Effects of pulse width, waveform and current direction in the cortex:

a combined cTMS-EEG study

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Abstract

Background: the influence of pulse width, pulse waveform and current direction on transcranial magnetic stimulation (TMS) outcomes is of critical importance. However, their effects have only been investigated indirectly with motor-evoked potentials (MEP). By combining TMS and EEG it is possible to examine how these factors affect evoked activity from the cortex and compare that with the effects on MEP.

Objective: we used a new controllable TMS device (cTMS) to vary systematically pulse width, pulse waveform and current direction and explore their effects on global and local TMS-evoked EEG response.

Methods: In 19 healthy volunteers we measured (1) resting motor threshold (RMT) as an estimate of corticospinal excitability; (2) global mean field power (GMFP) as an estimate of global cortical excitability; and (3) local mean field power (LMFP) as an estimate of local cortical excitability.

Results: RMT was lower with monophasic posterior-to-anterior (PA) pulses that have a longer pulse width ($p<0.001$). After adjusting for the individual motor threshold of each pulse type we found that (a) GMFP was higher with monophasic pulses ($p<0.001$); (b) LMFP was higher with longer pulse width ($p=0.015$); (c) early TEP polarity was modulated depending on the current direction ($p=0.01$).

Conclusions: Despite normalizing stimulus intensity to RMT, we found that local and global responses to TMS vary depending on pulse parameters. Since EEG responses can vary independently of the MEP, titrating parameters of TMS in relation to MEP threshold is not a useful way of ensuring that a constant set of neurons is activated within a cortical area.

Keywords: TMS, EEG, cTMS, pulse width, pulse waveform, motor cortex

Abbreviations: TMS, transcranial magnetic stimulation; EEG, electroencephalography; M1, primary motor cortex; GMFP, global mean field power; LMFP, local mean field power; RMT, resting motor threshold; MSO, maximum stimulator output; MEP, motor-evoked potential; TEP, TMS-evoked potential; EMG, electromyography; FDI, first dorsal interosseous; ICA independent component analysis

Introduction

Activity in several different populations of cortical neurons contribute to the MEP after TMS over the motor hand area. Some neurons are involved in producing early I-wave inputs to corticospinal neurons whereas others appear to contribute to late I-waves. Inhibitory neurons are also activated in addition to these excitatory inputs. Each set of neurons appears to be preferentially sensitive to particular characteristics of the TMS pulse, such as the initial direction of induced current in the brain (posterior-anterior, PA; anterior-posterior, AP), the induced electric field waveform (monophasic or biphasic) or the pulse width (Figure 1). Early I-wave inputs are better activated by a monophasic pulse in the PA direction whereas late I-wave inputs are more sensitive to AP pulses (Di Lazzaro et al., 2001). Very low intensities of stimulation activate inhibitory neurons whereas narrow pulse widths tend to favor excitation of late I-wave inputs (D'Ostilio et al. 2016; Hannah & Rothwell, 2017). Biphasic pulses evoke a more complex sequence of inputs than monophasic pulses and this mixture of effects can be less effective than monophasic stimulation in some rTMS paradigms (Sommer et al., 2018).

The MEP only samples a fraction of the total activity evoked in motor cortex by TMS. Indeed we know that TMS activates not only corticospinal outputs from M1, but also transcallosal connections as well as outputs to thalamus, basal ganglia and cerebellum. The newly developed technique of TMS-EEG potentially allows us to sample a wider fraction of M1 activity than MEP methods. The aim of the present experiments was to test the effect of different TMS pulse parameters on evoked EEG activity. Note that the comparison of monophasic and biphasic pulse shapes (which refers to the relative asymmetry and symmetry in the amplitude of the first and second phases of the electric field waveforms, respectively), and their interaction with current direction, is complicated by the fact the biphasic pulse produced by most machines is a cosine waveform in which stimulation is thought to be dominated by the reverse phase, which is twice the duration of the first phase. To circumvent these problems, the present experiments combined electroencephalography (EEG) with a new controllable pulse parameter TMS device (cTMS) able to control pulse width and waveform.

The intensity of TMS pulses of different shape, pulse width and polarity was normalized to the individual MEP threshold, and then EEG activity was evoked by pulses at 90% of this threshold intensity. Even though the intensity was equivalent in MEP terms, the evoked EEG activity was quite different, suggesting that normalization of stimulation parameters to MEP threshold may not be an appropriate way to ensure similar patterns of brain activation.

Methods

Participants and procedure

Nineteen healthy volunteers (six females, mean age 29±5 years) were enrolled in the study after giving written informed consent. All participants were tested for TMS exclusion criteria (Rossi et al., 2009). The experimental procedure was approved by the local ethics committee and was in accordance with the Declaration of Helsinki (Sixth revision, 2008). Each participant underwent four blocks of cTMS and one block of standard monophasic TMS (Magstim 200^2, see details below), all consisting of 70 TMS pulses delivered over the left primary motor cortex (M1) during recording of multichannel EEG and electromyography from the contralateral first dorsal interosseous (FDI) muscle. Among the five blocks, we modified three parameters of stimulation: (1) current direction (posterior-anterior, PA or anterior-posterior, AP); (2) M-ratio (i.e. the amplitude ratio of the second phase relative to the first phase of the pulse; monophasic, 0.1 or biphasic, 0.5) and pulse width (30 or 80 μs), so that the five blocks were termed PA$_{mono\_30}$, PA$_{bi\_30}$, AP$_{mono\_30}$, AP$_{bi\_30}$ and PA$_{mono\_80}$ (figure 1). The order of the five blocks was counterbalanced across the participants. During the stimulation participants were seated on a comfortable armchair and wore earplugs which continuously played an *ad-hoc* white noise to mask the TMS click (Massimini et al., 2005).

