Human brain cortical correlates of short-latency afferent inhibition: a combined EEG–TMS study

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Ferreri F, Ponzo D, Hukkanen T, Mervaala E, Könönen M, Pasqualetti P, Vecchio F, Rossini PM, Määttä S. Human brain cortical correlates of short-latency afferent inhibition: a combined EEG–TMS study. J Neurophysiol 108: 314–323, 2012. First published March 28, 2012; doi:10.1152/jn.00796.2011.—When linking in time electrical stimulation of the peripheral nerve with transcranial magnetic stimulation (TMS), the excitability of the motor cortex can be modulated to evoke clear inhibition, as reflected by the amplitude decrement in the motor-evoked potentials (MEPs). This specific property, designated short-latency afferent inhibition (SAI), occurs when the nerve–TMS interstimulus interval (ISI) is approximately 25 ms and is considered to be a corticothalamic phenomenon. The aim of the present study was to use the electroencephalographic (EEG) responses to navigated-TMS coregistration to better characterize the neuronal circuits underlying SAI. The present experimental set included magnetic resonance imaging (MRI)-navigated TMS and 60-channel TMS-compatible EEG devices. TMS-evoked EEG responses and MEPs were analyzed in eight healthy volunteers; ISIs between median nerve and cortical stimulation were determined relative to the latency of the individual N20 component of the somatosensory-evoked potential (SEP) obtained after stimulation of the median nerve. ISIs from the latency of the N20 plus 3 ms and N20 plus 10 ms were investigated. In all experimental conditions, TMS-evoked EEG responses were characterized by a sequence of negative deflections peaking at approximately 7, 44, and 100 ms alternating with positive peaks at approximately 30, 60, and 180 ms post-TMS. Moreover, ISI N20+3 ms modulated both EEG-evoked activity and MEPs. In particular, it inhibited MEP amplitudes, attenuated cortical P60 and N100 responses, and induced motor cortex beta rhythm selective decrement of phase locking. The findings of the present experiment suggest the cortical origin of SAI that could result from the cortico–cortical activation of GABAergic-mediated inhibition onto the corticospinal neurons modulated by cholinergic activation able to reducing intralaminar inhibition and promoting intracolumnar inhibition.

