements during MI that might not be detectable with SEMG.

MI presents an internal simulation of actual movement (1, 6) that also involves "computational equivalence," such as recruiting the forward models to predict the sensory consequences of imagined movements (7). A widely accepted view is that covert movements, and in particular MI, may improve motor performance in healthy people and in patients requiring physical therapy (8). There is also evidence that MI may have the effect on increasing muscle strength (9–11). This has been attributed to frequently observed electrical activity of muscles, recorded by SEMG, during imagination of movement. The electrical activity has been related to the incomplete inhibition of muscle activity during MI (1). The incomplete inhibition theory has been supported by studies showing increased H reflex (12), that is, corticospinal excitability during MI. Yao et al. (11) demonstrated a correlation between increased muscle strength following a prolonged MI practice and increased motor potential component of movement- related cortical potential (MRCP). Guillot et al. (13) suggested that the vividness of MI is directly proportional to the magnitude of a subliminal muscular activity, also showing that a larger muscle activity is recorded during kinesthetic, than during first person visual motor imagery. On the other hand, Naito et al. (14) proposed that kinesthetic sensation during kinesthetic imagery is entirely illusory and centrally generated. do Nascimento et al. (15) found no reafferentation potential in MRCP during MI of the foot, implying no proprioceptive component, that is, no muscle contraction.

Although some studies claimed to record no SEMG activity during MI (14, 16), there is an open question how often motor imagery in able-bodied people is accompanied by muscle activation and whether it is possible to quantify the contribution of subliminal muscle activation on the cortical activity.

It has been speculated that deeper muscle structures are active during MI (13) that cannot be detected by SEMG. Single motor unit animal model studies show that deeper muscles are active predominantly during movement preparation (17).

Due to the similarity of cortical activation during real and imagined movement, MI has been frequently used as a brain computer interface (BCI) experimental paradigm (18), where healthy people are asked to imagine movements to simulate motor processes in paralyzed people. The indented use of such BCI is for communication and control of environment (19). MI has also been suggested as an alternative to real movements for motor rehabilitation (8, 20). However, for the purpose of rehabilitation, patients have typically been asked to attempt, rather than just to imagine a movement that they cannot physically execute. It has been shown that patients with lower limb paralysis due to spinal cord injury can differentiate between the imagined and attempted action and that the latter produces stronger movement related potentials (21). In able-bodied people, a motor attempt (minimal muscle contraction required for a person to become aware of muscle activation) typically results in a visible muscle contraction and it takes considerable training to be able to attempt a movement with no visible SEMG (22). Still, an unanswered question is, whether there is a difference in cortical responses between MI with subliminal muscle contraction, that is, twitches and attempted movements (AM). Understanding differences in cortical activity between motor actions with different levels of central and peripheral contribution would not only contribute to understanding the central and peripheral component of motor action but would also help define EEG based neuroimaging biomarkers of various movement disorders.

Although most BCI applications rely on simple MI such as waving or a hand extension, a functional movement typically consists of an active muscle contraction followed by an active relaxation. For rehabilitation purposes, active contraction has been studied much more than active relaxation, because contraction is relevant for movement preparation and execution. However, although muscle contraction is a major concern following neurological injuries such as stroke or spinal cord injury, lack of relaxation can be a significant problem in some neurological conditions such as focal dystonia (23) or Parkinson's disease (24). In able-bodied people, MRCP has similar morphology during active contraction and relaxation (25), whereas in focal dystonia "Bereitshaft Potential" is diminished during relaxation (23).

In this study, we compared brain responses during voluntary muscle contraction, motor attempt, and imagined movement, with or without subliminal muscle activity, that is, muscle twitches. We hypothesize that imagined, attempted, and executed actions present a continuum of motor action with distinctive EEG responses. Furthermore, we also hypothesize that a subliminal muscle activity during MI is also reflected in brain activity in distinct phases of motor action as defined by (15, 26). We analyzed MRCP during isometric sustained contraction and active relaxation of the ankle to answer to the following research questions:

1) Which phase of movement (motor preparation, execution, or reafferentation) shows the largest differences between MI with or without subliminal muscle activity?
2) Which phase of movement of MRCP or frequency band in ERS/ERD best characterize the differences between attempted movement and MI with subliminal muscle activity?
3) Are the differences between different overt and covert motor actions larger during active contraction or active relaxation?

Defining differences in EEG biomarkers of motor conditions with different levels of central planning and proprioceptive contribution in able-bodied people, could serve as a baseline for defining pathological conditions.

METHODS

Ethical Approval

All participants provided a written informed consent. The study No. 300150025 was approved by the College of Science and Engineering Ethics Committee and was in accordance with the Declaration of Helsinki.

Experimental Setup and Protocol

Eighteen able-bodied participants (age 27.3 ± 6.8 yr, 11 males) right handed in self-reported good health with no known sensory or motor deficits took part in the study.

During the experiment, they were facing a 19 in. computer screen positioned at the eye level ~1 meter away, where the cues to initiate the tasks were displayed. The visual angle of stimuli was 9°. Participants were comfortably seated on a chair, with the dominant leg bent at the knee at ~90° and the foot resting on a force platform. The heel was supported and the foot was restrained with Velcro straps to restrict ankle movements.

For each task, a total of 90 cues, lasting 2.5 s each, were shown. Immediately after the cue appeared, the participant performed plantar flexion, sustaining the isometric contraction for 2.5 s and relaxing when the cue disappeared. A variable inter-trial (resting) time of 3.0–5.5 s was used to avoid preparation for movement due to habituation with fixed time intervals. The tasks were performed in ~2-min long subsessions (5 sessions of 18 trials for each task) which allowed the participants to remain alert and avoid fatigue (Fig. 1*A*).

Torque output, SEMG, ultrasound (US) videos from the gastrocnemius muscle of the dominant side and multichannel EEG were recorded simultaneously (Fig. 1*B* and Fig 2*A*). Initially, baseline measurements were recorded for 120 s when the participant was not performing any movements. Following this, their maximum voluntary contraction (MVC) was measured when pressing on the force platform as strongly as possible 3 times for 5 s. A middle 3 s portion of each trial was extracted and the maximum value out of the three trials was taken for MVC.

During the experimental session, participants performed four different cued motor tasks (MT): executed movements (EM), attempted movements (AM), kinesthetic motor imagery (KI), or visual motor imagery (VI). Different MT were performed in separate sessions and the order of sessions was randomized between participants to avoid the effect of fatigue on any particular type of MT. Before the experiment, participants had several minutes to familiarize themselves with each task, receiving feedback about the exerted force. Participants who performed motor imagery before motor action had a chance to practice real movements before the experiment, to facilitate motor imagery based on motor execution. The experimental protocol is shown in Fig. 1.

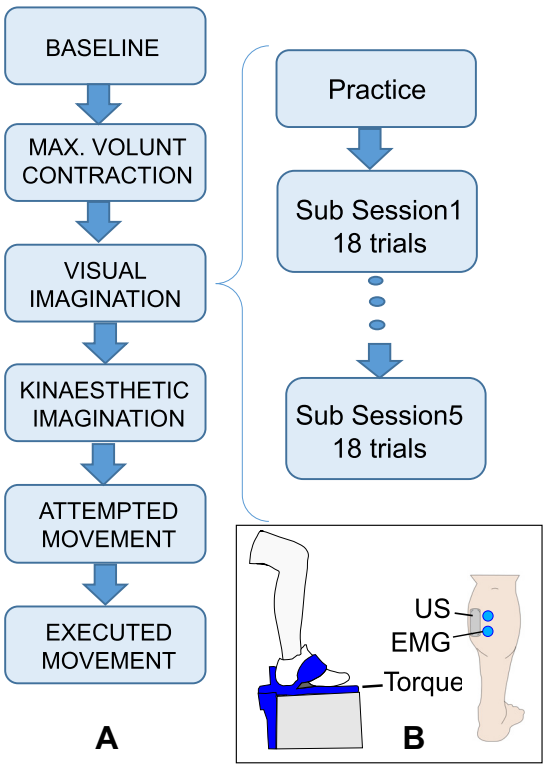

Figure 1. Experimental protocol (*A*), experimental setup (*B*) with EMG, ultrasound, and force platform. US, ultrasound.