Transcranial magnetic stimulation and Electromyography

Standard TMS was carried out with a Magstim 200^2 device connected to a 70-mm figure-of-eight coil (Magstim Company Limited, Whitland, UK), which produces monophasic pulses with a pulse width of ~80 μs. For cTMS, we used a prototype device (cTMS-3, Rogue Research Inc., Montreal, Canada) able to produce both monophasic and biphasic pulses with independent control of pulse width and ratio between the pulse phases (Peterchev et al., 2008; 2014). The cTMS was connected to a standard 70-mm figure-of-eight coil (Magstim Company Limited, Whitland, UK) and was controlled with an NI PCI-7831R control/acquisition board. The maximum capacitor voltage of the cTMS was 1984 V, corresponding to maximum pulse amplitude, whereas for the standard Magstim 200^2 was 2800 V (Peterchev et al., 2008). For the cTMS, we used a pulse width of 30 μs (D'Ostilio et al. 2016; Hannah & Rothwell, 2017) and two M-ratio values (0.1 and 0.5) to produce monophasic and more biphasic pulses, respectively. The M-ratio refers to the amplitude ratio of the second phase of the pulse relative to the first. The current was delivered in a PA direction (i.e. with the coil handle pointing backward with respect to the focus of stimulation) for standard monophasic TMS (PA$_{mono\_80}$) and two cTMS blocks (PA$_{mono\_30}$ and PA$_{bi\_30}$) and in an AP direction (i.e. with the coil handle pointing forward) for the other two cTMS blocks (AP$_{mono\_30}$ and AP$_{bi\_30}$). For M1 stimulation, the coil was positioned tangentially to the scalp at an angle of about 45° to the sagittal plane over the hand motor area of the

left M1, defined as the point where stimulation evoked the largest MEP from the contralateral FDI muscle. Stimulus intensity was set to 90% of resting motor threshold (RMT; Rossini et al., 1994). TMS pulses were delivered at an inter-stimulus interval (ISI) of 4-6 seconds. During TMS participants wore in-ear plugs which continuously played a white noise that reproduced the specific time-varying frequencies of the TMS click, in order to mask the click and minimize possible auditory ERP responses. Surface EMG was acquired from the right FDI muscle via Ag/AgCl electrodes in a belly-tendon montage using a Digitimer D360 Amplifier (Digitimer Ltd, Welwyn Garden City, UK); raw signal was sampled at 5000 Hz and band-pass filtered between 5 and 2000 Hz. EMG was continuously monitored during the entire TMS-EEG session to ensure that no muscle activity was evoked.

Somatosensory threshold

To exclude possible contamination by somatosensory evoked potentials (SEPs) evoked by the sensation resulting from TMS, we measured the somatosensory threshold (SST) due to TMS applied over M1 in a subsample of eight participants, in the five conditions of stimulation. SST was defined as the lowest TMS intensity necessary to induce a somatosensory sensation in three consecutive trials. For each pulse the participant had to report if s/he could feel the sensation. During stimulation the participant wore in-ear plugs and listened to the same white noise used for the experiment. This was done to ensure that the ratio between SST and RMT was similar for all the stimulation conditions (AP_{mono_30}=2.2; PA_{mono_30}= 1.9; AP_{bi_30}=2.14; PA_{bi_30}=1.89; PA_{mono_80}=1.89; Table 2). Since SEP intensity is usually calibrated using SST (IFCN guidelines, Nuwer et al., 1999) we can be confident that SEP amplitude was the same across the different conditions.

Electroencephalography

EEG was acquired using a TMS-compatible EEG DC amplifier (ANT, Enschede, Netherlands). The amplifier was optically connected to a PC for online EEG monitoring, and to a 32-channels EEG cap. EEG was continuously recorded from 30 TMS-compatible Ag/AgCl pellet electrodes. Electrode-skin impedance was kept below 5 kΩ. Recordings were referenced to the linked mastoids; the ground electrode was placed on POz. Hardware filter settings included a low pass FIR with a 1000 Hz cutoff frequency. Sampling frequency was 2048 Hz.

