Evidence for gamma inhibition deficits in the
dorsolateral prefrontal cortex of patients
with schizophrenia

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Previous studies have shown that patients with schizophrenia and bipolar disorder have deficits in cortical inhibition. Through the combination of interleaved transcranial magnetic stimulation and electroencephalography, we have recently reported on methods in which cortical inhibition can be measured from the dorsolateral prefrontal cortex, a cortical region that is more closely associated with the pathophysiology of schizophrenia. Furthermore, it is possible to index cortical inhibition of specific oscillatory frequencies including the gamma band (30–50 Hz) whose modulation has been related to higher order cortical processing. In this study, we show that patients with schizophrenia have significant deficits of cortical inhibition of gamma oscillations in the dorsolateral prefrontal cortex compared to healthy subjects and patients with bipolar disorder, while no deficits are demonstrated in the motor cortex. These results suggest that the lack of inhibition of gamma oscillations in the dorsolateral prefrontal cortex may represent an important frontal neurophysiological deficit, which may be responsible for the spectrum of deficits commonly found in schizophrenia.

Keywords: transcranial magnetic stimulation; electroencephalography; dorsolateral prefrontal cortex; schizophrenia; bipolar disorder

Abbreviations: CI = cortical inhibition; DLPFC = dorsolateral prefrontal cortex; GABA = γ-aminobutyric acid; LICI100 = long interval CI with 100 ms interstimulus interval; TMS = transcranial magnetic stimulation
Introduction

Cortical inhibition (CI) refers to a neurophysiological process in which cortical γ-aminobutyric acid (GABA) inhibitory interneurons selectively attenuate the activity of other neurons (e.g. pyramidal neurons) in the cortex. Several lines of evidence suggest that CI is impaired in patients with schizophrenia and bipolar disorder. For example, in the motor cortex, deficits in CI have been demonstrated in previous transcranial magnetic stimulation (TMS) studies in patients with schizophrenia (Daskalakis et al., 2002a; Fitzgerald et al., 2002, 2003) and more recently in bipolar disorder (Levinson et al., 2007). This is consistent with several studies that have provided neuroanatomic evidence for GABAergic impairments in the dorsolateral prefrontal cortex (DLPFC) of patients with schizophrenia and bipolar disorder (Benes and Berretta, 2001; Lewis et al., 2005; Akbarian and Huang, 2006; Straub et al., 2007; Hashimoto et al., 2008).

Disrupted gamma (γ) oscillatory activity (30–50 Hz) in the cortex, as indexed through electroencephalography (EEG), has also been demonstrated in patients with schizophrenia (Lee et al., 2003; Spencer et al., 2003; Lewis et al., 2005; Cho et al., 2006; Light et al., 2006; Ferrarelli et al., 2008). For example, it has been shown that patients with schizophrenia have deficits in γ oscillatory activity in response to 40 Hz auditory stimulation (Light et al., 2006) or during perception of gestalt objects (Spencer et al., 2003) as compared to healthy individuals, while other studies have shown an impairment in modulation of γ oscillations in the DLPFC during working memory tasks (Cho et al., 2006). Also, both increase and reduction of γ oscillations have been reported in patients with schizophrenia (Lee et al., 2003; Ferrarelli et al., 2008). Several reports have implicated GABAergic inhibitory neurotransmission in both the generation and modulation of γ oscillations in the cortex (Whittington et al., 1995, 2000; Traub et al., 1996; Wang and Buzsaki, 1996; Bartos et al., 2007; Brown et al., 2007b; Leung and Shen, 2007). Specifically, these studies suggest that the GABA A receptor mediated inhibitory post-synaptic potentials contribute to generation of γ oscillations (Whittington et al., 1995; Wang and Buzsaki, 1996; Bartos et al., 2007), whereas GABA A receptor mediated inhibitory post-synaptic potentials have been suggested to be key to the modulation of γ oscillations (Whittington et al., 1995; Brown et al., 2007b; Leung and Shen, 2007). While several studies have examined the association between the generation of γ oscillatory activity with frontal cognitive deficits in schizophrenia (Cho et al., 2006), to our knowledge, no human studies have yet investigated the role of modulation of γ oscillations in this disorder. Such knowledge may provide valuable insight into the neurophysiological underpinnings of frontal deficits in this disorder.

