Human brain cortical correlates of Short-latency Afferent Inhibition (SAI): a combined EEG-TMS study

Florinda Ferreri1,2, David Ponzo2,3, Taina Hukkanen1, Esa Mervaala1, Mervi Könönen1,4, Patrizio Pasqualetti2,3, Fabrizio Vecchio3, Paolo Maria Rossini5,6 and Sara Määttä1

1) Department of Clinical Neurophysiology, Kuopio University Hospital, University of Eastern Finland, Kuopio, Finland
2) Department of Neurology, University Campus Biomedico, Rome, Italy
3) AFaR, Department of Neuroscience, Hospital Fatebenefratelli Isola Tiberina, Rome, Italy
4) Department of Clinical Radiology, Kuopio University Hospital, Kuopio, Finland
5) Neurology, Catholic University, Rome, Italy
6) IRCCS S. Raffaele Pisana, Rome & Casa di Cura S. Raffaele, Cassino, Italy

Abbreviated title: Cortical correlates of SAI
Number of figures: 5
Number of pages: 20
Number of words for Abstract: 271

Corresponding Author
Florinda Ferreri, MD; Dipartimento di Neuroscienze; Università Campus Biomedico di Roma; Via Alvaro del Portillo, 00100 Roma; +39066837300 – FAX +39066837360; Email: f.ferreri@unicampus.it

Acknowledgements
Florinda Ferreri, David Ponzo and Fabrizio Vecchio were partially supported by the grant GR-2008-1143091 from the Italian Institute of Health.
Abstract

Purpose: When linking in time electrical stimulation of 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, named short latency afferent inhibition (SAI), occurs when the nerve-TMS interstimulus interval is around 25 ms and is considered to be a cortico-thalamic phenomenon. The aim of the present study was to use the electroencephalographic (EEG) responses to navigated-TMS co-registration to better characterize the neuronal circuits underlying SAI.

Method: The present experimental set included MRI-navigated TMS and 60-channel TMS-compatible EEG devices. TMS-evoked EEG responses and MEPs were analyzed in 8 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.

Results: In all experimental conditions, TMS-evoked EEG responses were characterized by a sequence of negative deflections peaking at approximately 7, 44, 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. Particularly it inhibited MEPs amplitude, attenuated cortical P60 and N100 responses and induced motor cortex beta rhythm selective decrement of phase locking.

Conclusion: The findings of the present experiment suggest 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 intra-laminar inhibition and promoting intra-columnar inhibition.

Keywords:
- EEG
- Navigated Transcranial Magnetic Stimulation
- EEG-TMS coregistration
- SAI
Introduction

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 (Mariorenzi 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 named short latency afferent inhibition (SAI) and is thought to depend on neural interactions within the cerebral cortex (Tokimura et al., 2000; Di Lazzaro et al., 2004) 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 non-invasive way of evaluating cholinergic activity in the cerebral cortex (Di Lazzaro et al., 2004, Di Lazzaro et al., 2005a, Di Lazzaro et al., 2005b). 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 (Di Lazzaro et al., 2004, Di Lazzaro et al., 2005b; Bikmullina et al., 2009a). 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 non-invasive and direct method for evaluating cortical excitability and connectivity as well as their experimental modulation (Ilmoniemi et al., 1997; Paus et al., 2001; Kahkonen et al., 2003; Massimini et al., 2005; Paus 2005; Bonato et al., 2006; Daskalakis et al., 2008; Huber et al., 2008; Farzan et al., 2009; Ferreri et al., 2011a). The amplitude, latency and scalp topography of single-pulse TMS-evoked EEG responses have been clearly described (Komssi et al., 2004; Bonato et al., 2006; Veniero et al., 2010; Ferreri et al., 2011a) and are thought to be dependent on the minute current states of the stimulated areas of the cortex (Nikulin et al., 2003; Thut et al., 2003; Fuggetta et al., 2005; Paus 2005; Ilmoniemi and Kicic 2010). 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 (Paus et al., 2001; Fuggetta et al., 2005). 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 focussed on cortical mechanisms integrating afferent information (Raij et al., 2008; Bikmullina et al., 2009b; Spieser et al., 2010), the purpose of present study was to confirm and extend previous preliminary results (Bikmullina et al., 2009b), showing that SAI is associated with the attenuation of the TMS induced N100 component (Bikmullina et al., 2009b). Particularly we further endeavored to evaluate the modulatory influence of SAI on cortical oscillations between 4 and 50 Hz in the healthy human sensorimotor networks, with particular attention to the beta oscillations in the light of recent studies suggesting that their supporting networks depend critically on the inhibitory neurotransmitters activity (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 Handedness 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 which was approved by the ethical committee of Kuopio University Hospital. There was strict adherence to the exclusion criteria established by international safety standards for TMS (Rossini et al., 1994; Rossi et al., 2009).

