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Transcranial magnetic stimulation and cortical evoked potentials: A TMS/EEG co-registration study

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Abstract

Objective: In recent years, a promising tool has been introduced which allows the co-registration of electroencephalographic (EEG) activity during brain transcranial magnetic stimulation (TMS). The aims of the present study are to identify eventual stimulus-related artefacts, and to confirm and extend previous EEG/TMS findings about the possible networks generating EEG responses evoked by TMS.

Methods: Focal TMS was delivered to the left primary motor cortex (MI), with different coils (real and sham) and orientations (45 and 135° in respect to the sagittal plane), in six healthy subjects. EEG and motor evoked potentials (MEPs) were simultaneously recorded from 19 scalp electrodes.

Results: TMS, with coil oriented at 45°, induced EEG responses characterized by a sequence of positive deflections peaking at approximately 14, 30, 60 and 190 ms and negative deflections peaking at approximately 10, 18, 40 and 100 ms post-TMS. The negative components were recorded at the recording electrode corresponding with the stimulation site (N10, N18), as well as at recording electrodes over the frontal region of the contralateral, unstimulated, hemisphere (N40) and bilaterally over the central hemispheres with its maximal representation at the stimulation site (N100). The positive components were instead detected at the frontal region of the right, unstimulated, hemisphere (P14), over the central electrodes Cz, Fz and the frontal region of the right hemisphere (P30), at the stimulation site (P60), and over the frontal regions of both hemispheres. When TMS was delivered with the coil oriented at 135°, no MEPs were recorded from the right target muscle. Nonetheless, all the TMS-induced EEG components were still evoked apart from the N20–P30. Finally, TMS with the sham coil over left MI did not induce either significant EEG responses or MEPs.

Conclusions: In conclusion, the TMS evoked components we have obtained by recording in continuous mode strikingly fit with those already described by other authors for both their latencies and the spatio-temporal pattern of scalp distribution.

Significance: This experiment is a farther validation of the combined EEG/TMS recording technique as a promising tool for experimental and clinical purposes.

Keywords: TMS; rTMS; Low frequency; Motor cortex; Electroencephalography; EEG

1. Introduction

Transcranial magnetic stimulation (TMS) is an electrophysiological technique which allows the investigation of the functional state of the human cerebral cortex. By means of rapidly changing magnetic fields, electric currents are induced in the brain and these, in turn, produce transsynaptic depolarisation of pyramidal neurones located in the superficial cortical layers (Heller and van Hulsteyn, 1992). When delivered over the primary motor cortex (MI) with adequate intensity, magnetic stimuli induce neural efferent volleys along the corticospinal pathway and electromyographic responses, termed motor evoked potentials (MEPs),

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can be recorded from the muscles contralateral to the site of stimulation (Barker et al., 1985). Amplitudes and latencies of MEPs are parameters that allow the evaluation of the functional state of the corticospinal pathway, thus providing valuable information about the functioning of motor pathways in both physiological and pathological conditions (Barker et al., 1986; for review see Rossini and Rossi, 1998). In general, MEPs induced by TMS are the result of a combination of excitatory/inhibitory events occurring at different neural levels along the motor pathway and the relative contribution of these events in determining the characteristics of MEPs is far from clear.

In recent years, TMS has also been used for the stimulation of non-motor cortical areas and it has been shown that it can influence the activity of brain centres distant from the stimulated site, presumably via cortico–cortical connections—e.g. cerebello–cortical and transcallosal (Amassian and Cracco, 1987; Meyer et al., 1998). In the same vein, it has been demonstrated that individual stimuli on parietal cortex can affect sensory perception from the hands (Cohen et al., 1991; Seyal et al., 1995, 1997; Oliveri et al., 1999, 2000), while trains of repetitive TMS of the prefrontal cortex (PFC) can influence the clinical outcome of patients affected by mood disorders (George et al., 2000; Miniussi et al., 2005; Pascual-Leone et al., 1996; Schlaepfer et al., 2003) and/or can modulate higher cognitive performances in healthy humans (Walsh and Cowey, 2000). However, there is much less evidence concerning the specific effects of TMS when delivered to cortical areas other than MI because, in those instances, no direct behavioural (i.e. muscle twitch) and electrophysiological (MEPs) information can be gathered as causal demonstration of TMS-induced changes. In summary, traditional recordings during TMS completely miss all the 'corollary' discharges of distant neurons that are connected to those primarily depolarised by the TMS. Indeed, the possibility to disentangle focal from distant effects induced by TMS upon different structures of the CNS may have valuable implications for both clinical and experimental purposes. For example, it might provide information about the pathophysiology of neurological diseases which are supposed to be cortical in origin, and/or have valuable implications in the field of behavioural sciences where TMS is used for mapping higher cognitive functions. Recently, neuronavigated TMS systems have been introduced that can localise with a high degree of precision the cerebral site first impacted by the stimulation. Thus, the exact location of the stimulating coil can be computed with respect to the underlying brain structure. However, this does not imply direct knowledge of the volume, position in space and the timing of activation of those brain areas that are directly or indirectly influenced by the stimulus. Therefore, investigating the facilitation or inhibition of a given brain area by TMS should provide valuable opportunities for better understanding TMS-induced effects.