During the EM task, participants were asked to aim for a contraction force of 30% of their maximum voluntary contraction. A relatively low levels of muscle activity was selected empirically to avoid fatigue due to lager number of repeated contractions (90 repetitions within 5 subsessions) while getting a clearly visible EMG response. The AM task was defined as a movement with a minimum bodily awareness of performing a physical action during which participants were instructed to initiate the overt action only up to a point when they became aware of exerting force on a plate or contracting the gastrocnemius medialis (GM) muscle without a visual feedback. The main difference between AM and KI was the awareness of motor execution during AM. For KI the participants were instructed to imagine the feeling in their muscles alongside with imagining movement from the first person perspective, while for VI they were instructed to imagine the movement from the first person perspective (though not necessarily looking at their feet) (27).

A Kinesthetic and Visual Motor Imagery Questionnaire (KVIQ) (28) was administered before the experiment to rate participant's kinesthetic and visual motor imagery ability and to explain to them the difference between these two types of MI.

Ultrasound Data Acquisition and Processing

During the experiments, an ultrasound probe (linear array LV7.5/60/96, central frequency of 6 MHz connected to Echoblaster128, Telemed, Lithuania) was positioned over the belly of the GM muscle. It was aligned with the mediolateral midline of the muscle at the level of the mid-belly to minimize errors due to probe orientation. The probe was placed in a custom-made holder and secured with a Velcro strap around the leg to minimize its movement relative to the skin. All recordings were performed in B-mode at an average rate of 40 frames/s (i.e., a frame was recorded every 25 ms) with EchoWave II software (Telemed, Lithuania). No image enhancing settings or compilations were used, as they rely on averaging information over time to create a more stable image and might therefore smooth away subtle muscle movements.

The videos were converted to a compressed AVI file format, which was used for processing with a features tracking method. The videos were cropped to show only the area containing the US image with the GM muscle, then the individual frames were extracted and converted to grayscale values between 0 and 1 in MATLAB (v. 2014a, The MathWorks Inc.). The automated feature tracking method that uses an Active Shape Model (ASM) (29) for image segmentation and the Kanade–Lucas–Tomasi (KLT) algorithm (30) for feature tracking were implemented (5).

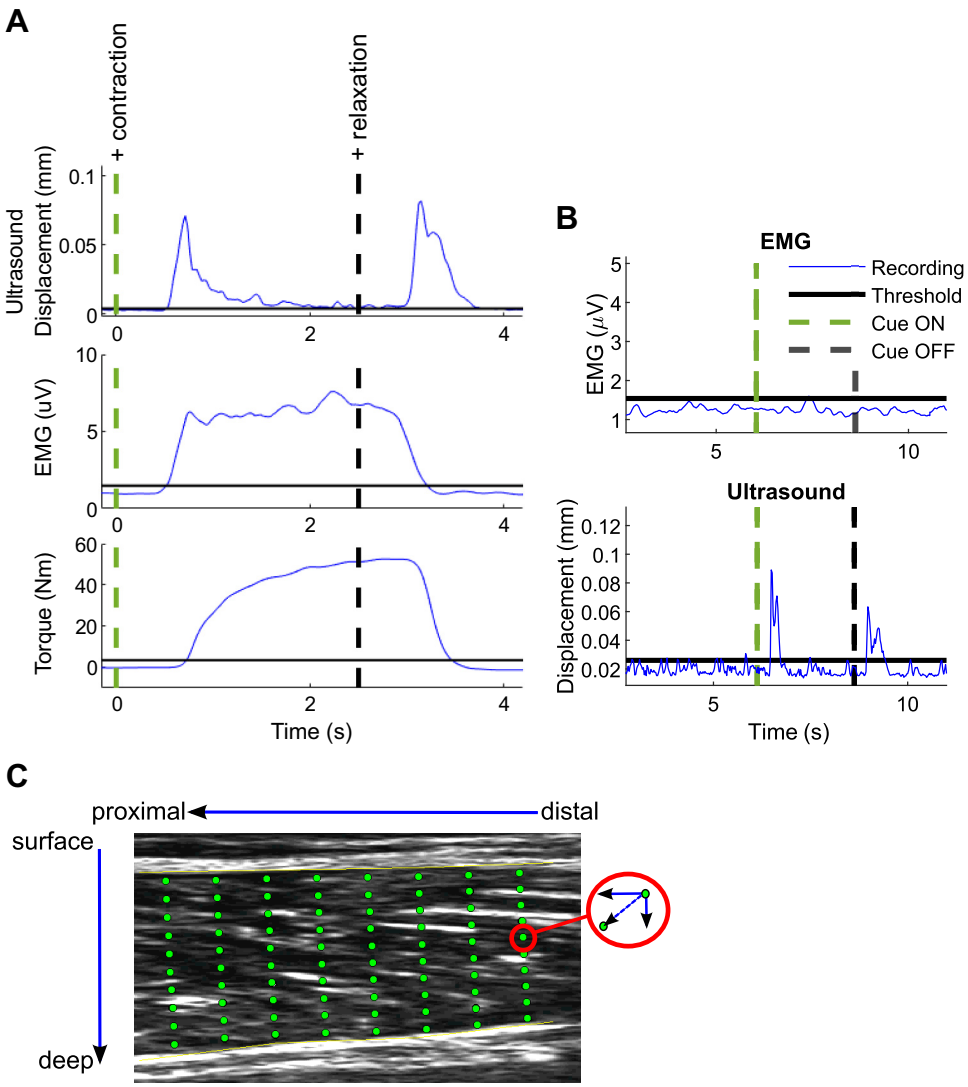

Figure 2. *A*: an example of measurement during one trial in an executed movement subsession. *B*: an example of muscle twitch detected with USI but not by SEMG. *C*: one USI frame with 80 probes presented with green dots. SEMG, surface electromyography; USI, ultrasound imaging.

As the features can appear and disappear in the US image, ghost markers (probes), were placed along the image, to make tracking more reliable and resistant to feature drift. For implementing the ASM, 80 probes (10 rows, 8 columns) were placed in the central part of the gastrocnemius medialis muscle. The probes were placed by averaging a region of KLT points through triangular interpolation between frames of the video. After the segmentation, features were identified by the KLT algorithm within the image segment to enable tracking them over time. As the interest was in the overall changes in the muscle activation, the magnitude of displacement was averaged across all 80 probes to detect instances of muscle activation between consecutive frames (Fig. 2*C*). This enabled plotting the muscle activity against time (Fig. 2, *A* and *B*). For more information about the method see Darby et al. (5).

Muscle activation threshold detection using ultrasound imaging.

The baseline video recorded for 2 min before the experiment was processed to determine the value of displacement of the features when the muscle was relaxed and to establish a threshold for detecting muscle activation. There was always some instability of the signal due to a small pulsation of the USI image of the muscle, phasic activity of the capillaries, and physiological changes in the muscles even when at rest. To account for these, the threshold for muscle activation was defined as a means + 3SD (standard deviations) across the resting phase. This value was selected upon verification that no more than 1.5% of data points during the rest exceeded the threshold (average of 1.29% ± 0.23%).

There were two types of muscle activations, namely a muscle contraction and a muscle twitch. In this study, a muscle contraction is defined as a muscle activation during which clear contraction and relaxation phases of the movement can be distinguished. In USI videos, during muscle contraction, it could be seen that the aponeuroses shear against each other and the pennation angle of muscle fascicles changes. Muscle contraction could also be detected by SEMG and force platform. The muscle activations observed with USI were qualified as contractions if two consecutive peaks exceeding the threshold were registered following the

action execution cue (first peak) and disappearance of the cue (second peak) (see Fig. 2*A*). The first peak appeared due to the muscle contraction resulting in a significant change between the positions of the features. When the muscle was in a state of sustained isometric and isotonic contraction, there was little activity in the image due to the tension being maintained, while relaxing the muscle (returning to the initial stage) results in the second peak. This is in contrast to SEMG and torque which show a plateau during a sustained contraction (Fig. 2*A*).

The muscle twitch is a faster muscle activation when fibers displace and return to the original position very quickly. It is characterized by a smaller displacement of the features in USI, that cannot always be detected by the other two methods. The singular peaks in USI recording, present only following the appearance or disappearance of a cue, corresponded to short lasting muscle fibers activations and were defined as twitches (Fig. 2*B*). Twitches had a distinctive temporal and spatial dynamic that was distinguishable from the artefacts coming from the activity of blood vessels seen in baseline recordings. It was empirically verified with USI recording that this activity was not due to imperfections of the video, such as small pulsations of the image.

