

## Low-frequency rTMS inhibitory effects in the primary motor cortex: Insights from TMS-evoked potentials



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### ABSTRACT

The neuromodulatory effects of repetitive transcranial magnetic stimulation (rTMS) have been mostly investigated by peripheral motor-evoked potentials (MEPs). New TMS-compatible EEG systems allow a direct investigation of the stimulation effects through the analysis of TMS-evoked potentials (TEPs).

We investigated the effects of 1-Hz rTMS over the primary motor cortex (M1) of 15 healthy volunteers on TEP evoked by single pulse TMS over the same area. A second experiment in which rTMS was delivered over the primary visual cortex (V1) of 15 healthy volunteers was conducted to examine the spatial specificity of the effects.

Single-pulse TMS evoked four main components: P30, N45, P60 and N100. M1-rTMS resulted in a significant decrease of MEP amplitude and in a significant increase of P60 and N100 amplitude. There was no effect after V1-rTMS.

1-Hz rTMS appears to increase the amount of inhibition following a TMS pulse, as demonstrated by the higher N100 and P60, which are thought to originate from GABAb-mediated inhibitory post-synaptic potentials.

Our results confirm the reliability of the TMS-evoked N100 as a marker of cortical inhibition and provide insight into the neuromodulatory effects of 1-Hz rTMS. The present finding could be of relevance for therapeutic and diagnostic purposes.

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### Introduction

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive technique that can produce after-effects on cortical excitability lasting 30 min or more (Ridding and Rothwell, 2000). Over the years, its use for research and therapeutic purposes has increased even though its mechanism of action is still only partially understood (Pascual-Leone et al., 1998; Ridding and Rothwell, 2000; Rossi et al., 2009). In the majority of the TMS/EMG literature, neuromodulatory effects of rTMS have been investigated by analysing motor-evoked potentials (MEPs). However, this is a complex measure reflecting excitability of the whole corticospinal pathway which can be influenced not only by

excitability of cortex, but also of spinal cord (Barker et al., 1985). Nowadays, with the current development of TMS-compatible electroencephalography (EEG) systems it is possible to record the cerebral activity evoked by TMS from the entire scalp (Ilmoniemi et al., 1997). These responses, collectively known as TMS-evoked potentials (TEPs), are unaffected by spinal excitability so they may be a more reliable measure of the response of the brain to TMS and give information about widespread effects throughout the cortex (Ilmoniemi and Kičič, 2010). Indeed, studies have shown that TEPs are sensitive to differences in intensity of stimulation and are reproducible from day to day (Casarotto et al., 2010; Lioumis et al., 2009). Given these advantages, there is a growing interest in using EEG measures during TMS to clarify the effects of stimulation protocols such as: rTMS (Helfrich et al., 2013; Van Der Werf and Paus, 2006), paired-pulse TMS (Daskalakis et al., 2008; Ferreri et al., 2011), transcranial direct current stimulation (Pellicciari et al., 2013) and paired associative stimulation (Bikmullina et al., 2009; Veniero et al., 2013).

Many studies have focused on the time-locked EEG response evoked by stimulation of the primary motor cortex (M1). This consists of a

Abbreviations: rTMS, repetitive transcranial magnetic stimulation; MEP, motor-evoked potential; TEP, TMS-evoked potential; RMT, resting motor threshold.

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sequence of positive and negative components, usually labelled P30, N45, P60, N100 and P180 (Bender et al., 2005; Bonato et al., 2006; Ferreri et al., 2011; Komssi et al., 2002; Lioumis et al., 2009; Paus et al., 2001; Van Der Werf and Paus, 2006). Of these the N100 peak appears to be the most robust and well characterised with little clear evidence about the functional origin of the other components (Komssi and Kähkönen, 2006). Several lines of evidence suggest that the N100 reflects inhibitory processes following the TMS pulse (Bender et al., 2005; Bonnard et al., 2009; Bruckmann et al., 2012; D'Agati et al., 2013; Nikulin et al., 2003; Rogasch et al., 2013). In a simple reaction time task, the N100 is reduced in amplitude just prior to movement onset whilst the evoked MEP is increased (Nikulin et al., 2003); a similar reduction is seen in the late part of the foreperiod in a warned reaction time task (Bender et al., 2005). In both cases, the reduction was interpreted as removal of inhibition during excitatory preparation for a forthcoming movement. Bonnard et al. (2009) found that the N100 was larger during the warning period when participants were instructed to “resist” a forthcoming perturbation applied to the wrist compared with trials where the instruction was to “assist” the perturbation. At the same time, the duration of the cortical silent period in ongoing EMG activity was increased (Chen et al., 1999). Since the latter is thought to be a measure of cortical inhibition following a TMS pulse it was suggested that the increase in N100 also represented an inhibitory process primed by the instruction to “resist”. Additional evidence along the same lines have been provided by some very recent studies on ADHD children (Bruckmann et al., 2012; D'Agati et al., 2013). In contrast to this, some studies found an increase in the N100 amplitude evoked by occipital TMS in conditions that presupposed enhanced arousal (Murd et al., 2010; Stamm et al., 2011).

In this study we investigated in a group of healthy volunteers the effect of an rTMS protocol (1 Hz), which usually reduces motor cortical excitability (e.g. Chen et al., 1997; Maeda et al., 2000), on evoked MEPs and on the amplitude of TEPs evoked by single pulse TMS over the same area. In order to examine the spatial specificity of the effect we also tested whether applying rTMS over V1 would also affect the N100 evoked by stimulation of M1.