The first approach towards this objective evaluated cortical TMS-induced metabolic changes with positron emission tomography (PET) and blood oxygen level TMS-dependent changes with magnetic resonance imaging (MRI) (Baudewig et al., 2001; Bohning et al., 1999; Fox et al., 1997; Nahas et al., 2001; Oliviero et al., 1999; Paus et al., 1997, 1998; Siebner et al., 2000). Unfortunately, although these techniques have excellent spatial resolutions, they suffer from poor temporal discrimination since they can detect changes arising a few seconds (fMRI) or even minutes (PET) post-stimulus, but are insensitive to those occurring in the first tens of milliseconds post-TMS, i.e. the temporal window during which profound and function-related TMS-induced neural events take place.

In recent years, a promising tool has been introduced that permits the co-registration of the electroencephalographic (EEG) activity—which has a temporal resolution of a few milliseconds—during TMS, thus providing valuable information about the characteristics of cortical reactivity and connectivity in response to magnetic stimuli (Ilmoniemi et al., 1997; Virtanen et al., 1999). In this procedure, a sample-and-hold circuit locks the EEG signal for several milliseconds immediately post-TMS, thereby avoiding saturation of the recording amplifiers by the magnetic stimuli, and thus allowing the recording of EEG activity to take place very early in response to TMS. This technique has demonstrated that TMS produces evoked EEG responses peaking at precise and repeatable latencies after stimulation (Bender et al., 2005; Ilmoniemi et al., 1997; Kähkonen et al., 2004, 2005; Komssi et al., 2002, 2004; Nikulin et al., 2003; Paus et al., 2001). It has been suggested that the temporal evolution of the distribution of TMS-induced EEG components over the scalp reflects the spread of activation from the stimulated cortical site to ipsilateral and contralateral cortical areas via intra and interhemispheric cortico–cortical fibres, although the contribution of subcortical structures cannot be excluded (Ilmoniemi et al., 1997; Kähkonen et al., 2004, 2005; Komssi et al., 2002, 2004; Paus et al., 2001).

In the present study, we used a very similar approach to study the reactivity and connectivity of the cerebral cortex to TMS by using TMS-compatible EEG equipment that can work in very high, time-varying magnetic fields without saturation and does not make use of particular devices to pin the amplifier output to a constant level during and after stimulation. Further aims of the present study were to identify any stimulus-related artefacts and to confirm and extend previous EEG/TMS findings by manipulations designed to explore possible cortical generators and cortico-cortical networks responsible for the EEG-evoked components induced by TMS.

2. Materials and methods

2.1. Procedure and subjects

Six drug-free healthy subjects (three males and three females, mean age 28 years) were enrolled after giving

written informed consent. All experimental protocols had been approved by the local Ethics Committee.

Real and sham-TMS was applied over the left MI during multichannel EEG recording so that magnetic stimuli were delivered at the same time as EEG data collection. Each subject underwent a 30 min experimental session consisting of three 10 min blocks of TMS delivered at a frequency of 1 Hz. Subjects wearing ear plugs were seated in a comfortable armchair in an electrically insulated and sound-proof room with their hands pronated in a relaxed position and eyes open. The stimulating coil was fixed in the same position with respect to the site of stimulation throughout experiment by means of a mechanical support. Two blocks of sham magnetic stimuli (sham1-TMS and sham2-TMS, respectively) interleaved by one block of real stimulation (real-TMS) were delivered for each subject. Subjects were not informed about the type (sham or real) of TMS.

2.2. Stimulation

TMS was carried out by a Magstim SuperRapid magnetic stimulator and a figure-of-eight coil having an outer winding diameter of 70 mm (Magstim Company Limited, Whitland, UK). In the present protocol individual biphasic stimuli were employed. The coil was placed tangentially on the scalp with the handle pointing backwards and laterally at a 45° angle away from the midline, approximately perpendicular to the line of the central sulcus. With this coil orientation the lowest motor threshold is achieved, thus suggesting that the flow of the induced electric current in the brain is optimal for stimulation of MI (Brasil-Neto et al., 1992; Mills et al., 1992). The coil was moved in steps of 0.5 cm around the fronto-central regions of the scalp in order to find the best position—'hot spot'—for inducing maximal MEPs from the abductor pollicis brevis (APB) muscle of the right hand. After finding the hot-spot, the coil was stabilized and immobilized by means of a mechanical support and the motor threshold was determined as the lowest stimulus intensity which produced at least five MEPs of 50 μV out of ten consecutive stimuli (Rossini et al., 1994, 1999).