We have recently reported on methods (Daskalakis et al., 2008) through which CI can be measured directly from the DLPFC and motor cortex through interleaved TMS with EEG in a paired pulse TMS paradigm known as long interval CI. Long interval CI involves stimulation of the cortex with a conditioning stimulus within an interval of 50–200 ms prior to a test stimulus which results in suppression of cortical evoked activity of about 30% (Daskalakis et al., 2008) compared to a single test stimulus alone. Several lines of evidence suggest that such long interval CI induced suppression of the cortex is related to GABA A receptor mediated inhibitory neurotransmission (Sanger et al., 2001; McDonnell et al., 2006). In long interval CI, activation of GABA A receptors, evoked by the conditioning stimulus, inhibits excitation of cortex to a second pulse (test stimulus) that would otherwise excite the cortex if delivered alone. The GABA A receptor activation is suggested to peak around 150–200 ms post-stimulus (McCormick, 1989), as such, long interval CI is optimal when conditioning stimulus precedes the test stimulus by about 100–150 ms. We have recently investigated the modulatory influence of long interval CI on cortical oscillations across the five frequency bands [i.e. δ (1–3 Hz), θ (4–7 Hz), α (8–12 Hz), β (12.5–28 Hz), γ (30–50 Hz)] in the DLPFC and motor cortex in healthy subjects and reported that long interval CI selectively inhibits high frequency oscillations, particularly γ oscillations, in the DLPFC compared to the motor cortex (Farzan et al., 2009). However, it is unclear whether this pattern of inhibitory modulation of γ oscillations is preserved in illnesses whose pathophysiology may be related to GABAergic inhibitory deficits.

The aim of the current study, therefore, was to evaluate the effect of GABA A receptor mediated inhibitory neurotransmission on γ oscillations in the DLPFC and motor cortex of patients with schizophrenia compared to age- and sex-matched patients with bipolar disorder and healthy subjects. It was hypothesized that patients with schizophrenia would show selective deficits in the ability to inhibit γ oscillations in the DLPFC compared with healthy subjects and patients with bipolar disorder, in whom disruption of γ oscillations is inconsistent (for review, see Basar and Guntekin, 2008).

Materials and methods

Participants

This study included 14 patients with a Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV confirmed diagnosis of schizophrenia, 14 patients with a Structured Clinical Interview for DSM-IV confirmed diagnosis of bipolar disorder and 14 healthy subjects. Patients with schizophrenia did not differ from the other two groups in relation to age or sex (Table 1). Patients with schizophrenia also did not differ from patients with bipolar disorder for duration of illness (Table 1). Finally, patients with schizophrenia did not differ from patients with bipolar disorder in years of education (bipolar disorder: 14.8 ± 2.4 years, schizophrenia: 13.6 ± 2.5 years; t = 1.3, df = 26, P = 0.2). Two of the patients with schizophrenia were unmedicated (one medication-naïve; one medication-free for 6 months) and 12 patients were on medication (390.0 ± 54.8 mg clozapine, n = 5; 3.2 ± 2.5 mg risperidone, n = 3; 2 ± 1.4 mg haloperidol, n = 2; 100 mg of quetiapine, n = 1; 16 mg perphenazine, n = 1). Four of the patients with bipolar disorder were unmedicated (medication-naïve) and 10 patients were on medication (1100.0 ± 268.3 mg lithium, n = 3; 900.0 mg lithium and 1.0 mg risperidone, n = 2; 533.3 ± 115.5 mg quetiapine, n = 3; 20 mg olanzapine, n = 1; 200 mg clozapine and 200 mg buproprion, n = 1). Finally, 12 of the 14 patients with bipolar disorder had a history of psychosis.

In patients with schizophrenia, the Positive and Negative Syndrome Scale (Kay et al., 1987) was used to index the severity of
psychopathology (Table 1). Patients with bipolar disorder were diagnosed as bipolar I disorder, and Hamilton Depression Rating Scale and Young Mania Rating Scale were administered to assess the symptoms severity (Table 1). Patients with bipolar disorder had 6±14 major depressive episodes and 4±6 manic episodes. Ten patients with bipolar disorder were in remission (Young Mania Rating Scale ≤ 7, Hamilton Depression Rating Scale ≤ 8) (Chengappa et al., 2003), two had moderate manic symptoms (Young Mania Rating Scale ≤ 15), and two patients reported moderate depressive symptoms (Hamilton Depression Rating Scale ≤ 21). Ten patients with schizophrenia were mildly to moderately ill (Positive and Negative Syndrome Scale total score ≤ 71) (Leucht et al., 2005) and four patients were moderately ill (Positive and Negative Syndrome Scale total score ≤ 96) (Leucht et al., 2005). Additionally, five patients with schizophrenia were in remission (scored 3 or less on eight symptoms in the Positive and Negative Syndrome Scale) (van Os et al., 2006). Finally, one patient with schizophrenia and two patients with bipolar disorder were inpatients, while all others were outpatients. Therefore, severity of symptoms was comparable between patients with schizophrenia and bipolar disorder. In healthy subjects, psychopathology was ruled out through the personality assessment screener (Psychological Assessment Resources, Inc.) and exclusion criteria included a self-reported co-morbid medical illness or a history of drug or alcohol abuse. All subjects were right-handed, and handedness was confirmed using the Oldfield Handedness Inventory (Oldfield, 1971). All subjects gave their written informed consent and the protocol was approved by the Centre for Addiction and Mental Health in accordance with the Declaration of Helsinki.