## Methods

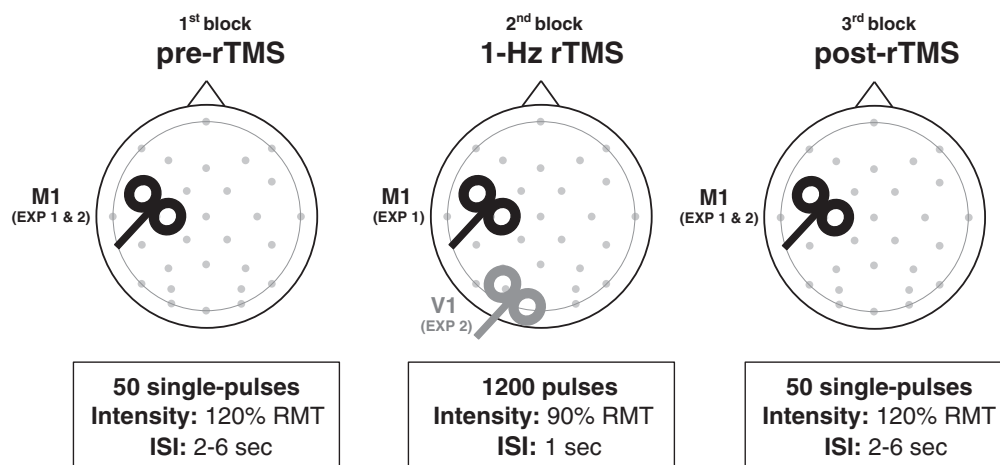
### Participants and procedure

Fifteen right-handed healthy volunteers (seven females, mean age  $25 \pm 5$  years) were enrolled for this experiment (experiment 1) after giving written informed consent. All participants were tested for TMS

exclusion criteria (Rossi et al., 2009) and had normal or corrected-to-normal vision. The experimental procedure was approved by the Institutional Review Board of the University of Padua, and was in accordance with the Declaration of Helsinki. Each participant underwent an experimental session consisting of three blocks of TMS during multichannel EEG and EMG recordings. The first and the third blocks of stimulation (“pre-rTMS” and “post-rTMS” respectively) consisted of 50 single-pulses delivered before and immediately after a 1-Hz rTMS block (Fig. 1). During the entire session participants were seated on a comfortable armchair in front of a monitor at 80 cm distance. They were asked to fixate on a white cross ( $6 \times 6$  cm) in the middle of a black screen and to keep their right arm in a relaxed position. 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 avoid possible auditory ERP responses (Massimini et al., 2005). The intensity of the white noise was adjusted for each subject by increasing the volume (always below 90 dB) until the participant was sure that s/he could no longer hear the click (Paus et al., 2001). To reduce the bone-conducted sound we used an EEG cap with a 4 mm plastic sheet that reduced the transmission of mechanical vibration produced by the coil (Esser et al., 2006; Nikouline et al., 1999).

### Transcranial magnetic stimulation (TMS)

TMS was carried out using a Magstim R<sup>2</sup> stimulator with a 70 mm figure-of-eight coil (Magstim Company Limited, Whitland, UK), which produced a biphasic waveform with a pulse width of  $\sim 0.1$  ms. The position of the coil on the scalp was functionally defined as the M1 site in which TMS evoked the largest MEPs in the relaxed first dorsal interosseous (FDI) muscle of the right hand. The coil was placed tangentially to the scalp at about  $45^\circ$  angle away from the midline, so that the direction of current flow in the most effective (second) phase was posterolateral–anteromedial. To ensure the same stimulation conditions during the entire experiment, coil positioning and orientation on the optimal hotspot were constantly monitored by means of theBrainsight neuronavigation system (using the ICBM152 template), coupled with a Polaris Vicra infrared camera (NDI, Waterloo, Canada). Stimulation intensity varied across the blocks of stimulation (see below) and was determined relative to the resting motor threshold (RMT), defined as the lowest TMS intensity which evoked at least five out of ten MEPs with an amplitude  $>50$   $\mu$ V peak-to-peak in the contralateral FDI at rest (Rossini et al., 1994). Single-pulses were delivered with an interstimulus interval (ISI) of 4–6 s, intensity was set at 120% RMT to obtain



**Fig. 1.** Schematic representation of the experimental procedure. Each participant underwent three blocks of stimulation. In the first block (pre-rTMS), 50 TMS single pulses were delivered over the left M1. In the second block (rTMS), 20 min of rTMS at 1 Hz of frequency were delivered over the left M1 (for the fifteen participants of experiment 1) or over the left V1 (for the fifteen participants of experiment 2). In the third block (post-rTMS), 50 TMS single pulses were delivered over the left M1, immediately after the rTMS block. rTMS, repetitive transcranial magnetic stimulation; RMT, resting motor threshold; ISI, interstimulus interval.

reliable MEPs. The rTMS block consisted of 1200 pulses delivered at 1 Hz using an intensity of 90% RMT.