**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:

1) 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 second, the subject’s primary motor cortex was mapped around the anatomical “hand knob” (Yousry et al., 1997; Denslow et al., 2005), to find the area evoking the largest response in right opponens pollicis muscle. Finally, rMT was defined according to international guidelines as the stimulator’s output able to elicit reproducible MEPs (at least 50 µV in amplitude) in 5 of 10 consecutive stimuli (Rossini et al., 1994).
2) In order 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 around 7 mA). Two hundred responses were averaged to identify the latency of N20 peak over C3 (common average reference; low pass filter 300 Hz, rate of stimulation 0.2-1 Hz).

3) SAI 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 supra-threshold magnetic stimulus alone (120% rMT, baseline condition) and to the peripheral conditioning 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)**

TMS was performed with an eXimia 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 anatomical structures (Nexstim Ltd., Helsinki, Finland). Three-dimensional individual T1-weighted MR images (TR 1980 ms, TE 3.93 ms, 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 Dicom 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, EMG and EEG systems.

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

**Median nerve stimulation**
Median nerve stimulation was performed at the wrist with 0.1 ms electrical rectangular pulses (Digitimer model DS7A, Digitimer, Welwyn 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 amplifier (Nextim Ltd., Helsinki, Finland) continuously throughout the experiments. In the EEG system, a sample-and-hold circuit was applied together with blocking of the amplifier input for 2 ms from the stimulus to avoid amplifier saturation. The data were recorded with a 1450 Hz sampling frequency 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**

1) **Motor evoked responses (MEPs).** In MEP analyses, the time window was from -50 ms to 100 ms from the onset of stimulus with a gain of 100 µV. Both baseline MEPs and SAI-MEPs were off-line inspected visually. 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.

2) **EEG.** Data analysis was conducted using MATLAB 2008b version 7.7 (MathWorks, Natick, Mass.) and the public license toolbox EEGLAB (Delorme and Makeig 2004). EEG data were divided in segments of 1000 ms including a 200 ms pre-stimulus 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 contaminated by environmental artifacts, muscle activity, or eye movement were rejected. Following this procedure, EEG signals were bandpass between 2 and 80 Hz, down sampled from 1450 Hz 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 square 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 semi-automatic amplitude/latency measurements of each component of the EEG evoked potentials were performed. On these data the General Estimating Equation 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) while 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), namely the ITC is an event-phase indicator function. While 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, while 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 inter-trial 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

1) The MEP amplitudes were analysed 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).

2) EEG data analyses were computed for ISI N20+3 and ISI N20+10 conditions, as we found (see later) that ISI N20+10 did not produce SAI on MEP amplitudes (Fig. 1, A) 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 TMS evoked responses’ amplitude was analyzed by means of General Estimating Equation model (hereafter, GEE; Ferreri et al., 2011a) to detect significant global effects of condition for each identified evoked peak. GEE was used as a generalization of General Linear Model, allowing to model correlated data (due to repeated measures within the same subject for each condition/position/latency). In other words, 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; Veniero et al., 2010; Ferreri et al., 2011a) with two factors: EEG electrodes (n=60) and conditions (2 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.