To localize the stimulated point on the subject's scalp before TMS, we used the SofTaxic Evolution Navigator system (E.M.S., Bologna, Italy), which works on the basis of digitised skull landmarks (nasion, inion and two pre-auricular points) from which 40 uniformly distributed points can be mapped out on the scalp (3D Fastrak Polhemus digitiser) and related to cerebral anatomy. Although individual radiological head images (MRIs) were not available, Talairach coordinates of cortical sites underlying coil locations were estimated for each subject by the SofTaxic Evolution Navigator system, on the basis of an MRI-constructed stereotaxic template (accuracy ∼1 cm, Talairach space).

For the sham-TMS conditions, the Magstim Placebo Coil System was used. Designed to replicate the standard figure-of-eight coil, the Placebo Coil provides slight sensory stimulation and discharge noise quite similar to the real TMS without stimulating cortical tissue. Its magnetic field output is about 10fold lower in respect to that delivered by the standard coil (maximal magnetic field strength 0.2 T for the Placebo Coil vs. 2.2 T for the Standard Coil).

Magnetic stimuli were delivered at 110% of resting motor threshold, and the same intensity of stimulation was maintained in the three TMS blocks. The frequency of stimulation was settled at 1 Hz constant rate so that a total of 600 stimuli were delivered for each block of stimulation. In this preliminary study, we decided to use many more magnetic pulses than in previous studies (Ilmoniemi et al., 1997; Kähkonen et al., 2004, 2005; Komssi et al., 2002, 2004; Paus et al., 2001) in order to reach an optimal signal-to-noise ratio of the TMS-evoked EEG responses and to confirm their stability in successive trials. In addition, the number we used (600 pulses) is very close to that used for therapeutic purposes in a single session (Miniussi et al., 2005), and we were interested in seeing what type of response this amount of stimuli could elicit directly from the brain (although the repetition rate used here was much lower than that used therapeutically). Given the constant rate of stimulation, subjects could easily predict the timing of TMS delivering in all conditions. Each subject underwent a block of sham-TMS first (sham1-TMS), followed by a block of real stimulation (real-TMS) and a second block of sham stimulation (sham2-TMS). The different blocks of stimulation were separated by stimulus-free intervals of 2 min duration to allow replacement of the coils.

In a separate session of the study, the same subjects were re-evaluated by delivering a single block of real-TMS. Again, the hot-spot was localized, parameters and procedures of stimulation being the same of those utilised in the first part of the study except for the positioning of the coil. In fact, in this part of the study, the coil was rotated and fixated at a 135° angle with respect to the sagittal plane with the handle pointing forward and laterally by keeping its centre over the optimal scalp position first determined with the coil oriented 45° away from the midline. This particular placement of the coil was chosen because Mills et al. (1992) previously demonstrated that it is not optimal for stimulation of MI. This was confirmed by the fact that, with this particular orientation of the coil, no MEPs could be elicited from the contralateral APB when the intensity of stimulation was set at the same value as that used in the first part of the study.

2.3. EEG recordings

TMS-compatible EEG equipment (BrainAmp 32MRplus, BrainProducts GmbH, Munich, Germany) was used for recording TMS-evoked potentials from the scalp. The EEG activity was continuously acquired from 19 scalp

sites using electrodes, mounted on an elastic cap, positioned according to the 10–20 International System (Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, O2). Additional electrodes were used as ground and reference. The ground electrode was positioned in OZ in order to have maximal distance from the stimulating coil. The linked mastoid served as the active reference for all electrodes.

The signal was bandpass filtered at 0.1–500 Hz and digitised at a sampling rate of 2.5 kHz. In order to minimize overheating of the electrodes located in the vicinity of the stimulating coil, TMS-compatible Ag/AgCl-coated electrodes were used. Skin/electrode impedance was maintained below 5 kΩ.

Horizontal and vertical eye movements were detected by recording the electrooculogram (EOG). The voltage between two electrodes located to the left and right of the external canthi recorded horizontal eye movements. The voltage between reference electrodes and electrodes located beneath the right eye recorded vertical eye movements and blinks. Magstim and BrainAmp were linked using TTL based trigger interfaces. Electrical correlates of the TMS field were recorded by the scalp electrodes. The design of the BrainAmp MRplus allows the fine adaptation to the TMS stimulus magnitude by selection of amplifier sensitivity and operational range. This was done by using a sensitivity of 100 nV/bit (signal range/resolution) and an analogue/digital-conversion range of 6553.5 μV (± 3.277 mV) which is sufficient to prevent saturation under the given stimulus conditions. Therefore, a continuous recording mode without any SSH-circuits was chosen (i.e. no sample and hold).

