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Long-Interval Cortical Inhibition from the Dorsolateral Prefrontal Cortex: a TMS–EEG Study

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Several studies have demonstrated that cortical inhibition (CI) can be recorded by paired transcranial magnetic stimulation (TMS) of the motor cortex and recorded by surface electromyography (EMG). However, recording CI from other cortical regions that are more closely associated with the pathophysiology of some neurological and psychiatric disorders (eg, dorsolateral prefrontal cortex (DLPFC) in schizophrenia) was previously unattainable. This study, therefore, was designed to investigate whether CI could be measured directly from the motor cortex and DLPFC by combining TMS with electroencephalography (EEG). Long-interval CI (LICI) is a TMS paradigm that was used to index CI in the motor cortex and DLPFC in healthy subjects. In the motor cortex, LICI resulted in significant suppression (32.8 ± 30.5%) of mean cortical evoked activity on EEG, which was strongly correlated with LICI recorded by EMG. In the DLPFC, LICI resulted in significant suppression (30.1 ± 26.9%) of mean cortical evoked activity and also correlated with LICI in the motor cortex. These data suggest that CI can be recorded by combining TMS with EEG and may facilitate future research attempting to ascertain the role of CI in the pathophysiology of several neurological and psychiatric disorders.
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INTRODUCTION

Cortical inhibition (CI) refers to a neurophysiological process in which γ-aminobutyric acid (GABA)-inhibitory interneurons attenuate the activity of other neurons in the cortex. Transcranial magnetic stimulation (TMS) represents a unique experimental modality that has been used to index several different cortico-cortical inhibitory processes. One such TMS inhibitory paradigm includes long-interval CI (LICI) (Valls-Sole *et al*, 1992). In LICI, when a suprathreshold conditioning stimulus (CS) precedes the suprathreshold test stimulus (TS) by 50–150 ms, the motor-evoked potential (MEP) is inhibited by approximately 50% (Daskalakis *et al*, 2002b) compared with a single TS alone (Figure 1a and b). Several lines of evidence suggest that LICI reflects $GABA_B$ receptor-mediated inhibitory neurotransmission. For example, the fact that LICI inhibits another inhibitory paradigm, short-interval CI (SICI) (Sanger *et al*, 2001), which relates to $GABA_A$ receptor-mediated inhibitory neurotransmission (Ziemann *et al*, 1996a), is consistent with the suggestion that presynaptic $GABA_B$ may inhibit the release of GABA with a concomitant decrease in $GABA_A$ receptor-mediated inhibition (Werhahn *et al*, 1999). Also, LICI is evoked with a high-intensity CS, which produces longer periods of CI (Valls-Sole *et al*, 1992), which is consistent with the finding that $GABA_B$ receptor-mediated responses have higher activation thresholds and their inhibitory influence is longer lasting (Deisz, 1999; Sanger *et al*, 2001). Further, the administration of the $GABA_B$ receptor agonist baclofen was found to potentiate LICI (McDonnell *et al*, 2006).

A significant limitation to recording LICI in its current form is that the motor cortex only can be studied by electromyography (EMG). This is problematic insofar as recording CI from other cortical regions that are more closely associated with the pathophysiology of some neurological and psychiatric disorders (eg, dorsolateral prefrontal cortex (DLPFC) in schizophrenia; Weinberger *et al*, 1986) was limited by the large artifact produced when TMS was combined with electroencephalography (EEG). Such limitations have recently been overcome (Komssi *et al*, 2004). To date, several published studies have combined TMS with EEG to evaluate the neurophysiological effects of TMS directly on the cortex (Komssi and Kahkonen, 2006).

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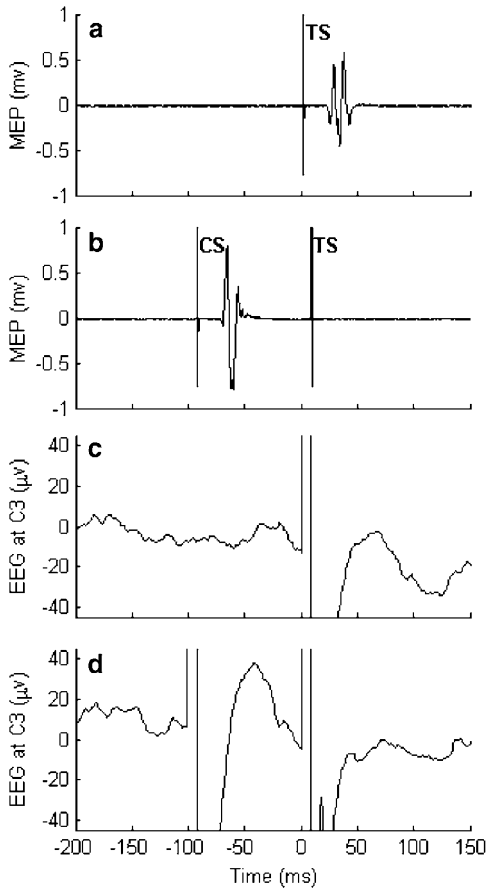

Figure 1 Single trials of EMG and EEG following stimulation of the left motor cortex. Traces represent single trials of EMG (a, b) recorded from right APB, and the corresponding EEG (c, d) recorded from the C3 electrode, during unconditioned (a, c) and conditioned stimulation (b, d) to left motor cortex in a single subject. (a, c) EMG and EEG waveforms following a TS that evokes a 1-mV MEP. (b, d) EMG and EEG waveforms following conditioned TS (CS, ISI 100 ms). Power of both EEG and EMG waveforms is attenuated following conditioned TS (b, d) compared with unconditioned TS (a, c).

For example, Komssi *et al* (Komssi and Kahkonen, 2006) demonstrated that TMS applied to the hand area of the motor cortex generates several evoked responses in global mean field amplitude waveforms, while the effect of the TMS stimulus artifact is negligible. Although these studies have been invaluable for assessing the effects of TMS on cortical excitability by single-pulse stimulation, assessment of CI by paired-pulse stimulation (eg, LICI) has yet to be demonstrated.

This study, therefore, had three objectives. The first was to evaluate LICI directly from the cortex using EEG. The second was to investigate whether EEG measures of LICI are related to the same mechanisms as those mediating EMG measures of LICI (Valls-Sole *et al*, 1992). The final objective was to determine whether LICI could be recorded from the DLPFC.

MATERIALS AND METHODS

Subjects

We studied 15 healthy, right-handed volunteers (mean age = 34.7 years, SD = 8.1 years, range = 23–47 years; 5 males and 10 females). Handedness was confirmed with the Oldfield Handedness Inventory (Oldfield, 1971). All subjects gave written informed consent and the protocol was approved by the Centre for Addiction and Mental Health in accordance with the Declaration of Helsinki Principles. Exclusion criteria included a self-reported comorbid medical illness or a history of drug or alcohol abuse. Moreover, psychopathology was ruled out using the personality assessment inventory (PAI; Psychological Assessment Resources Inc.). The PAI is a self-administered, objective inventory of adult personality and psychopathology (eg, personality depression, somatic disorders, anxiety, anxiety-related disorders, and schizophrenia), comprising non-overlapping clinical, treatment, interpersonal, and validity scales. Specifically, the PAI measures manifestation of clinical syndromes, providing information to assist diagnosis, treatment, and screening for all psychopathology corresponding DSM-IV categories (Morey, 1991, 1996).