### Experiment design

Active TMS was administered over the left motor cortex and DLPFC of all subjects. Inhibition was measured through long interval CI and indexed through EMG and EEG. Long interval CI involves the pairing of a suprathreshold conditioning stimulus followed by a suprathreshold test stimulus at long interstimulus intervals (e.g. 100 ms), which inhibits the motor evoked potential produced by the test stimulus (Valls-Sole et al., 1992). Long interval CI is reportedly optimal at 100 ms interstimulus interval (Sanger et al., 2001), and as such, in this experiment we evaluated long interval CI at this interval (i.e. LICI100). Both the conditioning stimulus and test stimulus were delivered at the same suprathreshold intensity that was adjusted to produce mean peak-to-peak motor evoked potential amplitudes of 1 mV. One hundred TMS stimuli were delivered per-condition (paired conditioning stimulus-test stimulus and test stimulus alone) every 5s. Moreover, to control for the effect of TMS click-induced auditory activation on the cortical evoked potentials, sham stimulation was administered using the same parameters as the active stimulation over the DLPFC and motor cortex of patients with schizophrenia, bipolar disorder and in a subset of 11 healthy subjects with the coil angled at 90° from the scalp, resting on one wing of the coil. All conditions were randomly counterbalanced between subjects to avoid order effects.

### Data recording

#### 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. We examined long interval CI in the motor cortex through simultaneous recording of both EMG and EEG, and in the DLPFC through EEG only. The intensity of TMS pulses was determined at the beginning of each experiment and it was set such that it elicited an average motor evoked potential of 1 mV peak-to-peak upon delivery of 20 pulses over the motor cortex; this corresponded to 65.8±17.1% of stimulator output in patients with schizophrenia, 72.1±15.2% in patients with bipolar disorder, and to 62.8±11.8% in healthy subjects.

#### Localization of motor cortex

In stimulating the motor cortex, the TMS coil was placed at the optimal position for eliciting motor evoked potentials from the right abductor pollicis brevis muscle, which typically corresponds to a region between FC3 and C3 electrodes using the international 10–20 EEG system.

#### Localization of DLPFC

Localization of DLPFC was achieved through neuronavigation techniques using the MINIBIRD system (Ascension Technologies) and MRicro/reg software using a T1-weighted 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 co-ordinates (x, y, z)=(-50, 30, 36)] corresponding with posterior regions of Brodmann area 9 that overlap with the superior section of Brodmann area 46. This site was chosen based on a recent meta-analysis of functional imaging studies of working memory and the DLPFC (Cannon et al., 2005; Mendrek et al., 2005; Tan et al., 2005). This ensured that assessment was targeted at

### Table 1  Demographic and clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>Schizophrenia (n=14)</th>
<th>Bipolar disorder (n=14)</th>
<th>Healthy (n=14)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.5 ± 10.4</td>
<td>32.6 ± 13.4</td>
<td>36.7 ± 7.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>10/4</td>
<td>9/5</td>
<td>9/5</td>
<td>0.9</td>
</tr>
<tr>
<td>Illness duration (years)</td>
<td>9.8 ± 7.3</td>
<td>7.7 ± 9.3</td>
<td>NA</td>
<td>0.5</td>
</tr>
<tr>
<td>PANSS scores</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>65.5 ± 18.3</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>16.6 ± 4.4</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>18.4 ± 6.3</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Global</td>
<td>30.6 ± 9.2</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>HAMD</td>
<td>NA</td>
<td>6.2 ± 5.9</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>YMRS</td>
<td>NA</td>
<td>4.0 ± 4.5</td>
<td>NA</td>
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</tr>
</tbody>
</table>

Data are given as mean ± SD. PANSS = Positive and Negative Syndrome Scale; HAMD = Hamilton Depression Rating Scale; YMRS = Young Mania Rating Scale; NA = not applicable.
a DLPFC site where functional neurophysiological abnormalities have been demonstrated.