EEG; navigated transcranial magnetic stimulation; EEG-TMS coregistration; SAI

MUSCLE RESPONSES RECORDED in the hand muscles after transcranial magnetic stimulation (TMS) of the primary motor cortex (M1) can be modulated in terms of their amplitude when TMS is preceded by an electrical stimulation of the peripheral nerve as long as the time interval between stimulation of the nerve and motor cortex is 2–8 ms longer than the time needed by the peripheral nerve afferent input to reach the cortex (Marionenzi et al. 1991; Tokimura et al. 2000). This effect has a specific time window when it is possible to evoke a clear-cut decrement of motor cortex excitability: this is designated short-latency afferent inhibition (SAI) and is thought to depend on neural interactions within the cerebral cortex (Di Lazzaro et al. 2004; Tokimura et al. 2000), either by direct inhibition of the motor cortex from fast conducting afferents or via withdrawal of tonic facilitation from other structures such as thalamus. Moreover, since this inhibitory phenomenon can be reduced or abolished by intravenous injection of the muscarinic antagonist, scopolamine (Di Lazzaro et al. 2000), it was postulated that SAI might be a noninvasive way of evaluating cholinergic activity in the cerebral cortex (Di Lazzaro et al. 2004, 2005a,b). Release of acetylcholine (ACh) within the neocortex and hippocampus profoundly alters cellular excitability, network synchronization, and behavioral state and, despite its diverse cellular and synaptic targets, the actions of ACh can be highly specific, altering the excitability of distinct inhibitory and excitatory cell types (Lawrence 2008). However, a significant limitation in our understanding of physiological basis of SAI stems from the fact that it has only been indirectly investigated by means of motor-evoked potentials (MEPs) modulation (Bikmulina et al. 2009a; Di Lazzaro et al. 2004, 2005b). Recently a technical device has been introduced that allows recording electroencephalographic (EEG) responses to TMS of a given scalp site within the millisecond temporal resolution range. Combining TMS with EEG enables a noninvasive and direct method for evaluating cortical excitability and connectivity as well as their experimental modulation (Bonato et al. 2006; Daskalakis et al. 2008; Farzan et al. 2009; Ferreri et al. 2011a; Huber et al. 2008; Ilmoniemi et al. 1997; Kahkonen et al. 2003; Massimini et al. 2005; Paus 2005; Paus et al. 2001). The amplitude, latency, and scalp topography of single-pulse TMS-evoked EEG responses have been clearly described (Bonato et al. 2006; Ferreri et al. 2011a; Komssi et al. 2004; Veniero et al. 2010) and are thought to be dependent on the minute current states of the stimulated areas of the cortex (Fuggetta et al. 2005; Ilmoniemi and Kicic 2010; Nikulin et al. 2003; Paus 2005; Thut et al. 2003). Moreover cortical synchronization in the beta range (15–30 Hz) has been observed after single-pulse TMS and associated with the slow evoked EEG responses (Fuggetta et al. 2005; Paus et al. 2001). However, the effect of peripheral stimulation on TMS-evoked EEG responses and induced oscillations is not well known. Therefore, in line with a few previous EEG–TMS studies focused on
cortical mechanisms integrating afferent information (Bikmul-
лина et al. 2009b; Raji et al. 2008; Spies et al. 2010), the purpose of
the present study was to confirm and extend previous preliminary
results (Bikmulлина et al. 2009b), showing that SAI is associated
with the attenuation of the TMS-induced N100 component (Bik-
mulлина et al. 2009b). In particular, we further endeavored to
evaluate the modulatory influence of SAI on cortical oscillations
between 4 and 50 Hz in the healthy human sensorimotor net-
works, with particular attention to the beta oscillations in the light
of recent studies, suggesting that their supporting networks de-
pend critically on the activity of inhibitory neurotransmitters
(Gaetz et al. 2011) as modulated by cholinergic activation.

MATERIALS AND METHODS

Subjects

Eight healthy adults (age 24–45 years; mean 33 years; four men)
were enrolled in the present study. According to the Waterloo Hand-
edness Questionnaire, in its revised and reduced form with 20 items
(Elias and Bryden 1998), all participants were right-handed. All
subjects signed an informed consent form before participating in the
study that was approved by the ethical committee of Kuopio Univer-
sity Hospital. There was strict adherence to the exclusion criteria
established by international safety standards for TMS (Rossini et al.
2009; Rossini et al. 1994).

Experimental Design

During the experiment, the subjects sat in an adjustable chair with
a headrest that ensured a stable head position, and were instructed to
keep their eyes open and to look at a fixation point on a screen in front
of them. The experiment was as follows:

The optimal cortical representation area and the individual resting
motor threshold (rMT) of the right opponens pollicis muscle were
determined. In the first step, using individual magnetic resonance
images, the hand area on the anterior bank of the left central sulcus
was identified. As a second step, the subject’s primary motor cortex
was mapped around the anatomic “hand knob” (Denslow et al. 2005;
Yousry et al. 1997), to find the area evoking the largest response in the
right opponens pollicis muscle. Finally, rMT was defined according to
international guidelines as the stimulator’s output able to elicit repro-
ducible MEPs (at least 50 μV in amplitude) in 5 of 10 consecutive
stimuli (Rossini et al. 1994).

To obtain the actual individual N20 latency for each subject, prior
to the SAI protocol, we recorded somatosensory-evoked potentials
(SEPs) by electric stimulation of the median nerve at the right wrist.
The stimulus intensity was adjusted to be slightly above the motor
threshold for evoking a visible twitch of the thenar muscles (usually
~7 mA). Two hundred responses were averaged to identify the latency of N20 peak over C5 (common average reference; low pass
filter 300 Hz, rate of stimulation 0.2–1 Hz).