#### Electromyographic recordings (EMG)

Surface EMG was recorded from the right FDI muscle via Ag/AgCl electrodes in a belly-tendon montage (Myohandy Matrix Line – Micromed Srl, Mogliano Veneto, Italy); raw signals were sampled at 2.5 kHz and band-pass filtered at 50–1000 Hz. EMG signal was on-line monitored and off-line analysed by software Brain-Quick System Plus using epochs of 50 ms. MEP amplitudes were measured peak-to-peak.

#### Electroencephalographic recordings (EEG)

EEG was recorded using a TMS-compatible AC amplifier (Micromed SD MRI, Micromed Srl, Mogliano Veneto, Italy) designed to work in the presence of high external magnetic fields as used in TMS or MRI (Morbidi et al., 2007). The amplifier was optically connected to a PC with software Brain-Quick System Plus through which EEG was on-line monitored, and to a 64-channels customised EEG cap (EasyCap Inc., Herrsching, Germany). EEG was continuously recorded from 31 TMS-compatible Ag/AgCl pellet electrodes mounted on the cap according to the 10–20 international system including: Fp1, Fpz, Fp2, F7, F3, Fz, F4, F8, FC5, FC1, FC2, FC6, T3, C3, Cz, C4, T4, CP5, CP1, CP2, CP6, T5, P3, Pz, P4, T6, PO3, PO4, O1, Oz, O2. Skin impedance was kept below 5 k $\Omega$ . Recordings were referenced to AFz electrode; the ground electrode was placed on POz. EEG signal was bandpass filtered at 0.1–500 Hz and the sampling frequency was 2048 Hz. Off-line analysis was performed with EEGLAB 10.2.2.4b (Delorme and Makeig, 2004), running in a MATLAB environment (Version 7.9.0, MathWorks Inc., Natick, USA). The continuous EEG signal was segmented into epochs starting 50 ms before the TMS pulse and ending 250 ms after it. After this, data from 5 ms before the TMS pulse to 22 ms after were removed from each trial to exclude the TMS artefact through the cubic interpolation function of MATLAB (Thut et al., 2011). The identification of artefacts unrelated to TMS (e.g. eye blinks, muscle activity, electric current, alpha activity) was made using the independent component analysis (ICA) function on EEGLAB. Identified components were then visually inspected in terms of scalp distribution, frequency, timing and amplitude and removed with ICA (Johnson et al., 2010; Mattavelli et al., 2013; Veniero et al., 2013). Afterwards, all the epochs were visually inspected and those with excessively noisy EEG were excluded from the analysis (resulting in less than 5% for each participant). A baseline correction, taken as the interval starting 500 ms before the TMS pulse, was applied on all the epochs. For the TEP analysis, all the epochs of each participant were averaged separately for the pre-rTMS and the post-rTMS conditions. Based on the recent literature (for a review, Ilmoniemi and Kičić, 2010), we chose five time windows to determine the TEPs amplitudes and latencies, computed as the highest peaks in the following intervals: 23–45 ms (positive peak); 30–60 ms (negative peak); 45–70 ms (positive peak); 70–130 ms (negative peak); 130–230 ms (positive peak). To assess the total brain activation induced by TMS over M1, we performed a local mean field power analysis (LMFP), computed as the square root of the signal across the electrodes surrounding the two motor cortices (Lehmann and Skrandies, 1980; Pellicciari et al., 2013).

#### Control experiment

A control experiment (experiment 2) was conducted to examine the spatial specificity of the effects observed in experiment 1. Fifteen right-handed healthy volunteers (different from those who participated in experiment 1) were enrolled and underwent the same experimental procedure as experiment 1, except that the rTMS stimulation was delivered over V1 (3 cm anterior and 1 cm lateral from theinion (Silvanto et al., 2007). The coil was held with the handle pointing towards the

left side, so that the current flow direction of the second, most effective, phase wave was latero-medial.

#### Statistical analyses

All data were analysed using SPSS version 19 (SPSS Inc., Chicago, USA). Prior to undergoing ANOVA procedures, normal distribution of EEG and EMG data was assessed by means of Shapiro–Wilks' test. Level of significance was set at  $\alpha = .05$ . Extreme outliers (i.e. 3 standard deviations or more) within individual trials were identified and excluded from the analysis (resulting in less than 4% for each participant). MEP amplitudes were first log-transformed to limit the effect of outliers and then analysed with a 2 (group: experiment 1, experiment 2)  $\times$  2 (rTMS: pre-rTMS, post-rTMS) mixed ANOVA. TEP analysis was performed considering ten electrodes: FC1, C3, T3, CP1 and CP5 for the left stimulated side; FC2, C4, T5, CP2 and CP6 for the right one. TEP amplitudes and latencies were analysed with a 2 (group: experiment 1, experiment 2)  $\times$  2 (rTMS: pre-rTMS, post-rTMS)  $\times$  2 (laterality: left, right)  $\times$  5 (electrode: FC1/FC2, C3/C4, T3/T4, CP1/CP2, CP5/CP6)  $\times$  5 (peak: P30, N45, P60, N100, P180) mixed ANOVA. The same electrodes were considered for the LMFP analysis that was performed comparing the LMFP differences of the two conditions (post-pre) between the two groups with paired t-tests, separately for each hemisphere. The sphericity of the data was tested with Mauchly's test; when sphericity was violated (i.e. Mauchly's test < 0.05) the Greenhouse–Geisser correction was used. Pairwise comparisons were corrected by the Bonferroni method.