3) 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**

As regard to the first assessment (SAI effects on MEP data) we relied on previous evidence of large effect size (conventionally, 0.8*SD, as that shown by Tokimura et al., 2000). With such assumptions, even a small sample of eight subjects provide an adequate power (86%) of detecting as significant (at bilateral alpha level of 0.05) any change between conditions equal or above 0.8*SD.

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 frequency bands (theta, alpha, beta and gamma). With such a design and in agreement with the advices of Friston et al., 1999, we should
be able -with eight subjects- to address the issue of the identify a “typical characteristic” of brain functionality (Friston et al., 1999).

Results

a. Motor Evoked Potentials

1. Resting Motor Threshold

The mean of measured rMTs was 48% (range 40%-57%) of the maximal stimulator’s output.

2. ISI and MEP

The characteristic relationship between ISIs and MEP ratios was observed (Tokimura et al., 2000; Di Lazzaro et al., 2004, Di Lazzaro et al., 2005b. Fig. 1, A and C). The mean values were found to be 1739 µV (S.D. 1157 µV) for a supra-threshold single-pulse with an intensity of 120% of the rMT, 790 µV (S.D. 603 µV) for ISI N20+3 and 1848 µV (S.D. 1134 µV) for ISI N20+10. Pairwise comparison with Bonferroni correction revealed a significant difference in MEPs amplitude between ISI N20+3 and supra-threshold 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 (supra-threshold single pulse) and ISI N20+10 (p >0.05), (Fig. 1, A).

b. TMS-evoked EEG responses amplitude modulation

In both experimental conditions, namely both in ISI N20+3 and N20+10 conditions, and in each subject, the EEG signals were composed at a vertex by a sequence of deflections as already described (Ilmoniemi et al., 1997; Ferreri et al., 2011a; for review 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 Figure 1 B and 2 A. Therefore, in the following, the latencies used in the statistical analyses will be the individual N7, P30, N44, P60, N100, P180.

The GMFP for ISI N20+3, that is 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 the Fig 2, B. 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) when compared to the ISI N20+10 condition (main effect of condition, Sidak’s p<0.001; Fig 3 and 4). No significant main effects of condition were found for the other latencies. In the topographical 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 ISIN20+3 (see above), the local attenuation of N100 component in the motor cortex did not reach significance when evaluated with the subsequent topographical analysis by SnPM, underlining the fact that N100 presented a more generalized attenuation.

c. 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 (about 14-30 Hz) and gamma (around 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, namely 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 2 channels localized in the fronto-central 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 phase locking of TMS evoked responses in SAI, especially in a fronto-central region closer to TMS application.

d. 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 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 while for ITC in the beta range a positive correlation was evident. The bottom left side of Fig 5 reports the scatterplot of the significant positive correlation with MEP at C1 (\( p<0.05 \), \( r=0.624 \)).
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 MEPs amplitude (Tokimura et al., 2000; Di Lazzaro et al., 2003) as well as attenuate cortical N100 (Bikmullina et al., 2009b), but it also reveals that SAI is accompanied by motor cortex P60 attenuation and motor cortex beta rhythm selective decrease of phase locking (decrease of intertrial synchronization). As a positive correlation has been also 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 inter-neurons modulation (Tokimura et al., 2000; Di Lazzaro et al., 2004; Di Lazzaro et al., 2007). Namely 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 sub-cortical areas. However, the synaptic mechanisms as well as the exact anatomical circuits responsible for the SAI as well as its functional role remain only partly clarified, even though it has be taken as a possible measure of sensori-motor interaction.