In order to remove the TMS artifact, we first removed and then replaced data using a cubic interpolation from 5 ms before to 20 ms after the TMS pulse from each trial. The continuous EEG was then re-referenced to the average signal of all the electrodes and band-pass filtered between 1 and 80 Hz (Butterworth zero phase filters). A 50 Hz notch filter was applied to reduce noise from electrical sources. Artifact removal was performed by using independent component analysis

(INFOMAX ICA) applied to the continuous EEG signal. Artifact-related components (e.g. eye blinks, muscle activity, residual TMS artifact) were identified following the criteria published by previous studies (Rogasch et al., 2016; Casula et al., 2017a). Importantly, the same ICA components were removed for each dataset to avoid any confounding due to the ICA procedures. The signal was then segmented into 70 epochs starting 1 s before the TMS pulse and ending 1 s after it. Afterwards, all the epochs were visually inspected and those with excessively noisy EEG were excluded from the analysis (less than 3% for each participant).To assess the TMS-evoked global cortical response, the global mean field power (GMFP) was computed as:

$$GMFP(t) = \sqrt{\frac{[\sum_i^k (V_i(t) - V_{mean}(t))^2]}{K}}$$

Where t is time, K the number of channels, V the voltage in channel i averaged across patients and V_mean is the mean of the voltage in all the channels (Lehmann and Skrandies, 1980). To assess the TMS-induced local cortical activation, we analyzed the local mean field power (LMFP) and TEP peaks over a cluster of electrodes surrounding M1. LMFP was computed in the same way as GMFP but only considering the three electrodes surrounding the site of stimulation, i.e. C3, FC1 and CP1. Both GMFP and LMFP were calculated in four time windows: 20-50 ms (T1); 50-100 ms (T2); 100-150 ms (T3); 150-250 ms (T4). TEP peaks were computed as the highest (positive peaks) and the lowest values (negative peaks) within the first two time windows (T1 and T2) over the electrodes where the TMS-evoked dipole was prominent (F3, FC1, C3, CP1, P3).

Statistical analysis

We first explored the effects of current direction (PA *vs.* AP) and M-ratio (0.1 *vs* 0.5) by comparing the RMT, the GMFP and the LMFP among the four cTMS blocks with the same pulse width (30 μs). To this end, we used two 3-way ANOVAs with factors "direction" (PA, AP), "waveform" (monophasic, biphasic) and "time window" (T1, T2, T3, T4) for GMFP and LMFP; a 2-way ANOVA with factors "direction" and "waveform" was used to investigate changes in RMT and SST/RMT ratio. To explore the effects of stimulation parameters on TEP polarity (see results section 3.2), we further conducted a 5-way ANOVA with factors "electrode" (F3, FC1, C3, CP1, P3), "direction", "waveform", "peak" (1, 2) and "polarity" (positive, negative).

To explore the effects of pulse width, we compared the standard monophasic TMS (i.e. PA_mono_80), which has a pulse width of ~80 μs, with the corresponding cTMS condition (i.e. PA_mono_30). To this aim, we conducted a paired t-tests to compare RMT and a two-way repeated measures ANOVA with factors "pulse width" (30 *vs.* 80 μs) and "time window" (T1, T2, T3, T4) to compare GMFP and LMFP. Sphericity of data was tested with Mauchly's test; when sphericity was violated (i.e.

Mauchly's test < 0.05) the Huyhn-Feldt correction was used. Pairwise comparisons were corrected using the Bonferroni method.

Results

Corticospinal excitability

Figure 2A depicts the mean RMT values for each condition. ANOVA conducted on the RMT values showed a significant main effect of waveform [$F_{(1,18)}$=19.193; p<0.001; ε=.516] and direction [$F_{(1,18)}$=24.670; p<0.001; ε=.578]. Post-hoc analysis revealed that RMT was significantly lower with monophasic pulses compared to biphasic (65±2 vs. 70±2 %; p <0.001; figure 2B) and with PA direction compared to AP (62±2.68 vs. 72±1.55 %; p <0.001; figure 2C). When RMT was compared between PA$_{mono\_80}$ and PA$_{mono\_30}$ to explore the effect of pulse width, the ANOVA revealed a significant effect of pulse width [$F_{(1,18)}$=26.633; p<0.001; ε=.597], with post-hoc analysis showing that RMT was significantly lower with a pulse width of 80 μs compared to 30 μs (51±1 vs. 59±2 %; p <0.001; figure 2D).

Cortical excitability

Single-pulse cTMS and standard TMS pulses evoked a well-known sequence of positive and negative deflections with amplitude ranging from -3 to 3 μV and lasting up to ~250 ms (figure 3). Four main GMFP peaks of activity were distinguishable within the following time windows: 20-50 ms (T1); 51-100 ms (T2); 101-150 ms (T3) and 151-250 ms after TMS (T4). Scalp maps of voltage distribution showed a dipole of activity focused over the site of stimulation for the first ~80 ms after TMS, i.e. within T1 and T2. The amplitude of these dipoles appeared to change depending on the current direction (figure 3). Specifically, stimulation with PA direction produced an evident positivity over the stimulated area (C3), involving both frontal (FC1, F3) and posterior electrodes (CP1, P3), with a prominent negativity over the contralateral fronto-central electrodes. When the stimulation was delivered with AP direction, the dipole appear to be reversed, i.e. a negative peak over the stimulated area and a contralateral positivity. ANOVAs conducted on TEP peaks showed a direction×polarity [$F_{(1,18)}$=15.499; p=0.001; ε=.463] and a direction×waveform×polarity interaction [$F_{(1,18)}$=5.656; p=0.029; ε=.239]. Post-hoc analysis revealed that PA pulses induced greater positive peaks compared to AP when monophasic (2.783±0.41 vs. 1.381±0.38 μV; p=0.01) but not when biphasic (2.02±0.24 vs. 1.437±0.19 μV; p=0.07); on the other hand., AP induced greater negative peaks compared to PA both when monophasic (-2.355±0.44 vs. -0.539±0.19 μV; p=0.002) and biphasic (-1.181±0.2 vs. -0.487±0.18 μV; p=0.022) (figure 4E). An evident negativity peaking ~100 ms was then observable bilaterally over central electrodes, followed by a positivity peaking around 200 ms.