To automatically detect muscle activation, the following rules were applied: for the first peak, when the contraction was expected, the algorithm detected the instances when the signal exceeded the threshold and monitored the signal for a subsequent 0.25 s (10 consecutive samples for 40 samples/s), that was a heuristically observed minimum duration of a peak when muscle was contracted (Fig. 3). If within that interval, 70% of the samples stayed above the threshold, then the first detected point was treated as the onset of the contraction. This approach was taken to ensure that the signal exceeding the threshold was not due to random variability of the recording that was seen during the baseline. The value of 70% of samples was based on analyzing weak contractions, because within 0.25 s from the first detection, some samples can still fall below the threshold. Using a higher value would omit detections of actual muscle activity (7.3% of trials) as verified by comparing with USI video frames during a feasibility study (31). When looking for the second peak, corresponding to the relaxation, the continuous interval when the signal stayed below the threshold was identified and then the same condition for peak detection was applied.

If, however, following a peak detection, between 40% and 70% of consecutive 10 samples reached the threshold or the first four consecutive samples reached the threshold, the activity was considered a muscle twitch. Trials with no movement detected in AM and EM or with muscle twitches in EM were discarded. Trials with EM in KI and VI were discarded. The maximum number of trials per person was 90 and with 18 participants this resulted in 1620 trials before removing noisy trials.

The algorithm for detection of muscle contraction and muscle twitch is shown in Fig. 3 and an example of muscle twitch and muscle contraction detected by USI but not by SEMG, is shown in Fig. 2*B*.

SEMG Data Acquisition and Processing

SEMG data were recorded at 1,200 Hz (g.USBamp, g.Tech, GmbH, Austria) using bipolar Ag/AgCl electrodes positioned over the GM muscle, whereas the reference electrode was positioned over the ankle. The SEMG signal was band-pass filtered between 5 and 500 Hz and notch filtered at 50 Hz with a 5th order Butterworth filter within the g.USBamp device, and acquired in Simulink, (MATLAB R2014a, The MathWorks Inc.). The raw SEMG data were full-wave rectified to produce a linear envelope of the original signal. The data were smoothed with a moving average filter over 0.01 s.

For the detection of muscle activation (contraction or twitch), SEMG during a motor task was compared with the baseline signal recorded at the start of the session. SEMG onset of the muscle activation was defined as the point in time when the enveloped SEMG signal exceeded a threshold defined as a means + 2SD of the SEMG signal at rest before a contraction (2, 32).

To detect muscle contraction, it was necessary that the signal remained above the threshold for 1 s, the time stamp of the first data point crossing the threshold was considered

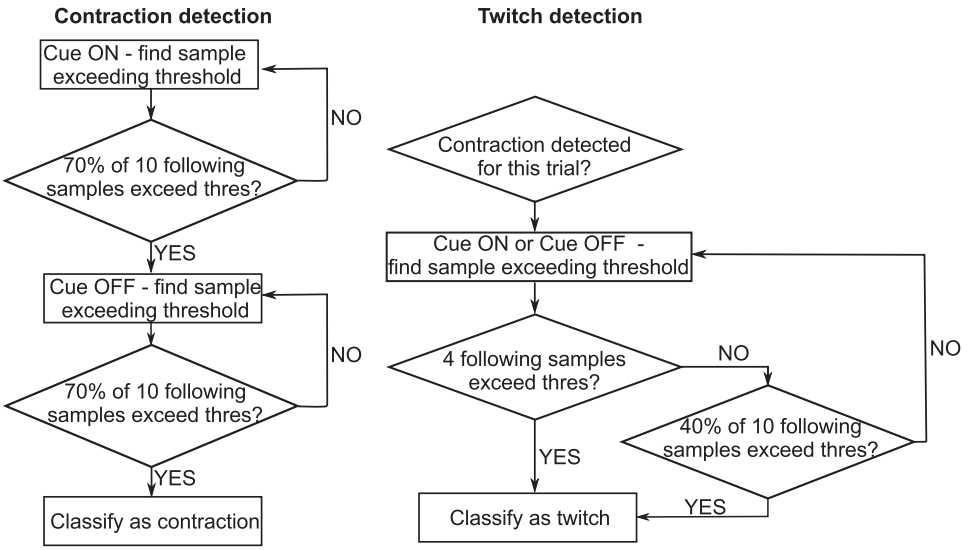

Figure 3. The algorithm for muscle contraction and muscle twitch detection.

as the moment of muscle contraction. A period of 1 s was selected to ensure that a lasting contraction occurred and that any noise from the signal was not falsely interpreted as muscle activation.

For the trials during which the contraction was not detected, the algorithm looked for any other muscle activation, that is, the muscle twitch. If the signal crossed the threshold, the corresponding sample number was registered and the following interval of 0.1 s was examined (similarly to USI analysis). A twitch was registered if within this 0.1 s, 70% of the data points remained above the threshold. This reasoning originated from the fact that since twitches were short-lasting and small in magnitude, not all of the consecutive samples would stay above the threshold, and also taking into account that a twitch can consist of separate muscle bursts. For each trial only the first registered twitch after the appearance of a cue was recorded. The analysis of SEMG detected twitches was performed to compare the sensitivity between SEMG and USI.

Force Platform Data Acquisition, Processing and Signal Synchronization

The ankle torque was recorded with a custom-made force platform at 1,000 Hz (DAQcard-6024E, National Instruments). The data were acquired in Simulink, MATLAB. Before the analysis, torque data were low-pass filtered with a 5th order Butterworth filter and smoothed with a moving average filter over 0.01 s, which is a symmetric and centered filter, thus the phase or timing of the signal was not distorted. The accuracy of the platform was 0.01 N. The torque onset was detected as smoothed torque signal exceeding a threshold defined as the means + 2SD of the torque baseline signal. It followed from the feasibility study (31) and similar approaches employed in the literature (2, 32). The time stamp of the first data point crossing the threshold was considered as the moment of torque onset.

It was predicted that the twitches would most likely not be seen in the torque signal, since such small muscle activations would not result in foot movement and thus torque would not be exerted on the force platform. The purpose of the ankle torque measurement was to teach participants to produce consistent normalized torque during 30% of MVC measured by SEMG for the executed movement and to estimate the average exerted torque during the attempted movements.

A digital output signal from the ultrasound system was used to synchronize data collection between the ultrasound, torque, and SEMG. The minimal time difference observed between USI frames was ~0.025 s for a frame rate of 40 frames/s, whereas it was 0.001 s and 0.00083 s for torque and SEMG recorded at sampling rates of 1 K samples/s and 1.2 samples/s, respectively. Thus, the temporal resolution of muscle activation was limited by the frame rate of the ultrasound imaging. To compare the moment of detection of the muscle activity, it was considered that all data points of SEMG or torque data within 0.025 s (average inter-frame interval of USI recording) corresponded to 1 USI frame and were considered to be detected at the same time. Difference in sampling rates did not affect EEG analysis because EEG across different trials were averaged with respect to the onset of the execution cue, to provide a common reference point for both executed and imagined movements.

EEG Recoding and Preprocessing

The EEG signals were recorded monopolarly at 27 locations according to the international 10/10 system (33) with Ag/AgCl scalp electrodes and using a modular amplifier (g. USBamp, .gTech, Austria). EEG was recorded with sampling frequency of 1,200 samples/s from Fp1, Fp2, F7, F3, Fz, F4, F8, FC1, FCz, FC2, T7, C3, C1, Cz, C2, C4, T8, CP1, CPz, CP2, P7, P3, Pz, P4, P8, O1, O2, and the reference and ground electrodes were located on the earlobes (left and right earlobes, respectively). EEG was resampled to 300 samples/s before the analysis, 1,200 samples/s were initially required because of simultaneous recording with SEMG. A notch filter at 50 Hz was applied and the signal was band-pass filtered between 0.1 and 100 Hz using 5th order infinite impulse response (IIR) Butterworth filter within the g.USBamp device. The recording was performed using Simulink and MATLAB. The impedance of the electrodes was kept below 5kΩ throughout the study.

Trials extraction.