## Results

#### Motor-evoked potentials (MEPs)

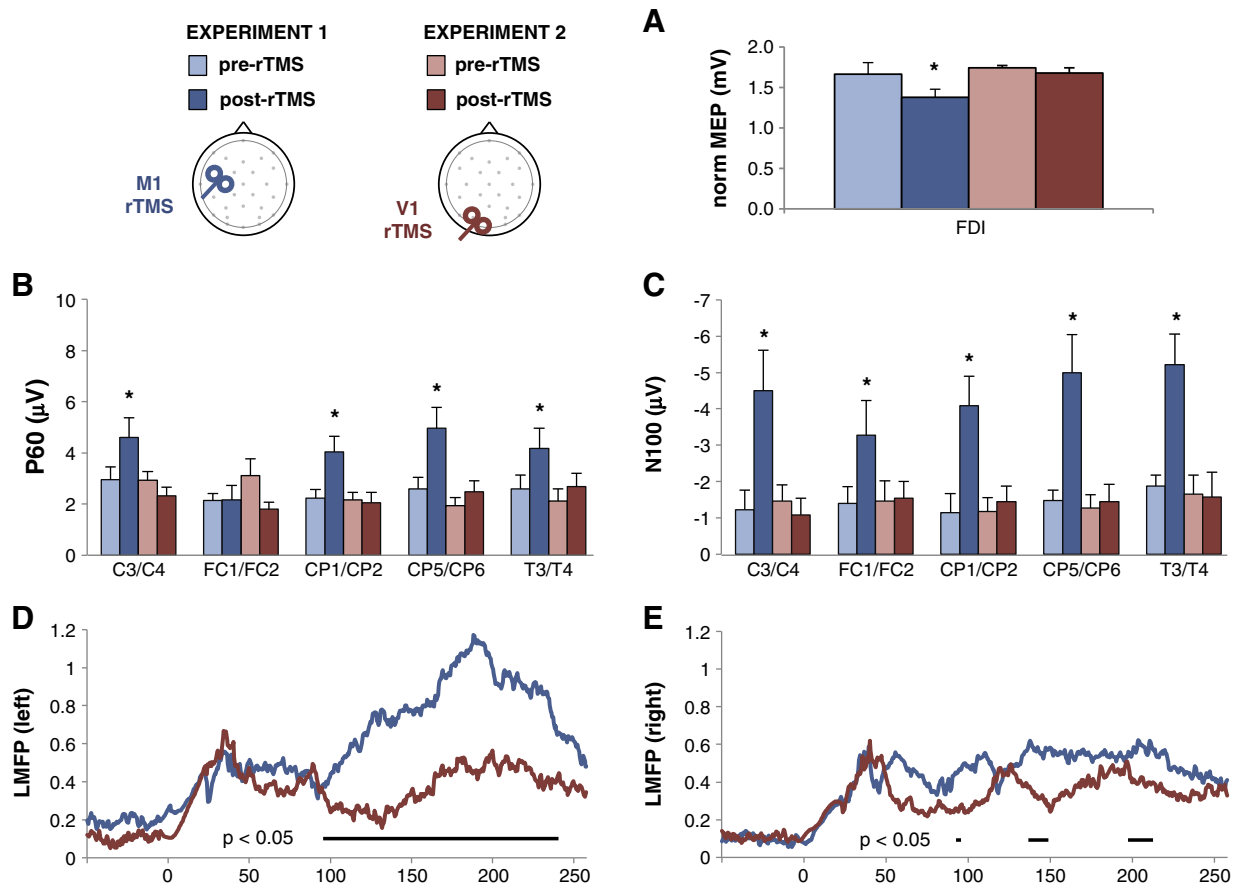
There was no difference in the baseline MEP amplitudes (i.e. pre-rTMS blocks) in the two groups of experiments 1 and 2 ( $p = 0.38$ ). However, there was a significant group  $\times$  rTMS interaction [ $F(1,28) = 4.247$ ;  $p = 0.049$ ] that post-hoc analysis revealed was caused by a significant reduction in the post-rTMS MEP amplitude in experiment 1 ( $1.37 \pm 0.14$  vs.  $1.66 \pm 0.09$  mV;  $p = 0.001$ ) but not in experiment 2 ( $1.67 \pm 0.06$  vs.  $1.72 \pm 0.03$ ;  $p = 0.47$ ; Fig. 2A).

#### TMS-evoked potentials (TEPs)

Single-pulse TMS over M1 evoked a sequence of positive and negative deflections lasting up to 180–200 ms. The grand-average waveform clearly shows four main peaks at approximately 30 ms (P30), 45 ms (N45), 60 ms (P60), 100 ms (N100) after the TMS pulse (Fig. 3).

The mixed ANOVA including all factors revealed that there was a significant group  $\times$  rTMS  $\times$  peak interaction [ $F(4,112) = 6.891$ ;  $p = 0.001$ ] as well as a significant group  $\times$  rTMS  $\times$  electrode  $\times$  peak interaction [ $F(16,448) = 1.738$ ;  $p = 0.037$ ].