ANOVA conducted on cTMS-GMFP showed a significant main effect of waveform [$F_{(1,18)}$=19.606; p<0.001; ε=.521] and a significant waveform×time window interaction

[F(2.27,40.93)=3.165; p=0.047; ε=.150]. Post-hoc analysis revealed that monophasic pulses induced a stronger cortical response compared to biphasic pulses, especially from 50 ms after TMS (T2: 2.475±0.25 vs. 1.858±0.17 µV, p = 0.002; T3: 1.823±0.21 vs. 1.301±0.10 µV, p = 0.001; T4: 1.783±0.19 vs. 1.315±0.11 µV, p = 0.001) (figure 4A). ANOVA conducted on cTMS-LMFP revealed no significant effect of direction (p = 0.39), waveform (p = 0.24) or other interactions (all p > 0.05).

When cortical responses were compared between PA_{mono_80} and PA_{mono_30} to explore the effect of the pulse width, ANOVA conducted on GMFP revealed a significant main effect of pulse width [F(1,18)=7.265; p=0.015; ε=.288]. Post-hoc analysis revealed a greater response evoked by PA_{mono_80} compared to PA_{mono_30} (2.008±0.15 vs. 1.755±0.18 µV, p = 0.015) (figure 4C). When the same analysis was conducted locally to M1 (i.e. LMFP), ANOVA showed a significant pulse width×time window interaction [F(3,54)=6.515; p=0.001; ε=.266]. Post-hoc analysis revealed that PA_{mono_80} induced an initial stronger response (i.e. from 15 to 50 ms) compared to PA_{mono_30} (2.003±0.35 vs. 1.206±0.17 µV, p = 0.047) (figure 4D).

Discussion

We used for the first time a novel cTMS stimulator in combination with EEG to test the effects of current direction, pulse waveform and pulse width.

Effects of current direction

Previous studies showed that the optimal direction to evoke MEPs from the hand is approximately perpendicular to the central sulcus, with the induced current in the brain flowing diagonally from back to front (Brasil-Neto et al., 1992; Porter and Lemon, 1996). This is in agreement with our RMT results. However, there was no significant effect of current direction on the GMFP or LMFP amplitude. This result is unexpected because GMFP has been reported to increase with increasing stimulus intensities (Komssi et al., 2004), and the absolute stimulus intensities were greater for AP pulses than PA pulses.

TMS over M1 activates a variety of excitatory and inhibitory neurons in the pre- and post-central gyrus (Laakso et al. 2014; Seo et al., 2017), including both local interneurons and projection neurons entering and exiting the sensorimotor cortex. The MFP reflects the strength of this net activity, which is expected to be higher for greater stimulus intensities. However, current orientation with respect to an axon influences its recruitment threshold and thus the size of the response for a given stimulus intensity. Some cortical responses to TMS, such as the GABA$_A$-ergic inhibition to two closely spaced TMS pulses (Kujirai et al. 1993), seem insensitive to current direction (Ziemann et al. 1996). The AP stimuli here would be expected to recruit a greater proportion of these neurons by virtue of the greater stimulus intensity, and contribute to a greater MFP. However, previous studies showed that monophasic PA and AP currents activate the corticospinal pathway in a different manner (Sakai et al. 1997; Di Lazzaro et al. 2001). One hypothesis is that there are sets of neurons in the pre- and post-central gyrus capable of activating the corticospinal tract that are sensitive to the orientation of currents induced by TMS and also have different thresholds for activation (Day et al. 1989; Sakai et al. 1997). Here we found that a greater intensity of AP directed stimulation was required to evoke similar LMFPs and GMFPs, which means that the proportion and threshold of directionally sensitive neurons is unlikely to be evenly distributed across the central sulcus.

Further evidence for the idea that PA and AP currents evoke different patterns of cortical activity comes from examining the topography of the TEP, which differed over the fronto-central scalp for the first 80ms or so. Studies modelling the mechanisms by which TMS activates the M1 output neurons suggest that PA currents are likely to activate layer II/III and V pyramidal neurons in the upper part of the sulcal wall in the anterior bank of the central sulcus (Thielscher et al. 2011; Seo et al., 2017). PA-oriented currents are, however, also predicted to activate some pyramidal neurons in

the posterior bank of the central sulcus corresponding to the somatosensory cortex (Seo et al., 2017). If this is true, the TMS-evoked cortical activity is likely to reflect a mixture of activity (opposing dipoles) generated on either side of the central sulcus, but biased towards activity emanating from M1. By contrast, AP-directed stimuli would be expected to recruit a larger portion of neurons in the posterior bank of the central sulcus than PA stimuli (Salvador et al. 2011; Laakso et al. 2014; Seo et al. 2015), though they would still activate a similar proportion of M1 output neurons as PA stimulation since they were matched for RMT. Our data are consistent with these ideas in that they imply TMS evokes a mixture of activity that is biased towards, but not exclusive, to the pre- or post-central sulcus depending on the TMS current orientation. Dendritic depolarization of pyramidal neurons in the posterior wall would tend to cause a frontal negativity (as in the N20 component of the SEP) whereas activation in the anterior wall would produce a frontal positivity.