Experimental Design

Active and sham TMS were administered over the motor cortex (experiment 1) and DLPFC (experiment 2). All 15 subjects participated in experiment 1, whereas a subset of nine subjects (mean age = 36.4 years, SD = 8.6 years, range = 23–47 years; 3 males and 6 females) who were selected at random on a first come, first served basis, participated in experiment 2.

Transcranial Magnetic Stimulation

Monophasic TMS pulses were administered to the left motor cortex and DLPFC using a 7-cm figure-of-eight coil, and two Magstim 200 stimulators (Magstim Company Ltd., UK) connected via a Bistim module and MEP data were collected using commercially available software, Signal (Cambridge Electronics Design, UK). In experiment 1, we examined LICI in the motor cortex concomitantly by both EMG and EEG. The coil was placed at the optimal position for eliciting MEPs from the right abductor pollicis brevis muscle (APB) muscle, which typically corresponded to a region between FC3 and C3 electrodes (Herwig *et al*, 2003). In experiment 2, LICI was examined in the DLPFC by EEG only. In both experiments, the optimal position was marked on the EEG cap with a felt pen to ensure identical placement of the coil throughout the experiment, and the handle of the coil pointed backward, perpendicularly to the presumed direction of the central sulcus, approximately 45° to the mid-sagittal line.

Resting motor threshold was defined as the minimum stimulus intensity that elicits an MEP of more than 50 μV in 5 of 10 trials (Rossini *et al*, 1994). This corresponded to 42.9 ± 7.7% of stimulator output in 15 subjects who participated in experiment 1, and to 39.6 ± 6.2% of stimulator output in nine subjects who participated in experiment 2.

Measurement of CI

The LICI paradigm involves pairing of a suprathreshold CS followed by a suprathreshold TS at long interstimulus

intervals (ISIs) (eg, 100 ms), which inhibits the MEP produced by TS (Valls-Sole *et al*, 1992; Figure 1). LICI is reportedly optimal at 100 ms ISI (Sanger *et al*, 2001), and as such, in this experiment we evaluated LICI at this interval (ie, $LICI_{100}$). Both CS and TS were suprathreshold and adjusted to produce a mean peak-to-peak MEP amplitude of 1 mV (Valls-Sole *et al*, 1992) that corresponded to $68.9 \pm 14.5\%$ of stimulator output in all 15 subjects who participated in experiment 1, and $63.2 \pm 13.7\%$ of stimulator output in all nine subjects who participated experiment 2. One hundred TMS stimuli were delivered per condition (ie, paired CS–TS and TS alone) every 5 s, an interval conventionally used in most TMS studies evaluating CI that has not been shown to result in habituation with repeated stimulation (Kujirai *et al*, 1993; Nakamura *et al*, 1997; Sanger *et al*, 2001; Ziemann *et al*, 1996a), and all conditions (ie, active/sham, motor/DLPFC) were randomized and counterbalanced to avoid order effects.

Sham Stimulation

To control for the effect of TMS click-induced auditory activation on the cortical-evoked potentials, sham stimulation was administered to a subset of subjects in both the experiments (8 in experiment 1 and 6 in experiment 2), with the coil angled at 90° from the scalp resting on one wing of the coil.

MRI Identification of the DLPFC

In experiment 2, localization of the DLPFC was achieved by neuronavigation techniques using the MINIBIRD system (Ascension Technologies) and MRIcro/reg software using a T1-weight MRI scan obtained for each subject with seven fiducial markers in place. Stimulation was directed at the junction of the middle and anterior one-third of the middle frontal gyrus (Talairach coordinates $(x, y, z) = -50, 30, 36$) corresponding with posterior regions of Brodmann area 9 (BA9), which overlap with the superior section of BA46. This site was chosen on the basis of a recent meta-analysis of functional imaging studies of working memory and the DLPFC in schizophrenia (Glahn *et al*, 2005; Mendrek *et al*, 2005; Tan *et al*, 2005). This ensured that assessment was targeted at a DLPFC site where functional neurophysiological abnormalities have been demonstrated. A spatial resolution of 1 mm has been reported with such techniques (Verlinden *et al*, 2006).

Electromyography

EMG was captured by placing two disposable disc electrodes over the right APB in a tendon–belly arrangement, and LICI was derived according to our previously published methods (Daskalakis *et al*, 2002b).

Electroencephalography

To evaluate TMS-induced cortical evoked activity, EEG was recorded concurrently with the EMG recordings (Figure 1). EEG recordings were acquired with a 64-channel Synamps2 DC-coupled EEG system (Compumedics). A 64-channel EEG cap was used to record the cortical signal, and four electrodes were placed on the outer side of each eye and above and below the left eye to closely monitor the eye movement artifact. All electrodes were referenced to an electrode placed on the vertex positioned posterior to the CZ electrode. EEG signals were recorded DC at 20 kHz sampling rate and with a low-pass filter of 100 Hz. The two unique recording features of this amplifier, which limit the effect of the TMS stimulus artifact relate to (1) DC coupling and (2) recording at high sampling frequencies. Vis à vis the EEG system, in an AC-coupled amplifier, a typical 500-mV TMS pulse prevents the signal from returning to zero immediately after the pulse has terminated. Rather the signal that is recorded is followed by a negative deflection that can take up to 5 s to return to its initial state, with a 100-ms wide pulse. With a 50-μs TMS pulse, this return would be shorter, but the signal artifact that is produced still precludes meaningful recordings in the time range required to record LICI. By contrast, with a DC-coupled EEG amplifier, the prolonged negative swing is eliminated or 'clipped' and immediately returns to its linear range after the stimulus stops. DC coupling has only become available in recent years with the introduction of fast 24-bit analog digital converter (ADC) resolution (ie, 24 nV/bit) that is superior to the older 16-bit ADC resolution that was limited to 6.1 μV/bit, a resolution that fails to limit the TMS stimulus artifact. Vis à vis recording at high sampling frequencies, as TMS pulses have a fairly high rise time, they contain a fair amount of high-frequency activity. As a result, sampling at a high rate fully characterizes the TMS pulse and limits the stimulus artifact that is produced. The EEG recordings were first processed offline by the commercially available software, Neuroscan (Compumedics). The EEG data were downsampled to 1 kHz sampling frequency, and segmented with respect to the TMS TS such that each epoch included a 1000-ms pre-stimulus baseline and a 1000-ms post-stimulus activity. Epochs were baseline corrected with respect to the TMS-free pre-stimulus interval (1000–110 ms prior to TS). The baseline-corrected post-TS intervals (~25–1000 ms), which were not contaminated by TMS artifact, were extracted and digitally filtered using a zero-phase shift 1- to 100-Hz band-pass filter (48 dB/Oct). At this stage, epochs were manually reviewed and trials contaminated with muscle activity, movement, and TMS artifacts were excluded from further analysis. Finally, the averaged signals at each recording site were computed from the movement-free epochs and were fed into an automated eye-blink correction algorithm (Croft *et al*, 2005). The eye-blink-corrected average EEG waveforms were then imported into MATLAB (The MathWorks Inc., Natick, MA) and further analyses were carried out with EEGLAB toolbox (Delorme and Makeig, 2004)