Based on the muscle activity detection performed with USI, the following trials were used for the EEG analysis: *1*) for the EM and AM tasks, only the trials when muscle contraction was detected within 1 s from the experimental cue, rejecting the ones when participants failed to react to the cue, due to performing the movement too late or only muscle twitch appearing; *2*) for the motor imagination tasks involving visual and kinesthetic imagination, the trials were classified depending on whether a muscle twitch was detected with USI within 1 s from the execution cue or the trial was purely imagined without any muscle activation. A period of 1 s was selected to ensure that twitches were associated with a cue rather than with involuntary, random muscle activations. The trials during which real muscle contraction was detected during MI were rejected, as it was considered that in such circumstances the participant failed to follow the instructions. Following this, trials with visual and kinesthetic imagination were combined together due to a smaller number of twitches during imagination compared with muscle contractions during actual movement tasks, to get a MRCP with a clearly visible morphology. In this way, the total number of trials for each analyzed condition were similar. Both visual and kinesthetic imagination resulted in a similar number of twitches (395 for KI and 409 for VI, identified for all 18 participants) detected with USI. Therefore, instead of analyzing separately KI and VI, the motor imagery tasks were grouped based on the presence/absence of twitches.

Throughout this paper four conditions are referred to as: EM (executed movements), AM (attempted movements), IT (imagination tasks when muscle twitches occurred within 1 s from the cue as detected with USI), and I (imagination tasks without any muscle activation).

Because of problems with data acquisition and low quality EEG recording across the majority of channels, data from two participants were rejected. Thus, the EEG analysis presented here refers to 16 able-bodied volunteers. After data cleaning and organizing the trials as described in the

previous paragraphs, for group analysis the datasets for EM, AM, and I conditions included 1,329, 1,270, and 1,261 trials, respectively, whereas IT condition included 485 trials. This significantly lower number of trials in IT task will be addressed in the description of statistical methods below.

The EEG signal was divided into epochs, starting at t = −2 s and finishing at t = 3.5 s (5.5 s long) referring to the appearance of the execution cue (cross with an arrow pointing down) at time t = 0 s. For a real contraction tasks, the movement onset was detected on average at t = 0.51 ± 0.15 s for EM and at t = 0.61 ± 0.18 s for AM, with respect to the cue onset t = 0 s. The movement offset was detected on average at t = 0.44 ± 0.19 s for EM and at t = 0.52 ± 0.28 s for AM with respect to the cue offset at t = 2.5 s.

For the analysis of MRCP in time domain, the epoched signal was high pass filtered at 0.1 Hz with IIR filter (12 dB cutoff frequency) and a band-stop filter was also applied at 48–52 Hz to eliminate the baseline shift generated by DC recordings and to remove any line noise present at 50 Hz frequency. EEG recordings were visually inspected and trials with artifacts due to eye movements, facial muscle activity, or those in which the amplitude exceeded 100 μV across all channels were rejected. On average no more than 9% of trails were removed. Following artefact removals, EEG signals were rereferenced to an average reference and independent component analysis (ICA) decomposition (34) was performed on data that was high pass filtered at 1 Hz for further noise removal. The ICA weights obtained from the decomposition of 1 Hz filtered EEG were applied to 0.1 Hz datasets as well. This was done as recommended by EEGlab, because ICA does not perform well on signal with baseline shifts, due to sweating or similar artefacts (35). After the decomposition, the non-EEG components containing biological or instrumental noise were identified and removed from the datasets filtered at 0.1 Hz and 1 Hz by considering their characteristic morphology, spatial distribution and frequency content. It was necessary to apply ICA for removal of artefacts because many participants were consistently blinking upon cue appearance while preparing for a motor action.

Movement related cortical potentials.

During the analysis of MRCP, the trials were averaged for each condition over the whole group. The focus of MRCP analysis was on Cz electrode as it is located over the motor cortex of legs (25). A surrogate channel C_{zLAP} was computed based on a large Laplacian spatial filter to improve the signal recorded from the local sources:

$$C_{zLAP} = C_z - (F_3 + F_4 + F_z + C_3 + C_4 + P_3 + P_4 + P_z)/8. \quad (1)$$

Different phases of MRCP may be observed in different parts of the brain therefore for further analysis the electrodes were grouped together representing the frontal (F7, F3, Fz, F4, F8), central (C3, C1, Cz, C2, C4), parietal (P7, P3, Pz, P4, P8), and occipital (O1, O2) regions of the scalp to cover premotor and supplementary motor cortex, primary sensorymotor cortex, somatosensory association cortex, and the visual cortex, respectively (36). For this analysis, the EEG signals were low-pass filtered at 10 Hz with a second order Butterworth filter.

The spatial distributions (scalp maps) of the group averaged MRCP amplitudes were found by the interpolation method (inverse distance weighting), which also estimates intensities at locations besides the measured region. The amplitudes and latencies of dominant peaks were determined and compared between conditions.

Statistical Analysis

To assess the differences in cortical potentials between the conditions, the statistical non-parametric bootstrap test (37) was used. The null hypothesis was that for each condition, the MRCPs at a specific location had the same average value at the same temporal location. The statistical significance level was set to P = 0.05. For the statistical comparison of peaks, a height and a latency between conditions in different scalp regions, and maximum values in the identified intervals were compared. For C_{zLAP}, the normality of the data was verified with Shapiro–Wilk test (38) and sphericity assumption was tested with the Mauchly's test (39), therefore one-way analysis of variance (ANOVA) with repeated measures was used. The null hypothesis was that for each condition, the peaks of MRCPs had the same amplitude and latency. The outcomes were considered significant for $P <$ 0.05. If the sphericity assumption was violated, the results with the Greenhouse–Geisser correction were presented. If the statistical difference was found between four conditions, the Bonferroni post-hoc test with Holm–Bonferroni correction for multiple comparisons (40) was performed to compare between each pair.

To test the hypothesis that there was no difference in the number of detected contractions and twitches between USI, SEMG and force platform, and in the number of detected contraction and twitches between different MTs, a post hoc analysis with Wilcoxon signed-rank test was conducted with Bonferroni correction for multiple comparison (EM, AM, VI, KI) and two conditions (twitches and full contractions).

RESULTS

In this section, the analysis of contraction and twitches detected by USI, SEMG, and force platform (FP) data are presented first, followed by the analysis of MRCP during isometric contraction/plantar flexion and relaxation.

Analysis of USI, SEMG and FP Data

During EM, all three methods showed excellent agreement, detecting 99.07% trials as contractions. During AM, 93.15% trials were detected as contractions based on all three methods of measurement, and USI detected additional 1.91% contractions. During the executed movements, muscle twitches were detected during AM (2.35% USI and 0.62% USI and SEMG) and during EM (0.12% USI and SEMG). There was a significant difference between the number of detected contraction between EM and AM (Z = −2.805, P = 0.005), but no significant difference in the number of identified twitches (Z = −2.524, P = 0.012). The average torque during AM was 6.45% ± 7.92% of MVC, that shows that people may exert a considerable muscle activity before becoming aware of performing the movement.

During imagined movements, 35.50% (KI) and 25.92% (VI) trials were detected as contraction by either USI alone or USI

and some other method. These were discarded from the EEG analysis. Further 20.68% and 21.17% of trials were detected as twitches during KI and VI, respectively, only using USI and further 3.70% and 4.01% were registered by both USI and SEMG. In addition twitches were detected in 0.93% of VI and 1.17% of KI trials with SEMG only, indicating that occasionally the position of the USI probe was not adequate to detect a twitch. In a small percentage of motor imagery trials, 1.79% VI and 1.23% KI, torque was detected in the absence of USI or SEMG activity indicating that this was most likely caused by participants shifting their whole body weight towards the force platform. There was no statistically significant difference between the number of full contractions or twitches detected by USI during VI and KI ($Z = -1.894$, $P = 0.058$). Likewise there was no significant difference between the number of twitches detected by USI during VI and KI ($Z = -0.024$, $P = 0.981$), indicating that imagination of muscle contraction during KI on the group level does not necessarily lead to more twitches than visual imagination only. Results of KVIQ-20 questionnaire showed that average score for the VI was 39.9 ± 6.1 and for KI was 34.9 ± 8.2 (max score 50) indicating a comparable average visual and kinesthetic imagery on a group level.