The epoching of the TMS-related scalp EEG responses was performed off-line. Epochs started 100 ms before and ended 1000 ms after TMS onset. Epochs with excessively noisy EEG or eye-movement artefacts (blinks or saccades) were rejected. Separate averaged ERP waveforms were constructed for each condition of stimulation. The actual mean number of trials contributing to final averages ranged between 450 and 500. EMG activity and MEPs from the right APB were recorded via surface electrodes in belly tendon montage; signals were sampled at 2.5 kHz and bandpass filtered at 50–1000 Hz.

3. Results

Real TMS of the left MI with the coil oriented at 45° with respect to the sagittal plane evoked EEG activity lasting up to 200 ms. This TMS-induced EEG activity resulted in a sequence of evoked-EEG responses consisting of a series of deflections of alternating positive and negative polarity starting a few milliseconds post-stimulation. Fig. 1a illustrates the distribution of the TMS-induced EEG evoked components at the recording sites. It can be seen that, after an initial large artefact probably due to currents induced by the magnetic field, the strongest EEG responses to TMS were recorded at the left-central electrodes, which are more proximal to the stimulation site. The amplitudes of the EEG responses evaluated with respect to their maxima varied in all subjects between ±20 μV, while the artefact varied on average between −2500 and +1750 μV.

As illustrated in Fig. 1b, the EEG signals were composed of downgoing (positive polarity) deflections which occurred at approximately 14, 30, 60 and 190 ms post-stimulation (P14, P30, P60, P190, respectively) and negative deflections peaking at approximately 10, 18, 40 and 100 ms post-TMS (N10, N18, N40 and N100, respectively).

The equipotential maps (Fig. 2a), after an initial large artefact (4–6 ms) corresponding to the stimulation site, showed an immediate localized response of negative polarity peaking at 9.5±0.5 ms. At 14±1 ms, a positive component appeared more anteriorly (approximately at the F3 electrode) rapidly expanding to the frontal region of the contralateral hemisphere, thus forming a dipolar field with positive front located anteriorly and the negative front located posterior to it. The negative component peaked at 18±1 ms corresponding to the stimulation site. This dipolar scalp distribution then evolved into a widespread positivity that had its maximum over the central electrodes Cz and Fz and corresponded to the frontal regions of the hemisphere contralateral to the stimulation site, peaking at 28±2 ms. At 41±2 ms a frontal negativity with its maximum at the hemisphere contralateral to the stimulation developed, followed by a large positivity corresponding to the stimulation site peaking at 56±3 ms. The latter two TMS-induced EEG components were represented by a large negativity occurring at 105±15 ms followed by a positive field occurring at 187±8 ms. The former component had a wide distribution in particular over the central electrodes of both hemispheres and peaked at the stimulation site while the latter component was essentially distributed over the anterior scalp regions peaking at the frontal electrodes of the stimulated hemisphere. In this set of stimuli, MEPs of variable amplitude were elicited in the target APB muscle.

In order to verify whether any correlation exists between the amplitude of MEPs and the amplitude of the early TMS-induced EEG deflections (N10, N18 and P30), five series of 100 responses obtained in response to magnetic stimuli were performed for each subject and subjected to regression analysis. No significant correlation was found between the mean amplitude of MEPs and the mean amplitude of any of these TMS-induced EEG potentials ($r=0.01$ for MEPs/N10; $r=0.13$ for MEPs/N20; $r=0.46$ for MEPs/P30).

In contrast to real-TMS, both Sham1 and Sham2 stimulation of the left MI neither elicited recordable MEPs nor relevant EEG responses (Fig. 1a and b) except for a clear positive component recorded at 14 ms post-stimulation and which was observed at all the recording electrodes. Nevertheless, as can be seen from Fig. 1a and b, a large artefact was present in sham conditions resembling