Data Analysis

EMG measures of $LICI_{100}$. EMG recordings were imported into MATLAB (The MathWorks Inc.) and for each subject, MEPs following single and paired-pulse stimulations were averaged for each condition (100 trials per condition). We then measured the ratio of the area under the rectified curve of the average conditioned MEPs over the average unconditioned MEPs, and for each subject the EMG measure of $LICI_{100}$ was represented by the following equation:

Equation A

$$\left[1-\frac{\text{Area under rectified curve (conditioned)}}{\text{Area under rectified curve (unconditioned)}}\right]\times 100$$

EEG measures of LICI$_{100}$. For EEG measures of LICI$_{100}$, eye-blink-corrected average waveforms were band-pass filtered (1–50 Hz) and the mean area under the rectified curve for the unconditioned and conditioned waveforms were generated and LICI$_{100}$ was defined using equation A (ie, the area under the rectified curve for averaged EEG recordings between 50 and 150 ms post TS; Figure 2). The first interval (ie, 50 ms post stimulus) was chosen as it represents the earliest artifact-free data that can be recorded post stimulus, and the second interval (ie, 150 ms post stimulus) was chosen as it represents the duration of GABA$_B$ receptor-mediated inhibitory postsynaptic potentials (IPSPs) (Deisz, 1999) (ie, 250 ms) elicited by the CS (Sanger *et al*, 2001). To evaluate LICI directly from the motor cortex, C3 electrode was used as it has been shown to be the electrode best representing evoked activity in the hand area of motor cortex and being closest to the optimal site of APB activation by TMS (Cui *et al*, 1999). To capture LICI in the DLPFC, the recording electrode of interest was AF3, which optimally represents the overlap of BA9 and BA46 of the DLPFC (Herwig *et al*, 2003).

In combining TMS with EEG, two additional potential sources of artifact may further contaminate recordings and possibly lead to spurious findings. The first relates to the effect of the late evoked response of the CS on the early evoked response of the TS. That is, the late evoked response of the CS (ie, 150–250 ms) may produce a signal sufficient to modify or attenuate the early evoked response to the TS (50–150 ms) that may, in part, be responsible for the inhibitory signal anticipated in this conditioning-test paradigm. To control for this, the following method was used: in each subject, the average cortical evoked potential elicited by the TS alone was shifted by 100 ms and subtracted from the average cortical-evoked potentials elicited by the TS in LICI paradigm. The second relates to the possibility that suppression of cortical evoked activity measured by LICI$_{100}$ was due to suppression of auditory evoked potentials (eg, N100 suppression following presentation of paired auditory clicks separated by 100 ms; Muller *et al*, 2001). To control for this effect, we applied sham LICI for both the motor and the DLFPC. In this way, sham stimulation preserves the auditory stimulation produced by TMS clicks, without eliciting direct brain stimulation. We then subtracted the contribution of these auditory evoked potentials from the cortical evoked potentials produced by active stimulation.

RESULTS

All subjects tolerated the procedure without adverse events. EEG activity following single-pulse TMS (Figure 3) to the left motor cortex at an intensity required to produce a 1-mV peak-to-peak MEP amplitude ($\sim$120 percent of MT), and recorded from the CZ electrode produced recordings that were consistent with those published previously (eg, Figure 1a from Komssi *et al*; Komssi and Kahkonen, 2006).

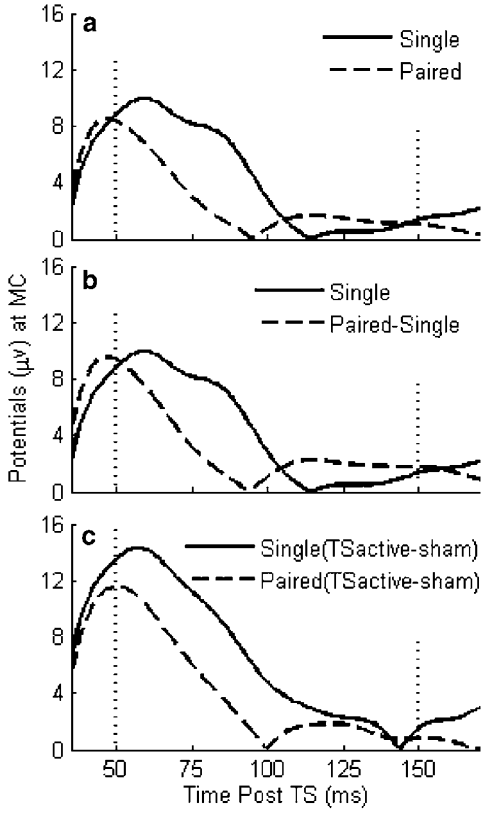

Figure 2 EEG activity following single and paired-pulse TMS of the left motor cortex. Comparison of mean rectified waveforms recorded from the C3 electrode in all 15 subjects (a, b) who received active TMS to the motor cortex, and in eight subjects (c) who also received sham TMS over this region. In all figures, the *x*-axis represents the time from 50 to 150 ms (marked by hash lines) after delivery of TS. The *y*-axis represents evoked potentials recorded from the C3 electrode, which lies nearest the motor cortex. (a) The black waveform represents cortical evoked activity in response to TS alone, while the dashed waveform represents cortical evoked activity following paired-pulse LICI paradigm using an interstimulus interval of 100 ms (ie, LICI$_{100}$). Overall, there was significant attenuation (32.8 ± 30.5%, $t = 3.60$, $p = 0.003$) in mean cortical evoked activity by LICI$_{100}$ compared with that by TS alone. (b) The black waveform represents cortical evoked activity in response to TS alone, while the dashed waveform represents LICI$_{100}$ after controlling for the late evoked response of the CS. In this condition, suppression of cortical evoked activity remained significant ($t = 2.21$, df = 14, $p = 0.045$). (c) The black and dashed waveform represents cortical evoked activity in response to TS alone and LICI$_{100}$, respectively, after controlling for sham-induced auditory evoked responses. In this condition, suppression of cortical evoked activity also remained significant ($t = 2.96$, df = 7, $p = 0.021$).