Analysis of Movement-Related Cortical Potential during Active Contraction and Relaxation

For EEG analysis we included both components of the motor task: a sustained plantar flexion followed by an active relaxations. Because the motor task was cue based, visually evoked potentials were superimposed to MRCP within 300–400 ms post cue. Early phase of the contingency negative variation (41) was absent because there was no preparation cue (42). We varied the duration between trials to minimize the anticipation, but the late component of the CNV might be present just before the onset of a cue, overlapping with the readiness potential.

The analysis consist of two approaches: The first one is a traditional MRCP analysis identifying several clearly visible peaks and comparing their peak values and latency between the conditions. In the second approach, we identified different phases of movements as defined in literature (15, 26) averaged MRCPs over each phase and compared these values between motor conditions.

Analysis of dominant MRCP peaks.

During cue based overt or covert dorsiflexion, the following peaks were identified (43): the most pronounced positive peak (P1) located approximately at $t = 0.4$ s post cue, related to movement preparation; negative peak (N1) associated with action execution (motor related potential), located at approximately $t = 0.9$ s; positive overshooting U1, a reafferentation potential of kinesthetic sensory origin also known as movement-monitoring potential; and P2 and N2 following disappearance of a cue, related to the movement preparation and execution of the active relaxation.

Figure 4 shows an example of group MRCP at Cz with a large Laplacian derivation. Overt and covert movements have similar MRCP morphology and timing but different amplitudes, in particular during N1. A bar underneath each plot represents statistically significant differences (bootstrapping, $P = 0.05$). Largest difference between different conditions can be observed around N1, most notably between EM and I, AM and I, and AM and IT. A period of largest difference between IT and I was during P2 following disappearance of a cue and voluntary relaxation. Negativity N2 was smaller than N1 indicating that voluntary relaxation from sustained contraction requires different level of motor cortico-spinal drive (25). Both IT and I conditions do not have a clearly visible U1 component.

Figure 5 shows a group average MRCP (ear reference) over each single electrode location for all four types of movement. The overall morphology depended on the electrode location. Active contraction and relaxation had a similar amplitude of P1 and P2 in the central and frontal region. P2 decreased in the parietal regions as compared to P1 and was absent in the occipital region. N2 was smaller than N1 in the frontal and central regions and was absent in the parietal and central regions.

To analyze the difference between conditions, we averaged electrodes across the frontal, central, parietal, and occipital area averaging over the following electrode locations: frontal (F7, F3, Fz, F4, and F8), central (C3, C1, Cz, C2, and C4), parietal (P7, P3, Pz, P4, and P8) and occipital (O1 and O2). This approach is supported by the fact that MRCP during foot contraction and relaxation shows no lateralization

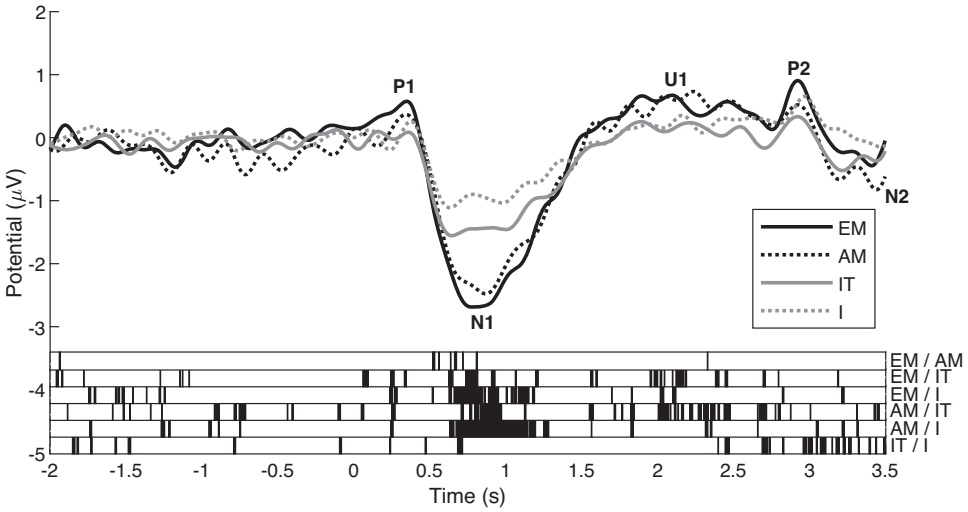

Figure 4. Movement related cortical potentials averaged between the participants at Cz with Laplacian derivation (Cz_{LAP}). The black bars under the plots indicate statistically significant difference (bootstrap test, $P < 0.05$); $n = 16$ (10 males, 6 females). AM, attempted movements; EM, executed movements; I, imagination tasks without detectable muscle twitches; IT, imagination tasks without detectable muscle twitches.

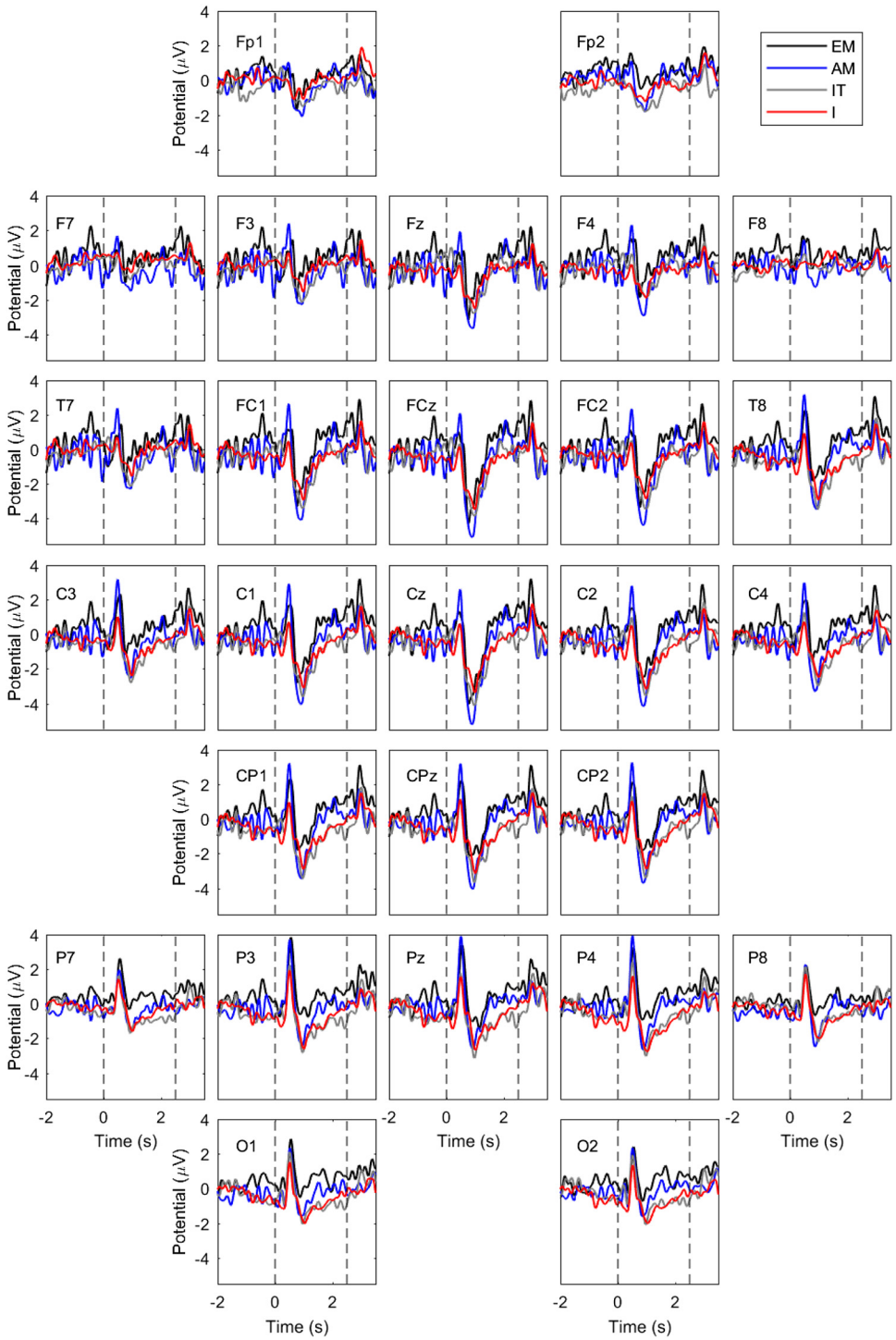

Figure 5. Movement related cortical potentials averaged between the participants for each single electrode locations. Cue appears at $t = 0$ s and disappears at $t = 2.5$ s. $n = 16$ (10 males, 6 females). AM, attempted movements; EM, executed movements; I, motor imagination tasks without detectable muscle twitches; IT, imagination tasks without detectable muscle twitches.