The post-hoc analysis of the first interaction did not reveal any differences between the pre-rTMS blocks of any of the peaks of the two experiments (all  $ps > 0.15$ ). On the other hand, significant differences between post- and pre-rTMS blocks were detected for the N100 ( $2.99 \pm 0.67$   $\mu$ V;  $p < 0.001$ ) and for the P60 ( $1.47 \pm 0.59$   $\mu$ V;  $p = 0.02$ ) of experiment 1, which appeared to be larger in the post-rTMS condition. No differences were detected in experiment 2 (all  $ps > 0.25$ ). The post-hoc analysis of the second interaction showed that the N100 was larger in the post-rTMS block compared to the pre-rTMS one at the following sites: C3/C4 ( $3.27 \pm 0.86$   $\mu$ V;  $p = 0.001$ ), FC1/FC2 ( $1.88 \pm 0.66$   $\mu$ V;  $p = 0.009$ ), CP1/CP2 ( $2.95 \pm 0.60$   $\mu$ V;  $p < 0.001$ ), CP5/CP6 ( $3.52 \pm 0.83$   $\mu$ V;  $p < 0.001$ ) and T3/T4 ( $3.35 \pm 1.02$   $\mu$ V;  $p = 0.003$ ). The P60 was larger in the post-rTMS block compared to the pre-rTMS at the following sites: C3/C4 ( $1.56 \pm 0.69$   $\mu$ V;  $p = 0.033$ ), CP1/CP2 ( $1.80 \pm 0.63$   $\mu$ V;  $p = 0.008$ ), CP5/CP6 ( $3.52 \pm 0.83$   $\mu$ V;  $p = 0.005$ ) and T3/T4 ( $3.35 \pm 1.02$   $\mu$ V;  $p = 0.047$ ). The P60 and N100 amplitudes are



**Fig. 2.** (A) MEPs amplitudes before and after rTMS in experiment 1 (pre-rTMS: light blue; post-rTMS: dark blue) and in experiment 2 (pre-rTMS: light red; post-rTMS: dark red). A decrease of MEP amplitude was observed in the post-rTMS block of the experiment, compared to the other conditions. (B, C) P60 and N100 amplitude before and after rTMS for experiment 1 (pre-rTMS: light blue; post-rTMS: dark blue) and for experiment 2 (pre-rTMS: light red; post-rTMS: dark red). The rTMS over M1 (experiment 1, dark blue line) produced an increase in the amplitude of the P60 and of the N100 compared to the other conditions. Error bars indicate standard error. Asterisks indicate  $p < 0.05$ . (C, D) Local mean field power in the left and in the right hemisphere of the difference post-rTMS – pre-rTMS in the two experiments (experiment 1: blue; experiment 2: red). In experiment 1, rTMS caused an increase of the late LMFP in the left hemisphere (96–231 ms after the TMS pulse) and in the right hemisphere (86–90, 132–146 and 196–216 ms after the TMS pulse); compared to experiment 2. Black line at the bottom indicates when the difference between the two experiments was significant. MEPs, motor-evoked potentials; rTMS, repetitive transcranial magnetic stimulation; LMFP, local mean field power.

reported in Figs. 2B and c. Finally, there were no significant differences in the P30, N45 and P180 amplitudes for experiment 1 (all  $ps > 0.15$ ), and no effects on any component for experiment 2 (all  $ps > 0.07$ ).

The analysis of TEP latencies failed to reveal any significant effects (all  $ps > 0.05$ ). Following these results, we tested for possible correlation between the rTMS-induced modulations in MEP and in N100/P60 amplitudes using the Pearson correlation coefficient. No significant correlations were revealed (all  $ps > 0.05$ ).

#### Local mean field power (LMFP)

No differences were detected in the LMFP of the two groups from 50 ms before the TMS pulse to 72 ms after (all  $ps > 0.05$ ). Paired sample t-tests revealed a significantly higher LMFP for experiment 1, compared to experiment 2, in the following temporal windows: 96–231 ms in the left hemisphere (Fig. 2D); 86–90 ms, 132–146 ms and 196–216 ms in the right hemisphere (Fig. 2E).