LICI in the Motor Cortex

Overall, 10.4% of the trials were discarded due to movement artifact immediately after data collection. Consistent with previous studies (Sanger *et al*, 2001; Valls-Sole *et al*, 1992), there was a significant suppression (72.0 ± 21.1%) of the mean area under the rectified EMG curve by LICI$_{100}$ compared with that by TS alone ($t = 5.52$, df = 14, $p < 0.0001$), and also a significant suppression (32.8 ± 30.5%) of mean cortical evoked activity by LICI$_{100}$

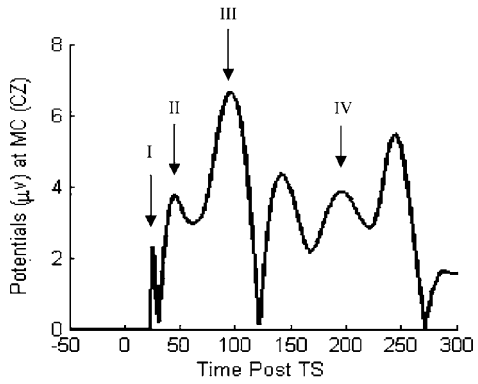

Figure 3 EEG activity following single-pulse TMS to the left motor cortex at an intensity required to produce a 1 mV peak-to-peak MEP amplitude (~120% of MT) and recorded from the CZ electrode. Arrows indicate peaks that are consistent with those published previously (eg, Figure 1a from Komssi *et al*; Komssi and Kahkonen, 2006).

compared with that by TS alone ($t = 3.60$, df = 14, $p = 0.003$; Figure 2a). The suppression of cortical evoked activity remained significant after controlling for both late evoked response of the CS ($t = 2.21$, df = 14, $p = 0.045$; Figure 2b) and the evoked responses from sham LICI ($t = 2.96$, df = 7, $p = 0.021$; Figure 2c). Figure 4a represents a topographic illustration of the averaged cortical evoked responses measured at various latencies (chosen for illustrative purposes to optimally demonstrate the topography of LICI recorded by EEG) following single- and paired-pulse TMS (ie, $LICI_{100}$) measured over motor cortex, and Figure 5 indicates that suppression of cortical evoked activity is maximal over a region between FC3 and C3 electrodes, and becomes progressively reduced as the distance from C3 is increased.

Association Between EMG and EEG Measures of LICI in the Motor Cortex

There was a significant correlation ($r = 0.88$, $p < 0.0001$) between EEG and EMG measures of $LICI_{100}$ (Figure 6a). EEG and EMG measures of $LICI_{100}$ were also significantly correlated after controlling for both the late evoked response of the CS ($r = 0.81$, $p < 0.001$; Figure 6b) and evoked responses from sham stimulation ($r = 0.94$, $p < 0.001$; Figure 6c).

LICI in the DLPFC

There was a significant suppression in mean cortical evoked activity (30.1 ± 26.9%) by $LICI_{100}$ compared with that by TS alone ($t = 3.50$, df = 8, $p = 0.008$; Figure 7a). Suppression of cortical evoked activity remained significant after controlling for both the late evoked response of the CS ($t = 3.05$, df = 8, $p = 0.02$; Figure 7b) and auditory evoked responses from sham stimulation in the $LICI_{100}$ condition ($t = 2.61$, df = 5, $p = 0.048$; Figure 7c). Figure 4b presents a topographic illustration of the averaged cortical evoked responses measured at various latencies (chosen for illustrative purposes to optimally demonstrate the topography of LICI recorded by EEG) following single- and paired-pulse TMS (ie, $LICI_{100}$) measured over DLPFC, and indicates that EEG suppression occurs between

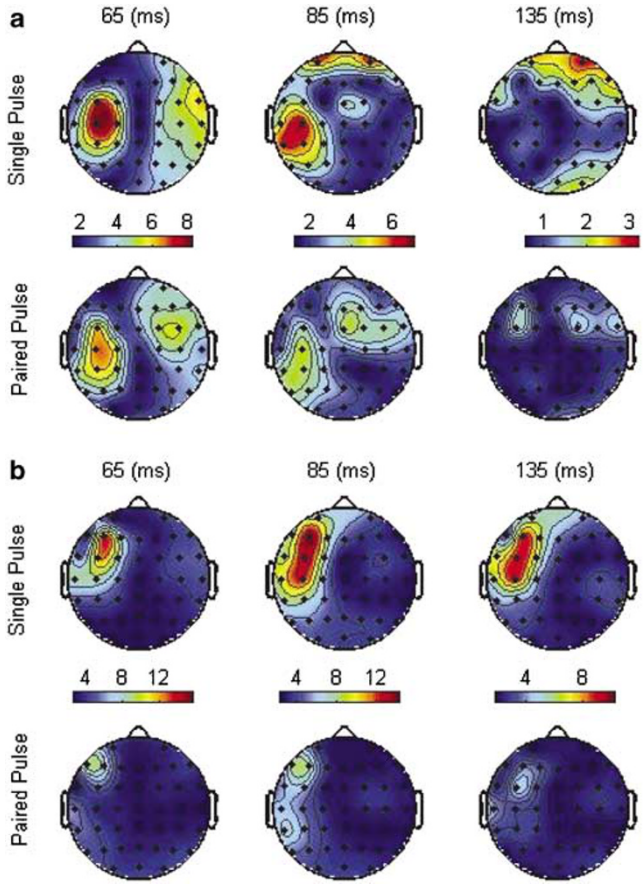

Figure 4 (a) Cortical evoked activity following single- and paired-pulse stimulation over the left motor cortex. Rectified cortical evoked activity averaged across 15 subjects at 65, 85, and 135 ms after presentation of TS alone (top) and LICI (bottom). These intervals were chosen for illustrative purposes to optimally demonstrate the topography of LICI recorded by EEG. To control for the effect of the late evoked response of the CS (ie, >100 ms) on the early evoked response of the TS for LICI (Bottom), the average cortical evoked potential following TS alone was shifted by 100 ms and subtracted from the average cortical evoked potential elicited by LICI. Cortical evoked activity is suppressed following delivery of $LICI_{100}$ (bottom) compared with TS alone (top). Topographic head plots were obtained with EEGLAB toolbox (Delorme and Makeig, 2004). (b) Cortical evoked activity following single- and paired-pulse TMS over the left DLPFC. Rectified cortical evoked activity averaged across nine subjects at 65, 85, and 135 ms after presentation of unconditioned TS (top) and conditioned TS (bottom) over the left DLPFC. These intervals were chosen for illustrative purposes to optimally demonstrate the topography of LICI recorded by EEG. For each subject, the average cortical evoked potential following the TS alone (single pulse) was shifted by 100 ms and subtracted from the average cortical evoked potential elicited by the conditioned TS. This method was carried out to account for the effect of conditioning stimulus on the early EEG response following the conditioned TS. Cortical evoked activity is suppressed following delivery of paired-pulse TMS (ie, $LICI_{100}$) compared with TS alone. Topographic head plots were obtained by EEGLAB toolbox (Delorme and Makeig, 2004).