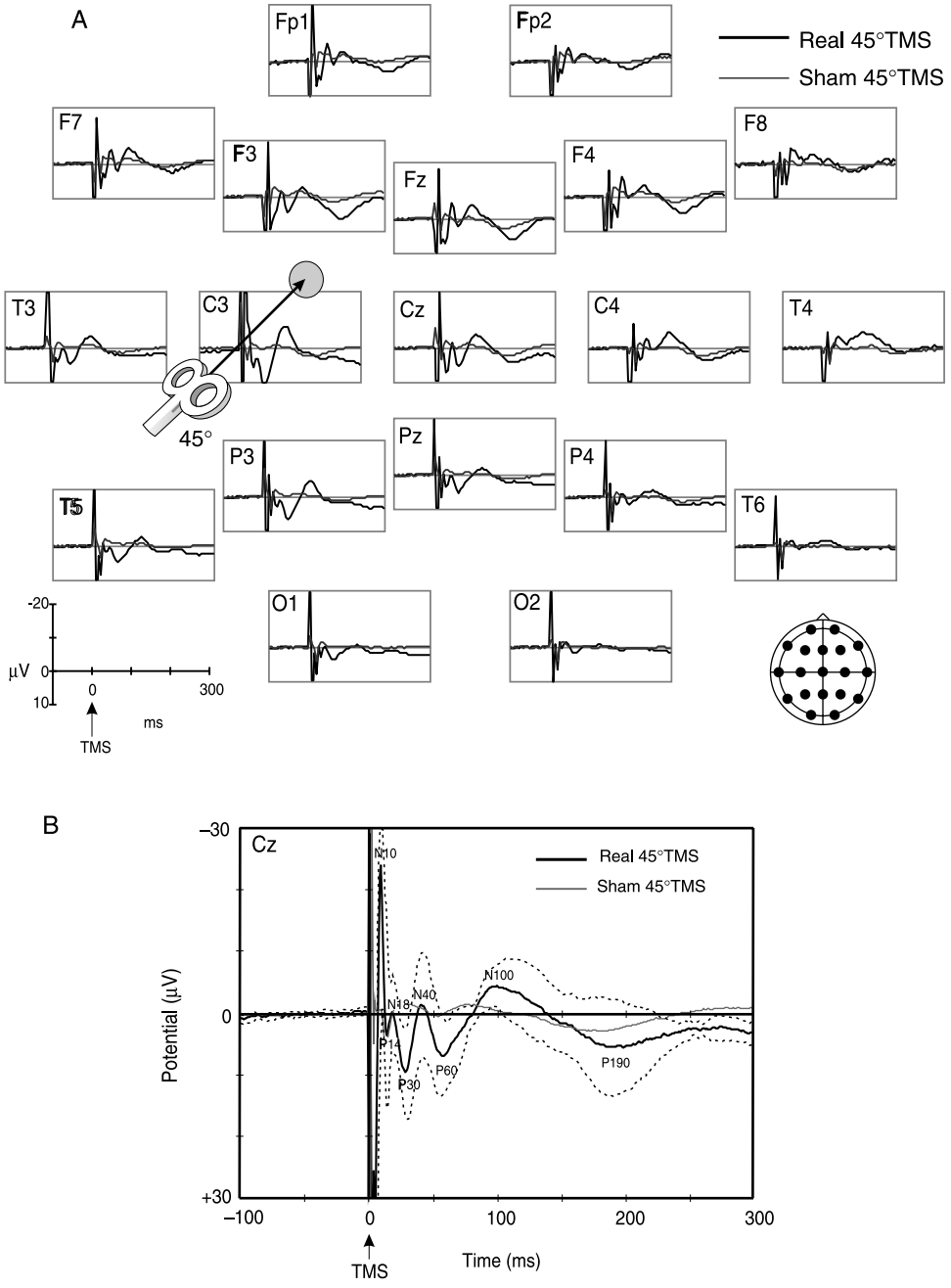

Fig. 1. (A) Grand average of the electroencephalographic (EEG) responses from 100 ms pre to 300 ms post-transcranial magnetic stimulation (TMS) at all scalp locations recorded during real-TMS and Sham-TMS. This figure refers to stimulation of the left primary motor cortex (MI) performed with the coil oriented 45° away from the midline and with the handle pointing backwards and laterally. The grey point indicates the site of stimulation (between F3 and C3), while the arrow indicates the orientation of the coil in respect to the stimulation site (45° to the sagittal plane). The electrode montage used for the experiment is shown at the bottom. Polarity of the waveforms is plotted with negative values upward in this and subsequent figures. The two Sham-TMS conditions (Sham 1-TMS and Sham 2-TMS) have been averaged. (B) Grand average of the EEG responses recorded at the vertex (Cz) during the real-TMS (thick solid line) and the Sham-TMS (thin solid line) conditions of the left MI performed with the coil oriented 45° away the midline and with the handle pointing backwards and laterally. Standard deviation of real TMS is also shown (dashed line). The onset of the TMS stimulus (at 0 ms) is labelled. Main features are marked in these sample waveforms for orientation. The two Sham-TMS conditions (Sham 1-TMS and Sham 2-TMS) have been averaged.

the one induced by real stimulations, and several small sham-evoked deflections were present at about 25, 50, 80, and 180 ms. This was probably due to the magnetic field and click induced by sham coil that elicited a barely detectable cortical response. No differences in latency and amplitude of this component were observed between Sham1 and Sham2-TMS.