So far, only two studies have recorded TEPs using different coil orientations (Bonato et al., 2006; Casarotto et al., 2010). Casarotto and colleagues found that stimulation of the left frontal, parietal and occipital lobe with different coil orientations produced different morphology of cortical responses (Casarotto et al., 2010). Notably, in their study, Casarotto and colleagues did not compare the standard PA and AP direction, since they used different angles of stimulation respect to the midline. Bonato and colleagues (2006) stimulated M1 with two coil orientations (45 and 135° respect to the midline) and reported some differences in early TEP polarity (i.e. P30 and N45), although they did not provide any physiological interpretation. Notably, the authors used a supra-threshold intensity (110% of RMT) that can contaminate the TEP with sensory feedback from the contraction induced in peripheral muscle (Komssi et al., 2004). Furthermore both studies used a biphasic stimulus, which complicates the effect of coil orientation since in these devices the current flows in two opposite directions. Notably, when we used a biphasic stimulation differences in topography were minimal, probably due to the fact that they reflect summation of the effects of two opposite current directions.

Some studies have reported a correlation between the amplitudes of MEPs and early TEPs suggesting that the latter may reflect activity of EPSPs in corticospinal pyramidal neurons involved in producing the MEP (Maki and Ilmoniemi, 2010). Along the same lines, it has been shown that varying the coil angle modifies the amplitude of both MEP and TMS-evoked P30 (Bonato et al., 2006). This would be consistent with the idea that a change in peak amplitudes due to coil orientation is due to activation of different populations of interneurons and potentially corticospinal neurons with different physiological properties within M1 (Hannah et al., 2016).

Effects of pulse waveform

We found that the RMT was greater for biphasic pulses compared to monophasic pulses, consistent with previous studies (Kammer et al., 2001; Sommer et al., 2006). However, despite their lower absolute stimulus intensity, monophasic pulses produced a greater GMFP confined to late activity. This again highlights differences in the cortical activity evoked by different pulses even when the stimulus intensity is normalized to the RMT.

Several studies have shown that longer latency components of the TEP can be modulated by drugs that affect slower GABA(B)-mediated IPSPs (Ferreri et al., 2010; Rogasch et al., 2013; Premoli et al., 2014; Casula et al., 2016; 2017b). Administration of GABA-agonists increases the N100 and P180 components of the TEP (Kähkönen and Wilenius, 2007; Premoli et al., 2014a) and increases indirect measures of GABA(B) activity such as long interval intracortical inhibition (Daskalakis et al., 2008; Fitzgerald et al., 2008; Premoli et al., 2014b). Importantly, while early TEPs are distributed locally to the stimulated area, likely representing the activation of local M1 interneurons, later TEPs showed a bilateral distribution over central (N100) and fronto-central (P180) areas, presumably mediated by transcallosal interhemispheric connections (Voineskos et al., 2010; Casula et al., 2014; Määttä et al., 2017). We postulate that although monophasic pulses activated an equivalent amount of local input to corticospinal (and perhaps other) neurons in M1, they were more effective in recruiting outputs to other cortical areas as reflected in the larger late, widespread, activity. Alternatively, it is possible that the reverse phase of biphasic pulses could have partially impeded the activation of neurons involved in the origin of late TEPs.

Effects of pulse width

The threshold for activating an axon by electrical or magnetic stimulation depends on the duration of the pulse. This strength-duration behavior has been characterized by stimulation of peripheral nerves showing that different sets of axons have different strength-duration properties (Mogyoros et al., 1996). Peterchev and colleagues (2011; 2013) used cTMS to manipulate the duration of stimuli applied to M1. They found that longer pulse widths were associated with a lower RMT and steeper input/output curves, and they used this information to quantify the strength-duration behavior of axons in the corticospinal pathway. Furthermore, by concurrently manipulating pulse duration and current direction it is possible to enhance the difference in onset latencies of MEPs evoked by AP and PA current pulses. This suggests that different pulse widths alter the proportion of early and late synaptic inputs to corticospinal neurons (D'Ostilio et al. 2016; Hannah & Rothwell, 2017). These data suggest that the cortical response to TMS should also depend on the pulse duration.

In the present study we compared the effect of a stimulus with a pulse width of 30 μs with that of an 80 μs pulse from a standard Magstim 200 monophasic stimulator. The longer pulse width was associated with lower RMT and higher LMFP, but not GMFP. This suggests that longer pulse width influences the activity of local neurons of the motor cortex, but it does not seem to affect the response over the distal cerebral areas. The early TMS-evoked response, between 20 and 50 ms, originates or is modulated by fast GABA(A)-mediated IPSPs (Premoli et al., 2014; Premoli et al., 2016). This is interesting because we recently suggested that GABA-ergic inhibition between two closely spaced TMS pulses was influenced by pulse duration (Hannah et al. 2017), even though stimulus intensity was similar in relative terms (i.e. %RMT). This implies that the GABA-ergic inhibitory interneurons exhibit a different strength-duration behavior from the excitatory interneurons responsible for the MEP. Together with our present data this might imply that the greater LMFP for long versus short duration pulses, of similar relative intensity, is partly a consequence greater recruitment of GABA$_A$-ergic interneurons in M1. The implication is that normalizing stimulation intensity to motor threshold across different pulse widths only controls for the activation of excitatory neurons generating the MEP, but cannot control for the activation of other populations of neurons whose strength-duration behavior does not scale with those excitatory neurons.