ASI was studied using the standard technique (Tokimura et al.
2000). The intensity of the TMS was 120% of the rMT. ISIs between
median nerve and cortical stimulation were determined relative to the
latency of the N20 so that the ISIs corresponding to the latency of the
individual N20 plus 3 ms and plus 10 ms were used (from now on
these will be termed ISI N20+3 and N20+10, respectively). MEPs and
TMS-evoked EEG responses to suprathreshold magnetic stimulus
alone (120% rMT, baseline condition) and to the peripheral condi-
tioning stimulus preceding the cortical magnetic stimulus at ISI
N20+3 and N20+10 were collected. A total of 120 trials were
collected for each condition, with an interstimulus interval jittering
randomly between 4 and 5.5 s. The order of the conditions was
randomized.

MRI-Navigated Transcranial Magnetic Stimulation

TMS was performed with an eXima stimulator and a biphasic
figure-of-eight 70-mm coil combined with a navigation system that
enables continuous visualization of the stimulation site in relation to
the individual cortical anatomic structures (Nexstim, Helsinki, Fin-
land). Three-dimensional individual T1-weighted MR images (repe-
tition time [TR] 1980 ms; time to echo [TE] 3.93 ms; field of view
[FOV] 256 mm; matrix 179 × 256; slice thickness 1.0 mm; Siemens
Avanto 1.5 T, Erlangen, Germany) were used for the navigation.
Three-dimensional scalp surface segmentation of high-resolution Di-
com MR images at the region of the motor knob was used as the
mapping surface. The TMS system delivered trigger pulses that
synchronized the TMS, electromyography (EMG), and EEG systems.

During TMS, muscle activity was monitored online and recorded
by continuous EMG (ME 6000; Mega Electronics, Kuopio, Finland).
Disposable surface electrodes (circular, diameter 24 mm, Ag-AgCl)
were positioned on the right opponens pollicis (OP) and referred to
the first metacarpal bone in the metacarpophalangeal joint. The EMG
signal was recorded at 1 kHz and filtered to the 8- to 500-Hz band for
analysis, amplified, and stored for offline analyses.

Median Nerve Stimulation

Median nerve stimulation was performed at the wrist with 0.1-ms
electrical rectangular pulses (Digitimer model DS7A; Digitimer, Wel-
wyn Garden City, Herts, UK) using a bipolar electrode and an
intensity inducing a painless thumb twitch.

EEG Recordings

The EEG was recorded with a 60-channel TMS-compatible ampli-
dier (Nexstim) continuously throughout the experiments. In the EEG
system, a sample-and-hold circuit was applied together with block-
ing of the amplifier input for 2 ms from the stimulus to avoid amplifier
saturation. The data were recorded with a 1,450-Hz sampling fre-
quency and 16-bit precision. A trigger signal marking the exact
stimulation moments was recorded with the EEG. All electrodes were
referred to an electrode placed on the right mastoid. To mask coil-
generated clicks, a white noise (obtained from the waveform of the
TMS click, digitized, and processed to produce a continuous audio
signal with specific time-varying frequencies [Massimini et al. 2005]),
was continuously delivered through earphones. We adjusted the
masking volume until the subjects reported that the TMS click was no
longer audible.

Data Analysis

Motor-evoked responses. In MEP analyses, the time window was
from −50 to 100 ms from the onset of stimulus with a gain of 100 μV.
Both baseline MEPs and SAI-MEPs were visually inspected offline. If
there was muscle activation preceding the response, the MEP was
excluded. Amplitudes of MEPs for each included trial for each subject
were measured between the two major and stable peaks of opposite
polarity. Average amplitude was calculated for each subject.