When TMS was delivered to the left MI with the coil oriented at a 135° angle with respect to the sagittal plane and with the handle pointing forward and laterally, no MEPs were

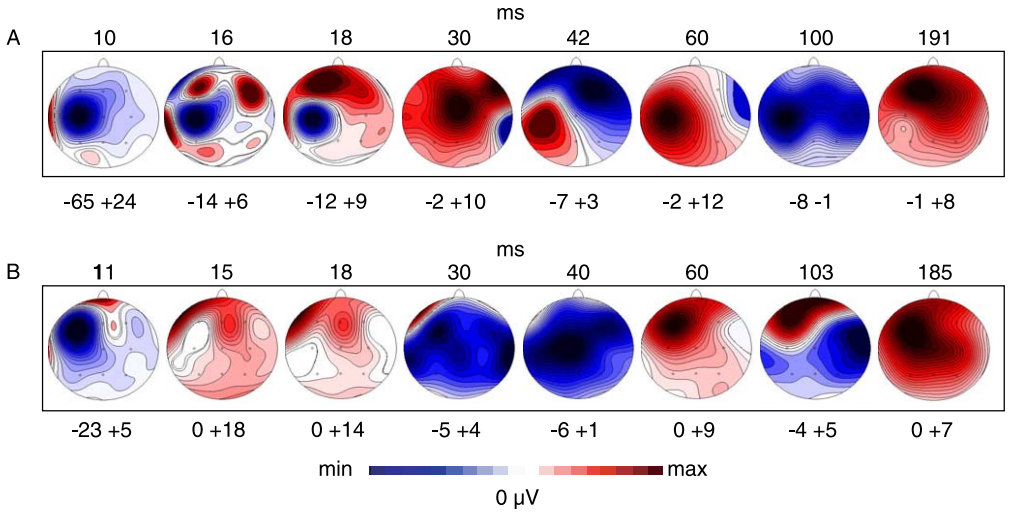

Fig. 2. Scalp distribution maps of the grand average potentials recorded at selected intervals during real-TMS of the left MI with the coil oriented 45° (A) and 135° (B) away from the sagittal plane. Numbers at the top of each map indicate time after TMS and numbers at the bottom indicate voltage of evoked responses. Red colour represents maximum relative positive voltage and blue represents maximum relative negative voltage.

recorded from the right APB target muscle (Fig. 3b). Nonetheless, some TMS-induced EEG components were still evoked in all subjects (Fig. 3a). In particular, the EEG signals were composed of positive deflections occurring at about 14, 55 and 170 ms (P14, P6 and P180, respectively), and negative deflections peaking at about 10, 35 and 100 ms (N10, N40, and N100, respectively). With respect to the condition where TMS was delivered with the coil oriented with the handle pointing backward and laterally at 45° to the sagittal plane, components N20–P30 were missing. As shown in Fig. 2b, after an initial huge artefact at 4–6 ms post-stimulus, a negative response was induced at 8–10 ms (9 ± 2 ms). At 14–16 ms a positive field was recorded reaching its maximal representation at the more anterior frontal electrodes (FP1 and F7) of the stimulated hemisphere. This positivity was then followed by a negative potential which was distributed over the central electrodes of both hemispheres and peaked at about 40 ms post-stimulation (39 ± 3 ms). A positive potential was then recorded at about 60 ms post-stimulus with maximal distribution at the stimulation site. The latter two components were recorded at about 100 and 180 ms post-stimulation and were composed of a weak negative deflection peaking at the central regions of the right, unstimulated hemisphere, and a more pronounced positive field distributed at the frontal and central electrodes with maxima representation over the left, stimulated, hemisphere. As can be seen from Fig. 3a, starting from 60 ms the response evoked by this condition somehow resembled those of the sham condition.

4. Discussion

When real TMS is delivered with the optimal coil orientation for activation of the left MI (45° in respect to the sagittal plane with the handle pointing backward and laterally), it elicits a series of positive and negative deflections on the scalp EEG, whose latencies and scalp distributions closely fit those already described by other authors using a different recording apparatus and experimental set-up (Ilmoniemi et al., 1997; Komssi et al., 2002, 2004; Paus et al., 2001). Of interest, these TMS-evoked EEG potentials have been obtained by using a recording apparatus which allows continuous data recording without saturation of the EEG signals and does not require pinning the preamplifier output to a constant level during TMS delivery. Thus, the technique used here, the continuous recording of the TMS, enables one to observe not only the evoked brain reaction but also the temporal variation and spatial distribution of the TMS field.

The origin of these TMS-evoked cortical potentials has been little explored. The immediate localized response occurring at 8–10 ms post-stimulus likely reflects activation of the stimulation site although its selective origin from neural structures of the cerebral cortex is hard to explore because of possible muscular contamination due to direct stimulation on scalp muscles or nerves or, perhaps, because of residual current induced in the electrodes.