(25) and may be averaged over the left and right hemisphere. We analyzed peak values and latencies of three clearly visible peaks, P1, N1, and P2 (Table 1).

The comparison between latencies and heights of the peaks showed a significant difference in the central region for the amplitude of N1 peak ($F(3, 45) = 4.498$, $P = 0.008$) and after applying Bonferroni correction, a significant difference was found between EM and I conditions ($P = 0.049$). The latency of N1 peak was also significantly different between four conditions ($F(3, 45) = 3.977$, $P = 0.013$), whereas post hoc test showed a statistical difference in latency specifically between EM and IT conditions ($P = 0.03$).

Another difference was recorded in the parietal region for the amplitude of the first positive peak P1 ($F(3, 45) = 9.122$, $P < 0.001$). Post hoc tests with Bonferroni corrections showed differences between the following pairs of conditions: EM and AM ($P = 0.022$), AM and I ($P < 0.001$), and IT and I ($P = 0.532$).

Table 1. *Results of one-way repeated measure ANOVA across four experimental conditions (EM, AM, IT, and I) when analyzing visible peaks P1, N1, and P2 across different scalp regions*

Area	Peak Amplitude (μV)		Latency (ms)	
	Test Results	*P* Value	Test Results	*P* Value
Frontal				
P1	$F(3,45) = 1.147$	0.341	$F(3,45) = 2.380$	0.082
N1	$F(3,45) = 0.786$	0.508	$F(3,45) = 1.009$	0.398
P2	$F(3,45) = 1.743$	0.172	$F(3,45) = 0.492$	0.690
Central				
P1	$F(3,45) = 1.106$	0.357	$F(3,45) = 2.389$	0.081
N1	$F(3,45) = 4.498$	0.008*	$F(3,45) = 3.997$	0.013*
P2	$F(3,45) = 0.967$	0.417	$F(3,45) = 0.591$	0.624
Parietal				
P1	$F(3,45) = 9.122$	0.000*	$F(3,45) = 0.354$	0.786
N1	$F(3,45) = 0.810$	0.496	$F(3,45) = 0.834$	0.482
P2	$F(3,45) = 1.247$	0.412	$F(3,45) = 1.020$	0.393
Occipital				
P1	$F(3,45) = 0.600$	0.618	$F(3,45) = 0.400$	0.754
N1	$F(3,45) = 1.522$	0.222	$F(3,45) = 0.573$	0.636
P2	$F(3,45) = 2.214$	0.100	$F(3,45) = 1.637$	0.194

Results with an asterisk (*) indicate the significant differences between the conditions. AM, attempted movements; EM, executed movements; I, imagination tasks without detectable muscle twitches; IT, imagination tasks without detectable muscle twitches.

Analysis of different phases of MRCP.

The experimental paradigm in this study was cue based, with the type of movement known in advance. Unlike tasks resulting in a Contingent Negative Variation (CNV) as defined by Walter et al. (41), our tasks had only one cue, rather than a warning and an execution cue. Because the task was known in advance, it also had some similarities with MRCP as defined by Deecke et al. (26) but was not purely defined by free will. From that reason the task was neither MRCP but also not a typical CNV. We found the phases most similar to phases defined for MRCP (26) with main difference related to the readiness potential before the onset of a cue. The following intervals were identified to examine cortical potentials related to movement preparation, execution, and control (15, 26), as shown in Fig. 6: *1*) readiness potential combined with the late phase of the cognitive negative variation (CNV1) measured in the interval from 0.4 s to 0.0 s before movement onset; *2*) visual potential (VP1) corresponding to the reaction to cue appearing on the screen and measured as peak positivity

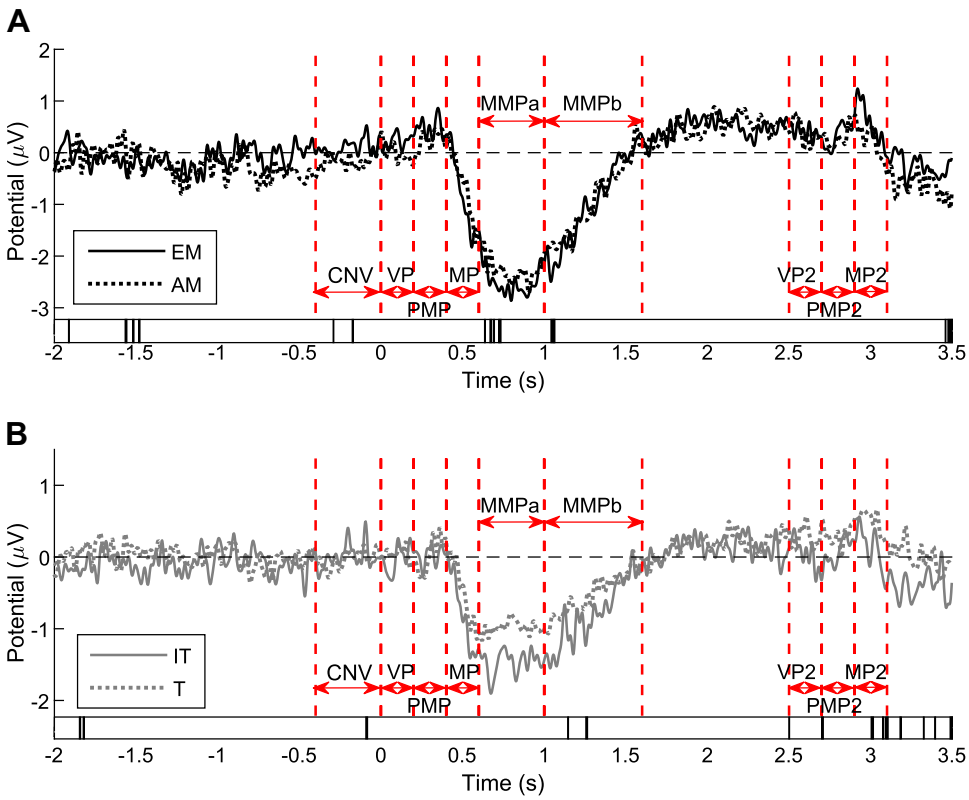

Figure 6. Movement related cortical potential (MRCP) averaged across all trials for Laplacian derivation at Cz, showing different phases for EM and AM (*A*) and IT and I (*B*). $n = 16$ (10 males, 6 females). AM, attempted movements; CNV, cognitive negative variation; EM, executed movements, I, imagination tasks without any muscle activation; IT, imagination tasks when muscle twitches occurred within 1 s from the cue as detected with ultrasound imaging; MP, motor potential; MMP, movement monitoring potential; PMP, premotor positivity; VP, visual potential.

from t = 0 s to 0.2 s; *3*) premotor positivity (PMP1) which is a small voltage difference preceding the movement at time 0.2–0.4 s; *4*) motor potential (MP1) indicating action execution around movement onset between 0.4 and 0.6 s; *5*) movement monitoring potential (MMP1), following movement execution and entering the hold phase of the contraction or continuing imagination divided into two phases: MMP1a from 0.6 s to 1.0 s, where peak negativity N1 is located and MMP1b from 1.0 s to 1.6 s in the area of reafferentation potential U1.

For relaxation following a disappearance of the cue at 2.5 s four intervals were analyzed: *1*) readiness potential combined with CNV2 proceeding a disappearance of the cue from 2.1 to 2.5 s, *2*) visual potential (VP2) corresponding to the reaction to cue disappearing between 2.5 and 2.7 s, *3*) a peak premotor positivity (PMP2) at time 2.7–2.9 s; and *4*) motor potential (MP2) indicating action execution of relaxing the muscle and finishing the imagination between 2.9 and 3.1 s considering maximum negativity.

Scalp map analysis (Fig. 7) was obtained by averaging MRCP over predefined time windows, related to the MRCP morphology. Based on Fig. 6, statistically significant differences were calculated between groups (Fig. 8).