EEG. Data analysis was conducted using MATLAB (2008b version
7.7; The MathWorks, Natick, MA) and the public license toolbox
EEGLAB (Delorme and Makeig 2004). EEG data were divided in
segments of 1,000 ms including a 200-ms prestimulus baseline. Both
in the TMS alone trials and in the SAI trials, all TMS-evoked EEG
activity was visually inspected in each channel and trials contami-
nated by environmental artifacts, muscle activity, or eye movement
were rejected. Following this procedure, EEG signals were bandpass
filtered between 2 and 80 Hz, downsampled from 1,450 to 725 Hz,
baseline corrected (100 ms prestimulus), average referenced, and
averaged for each subject.
To examine responses in the time domain and to identify global differences in TMS-evoked activity between the conditions, the global-mean field power (GMFP), which is a measure of global brain activation (Lehmann and Skrandies 1980), was first assessed as the root-mean-squared value of the signal across all electrodes. Then for the analysis of the evoked responses, averaged TMS–EEG responses over all the included trials for each electrode and each subject were used, and semiautomatic amplitude/latency measurements of each component of the EEG-evoked potentials were performed. On these data the General Estimating Equation (GEE) model was applied (see the Statistical Analyses section).

To examine responses in the frequency domain, event-related spectral perturbation (ERSP) and intertrial coherence (ITC) between 4 and 50 Hz were investigated for each subject and for each channel. ERSP measures the modulation of amplitude induced by a specific event (e.g., TMS pulse), relative to a baseline (e.g., prestimulus condition), whereas ITC provided a measure of the synchronization of the TMS-evoked potentials across different trials, independent of signal amplitude (Delorme and Makeig 2004; Ferrarelli et al. 2008); that is, the ITC is an event-phase indicator function. Although the usual averaging measures can reveal information about event-related EEG dynamics that ERP measures neglect, they are also averages and thus ignore trial-to-trial activity differences. The idea behind averaging is that event-relevant brain dynamics that are consistently time-locked to a class of events will be recovered by response averaging, whereas other processes unaffected by the same events will be filtered out by phase cancellation. The response-averaging approach, applied blindly to a set of single-trial data, does tend to reveal dominant, time-, and phase-locked activity (with respect to the time-locking trial events) that is consistent across trials, but it ignores the possible relevance of intertrial variability due to trial-to-trial variations in cognitive processing.

Statistical Analyses

Statistical analyses were computed with Matlab and SPSS for Windows 7 statistical program. The significance of the results was defined as P < 0.05. The following assessments were performed: 1) SAI effects on MEP data, 2) SAI effects on EEG data, and 3) correlation between EEG modulation and MEP modulation.

The MEP amplitudes were analyzed with Bonferroni corrected t-tests comparing MEPs without and with preceding median nerve stimulation (baseline vs. ISI N20 +10; baseline vs. ISI N20 +3; ISI N20 +10 vs. ISI N20 +3).

EEG data analyses were computed for ISI N20 +3 and ISI N20 +10 conditions, given that we found (see the following text) that ISI N20 +10 did not produce SAI on MEP amplitudes (Fig. 1A) and was therefore suitable to be used as a control condition for the ISI N20 +3 (test condition). By using ISI N20 +10 as a control condition instead of baseline condition, we aimed to eliminate potential confounding effects induced by the SEPs (Bikmullina et al. 2009b).

First, we calculated the total brain activation evoked by TMS in both the conditions under evaluation by means of the GMFP. Then the amplitude of TMS-evoked responses was analyzed by means of the GEE model (Ferreri et al. 2011a) to detect significant global effects of condition for each identified evoked peak. GEE was used as a generalization of the General Linear Model, allowing us to model correlated data (due to repeated measures within the same subject for each condition/position/latency). In other words, the GEE approach uses weighted combinations of observations to extract the appropriate amount of information from correlated data. Sidak’s post hoc was applied for the peaks identified by a visual inspection of the TMS-evoked responses (N7, P30, N44, P60, N100, P180; Ferreri et al. 2011a; Veniero et al. 2010) with two factors: EEG electrodes (n = 60) and conditions (two levels: ISI N20 +10 vs. ISI N20 +3). This procedure allowed us to evaluate whether the peaks were being modulated by ISI. Only results indicating statistically significant between-condition main effects are reported. Next, significant local topographic differences in EEG data were assessed by statistical nonparametric mapping (SnPM) (Nichols and Holmes 2002). For ERSP and ITC, in addition to the SnPM, two-tailed bootstrap statistics were applied.