There are confounds to our interpretation of the data. For example, it could be argued that the enhanced early activity could be produced by artefactual muscular activity, which may affect early TEPs (Rogasch et al., 2014). However, we can exclude this hypothesis for two reasons: first, the topographical distribution of the activity is not compatible with the typical TMS-evoked muscular response (Rogasch et al., 2013); second, we used a 20 ms interpolation to avoid this kind of artefact. A second possibility is that activity in this period is contaminated by activity evoked in sensory cortex from the sensation of stimulation on the scalp. Indeed, a previous cTMS study demonstrated that scalp sensation is more uncomfortable when brief pulse widths are used (Peterchev et al., 2013). However, when we measured SST on the scalp we found no difference in the SST/RMT ratio, thus excluding possible interference of SEPs in our results.

Conclusions

This is the first study in which the cortical effects of pulse width, pulse waveform and current direction are directly explored. Our main finding is that local and global responses to TMS vary according to the pulse parameters, despite stimulus intensity being normalized to RMT. We suggest that this is because pulse parameters influence not only "what" is stimulated, i.e. different neuronal populations, but also "where" the stimulation is effective within the cortex.

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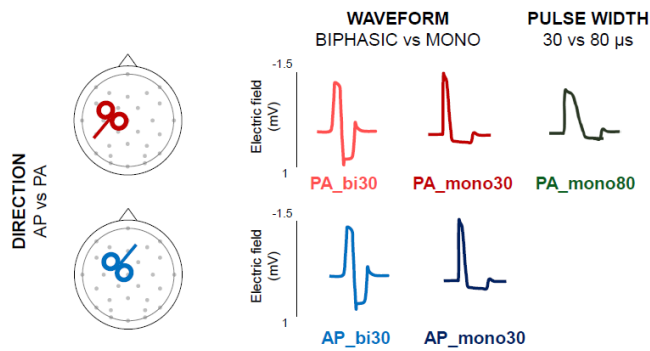
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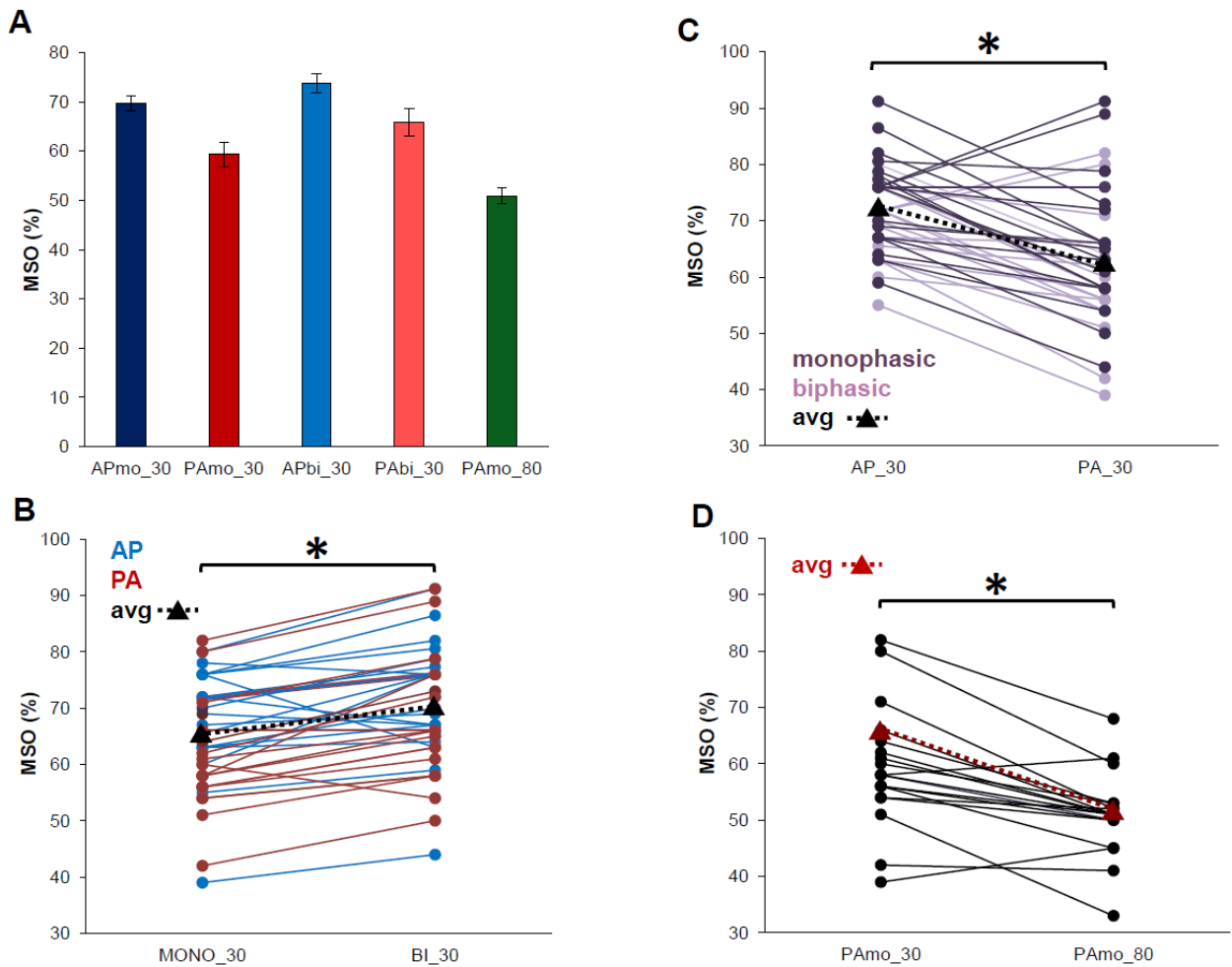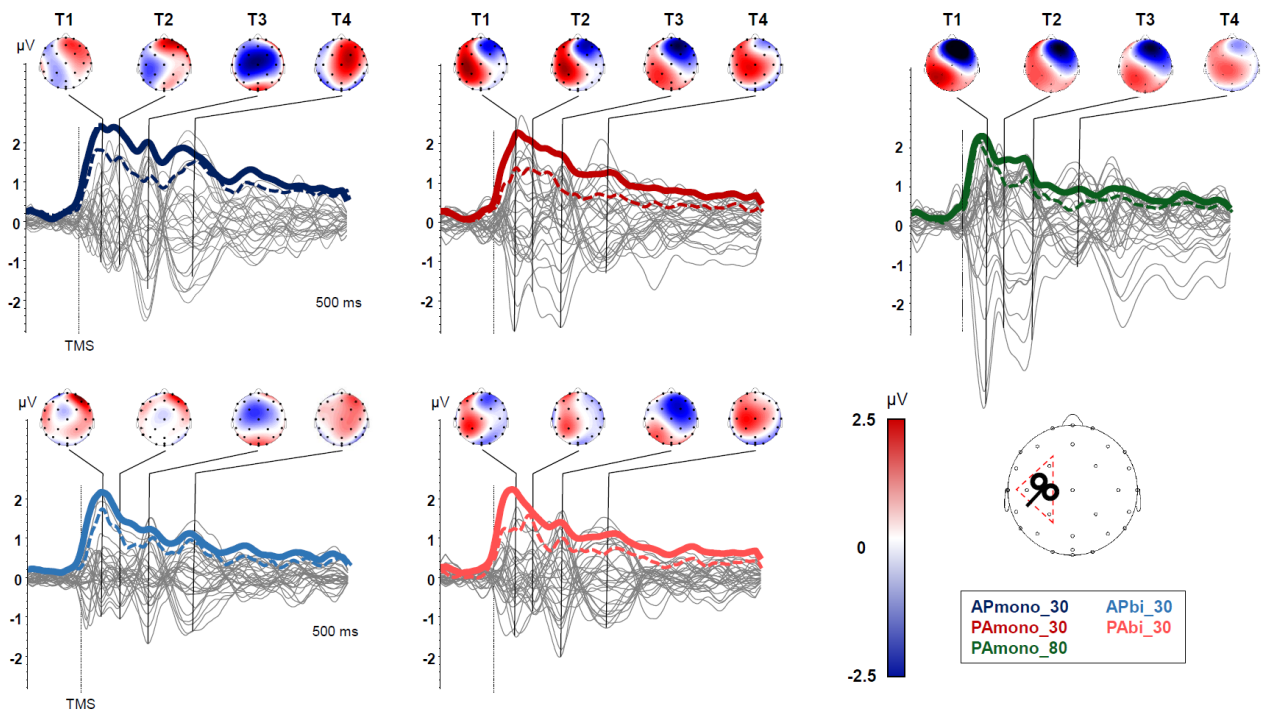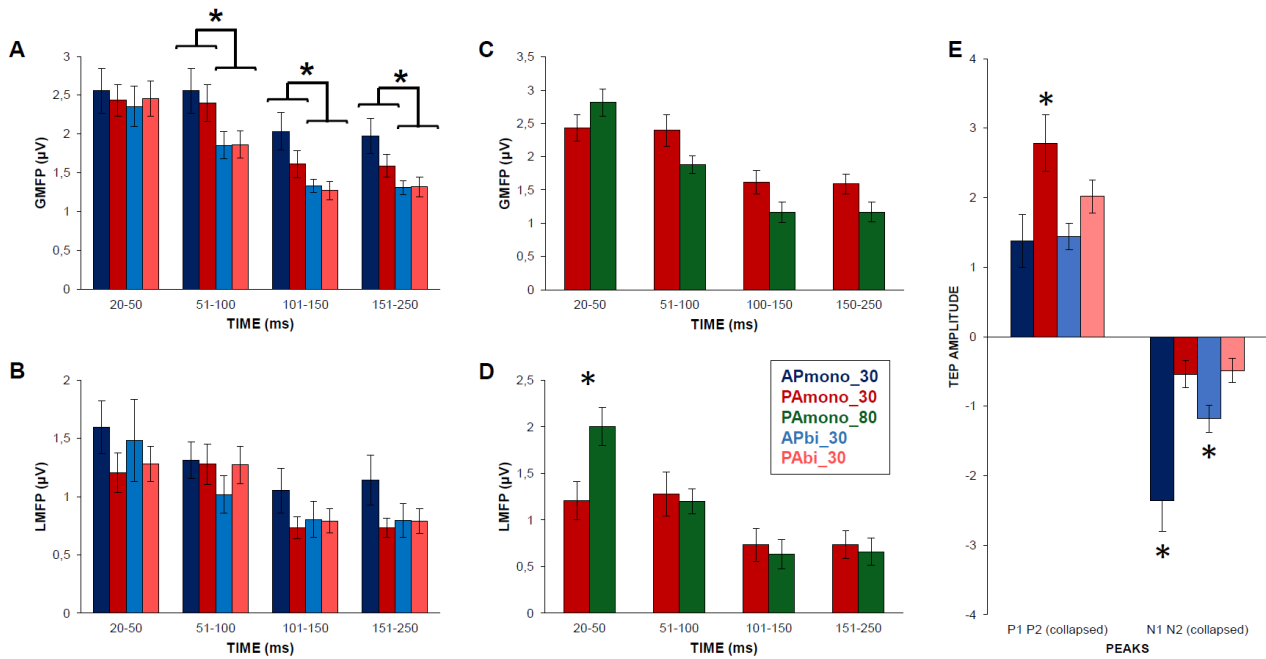
Figure captions