During contraction preparation period in CNV1, VP1, and PMP1 phases, the potentials were relatively similar among the conditions with slightly stronger positivity for EM condition during CNV1 over the frontocentral region and stronger negativity in occipitoparietal region for imagination without muscle twitch (I) during VP1 and PMP1 phases. PMP1 was largest for AM in the frontal area. During a motor execution, all four tasks showed the areas of positive potential in MP1, being wide spread for EM and located towards the parieto-occipital area for IT and I, notably smaller during I. Likewise all four tasks showed a negative, wide spread, potential during reaffrentation phase in MMP1a and MMP1b but notably smallest during MMP1b for ME.

During active relaxation during both preparation and execution of movement, topography was similar like during contraction.

A comparison between tasks (Fig. 8) over different cortical regions shown in Fig. 7 demonstrates that during the preparation for movement (CNV1, *top*) there was no statistically significant difference between EM and AM but frontal areas showed differences between AM and IM, as well as IM and I, due to positive potential in IM condition as opposed to AM and I. Similar differences were found during VP1 that could also be observed in scalp maps in Fig. 7. A PMP1 period is characterized by significant differences between all compared tasks. Notably, this is a phase with largest differences between EM and AM, mostly in the left frontocentral cortex, due to larger positivity for AM. Largest differences between AM and IT could be observed on the dominant, left hemisphere while largest difference between I and IT could be noticed over the right nondominant hemisphere, wide spread across frontal, central and parietal areas. These results indicate that there was a significant difference in the level of motor planning between all four MT, suggesting that IT is a task separate from both AM and I.

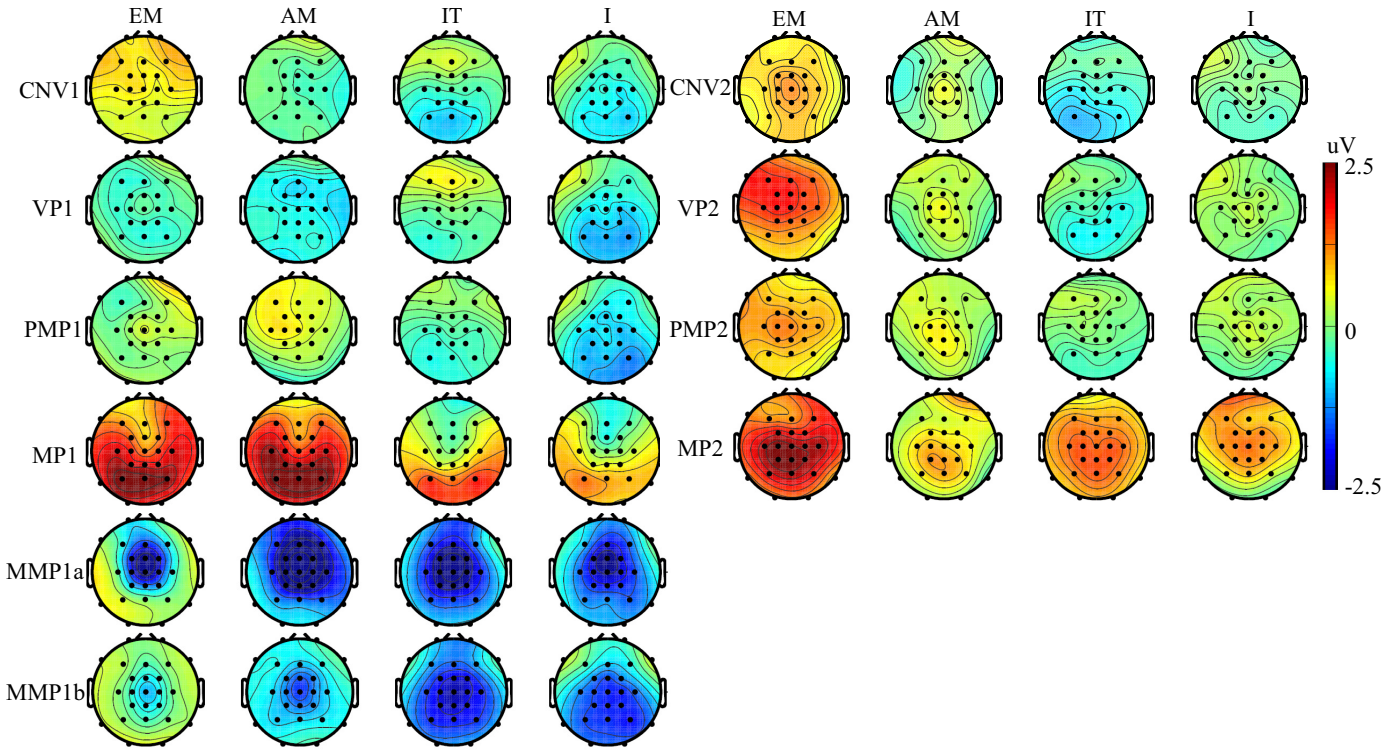

Figure 7. Scalp maps of all four conditions for different phases of MRCP. Labels with 1 are related to active contraction whereas labels with 2 are related to active relaxation. n = 16 (10 males, 6 females). AM, attempted movements; CNV, cognitive negative variation; EM, executed movements, I, imagination tasks without any muscle activation; IT, imagination tasks when muscle twitches occurred within 1 s from the cue as detected with ultrasound imaging; MP, motor potential; MMP1a and MMP1b, early and late phases, respectively, of movement monitoring potential during active contraction; PMP, premotor positivity; VP, visual potential.