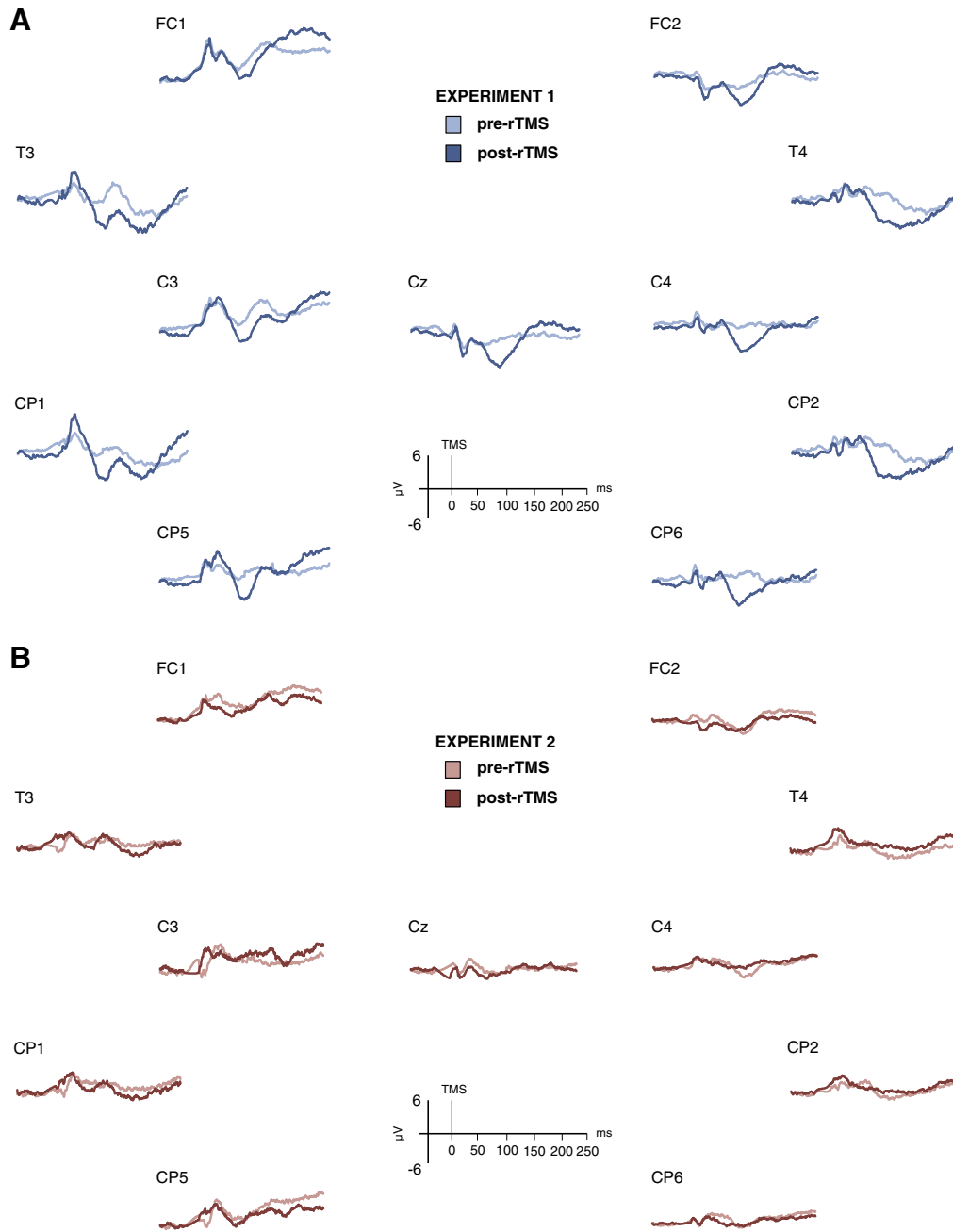
#### Scalp distribution maps

Fig. 4 shows the scalp distribution maps of the difference before and after rTMS in the two experiments. In experiment 1, starting from 50 ms after the TMS pulse, a positivity, representing higher post-rTMS P60, was visible over the site of stimulation which tended to return to baseline levels at 75–80 ms. From 85–90 ms, the higher post-rTMS N100 was

evident over the site of stimulation. From 105–110 ms, the negativity amplitude was prominent and tended to gradually spread over the adjacent regions and, interestingly, to the opposite hemisphere at 115–130 ms before returning to baseline levels at 145–150 ms. Starting from 160 ms, a sustained positivity is evident over the site of stimulation before returning to baseline levels at 240–250 ms. No differences were appreciable in experiment 2.