50–150 ms and is maximum over the region being stimulated (eg, AF3).

Association Between LICI and Cortical Evoked Activity in the Motor and DLPFC

EEG measures of LICI in the motor cortex (ie, C3) and DLPFC (ie, AF3) were of similar magnitude (ie, 32.8 ± 30.5%

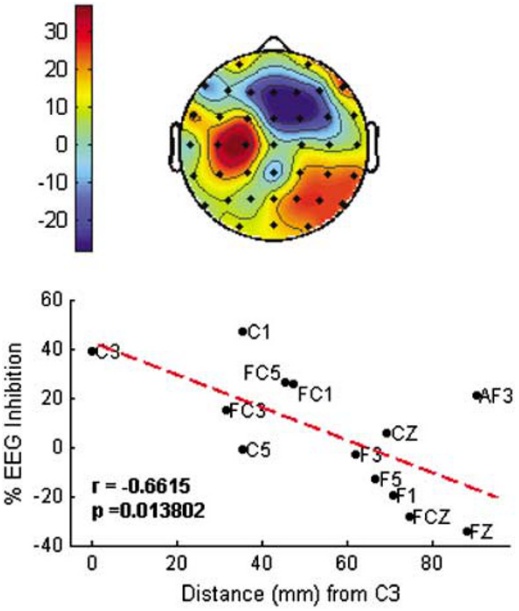

Figure 5 Suppression of cortical evoked activity in the motor cortex. Top: Topographic illustration of the suppression of cortical evoked activity measured through LICI paradigm applied to the motor cortex (obtained by equation a, see Materials and Methods) across all 15 subjects. Maximal suppression of cortical evoked activity (represented by hot colors) is observed in the vicinity of the stimulated area. Bottom: Mean suppression of cortical evoked activity recorded from left frontal electrodes as a function of relative distance from the C3 electrode in all 15 subjects. This correlation suggests that LICI is progressively reduced as the distance from the motor cortex (C3) increases. Topographic head plots were obtained by EEGLAB toolbox (Delorme and Makeig, 2004).

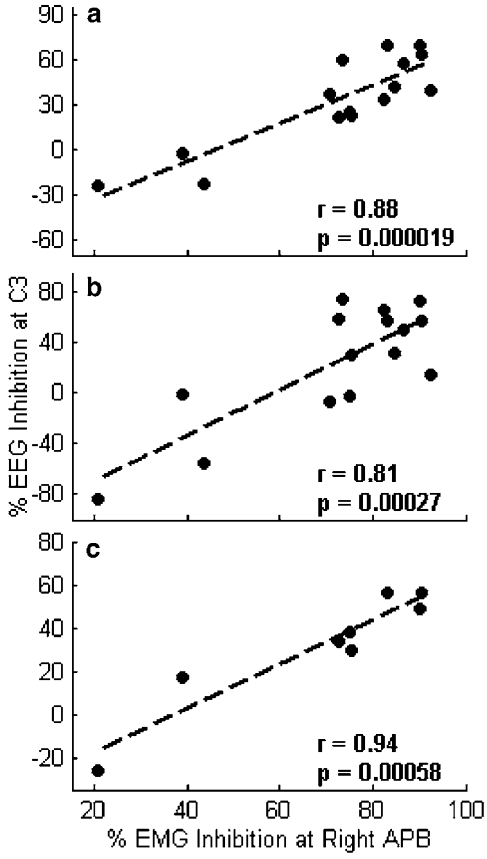

Figure 6 The relationship between EMG and EEG suppression measures of LICI. Data from experiment 1 and each point represent EEG and EMG measures of $LICI_{100}$ calculated by equation A (see Materials and Methods) for each subject. For all figures, the *x*-axis represents CI as indexed through EMG suppression recorded from right abductor APB, and the *y*-axis represents CI as indexed by EEG recorded from the C3 electrode following suprathreshold TMS to the left motor cortex. Overall, there was significant correlation ($r = 0.88$, $p < 0.0001$) between EMG and EEG indices of $LICI_{100}$ (a). This correlation remained significant ($r = 0.81$, $p < 0.001$) following subtraction of EEG response to TS alone (shifted by 100 ms) from the EEG response to the conditioned TS (b). EEG–EMG correlation was also significant ($r = 0.94$, $p < 0.001$) once EEG response to sham stimulation was subtracted from the EEG response recorded in active stimulation of the motor cortex, suggesting that TMS-induced auditory evoked potentials have insignificant effect on EEG suppression measured from the C3 electrode (c).

in motor cortex and $30.1 \pm 26.9\%$ in DLFPC) and correlated significantly ($r = 0.71$, $p = 0.03$; Figure 8). This correlation remained significant after controlling for evoked responses from sham stimulation ($r = 0.88$, $p = 0.021$). These data provide compelling evidence to suggest that LICI can be demonstrated directly from the DLPFC and that the extent of inhibition in the DLPFC is similar to that demonstrated in the motor cortex. Finally, we compared cortical evoked activity over the motor and DLPFC delivered at a TMS intensity to produce a 1-mV peak-to-peak MEP amplitude. Our results demonstrate that TMS over the motor cortex-induced cortical evoked activity of $695.52 \pm 367.78\,\mu V$ and that over the DLPFC-induced evoked activity of $953.61 \pm 496.14\,\mu V$, which were not significantly different ($t = 1.46$, $df = 22$, $p = 0.16$). These data suggest that TMS intensities sufficient to elicit activation of the motor cortex are also sufficient to elicit activation of the DLPFC.

DISCUSSION

In this study, we have demonstrated that CI can be recorded in both the motor and DLPFC through EEG by measuring the suppression of cortical evoked activity through paired-pulse TMS (ie, LICI). The evidence suggesting that suppression of cortical evoked activity is indeed related to CI and not to other factors (eg, N100 suppression following presentation of paired auditory TMS-induced clicks) is confirmed by several additional findings. First, in the motor cortex, suppression of cortical evoked activity in the C3 electrode was strongly correlated with suppression of MEPs in the APB. Second, suppression of cortical evoked activity in the motor cortex decreased as the distance from the C3 electrode increased, suggesting that inhibition is maximal over the area being stimulated and decreases as the distance from the stimulating coil center is increased. Third, neither the late evoked response elicited by the CS (ie, >100 ms) on the early evoked response caused by the TS nor the effect of sham-induced auditory evoked potentials (eg, N100 suppression following presentation of paired auditory clicks separated by 100 ms) had a significant effect on either the extent of inhibition or the relationship between EEG and EMG measures of inhibition. Finally, the fact that there was a relationship between the extent of inhibition in the motor and DLPFC at the same TMS intensities, argues strongly for