The high-amplitude deflection of negative polarity at about 18 ms post-stimulus under the coil that evolved into a dipolar scalp distribution with positivity located anterior to the stimulation site a few milliseconds thereafter may represent excitatory events at the precentral gyrus, thus reflecting sustained activation of MI induced by TMS. This hypothesis, already proposed by Komssi and collaborators (Komssi et al., 2004) would be supported by the observation that no such activity was induced when TMS was delivered over the left MI with the coil oriented at an 135° angle and with an antero-lateral handle in respect to the sagittal plane, i.e. with a placement that induces electric currents not suited

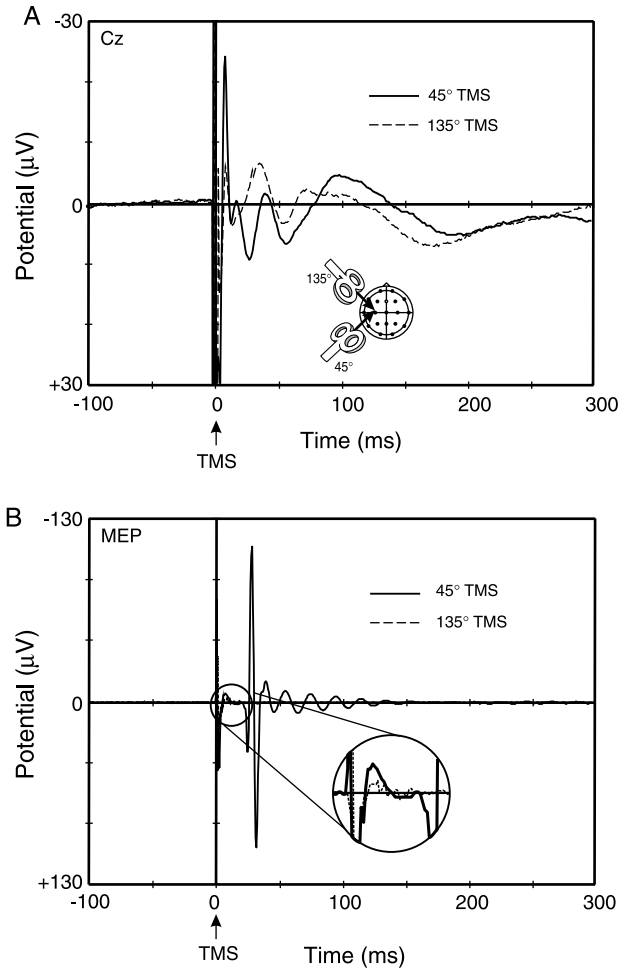

Fig. 3. (A) Grand average of the TMS-evoked responses recorded at the vertex (Cz) during real-TMS of the left MI performed with the coil oriented 45° away from the midline and with the handle pointing backwards and laterally (solid line) and with the coil oriented 135° away from the sagittal plane and with the handle pointing forwards and laterally (dashed line). The onset of the TMS stimulus is labelled. Note the absence of the N18-P30 component in this latter condition. Solid curve is the same as in Fig. 1B. (B) Grand average of the Motor Evoked Potential during real-TMS of the left MI performed with the coil oriented 45° (solid line) and with the coil oriented 135° (dashed line). Note the absence of response in the 135° condition. Modulation of the MEP component is magnified.

for activation of the MI, leading to disappearance of MEPs from the 'target' muscle (Brasil-Neto et al., 1992; Mills et al., 1992).

The positive deflections we observed to occur at about 14–16 and 26–30 ms post-stimulus and which were maximal at the fronto-central electrodes of the right, non-stimulated hemisphere, may correspond to those described to occur at the central electrodes contralateral to the site of stimulation (Komssi et al., 2002). These authors found that maximal activation of the contralateral cortex appeared at 17–28 ms post-stimulus and concluded that it may be expression of an interhemispheric spread of activation via the corpus callosum or a subcortical pathway. An alternative hypothesis may be that the first contralateral positive signal 14–16 ms post-stimulation may indeed represent an engagement of the contralateral cortex via callosal connections directly excited by magnetic stimuli, while the second positive deflection peaking at 26–30 ms might involve subcortical pathways, perhaps the non-specific thalamic nuclei and/or basal ganglia which, in turn, project back diffusely to the cortex. This hypothesis would best agree with studies on interhemispheric nervous propagation using electrical and TMS techniques and where conduction times of 12–15 ms on average were found (Amassian and Cracco, 1987; Boroojerdi et al., 1998; Cracco et al., 1989; Ferbert et al., 1992; Meyer et al., 1998). In any case, it has to be pointed out that the former positive component (P14) is also recorded during Sham-TMS, thus suggesting that it represents an artefact—muscular and/or acoustic in origin—rather than a real neural response directly induced by TMS. In contrast, the fact that the latter P30 component was obtained only with optimal stimulation of MI (coil oriented 45° to the sagittal plane), and not with suboptimal MI stimulation (coil orientated 135° to the sagittal plane) or during Sham-TMS, strongly supports the contention that it has a cortical origin.