MEP amplitudes were correlated (Pearson, one-tailed) with significant EEG parameters. The amplitudes of the EEG responses and ITC and MEPs in ISI20 +3 were expressed as a percentage of the corresponding values at ISI20 +10.

Sample Size Justification

With regard to the first assessment (SAI effects on MEP data) we relied on previous evidence of large effect size (conventionally, 0.8SD, as that shown by Tokimura et al. 2000). With such assumptions, even a small sample of eight subjects provided an adequate power (86%) of detecting as significant (at bilateral alpha level of 0.05) any change between conditions equal to or above 0.8SD. With respect to the second assessment (SAI effects on EEG data), no formal computation of appropriate sample size was performed. However, each of the eight subjects received 120 stimuli for each condition and a measure was obtained for each of the 60 electrodes and defined latencies (N7, P30, N44, P60, N100, P180) as well as frequency bands (theta, alpha, beta, and gamma). With such a design and in agreement with the counsel of Friston et al. (1999), we should be able (with eight subjects) to address the issue of identifying a “typical characteristic” of brain functionality (Friston et al. 1999).

RESULTS

Motor-Evoked Potentials

Resting motor threshold. The mean of measured rMTs was 48% (range 40%–57%) of the maximal stimulator’s output. ISI and MEP. The characteristic relationship between ISIs and MEP ratios was observed (Di Lazzaro et al. 2004, 2005b; Tokimura et al. 2000; Fig. 1, A and C). The mean values were found to be 1,739 μV (SD 1,157 μV) for a suprathreshold single pulse with an intensity of 120% of the rMT, 790 μV (SD 603 μV) for ISI N20 +3, and 1,848 μV (SD 1,134 μV) for ISI N20 +10. Pairwise comparison with Bonferroni correction revealed a significant difference in MEP amplitudes between ISI N20 +3 and suprathreshold single pulse (P = 0.009) as well as between ISI N20 +3 and ISI N20 +10 (P = 0.031). No difference was observed between baseline condition (suprathreshold single pulse) and ISI N20 +10 (P > 0.05; Fig. 1A).

TMS-Evoked Amplitude Modulation of EEG Responses

In both experimental conditions (i.e., both in ISI N20 +3 and in N20 +10 conditions) and in each subject, the EEG signals were composed at a vertex by a sequence of deflections as already described (Ferreri et al. 2011a; Ilmoniemi et al. 1997; for review, see Komssi and Kahkonen 2006) of negative polarity peaking at approximately 7, 44, and 100 ms, alternating with positive polarity peaking at approximately 30, 60, and 180 ms post-TMS, as illustrated in Figs. 1B and 2A. Therefore, in the following, the latencies used in the statistical analyses will be the individual N7, P30, N44, P60, N100, and P180.

The GMFP for ISI N20 +3 (i.e., SAI) revealed a decrease in amplitude between 50 and 130 ms poststimulus relative to ISI N20 +10. This decrease was maximal between 60 and 110 ms as highlighted in Fig. 2B.

According to the output of the GEE procedure, the ISI N20 +3 condition produced a significant global attenuation of N100 amplitude (a trend for P60; P < 0.06) compared with the ISI N20 +10 condition (main effect of condition, Sidak’s P <
No significant main effects of condition were found for the other latencies. In the topographic analysis of the TMS-induced responses, the N7, P30, N44, P60, N100, and P180 amplitudes were assessed by SnPM. After ISI N20 + 3, we found a significant local decrement in P60 amplitude over C1 (left motor cortex; \( P < 0.05 \), SnPM, bottom of Fig. 3). Nevertheless, the significant global attenuation of N100 amplitude found according to the GEE procedure after ISI N20 + 3 (see earlier text), the local attenuation of the N100 component in the motor cortex did not reach significance when evaluated with the subsequent topographic analysis by SnPM, underlining the fact that N100 presented a more generalized attenuation.