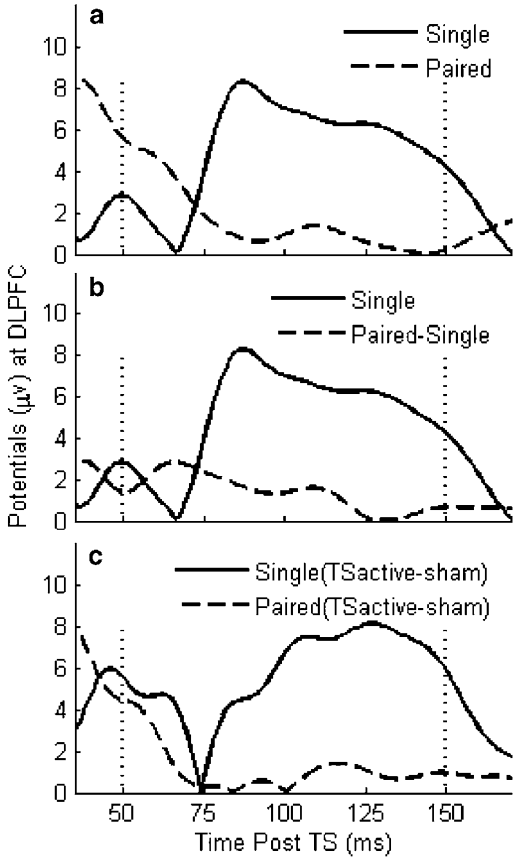

Figure 7 EEG activity following single- and paired-pulse TMS of the left DLPFC. Mean suppression of cortical evoked activity recorded following stimulation of the DLPFC in nine healthy subjects (a, b) who participated in stimulation of the DLPFC. In all the figures, the *x*-axis represents time from 50 to 150 ms (marked by hash lines) after delivery of TS. The *y*-axis represents evoked potentials recorded from the AF3 electrode, which optimally represents the overlap of BA9 and BA46 of the DLPFC (Herwig *et al*, 2003). (a) The black waveform represents cortical evoked activity in response to TS alone, while the dashed waveform represents cortical evoked activity following paired-pulse LICI paradigm using an interstimulus interval of 100 ms (ie, $LICI_{100}$). Overall, there was significant attenuation (30.0 ± 26.9%) of mean cortical evoked activity by $LICI_{100}$ compared with that by TS alone. (b) The black waveform represents cortical evoked activity in response to TS alone, while the dashed waveform represents $LICI_{100}$ after controlling for the late evoked response of the CS. In this condition, suppression of cortical evoked activity remained significant ($t = 3.0511$, df = 8, $p = 0.016$). (c) The black waveform represents cortical evoked activity in response to TS alone, while the dashed waveform represents $LICI_{100}$ after controlling for the auditory evoked responses. In this condition, suppression of cortical evoked activity remained significant ($t = 2.61$, df = 5, $p = 0.048$).

the fact that similar mechanisms mediate these forms of inhibition and are neurophysiologically consistent with $GABA_B$-receptor mediated inhibitory neurotransmission.

Initial studies recording TMS-evoked potentials directly from the cortex have demonstrated that TMS generates four peaks at 15, 55, 102, and 185 ms post stimulus (Komssi *et al*, 2004). It was suggested that peaks I and II reflect excitability (eg, voltage-gated mechanisms; Chen *et al*, 1997; Ziemann *et al*, 1996b) in the cortex, whereas later peaks may be associated with GABAergic inhibitory processes in the cortex. This study, albeit seminal in demonstrating that TMS-evoked activity can be recorded directly from the

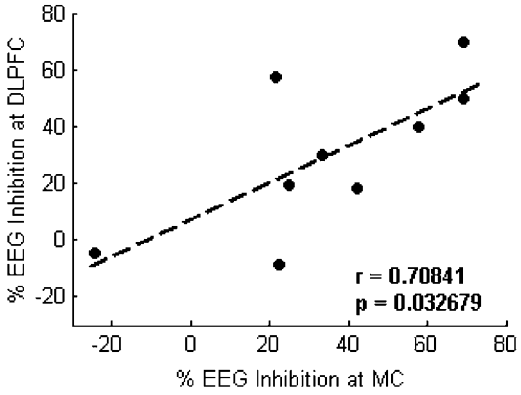

Figure 8 The relationship between LICI in the DLPFC and motor cortex. Data from nine subjects and each point represent suppression of cortical evoked activity following LICI delivered to the motor cortex (C3) and DLPFC (AF3) for each subject. There was a significant correlation ($r = 0.71$, $p = 0.033$) in suppression of cortical evoked potential between these regions, suggesting that these inhibitory indices are related neurophysiologically.

cortex, did not directly evaluate the suppression of such cortical evoked activity, that may be a more direct index of GABAergic inhibitory neurotransmission (Kujirai *et al*, 1993; Valls-Sole *et al*, 1992). Additionally, as activation of $GABA_B$-mediated IPSPs produces sustained inhibition of the cortex that peaks at approximately 150–200 ms (Davies *et al*, 1990; Deisz, 1999; Kang *et al*, 1994; McCormick, 1989), TMS-induced suppression of cortical evoked activity is unlikely to correspond with any one single peak, but affect the entire waveform generated by the TS for up to 250 ms (ie, duration of $GABA_B$ receptor-mediated IPSPs). That is, a CS delivered 100 ms prior to the TS activates $GABA_B$ receptor-mediated IPSPs. When TS arrives in the middle of this inhibition, its ability to generate evoked activity in the cortex is diminished for an additional 150 ms, reflected as waveform suppression on EEG compared with the TS alone.

Our finding of a significant relationship between reduction of the area under the EEG and EMG rectified waveforms by LICI recorded from the C3 and APB, respectively, strongly suggest that the mechanisms mediating these two events are similar and may indicate that cortical $GABA_B$ inhibitory neurotransmission underlies both forms of waveform suppression. As indicated, several lines of evidence suggest that $GABA_B$ receptor-mediated inhibitory neurotransmission is responsible for the waveform suppression, as indexed through both LICI and cortical silent period (CSP) TMS paradigms. For example, subjects who were administered baclofen, a $GABA_B$ agonist, demonstrated increased LICI (McDonnell *et al*, 2006) and increased CSP duration (Siebner *et al*, 1998). Further, slow IPSPs mediated by $GABA_B$ receptors peak at 150 ms, corresponding to the duration of the LICI and CSP (Sanger *et al*, 2001). Moreover, high stimulus intensities are required to activate the LICI and CSP, corresponding to the high thresholds of the slow IPSPs mediated by the $GABA_B$ receptors (Nakamura *et al*, 1997). Finally, studies have demonstrated that $GABA_B$ IPSPs inhibit $GABA_A$ IPSPs, consistent with the finding that LICI inhibits SICI (Sanger *et al*, 2001). Therefore, our finding of a strong correlation between LICI, as indexed though

both EEG and EMG, suggests that the mechanisms mediating suppression in EEG activity from the cortex are also related, in part, to $GABA_B$ receptor-mediated inhibitory neurotransmission.