Figure 1. Experimental design. Three stimulation parameters were systematically compared: current direction (PA vs. AP direction); pulse waveform (monophasic vs. biphasic) and pulse width (30 vs. 80 μs). Pulse waveforms reflect the induced electric field. Monophasic and biphasic are used to refer to electrical field waveforms that are predominantly unidirectional (i.e. monophasic, where the amplitude of the first phase is much greater than that of the second creating an asymmetry) or bi-directional (i.e. where there is greater symmetry in the amplitude of the first and second phase of the electric field waveform).

Figure 2. Analysis of resting motor threshold (RMT). *Panel A:* mean RMT for each for each stimulation condition. *Panel B:* effect of pulse waveform, mean RMT was generally lower when tested with monophasic cTMS pulses compared to biphasic, regardless the current direction (AP, blue lines; PA, red lines). *Panel C:* effect of current direction, mean RMT was generally lower when tested with PA direction compared to AP direction, regardless the pulse waveform (monophasic, dark violet lines; biphasic, light violet lines). *Panel D:* effect of pulse width, mean RMT was generally lower when tested with longer pulse width (80 μs) compared to shorter pulse width (30 μs). *$p<0.05$, error bars depict SEM.

Figure 3. Spatiotemporal analysis of TMS-evoked potentials (TEP). Butterfly plots depict the TEPs (grey lines) recorded from each electrode with their relative distribution over the scalp in each stimulation condition. Thick lines depict the global mean field power (GMFP); dotted lines depict the local mean field power (LMFP) (AP_{mono30}, dark blue line; PA_{mono30}, dark red line; AP_{bi30}, light blue line; PA_{bi30}, light red line; PA_{mono80}, green line). Scalp maps show the topographic distribution of TEPs at 15-50, 51-100, 101-150 and 151-250 ms after TMS.

Figure 4. Analysis of global (GMFP) and local mean field power (LMFP) and TMS-evoked potentials (TEPs). *Panel A, B:* effect of pulse waveform and current direction on GMFP *(A)* and on LMFP *(B)*. GMFP from 51 to 250 ms after stimulation was higher with monophasic pulses (AP dark blue bar; PA, dark red bar) compared to biphasic pulses (AP, light blue bar; PA, light red bar), no difference were detected between the two coil directions. No effects of pulse waveform and current direction were detected on LMFP. *Panel C, D:* effect of pulse width on GMFP *(C)* and on LMFP *(D)*. LMFP from 20 to 50 ms after stimulation was higher with a pulse width of 80 µs (green bars) compared to 30 µs (dark red bars). No effects were detected on GMFP. *Panel E:* effect of current direction on TEPs amplitude (peak 1 and 2 collapsed). Positive peaks were significantly higher with monophasic PA pulses compared to the other conditions, whereas negative peaks were significantly higher with monophasic and biphasic AP pulses compared to PA.

DEMOGRAPHIC VARIABLE			RMT				
N_SOG	GENDER	AGE	APmo_30	PAmo_30	APbi_30	PAbi_30	PAmo_80
1	m	22	72	80	76	89	60
2	m	29	76	60	63	54	53
3	f	25	76	61	82	66	51
4	m	27	63	51	64	58	33
5	f	25	67	66	69	66	51
6	m	22	72	82	76	91	68
7	m	25	76	71	80	79	52
8	m	28	80	64	91	73	53
9	m	28	72	54	77	58	50
10	f	42	78	58	76	76	50
11	f	24	55	39	59	44	45
12	m	30	76	58	86	66	51
13	m	24	69	54	67	58	52
14	f	31	72	56	67	63	50
15	f	32	70	56	79	63	51
16	m	39	63	42	67	50	41
17	m	30	65	62	76	72	51
18	m	32	63	58	70	65	61
19	m	28	60	56	76	61	45
		MEAN RMT	60	70	66	74	51
		RMT/SST	1,9	2,2	1,9	2,1	2,1

Table 1. Participants' demographic, RMT values and RMT/SST ratio for each condition.