**Event-Related Spectral Perturbation and Intertrial Coherence**

ERSP measures changes in the amplitude of the EEG spectrum relative to an experimental event (e.g., TMS stimulation) and is independent from the phase of the EEG-evoked activity. Event-related spectral perturbation values were calculated for different spectrum frequency bands: that is, theta (4–7 Hz), alpha (8–12 Hz), beta (14–30 Hz), and gamma (40 Hz) and no significant difference was found for any frequency ranges or channels (data not shown).

ITC determines the reproducibility of the phase of the EEG-evoked responses across trials, regardless of the amplitude of the responses. Thus, intertrial coherence reflects the intertrial synchronization (phase locking) of the EEG-evoked responses to TMS. We found that ISI N20 + 3 (i.e., SAI) induced a clear reduction in the phase locking of the TMS-evoked responses across trials in the beta band (Fig. 5). Intertrial coherence impairment was present in SAI within the first 300 ms following TMS. This impairment was restricted to the beta band (\( P < 0.05 \), statistical nonparametric mapping) and peaked at two channels localized in the frontocentral region (C1 and Cz). No significant differences were detected in the other frequency ranges or in any other channel. In summary, these findings suggest that there may be impairment in
Correlation Between EEG Modulation and MEP Modulation

To assess the relationship between peripheral and central correlates of SAI, we calculated the correlation between the attenuation of MEPS in ISI N20+3 (as the percentage variation with respect to the ISI N20+10) and modulation of EEG (again in N20+3 with respect to N20+10) in those electrode sites where a significant difference in EEG responses between the two conditions had been found (C1 for P60 and C1 and Cz for ITC). For P60 amplitude, no correlation was found, whereas two conditions had been found (C1 for P60 and C1 and Cz for ITC) for P60 amplitude, no correlation was found, whereas two conditions had been found (C1 for P60 and C1 and Cz for ITC). For P60 amplitude, no correlation was found, whereas two conditions had been found (C1 for P60 and C1 and Cz for ITC). For P60 amplitude, no correlation was found, whereas two conditions had been found (C1 for P60 and C1 and Cz for ITC). For P60 amplitude, no correlation was found, whereas two conditions had been found (C1 for P60 and C1 and Cz for ITC). For P60 amplitude, no correlation was found, whereas two conditions had been found (C1 for P60 and C1 and Cz for ITC).

**DISCUSSION**

This study adds a piece of original information to the previously described electrophysiological cortical correlates of SAI (Bikmullina et al. 2009b). In fact, not only does it confirm previous results showing that SAI can inhibit the MEP amplitude (Di Lazzaro et al. 2003; Tokimura et al. 2000) as well as attenuate cortical N100 (Bikmullina et al. 2009b), but it also reveals that SAI is accompanied by motor cortex P60 attenuation and a motor cortex beta rhythm selective decrease of phase locking (decrease of intertrial synchronization). Because a positive correlation has also been revealed between this phase-locking loss and the MEP amplitude, it could be speculated that the mechanisms mediating TMS-evoked cortical measures of SAI could be in some way related to the same mechanisms mediating EMG measures of SAI. The present results complement, on the intertrial measures field (Onton et al. 2006), all the previous classical frequency domains studies in which the focus was pointed to the frequency variation instead to the phase shifting. Furthermore, our results seem to confirm the widely accepted view that SAI has a likely cortical origin and exerts its inhibitory effects on the corticospinal neurons through cortical interneuron modulation (Di Lazzaro et al. 2004, 2007; Tokimura et al. 2000). That is, it is affected by the activity of inhibitory or facilitatory circuits in the M1 as well as by the influence of other motor-related brain cortical or subcortical areas. However, the synaptic mechanisms as well as the exact anatomic circuits responsible for the SAI as well as its functional role remain only partly clarified, even though it has been taken as a possible measure of sensorimotor interaction.