Finding a suppression of cortical evoked activity that was similar in both the motor cortex and DLPFC suggests that recording inhibition in non-motor regions (eg, DLPFC) is attainable. Thus, the methods presented in this paper can be used to directly evaluate inhibition from specific cortical regions (eg, DLPFC) whose dysfunction has been associated with the pathophysiology leading to several neurological and psychiatric disorders. For example, it has previously been demonstrated that unmedicated patients with schizophrenia have impaired CI, and that such inhibitory deficits can be rectified through treatment with antipsychotic medications (Daskalakis *et al*, 2002a; Fitzgerald *et al*, 2002). However, these studies were limited by the fact that such findings were from the motor cortex, a cortical region that is perhaps less relevant to the pathophysiology of schizophrenia compared with the DLPFC. Consequently, being able to assay CI directly from these cortical regions may not only help to annex further pathophysiological targets in such disorders, but may also help to guide future pharmacological treatments.

Some limitations to the aforementioned findings should be noted. First, and perhaps most importantly, is the fact that a correlation between EEG and EMG measures does not necessarily imply that the mechanisms mediating these two forms of inhibition are causally linked. While it is likely that LICI, as indexed through EMG in hand muscles, is associated with $GABA_B$ receptor-mediated inhibitory neurotransmission (McDonnell *et al*, 2006), this study does not present direct evidence to support this contention. Future studies using a $GABA_B$ receptor agonist (ie, baclofen) in healthy subjects similar to those by McDonnell *et al* (2006) will be able to ascertain this relationship more directly. Second, although similar waveform suppression was demonstrated in the DLPFC and motor cortex, it remains uncertain whether the inhibitory mechanisms, which mediate both forms of waveform suppression, are related. In fact, although the extent of inhibition between these cortical regions was similar and strongly correlated, the morphology of these waveforms do indeed appear to be different (eg, Figure 2 *vs* Figure 7). Again, a pharmacological challenge similar to that described above will be helpful to clarify this relationship. Finally, although we have suggested that inhibition of TMS-induced EEG activity is cortical in nature, other sources may also contribute to this inhibitory effect. For example, TMS-induced APB activation generates hand movement that may be recorded on EEG as a sensory evoked potential. Through paired stimulation, inhibition of such hand contraction would be associated with a concomitant reduction of such evoked potentials. Although our findings cannot fully rule out this possibility, the fact that paired stimulation over the DLPFC produced inhibition of EEG activity that was correlated with the extent of inhibition in the motor cortex makes this possibility less likely. Similarly, it remains possible that some inhibition recorded over the DLPFC from paired stimulation may be related to direct stimulation of frontalis muscle. This possibility, however, is less likely due to the fact that the time course for such inhibition (ie, within the first 25 ms) is different from that used in our study (ie, 50–150 ms), and that direct activation of the frontalis muscle with two TMS pulses spaced 100 ms apart (10 Hz) is expected to produce a compound muscle action potential (ie, enhanced activity and not suppression) based on the principles of nerve conduction studies (Kimura, 2001). Finally, the thalamus may be activated through excitation of cortical–thalamic circuits generated by TMS (Daskalakis *et al*, 2002b). Such activation may, in turn, generate thalamocortical activity that may inhibit the cortex by activating inhibitory interneurons (Daskalakis *et al*, 2005). Therefore, non-cortical processes may play a significant role in the generation of this form of CI. One advantage to recording inhibition directly from the cortex, however, will be that future source localization studies may uncover the origins of such inhibitory potentials. We anticipate that such future work will also be invaluable in helping to identify the pathophysiological origins of a variety of disorders associated with disrupted CI (Cantello *et al*, 2002; Daskalakis *et al*, 2002a). In this regard, future pharmacological studies and molecular studies attempting to clarify the link between these EEG recordings of LICI to $GABA_B$ receptor-mediated mechanisms in the cortex are needed. Additionally, future studies parsing EEG measures of LICI into its component frequencies (eg, delta, theta, alpha beta), are also needed to further characterize its physiology, as several lines of evidence suggest that theta frequencies may be closely tied to GABAergic inhibitory potentials in the cortex (Amzica and Steriade, 1995; Buzsaki, 1997; Buzsaki and Chrobak, 1995; Buzsaki and Eidelberg, 1983; Patenaude *et al*, 2003; Whittington *et al*, 1995; Ylinen *et al*, 1995a, b).

In conclusion, the results of this study suggest that inhibition can directly be recorded from the cortex in at least two cortical areas, the DLPFC and motor cortex. Recording inhibition directly from the cortex was previously impossible due to technological limitations in EEG. Through these novel EEG techniques (eg, high digitization rate, DC filter), which permit recording of paired-pulse TMS directly from the cortex, we anticipate that our results will provide several major advances in our understanding of the pathophysiology and treatment of a variety of neurological and psychiatric disorders. We also expect that these findings may enhance our understanding of the anatomical targets through which novel therapeutic treatments, including repetitive transcranial magnetic stimulation (rTMS) and deep brain stimulation (DBS), may be directed.

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DISCLOSURE/CONFLICT OF INTEREST

None

REFERENCES

Amzica F, Steriade M (1995). Disconnection of intracortical synaptic linkages disrupts synchronization of a slow oscillation. *J Neurosci* **15**: 4658–4677.

Buzsaki G (1997). Functions for interneuronal nets in the hippocampus. *Can J Physiol Pharmacol* **75**: 508–515.

Buzsaki G, Chrobak JJ (1995). Temporal structure in spatially organized neuronal ensembles: a role for interneuronal networks. *Curr Opin Neurobiol* **5**: 504–510.

Buzsaki G, Eidelberg E (1983). Phase relations of hippocampal projection cells and interneurons to theta activity in the anesthetized rat. *Brain Res* **266**: 334–339.

Cantello R, Tarletti R, Civardi C (2002). Transcranial magnetic stimulation and Parkinson's disease. *Brain Res Brain Res Rev* **38**: 309–327.

Chen R, Samii A, Canos M, Wassermann EM, Hallett M (1997). Effects of phenytoin on cortical excitability in humans. *Neurology* **49**: 881–883.

Croft RJ, Chandler JS, Barry RJ, Cooper NR, Clarke AR (2005). EOG correction: a comparison of four methods. *Psychophysiology* **42**: 16–24.

Cui RQ, Huter D, Lang W, Deecke L (1999). Neuroimage of voluntary movement: topography of the Bereitschaftspotential, a 64-channel DC current source density study. *Neuroimage* **9**: 124–134.

Daskalakis ZJ, Christensen BK, Chen R, Fitzgerald PB, Zipursky RB, Kapur S (2002a). Evidence for impaired cortical inhibition in schizophrenia using transcranial magnetic stimulation. *Arch Gen Psychiatry* **59**: 347–354.