#### Discussion

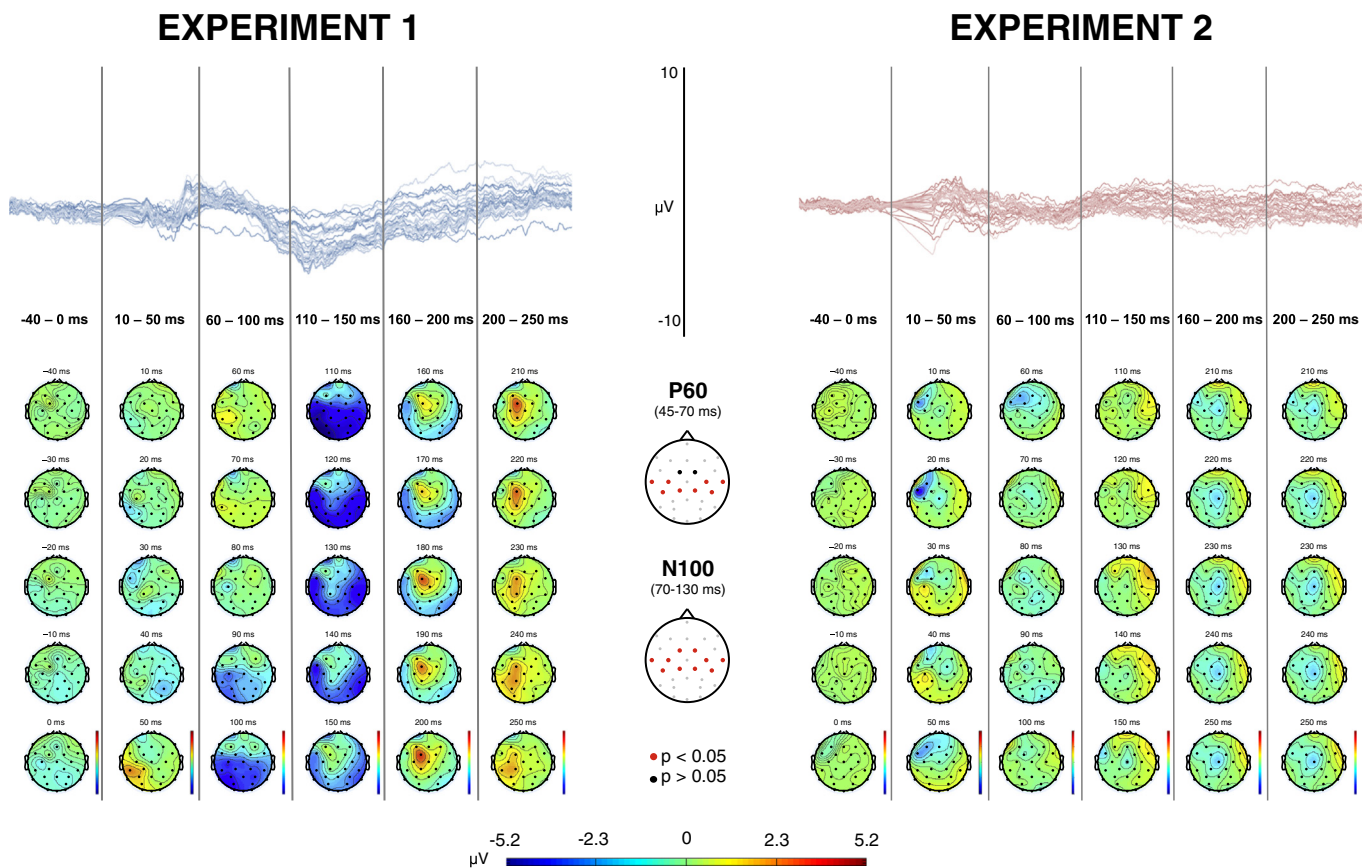
The present results show that 1-Hz rTMS over M1, but not V1, (a) reduces the amplitude of MEPs, (b) increases the amplitude of the N100 and P60 TEPs evoked by single pulse TMS over M1 and (c) induces a sustained increase in the late LMFP, especially in the stimulated hemisphere. The effect on MEP has been reported in a number of previous studies (e.g. Chen et al., 1997; Maeda et al., 2000; Siebner et al., 1999), whereas the effect on TEP has not been described previously in healthy volunteers. Interestingly, the largest changes were seen on the side of stimulation (left scalp), as clearly visible in the LMFP, indicating that their modulation was strictly related to TMS and not to other confounding factors. On the other hand, our TEP analysis failed to reveal a significant effect of laterality. This might be due to the interhemispheric spread of the N100 component from the left hemisphere (85–90 ms) to the right hemisphere (115–120 ms).



**Fig. 3.** (A) Grand average waveform recorded before and after rTMS in experiment 1 (pre-rTMS: light blue; post-rTMS: dark blue). (B) Grand average waveform recorded before and after rTMS in experiment 2 (pre-rTMS: light red; post-rTMS: dark red). Single-pulse TMS over M1 evoked four main peaks of activity in both experiments: P30, N45, P60, N100. In experiment 1, the P60 and N100 amplitudes were significantly modulated by the rTMS (A), especially in the stimulated left hemisphere. Such effect was not observed in experiment 2 (B). rTMS, repetitive transcranial magnetic stimulation.

It has been suggested that early TEPs, such as P30 and N45, originate or are modulated from fast GABA<sub>A</sub> (gamma-aminobutyric acid)-mediated inhibitory post synaptic potentials (IPSPs), whereas later TEPs, such as P60 and N100, are produced or modulated by slow GABA<sub>B</sub>-mediated IPSPs (Ferreri et al., 2011; McDonnell et al., 2006; Rogasch et al., 2013; Tamás et al., 2003). This latter hypothesis is supported by the fact that the N100 increases after consumption of alcohol, which is known to increase GABAergic transmission (Kähkönen and Wilenius, 2007). Moreover, the latencies of the P60 and N100 peaks coincide with the start of the inhibition (around 50–55 ms) and peak of inhibition (around 100–150 ms) produced by activation of GABA<sub>B</sub> receptors in both human and animal studies (Davies et al., 1990; Deisz, 1999). Finally, some recent works using paired-pulse TMS found a significant correlation between EMG measures of the depth of

long-interval cortical inhibition (LICI), a GABA<sub>B</sub>-ergic effect whose timing coincides with the P60/N100 peaks, and the total amount of EEG activity evoked by the TMS pulses (Daskalakis et al., 2008; Farzan et al., 2010; Fitzgerald et al., 2008). This is in line with what we observed in the LMFP, which became significantly higher after the administration of 1-Hz rTMS from about 75 ms to 230 ms after the TMS single pulse. On the other hand, our TEPs analysis did not reveal any significant change on the P180 component, and this might be explained by the absence of a real peak of activity in that temporal window. Given this reasoning, the increase in P60 and N100 may indicate that 1-Hz rTMS increases the amount of GABA<sub>B</sub>-ergic inhibition evoked by a TMS test pulse. The fact that P30 and N45 are unaffected would be compatible with a smaller or absent effect on GABA<sub>A</sub>-ergic activity.



**Fig. 4.** Scalp distribution maps of the difference (post-rTMS – pre-rTMS) in the activity changes induced by rTMS in experiment 1 (left) and in experiment 2 (right). In experiment 1 (left), after 40 ms from the TMS pulse, a higher positivity was observable near the site of stimulation, representing the increase in P60 amplitude produced by the M1-rTMS. Such positivity gradually returned to baseline levels at 75–80 ms. From 90–95 ms a negativity was observable near the site of stimulation representing the increase in N100 amplitude, which spread over the adjacent electrodes (100–105 ms) and over the contralateral hemisphere (115–130 ms) before returning to baseline levels (140–150 ms). A sustained positivity was also observable over the stimulated hemisphere starting from 160 ms. In experiment 2 (right), no significant difference between the post- and pre-rTMS blocks were appreciable.