Cholinergic neurons are known to participate in the initiation of SAI (Di Lazzaro et al., 2000) but 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 somato-sensory areas has been demonstrated to be 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- both via excitatory and inhibitory neurotransmission (Shimon 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; Marrosu et al., 2006; Roopun et
Particularly neuronal oscillatory activity at beta frequencies is observed in somatosensory, premotor, supplementary and primary motor cortex, namely in the cortico-thalamic 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 (Pfurtscheller et al., 1996; Alegre et al., 2002). They have also been strongly associated with cortical control and monitoring of the voluntary-activated descending pathways, namely with the cortical driver of spinal motoneurons activity inducing MEP (Salenius et al., 1997; Kilner et al., 2000; Jensen et al., 2005). 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 re-entrant cortico-basal ganglia-thalamo-cortical loop (Moruzzi and Magoun 1949; Paus et al., 2001; Fuggetta et al., 2005; Van Der Werf and Paus 2006; Van Der Werf et al., 2006). Now on however 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 is well known that motor output is influenced by sensory input and that SAI could be taken as a reliable measure of sensori-motor interaction (Raij et al., 2008; Bikmullina et al., 2009b; Spieser et al., 2010). As 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 sensori-motor networks level due to SAI, then beta band synchronization could be less pronounced (Reyns et al., 2008). Moreover as 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 trans-cortical 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 inter-neurons (Xiang et al., 1998; Kilb and Luhmann 2003; Jensen et al., 2005; Di Lazzaro et al., 2007; Lawrence 2008; Roopun et al., 2010). Then in view of our results when combined with previous studies from our own and other groups and considering the well-know interactions between cortical inhibitory circuits (Sanger et al., 2001; Ziemann 2003; Chen 2004; Stefan et al., 2002; Di Lazzaro et al., 2005c; Alle et al., 2009; Udupa et al., 2009), 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 rhythms decrement in the phase locking could results from the cortico-cortical activation of GABAergic mediated inhibition onto the corticospinal neurons modulated by cholinergic activation able to reducing intra-laminar inhibition and promoting intra-columnar inhibition (Xiang et al., 1998). These inhibitory interneurons could synapse on the pyramidal neurons, leading to a tighter negative control over them (Lytton and Sejnowski 1991; Di Lazzaro et al., 2007).

Conclusion

It is concluded that the EEG-TMS co-registration 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 anatomical circuits and the functional modulation of cortical inhibitory networks by neurotransmitters and peptides to clarify the physiological basis of human cerebral cortex functioning. 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 inter-neurons 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; Squitti et al., 2007; Ferreri et al., 2003; Ferreri et al., 2011b) 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 behavioural and cognitive symptoms encountered in that disease.
Figure Legend

Figure 1. A: Motor evoked potentials (MEP). Average of MEPs produced by stimulation at 120% of rMT and at ISI N20+3 and N20+10. Compared to baseline, MEPs were significantly decreased in amplitude at ISI N20+3 while no difference was seen at ISI N20+10. B: Butterfly plots. Grand average of TMS-evoked potentials recorded at all electrodes at ISI N20+3 and N20+10 superimposed in a butterfly diagram. All signals were aligned to the magnetic stimulus. The polarity of the waveforms is plotted with negative values downward in this and following figures. C: Grand average of the MEPs at ISI N20+3 and N20+10.

Figure 2. A: Single-Channel EEG Responses. Grand average of the EEG responses recorded at vertex (Cz) at ISI N20+10 and ISI N20+3. B: Global-Mean Field Power. Total activation produced by TMS as measured by the global-mean field power derived from all 60 electrodes. Relative to ISI N20+10, the global-mean field power was decreased at ISI N20+3, that is SAI, between 50 and 130 ms poststimulus (grey area). This decrease peaked at 60 and 110 ms (as evaluated in two-tailed unpaired t test).

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

Figure 4. Topographic distribution of the TMS evoked activity at N100. Average integrated evoked response at N100 in the two conditions. Contrasting the two conditions topography revealed a decreased activity approximately underlying the stimulation site at ISI N20+3, namely SAI.

Figure 5. Intertrial Coherence Topography. Intertrial coherence (ITC) in the two condition 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, namely SAI (p<0.05; statistical nonparametric mapping). Correlation between the ITC in the beta range at C1 and the MEP amplitude.


Neuper C, Wörtz M, Pfurtscheller GERD/ERS patterns reflecting sensorimotor activation and deactivation. Prog Brain Res. 2006;159:211-22