Daskalakis ZJ, Christensen BK, Fitzgerald PB, Fountain SI, Chen R (2005). Reduced cerebellar inhibition in schizophrenia: a preliminary study. *Am J Psychiatry* **162**: 1203–1205.

Daskalakis ZJ, Christensen BK, Fitzgerald PB, Roshan L, Chen R (2002b). The mechanisms of interhemispheric inhibition in the human motor cortex. *J Physiol* **543**(Pt 1): 317–326.

Davies CH, Davies SN, Collingridge GL (1990). Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. *J Physiol* **424**: 513–531.

Deisz RA (1999). GABA(B) receptor-mediated effects in human and rat neocortical neurones *in vitro*. *Neuropharmacology* **38**: 1755–1766.

Delorme A, Makeig S (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J Neurosci Methods* **134**: 9–21.

Fitzgerald PB, Brown TL, Daskalakis ZJ, Kulkarni J (2002). A transcranial magnetic stimulation study of inhibitory deficits in the motor cortex in patients with schizophrenia. *Psychiatry Res* **114**: 11–22.

Glahn DC, Ragland JD, Abramoff A, Barrett J, Laird AR, Bearden CE *et al* (2005). Beyond hypofrontality: a quantitative meta-analysis of functional neuroimaging studies of working memory in schizophrenia. *Hum Brain Mapp* **25**: 60–69.

Herwig U, Satrapi P, Schonfeldt-Lecuona C (2003). Using the international 10–20 EEG system for positioning of transcranial magnetic stimulation. *Brain Topogr* **16**: 95–99.

Kang Y, Kaneko T, Ohishi H, Endo K, Araki T (1994). Spatiotemporally differential inhibition of pyramidal cells in the cat motor cortex. *J Neurophysiol* **71**: 280–293.

Kimura J (2001). *Electrodiagnosis in Diseases of Nerve and Muscle: Principles and Practice*. Oxford University Press: New York.

Komssi S, Kahkonen S (2006). The novelty value of the combined use of electroencephalography and transcranial magnetic stimulation for neuroscience research. *Brain Res Brain Res Rev* **52**: 183–192.

Komssi S, Kahkonen S, Ilmoniemi RJ (2004). The effect of stimulus intensity on brain responses evoked by transcranial magnetic stimulation. *Hum Brain Mapp* **21**: 154–164.

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A *et al* (1993). Corticocortical inhibition in human motor cortex. *J Physiol (Lond)* **471**: 501–519.

McCormick DA (1989). GABA as an inhibitory neurotransmitter in human cerebral cortex. *J Neurophysiol* **62**: 1018–1027.

McDonnell MN, Orekhov Y, Ziemann U (2006). The role of GABA(B) receptors in intracortical inhibition in the human motor cortex. *Exp Brain Res* **173**: 86–93.

Mendrek A, Kiehl KA, Smith AM, Irwin D, Forster BB, Liddle PF (2005). Dysfunction of a distributed neural circuitry in schizophrenia patients during a working-memory performance. *Psychol Med* **35**: 187–196.

Morey LC (1991). *Personality Assessment Inventory Professional Manual*. Psychological Assessment Resources Inc.: Odessa, FL.

Morey LC (1996). *An Interpretative Guide to the Personality Assessment Inventory (PAI)*. Psychological Assessment Resources Inc.: Odessa, FL.

Muller MM, Keil A, Kissler J, Gruber T (2001). Suppression of the auditory middle-latency response and evoked gamma-band response in a paired-click paradigm. *Exp Brain Res* **136**: 474–479.

Nakamura H, Kitagawa H, Kawaguchi Y, Tsuji H (1997). Intracortical facilitation and inhibition after transcranial magnetic stimulation in conscious humans. *J Physiol* **498**(Pt 3): 817–823.

Oldfield RC (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* **9**: 97–113.

Patenaude C, Chapman CA, Bertrand S, Congar P, Lacaille JC (2003). GABAB receptor- and metabotropic glutamate receptor-dependent cooperative long-term potentiation of rat hippocampal GABAA synaptic transmission. *J Physiol* **553**(Pt 1): 155–167.

Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ *et al* (1994). Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalogr Clin Neurophysiol* **91**: 79–92.

Sanger TD, Garg RR, Chen R (2001). Interactions between two different inhibitory systems in the human motor cortex. *J Physiol* **530**(Pt 2): 307–317.

Siebner HR, Dressnandt J, Auer C, Conrad B (1998). Continuous intrathecal baclofen infusions induced a marked increase of the transcranially evoked silent period in a patient with generalized dystonia. *Muscle Nerve* **21**: 1209–1212.

Tan HY, Choo WC, Fones CS, Chee MW (2005). fMRI study of maintenance and manipulation processes within working memory in first-episode schizophrenia. *Am J Psychiatry* **162**: 1849–1858.

Valls-Sole J, Pascual-Leone A, Wassermann EM, Hallett M (1992). Human motor evoked responses to paired transcranial magnetic stimuli. *Electroencephalogr Clin Neurophysiol* **85**: 355–364.

Verlinden J, Imre H, Edelenbos, E (2006). Treatise of technologies for interactive augmented prototyping. In: Horvath, I, Duhovnik, J (eds.) *Proceedings of the Tools and Methods of Competitive Engineering* Organizing Committee of Tools and Methods of Competitive Engineering: Llubljana, Slovenia, pp. 1–14.

Weinberger DR, Berman KF, Zec RF (1986). Physiologic dysfunction of dorsolateral prefrontal cortex in schizophrenia. I. Regional cerebral blood flow evidence. *Arch Gen Psychiatry* **43**: 114–124.

Werhahn KJ, Kunesch E, Noachtar S, Benecke R, Classen J (1999). Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. *J Physiol (Lond)* **517**(Pt 2): 591–597.

Whittington MA, Traub RD, Jefferys JG (1995). Synchronized oscillations in interneuron networks driven by metabotropic glutamate receptor activation. *Nature* **373**: 612–615.

Ylinen A, Bragin A, Nadasdy Z, Jando G, Szabo I, Sik A *et al* (1995a). Sharp wave-associated high-frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechanisms. *J Neurosci* **15**(1 Pt 1): 30–46.

Ylinen A, Soltesz I, Bragin A, Penttonen M, Sik A, Buzsaki G (1995b). Intracellular correlates of hippocampal theta rhythm in identified pyramidal cells, granule cells, and basket cells. *Hippocampus* **5**: 78–90.

Ziemann U, Lonnecker S, Steinhoff BJ, Paulus W (1996a). The effect of lorazepam on the motor cortical excitability in man. *Exp Brain Res* **109**: 127–135.

Ziemann U, Lonnecker S, Steinhoff BJ, Paulus W (1996b). Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study [see comments]. *Ann Neurol* **40**: 367–378.