This conclusion would be compatible with the effect of 1-Hz rTMS on two EMG measures of GABAergic excitability in M1. These are (1) short interval intracortical inhibition (SICI) between a pair of TMS pulses, and (2) the cortical silent period (CSP) which describes the reduction in on-going EMG activity that follows the MEP. The former depends on GABA<sub>A</sub> activity whereas the latter depends on GABA<sub>B</sub>. Previous work has shown that when 1-Hz rTMS suppresses MEPs, it is usually accompanied by no change in the GABA<sub>A</sub>-ergic SICI (e.g. Daskalakis et al., 2006) whereas there may be an increase in duration of the GABA<sub>B</sub>-ergic CSP (Daskalakis et al., 2006; Lang et al., 2006). Thus the tentative conclusion would be that 1-Hz rTMS over M1 increases the depth and/or duration of the GABA<sub>B</sub>-ergic IPSP that follows a single pulse of TMS.

It is important to remember that MEPs are an indirect measure of pyramidal tract excitability, since they are affected by a combination of cortical, subcortical and spinal mechanisms, whereas TEPs are the direct result of activating excitatory and inhibitory postsynaptic potentials (Ilmoniemi et al., 1997). This could explain the absence of a significant correlation between changes in MEP and N100/P60 amplitudes in our data. Indeed, it has been found that reliable TEP patterns are evoked even at subthreshold intensities at which MEPs are not elicited, indicating that TEPs and MEPs reflect two separate indices of the neurophysiological state of a stimulated area (Komssi et al., 2004; Van Der Werf and Paus, 2006).

One possible confound to the present explanation is that since we used suprathreshold TMS pulses to evoke the TEPs, changes in afferent input from the induced muscle twitch following rTMS could contribute to changes in P60/N100 amplitude. However, this seems unlikely, since MEPs were smaller after rTMS so that the contribution of afferent input

should if anything decrease whereas the P60/N100 increased in size. Another possible confound is changes in general arousal or to an expectancy effect that can change EEG alpha and/or slower activity, and secondarily affect TEP amplitudes. As demonstrated by previous studies, low-frequency rTMS increases ipsilateral cortico-cortical and inter-hemispheric alpha coherence but not its amplitude (e.g. Strens et al., 2002). The effect was also spatially specific, and therefore unrelated to general arousal. Finally, if rTMS were having any general effects on expectation or arousal we might have expected to observe them equally well after V1 stimulation, but this was not the case.

Van Der Werf and Paus (2006) found that 0.6-Hz rTMS over M1 reduced the amplitude of the N45 potential without affecting MEPs. This effect was postulated to be due to activation of inhibitory interneurons that synapse on pyramidal neurons, an interpretation which is in line with the present study. They did not report any modulation of N100 although they interpreted it as being an auditory neural response to the TMS coil click. However, there are several reasons for excluding this hypothesis: (1) some studies found a TMS-evoked N100 in deaf participants (Kimiskidis et al., 2008; Ter Braack et al., 2013); (2) Bonato et al. (2006) did not find an N100 component with a placebo coil nor with a suboptimal orientation of a normal coil (135°); (3) several studies found no difference in the amplitude of TEPs with or without auditory masking, suggesting that the amplitude of the auditory response is negligible compared to the N100 TMS-evoked response (e.g. Nikulin et al., 2003; Komssi et al., 2004); (4) Lioumis et al. (2009) demonstrated that the amplitude of the N100 evoked over the motor cortex was five times larger than the potential evoked in the prefrontal cortex using the same intensity of stimulation (i.e. the same coil click),

and such difference cannot be explained in terms of auditory potentials; (5) the distribution of the N100 is not compatible with an auditory potential distribution (Näätänen and Picton, 1987; Ponton et al., 2001). Finally, a very recent study by Helfrich et al. (2013) reported a decrease in the TMS-evoked N100 after 15 min of 1-Hz rTMS in a group of ADHD children. This result was attributed to reduced inhibition following 1-Hz rTMS. At first glance, this result seems to be in contrast to our findings. However, Moll and colleagues (2000, 2003), testing LICI with paired-pulse TMS, had demonstrated that ADHD children a) show a significant reduction in intracortical inhibition and b) report an opposite effect of methylphenidate on the balance of intracortical facilitation/inhibition compared to healthy controls. It may therefore be that rTMS has a different effect on cortical inhibition in ADHD than in healthy adults and this could explain the discrepancy with the present results.

## Conclusions

In conclusion, we have shown that application of 1-Hz rTMS in healthy volunteers reduced MEPs whilst increasing the P60 and N100 components of the TEP. Previous work suggests that these EEG components may be caused by or modulated by GABA<sub>B</sub>-ergic activity evoked by the TMS pulse. We propose that 1-Hz rTMS over M1 increases the amplitude or duration of the GABA<sub>B</sub> IPSP evoked by single pulse TMS over the same area. The present findings could be of relevance for diagnostic and therapeutic purposes, particularly for pathologies characterised by disorders in cortical excitatory/inhibitory processes.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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