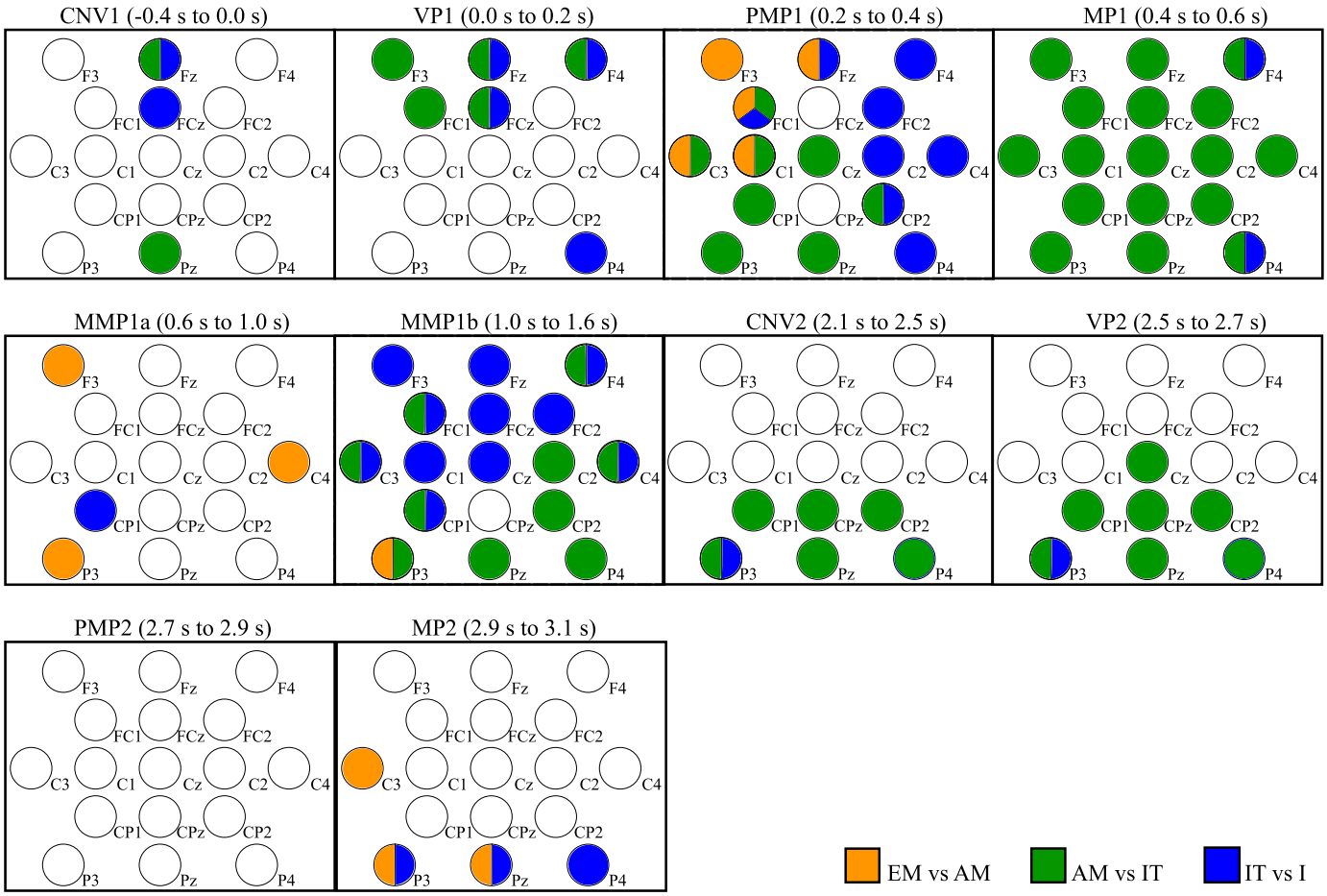

Figure 8. Electrode locations showing statistically significant differences in MRCP between conditions (bootstrap test, $P = 0.05$, after correction for multiple comparisons). Labels with 1 present active contraction while labels with 2 present active relaxation. $n = 16$ (10 males, 6 females). AM, attempted movements; CNV, cognitive negative variation; EM, executed movements, I, imagination tasks without any muscle activation; IT, imagination tasks when muscle twitches occurred within 1 s from the cue as detected with ultrasound imaging; MP, motor potential; MMP1a and MMP1b, early and late phases, respectively, of movement monitoring potential during active contraction; PMP, premotor positivity; VP, visual potential.

During MP1, there was no difference between the AM and EM and there were a small differences between IT and I tasks. However, differences between IT and AM were widespread over all electrode locations.

According to the scalp maps in Fig. 6, during MMP1a there was no difference between AM and IT and statistically significant differences between AM and EM were found on only a few electrodes, as well as between IT and I. This indicates that the MMP1a phase was very similar for all tasks. Although U1 is not clearly visible in Fig. 3 for IT and I, statistical analysis showed significant differences between IT and I tasks in frontocentral area related to the movement monitoring potential, MMP1b. MMP1b was also characterized by wide spread differences between AM and IT, and between AM and EM in the left parietal area.

During RP2 and VP2 of active relaxation, there were significant differences between AM and IT in a large portion of the centroparietal area. In the next phase, PMP2 (active planning of relaxation) there were no differences between the tasks. This was in a stark contrast to PMP1 during active contraction that showed differences between all motor conditions. Periods PMP1 and PMP2 correspond to peaks P1 and P2 in Fig. 4. During MP2, differences between AM and EM could be observed in the central and parietal regions, and between IT and I in the parietal region. This was also in contrast to MP1 that dominantly showed widespread differences between AM and IT.

DISCUSSION

The study showed that EM, AM, IT and I are distinguishable motor conditions with statistically significant differences in MRCP during both isometric contractions and active relaxations.

We used USI to detect both large and subliminal movements across both surface and deeper layers of gastrocnemius muscles. We showed that more than 20% of MI trials, were accompanied by muscle twitches. Motor imagery with twitches is a condition between pure mental simulation of action and attempted movements. The presence of muscle activity might explain why some previous studies found an increase in muscle strength following prolonged motor imagery practice (9–11).

Isometric Contraction

During isometric contraction, MRCP of executed (EM, AM) and imagined (IT, I) movements showed distinct spatial

topography. A spatially localized activity, in particular during EM, indicates less neural resources to accomplish the task as compared to other three tasks.

Attempted movement is a concept used mostly in rehabilitation, for people who cannot physically perform a movement with normal range and strength after, for example, spinal cord injury (44). Although studies on attempted movement in healthy individuals are rare, they could be compared with MRCP during new skill learning. AM requires more effort due to the higher level of control than EM, and therefore resulted in wider spread MRCP. Similar wide spread MRCP also existed during IT and I, indicating increased effort. This could be attributed to the fact that all participants were novices to AM, IT and I tasks. A noteworthy observation is that while the amplitude of the MRCP motor execution peak is proportional to force (15), the mental effort due to the novelty of the task did not affect the amplitude of MRCP, but resulted in a wider spread of MRCP outwit the central cortex.

The analysis of MRCP further revealed that, IT and I showed different levels of activation in all phases of movements, indicating differences in both central and peripheral components. It is believed that the amplitude of MRCP during movement execution is proportional to the number of motor units recruited (45), thus higher amplitude MRCP is to be expected during IT than during I. The sensory feedback was also stronger during IT due to subliminal activation of muscles, that resulted in higher reafferenation phase. In paralyzed people, the amplitude of MRCP during imagined movement is smaller than in able-bodied people (44); the difference might be partially contributed to subliminal twitches in able-bodied people.

The practical implication of this finding, for MRCP based BCI, is that even though no EMG can be recorded in able-bodied people during motor imagination, their MRCP will be different than in paralyzed patients for whom BCI might be developed. Sonsowska (31) also found differences between IT and I in the oscillatory brain activity i.e., in event related synchronization/desynchronization implying that BCI based on these phenomena are also affected by muscle twitching that escapes pure imagination.

Active Relaxation

It is believed that during the relaxation phase of a muscle, excitability of the corticospinal tract controlling that particular muscle is more suppressed than in the resting condition (46). In addition, muscle relaxation of one body part suppresses cortical activities controlling other body parts. Impairment of muscle relaxation is involved in a wide spectrum of movement disorders such as myotonic dystrophy, Parkinson's disease, dystonia, and stroke. This is typically reflected in reduced MRCP, as compared to able-bodied people (23).

In this study, MRCP had a similar morphology during the isometric contraction and active relaxation (25). Active relaxation dominantly involves activation of the primary and premotor cortex, which might explain more localized activity as compared to the active contraction.

Recent MRCP study (24) showed that active foot relaxation following a movement with lower force produces less Bereitschaft Potential (and less lateral inhibition) before active relaxation. A statistically significant difference between IT and I indicates a significant difference in the cortical activity when even the smallest amount of efferent activity reaches the muscles during active relaxation.

It should be noted here that not every IT trial had an identifiable muscle twitch in GM muscle during active relaxation. It is however possible that synergistic muscles were also involved in the relaxation (27) and that they produced twitches during active relaxation that was reflected on MRCP.

Limitations of the Study

Although kinesthetic and visual imagery were separated in different trials, due to the small number of trials with twitches, they were analyzed together. It is believed that kinesthetic and visual imagery activate different areas of the brain, with kinesthetic imagery activating more motor associated areas while visual imagery activating more occipital areas (27). Studies based on oscillatory brain activity showed that kinesthetic imagery produces activity closer to the sensory-motor area than visual motor imagery (47). In addition, during evoked motor responses, frontal areas are more active in the case of visual motor imagery (48). However we showed that a percentage of trials with twitches were similar in both types of MI although only kinesthetic imagery is supposed to involve stronger imagination of muscle contraction, leading to muscle twitches (27). This was despite training the participants to distinguish these two types of motor imagery while taking part in KVIQ questionnaire. For that reason, we believe that at least for trials with twitches, both types of imagination in this study are similar.

The other limitation of the study was that we measured the activity in GM muscle only to separate imagined movement based on the presence of twitches during active contraction. Importantly, we did not measure the activity in other muscles involved in the movement, which might have produced twitches during active relaxation, but previous studies have shown that imagined movements that result in subliminal SEMG during MI involve activation of all muscles which would be active during the executed movements (27), and thus the resultant cortical activity would reflect efferent and afferent contribution to and from all these muscles.

CONCLUSIONS

This study shows that EM, AM, IT, and I present different motor conditions with distinguishable MRCP. Movements with subliminal afferent components due to muscle twitches produced significantly different cortical responses as compared to movement imagination without muscle activation. Similar to active relaxation, active contraction showed distinguishable cortical activity between all four conditions. Results of this study could be used toward creating EEG diagnostic biomarkers of neurological conditions affecting both central and peripheral components of movements.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

H.G. and A.V. conceived and designed research; A.S. performed experiments; A.S. and A.V. analyzed data; A.S. and A.V. interpreted results of experiments; A.S. prepared figures; A.V. drafted manuscript; A.S. and H.G. edited and revised manuscript; A.S., H.G. and A.V. approved final version of manuscript.

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