The lack of any correlation between MEP amplitude and the amplitudes of the short-latency TMS-induced EEG potentials could reflect the contribution of neural structures located beyond the cortical level, especially at the spinal level, to MEP generation. In fact, while MEP amplitude is greatly affected by the excitability levels of the spinal motoneuronal pools, no spinal contributions are supposed to take place in determining the TMS-induced EEG-evoked responses. Indeed, it is well known that MEPs are extremely variable in amplitude when collected over time despite very stable experimental conditions in relaxed subjects (Kiers et al., 1993) and part of this variability is related to spontaneous changes in the excitability of the spinal motoneuron pools recruited by the cortical efferent volley induced by TMS.

At about 42 ms after TMS a negative component was recorded forming a dipole centred over the stimulation site with the positive pole lying posterior to the negative one. Such a latency might be compatible with somatosensory evoked potentials generated as proprioceptive and cutaneous feed-back from the TMS-induced twitch in the hand muscles, since the transmission time from both the cortex to hand muscles and from there back to the cortex is about 20 ms each way (40 ms in total). However, this hypothesis is contradicted by the fact that the same negative component, although with different scalp distributions, was also recorded when the coil was oriented 135° to the sagittal plane even thought this condition produced no recordable muscular activation. It has been proposed that the neural generator of the N40 is located in the sulcal part of the MI (Komssi et al., 2004) and that this component might represent the resetting of the ongoing rhythmicity from a local pacemaker activated by TMS (Paus et al., 2001). However, once again the finding that a negative component

with a similar latency was also obtained during suboptimal stimulation of MI does not support this hypothesis, unless one argues that such local rhythmicity is not directly linked with MEP elicitation in the target muscle. In any case, the 135° orientation stimulation presumably is not stimulating MI directly, so that without the knowledge of the actual site of stimulation it is difficult to rule out that potentials such as the N40 are not due to proprioceptive feed-back or resetting of the local pacemaker. On the other hand, it should be pointed out that the scalp distribution of N40 induced by suboptimal stimulation of MI is different from that obtained after effective stimulation of MI since the latter is distributed over the whole scalp with maximal representation over the central regions. It is therefore possible that different neural structures either separate from MI (e.g. cingulate areas) or functionally connected to it (e.g. pre-motor and supplementary motor cortices) recruited by the suboptimal stimulation may be responsible for this negative component.

In previous experiments the N100-P190 complex has been associated with the sound emitted by the discharging coil (Nikouline et al., 1999; Tiitinen et al., 1999), although later studies, that used white noise during stimulation to mask the coil click, have partially excluded such possible contamination (Kähkonen et al., 2005; Komssi et al., 2004; Paus et al., 2001). Our study adds further support to the possibility that these components might, at least in part, originate from cortically TMS-induced electric potentials as already proposed (Komssi et al., 2004). In fact, we did not find an N100 component during stimulation, neither with the placebo coil system nor in the 135° condition comparable to real 45° stimulation. Actually the sham coil produces a detectable click that it is not equal to the click produce by a real coil, nevertheless the coil oriented at 135° does produce the same click as the real coil. A different possibility is that during sham or real 135° conditions, the subjects were paying less attention to the 'stimulation apparatus' since no hand twitch was produced and therefore this component was reduced in amplitude. To verify such hypothesis we compared the responses to the first part of the experiment, where the attention of the subject should be higher, to the last part (an averages of 100 sweeps in each condition were considered). No differences were present between these two averages suggesting that an attentional hypothesis cannot account for the lack of this component. In contrast, the P190 component was of comparable amplitude in both real (45° and 135°) stimulation conditions. Nonetheless, the possibility that this late TMS-induced component can originate from bone-conducted sound cannot be excluded.

In conclusion, EEG responses to TMS were recorded in a continuous mode of signal acquisition. The TMS-evoked components we have obtained fit strikingly with those already described by other authors (Ilmoniemi et al., 1997; Kähkonen et al., 2004, 2005; Komssi et al., 2002, 2004; Paus et al., 2001) both for their latencies and the temporal evolution of their distribution over the scalp. Indeed, an anatomical-functional interpretation of the individual peaks has been proposed. Although some of these TMS-induced evoked components likely originated from the cortically induced electric potentials produced by TMS, their actual neural origin remains to be determined in detail.

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