Cholinergic neurons are known to participate in the initiation of SAI (Di Lazzaro et al. 2000), but it is still not clear which other neurotransmitters/neuromodulators and networks are involved in its regulation, even though a GABAergic influence is likely (Hallett 2011) and it was already proposed in the genesis and the modulation of the waves P60 and N100 (for details, see Ferreri et al. 2011). Acetylcholine (ACh) is an important neurotransmitter in the central nervous system (Xiang et al. 1998); a number of studies have looked into the effects of cholinergic signaling on sensory processing and ACh release in somatosensory areas has been demonstrated to be both region and modality specific, improving the signal-to-noise ratio and thus enhancing stimulus discrimination (Klinkenberg et al. 2011). To date, all data concerning cholinergic modulation in the brain have revealed that it is the result of a mixture of positive and negative modulations, implying that the cholinergic terminals must exert their effects (for instance, on the impact of the sensory input arriving to the neocortex) via both excitatory and inhibitory neurotransmission (Shimono et al. 2000; Yamawaki et al. 2008). Moreover, ascending cholinergic projections are clearly involved in temporal structuring and modulation of neuronal activity by population rhythms mainly in the beta frequency ranges (Kawaguchi 1997; Kohl and Paulsen 2010; Marrosu et al. 2006; Roopun et al. 2006, 2010). In particular, neuronal oscillatory activity at beta frequencies is observed in somatosensory, premotor, supplementary, and primary motor cortex, that is, in the corticothalamic networks involved in the motor control, which is highly tuned to oscillate at this characteristic rate (Neuper and Pfurtscheller 2001; Neuper et al. 2006). The source of these beta oscillations remains unclear, but it has been suggested that they are dependent on intact thalamocortical circuitry (Lopes da Silva 1991; Roopun et al. 2006) and indicative for the activity state of neural networks in the sensorimotor cortex (Alegre et al. 2002;
Pfurtscheller et al. 1996). They have also been strongly associated with cortical control and monitoring of the voluntary-activated descending pathways, that is, with the cortical driver of spinal motoneurons activity inducing MEP (Jensen et al. 2005; Kilner et al. 2000; Salenius et al. 1997). On the other hand, some studies also support a hypothesis whereby the beta oscillations modulation is involved in sensory reafferences, which are inhibitory for the motor cortex (Alegre et al. 2002; Cassim et al. 2001; Reyns et al. 2008). Finally, synchronization of beta activity has been observed after single-pulse magnetic stimulation on M1 in association with the slow TMS-evoked potentials (TEPs, such as P60 and N100) and has been linked to a form of resetting of the ongoing oscillatory activity (Paus et al. 2001; Rosanova et al. 2009) secondary to the artificial depolarization inducing synchronous activation of neurons of cortical and subcortical structures. It is very well known from animal studies that stimulation of subcortical structures (e.g., reticular system) in mesencephaly and pons induces a regional synchronization of the cortical oscillatory activity through a reentrant corticobasal ganglia-thalamo-cortical loop (Fuggetta et al. 2005; Moruzzi and Magoun 1949; Paus et al. 2001; Van Der Werf and Paus 2006; Van Der Werf et al. 2006); however,

Fig. 3. Topographic distribution of the TMS-evoked activity at P60. Average integrated evoked response at P60 in the two conditions. Contrasting the topography of these two conditions revealed decreased activity approximately underlying the stimulation site at ISI N20+3, that is, SAI. The white dot indicates the presence of significant differences ($P < 0.05$; statistical nonparametric mapping; see text for more details).

Fig. 4. Topographic distribution of the TMS-evoked activity at N100. Average integrated evoked response at N100 in the two conditions. Contrasting the topography of the two conditions revealed a decreased activity approximately underlying the stimulation site at ISI N20+3, that is, SAI.
only a few previous EEG–TMS studies focused directly on the effect of peripheral stimulation on motor cortex excitability and TMS-induced oscillations, even though it is well known that motor output is influenced by sensory input and that SAI could be taken as a reliable measure of sensorimotor interaction (Bikmullina et al. 2009b; Raij et al. 2008; Spieser et al. 2010). Because somatosensory afferents may reach precentral neurons either by cortico–cortical connections with the somatosensory cortex or by direct input from the thalamus (Jones 1983) in SAI whether the afferent input travels first to the sensory cortex and then via corticortical connections to the motor cortex, or whether the input reaches the motor cortex directly, is impossible to say, whatever the pathway of the inhibition is considered relatively direct. In our study assuming, on the basis of well-known previous studies, that synchronization of beta rhythm means inhibition or deactivation of the cortex underneath (Pfurtscheller et al. 1996; for review, see Alegre et al. 2002) and it is related to somatosensory inputs, which are inhibitory for the motor cortex (Cassim et al. 2001; for review, see Reyns et al. 2008), it may be hypothesized that, if less inhibition-deactivation is induced in M1 because a different amount of information is processed at the sensorimotor networks level due to SAI, then beta-band synchronization could be less pronounced (Reyns et al. 2008). Moreover, because it has been proposed that the thalamus influences the genesis and the magnitude of the beta oscillation in response to cortical stimuli, rather than its phase locking and we found that the phase-locking of the beta responses is highly significant, whereas the amplitude modulation is not, we could speculate on a transcortical route for the SAI modulation (Van Der Werf and Paus 2006; Van Der Werf et al. 2006), already preliminarily proposed (Bikmullina et al. 2009a). Finally, there are both in vivo and in vitro results indicating that acetylcholine plays an important role in modulating synchronous activity at high frequencies in neuronal oscillations in the neocortex and that this reflects the synchronized firing of different neuronal ensembles by a direct and complex activation of both glutamatergic and different subtype of GABAergic interneurons (Di Lazzaro et al. 2007; Jensen et al. 2005; Kilb and Luhmann 2003; Lawrence 2008; Roopun et al. 2010; Xiang et al. 1998). Then in view of our results when combined with previous studies from our own and other groups and considering the

Fig. 5. Intertrial coherence topography. Intertrial coherence (ITC) in the two conditions in the beta range between 15 and 25 Hz. The white dots represent the scalp position where intertrial coherence is significantly decreased at ISI N20+3, that is, SAI (P < 0.05; statistical nonparametric mapping). Correlation between the ITC in the beta range at C1 and the MEP amplitude.
well-known interactions between cortical inhibitory circuits (Alle et al. 2009; Chen 2004; Di Lazzaro et al. 2005c; Sanger et al. 2001; Stefan et al. 2002; Udupa et al. 2009; Ziemann 2003), we speculate that the suppressive effect of SAI on the excitability of the primary motor cortex as represented by the inhibition of MEP, P60, and N100 amplitude as well as beta-rhythm decrements in the phase locking could result from the cortico–cortical activation of GABAergic-mediated inhibition onto the corticospinal neurons modulated by cholinergic activation able to reduce intralaminar inhibition and promote intracolumnar inhibition (Xiang et al. 1998). These inhibitory interneurons could synapse on the pyramidal neurons, leading to a tighter negative control over them (Di Lazzaro et al. 2007; Lyton and Sejnowski 1991).

Conclusion

It is concluded that the EEG–TMS coregistration is a promising tool for exploring cortical mechanisms integrating afferent information and modulating motor cortex inhibitory phenomena such as SAI. It is important to understand the anatomic circuits and the functional modulation of cortical inhibitory networks by neurotransmitters and peptides to clarify the physiological basis of human cerebral cortex functionning. Different neurotransmitters interact with a variety of receptors whose activation can produce different effects on the excitability of a diverse group of cortical neurons. This differential modulation of subgroups of inhibitory interneurons by ascending cholinergic or other neurotransmitter systems is considered to represent a basis for fine control of functional state and information flow in cortical networks (Steriade et al. 1993). Cholinergic systems have been implicated in several important brain functions, including cortical arousal, sleep–wake cycles, visual information processing, learning, memory, and other cognitive functions. Alzheimer’s disease has also been associated at least in part with the loss of cortical cholinergic innervation (Dal Forno et al. 2006; Ferreri et al. 2003, 2011b; Squitti et al. 2007) and SAI abnormalities (Di Lazzaro et al. 2005a) and therefore detailed information about the effects of cholinergic innervation on the functioning of cortical circuits is needed if we are to understand the cellular mechanisms underlying the behavioral and cognitive symptoms encountered in that disease.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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