**EMG recording**

EMG was captured by placing two disposable disc electrodes over the right abductor pollicis brevis muscle in a tendon-belly arrangement and motor evoked potentials were filtered (band-pass 2 Hz to 5 kHz), digitized at 5 kHz (Micro 1401, Cambridge Electronics Design, Cambridge, UK), and collected through commercially available software Signal (Cambridge Electronics Design, UK) according to our previously published methods (Daskalakis et al., 2002b).

**EEG recording**

To evaluate TMS induced cortical evoked potentials, EEG was recorded concurrently with the EMG. EEG was acquired through a 64-channel Synamps 2 EEG system. A 64 channel EEG cap was used to record the cortical signals, 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 artefacts. All electrodes were referenced to an electrode positioned posterior to Cz electrode. EEG signals were recorded DC and a low pass filter of 100 Hz at 20 kHz sampling rate, which was shown to avoid saturation of amplifiers and minimize the TMS related artefact (Daskalakis et al., 2008).

**Data analysis**

In the motor cortex, LICI100 was evaluated through both EEG and EMG, which were referred to as EEG and EMG measure of LICI100. In the DLPFC, LICI100 was evaluated through EEG only.

**EMG measure of inhibition**

For each subject, the EMG measure of LICI100 was indexed by comparing the area under the curve of the rectified averaged motor evoked potential following the single pulse of TMS (unconditioned) with the area under curve following the paired pulses of TMS (conditioned) and the inhibition was obtained as follows:

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

**EEGLAB toolbox** (Delorme and Makeig, 2004). To quantify the EEG measure of LICI100 in all five frequency bands, for each subject, the average TMS evoked cortical potentials following single and paired pulses of TMS were decomposed into the \( \delta (1–3.5 \text{ Hz}), \theta (4–7 \text{ Hz}), \alpha (8–12 \text{ Hz}), \beta (12.5–28 \text{ Hz}) \) and \( \gamma (30–50 \text{ Hz}) \) frequency components by means of a Hamming-based zero phase-shift finite impulse response filter, and for each frequency band inhibition was obtained through Equation (1) (i.e. area under rectified curve for averaged EEG recordings between 50–150 ms post-test stimulus). The first interval (i.e. 50 ms post-stimulus) was chosen as it represents the earliest artefact free data that can be recorded post-stimulus and the second interval (i.e. 150 ms post-stimulus) was chosen as it represents the duration of GABA\(_b\) receptor inhibitory post-synaptic potentials (McCormick, 1989; Deisz, 1999) (i.e. 250 ms) elicited by the conditioning stimulus (Sanger et al., 2001). We referred to these measures as CI\(_\delta\), CI\(_\theta\), CI\(_\alpha\), CI\(_\beta\) and CI\(_\gamma\) to represent the extent of inhibition in each frequency band. Furthermore, to obtain the total amount of EEG inhibition, for each subject the TMS evoked cortical potentials following the single and paired pulse of TMS were band pass filtered (1–50 Hz) and CI\(_\text{total}\) was calculated through Equation (1). Finally, to evaluate long interval CI directly from the motor cortex, C3 electrode was used as it has been shown to be the electrode that best represents evoked activity in the hand area of motor cortex and is closest to the optimal site of abductor pollicis brevis muscle activation through TMS (Cui et al., 1999). To capture long interval CI in the DLPFC, the recording electrode of interest was AF3, which optimally represents the overlap of Brodmann areas 9 and 46 of the DLPFC.

**Statistical analysis**

The effects of subject group and region were tested separately for each frequency band. A mixed model which included Group (schizophrenia, bipolar disorder and healthy subjects), Region (motor cortex and DLPFC) and the Group \( \times \) Region interaction was used in each case. A heterogeneous compound symmetric covariance structure was used to model the relatedness between measures taken from different regions for the same subject. Pair-wise comparisons were two-tailed, and \( P \)-values were Bonferroni adjusted. The Chi-Square test was used for the categorical variables (i.e. gender). Finally, a Pearson’s correlation coefficient was used to determine the relationship between CI\(_\gamma\) and anti-psychotic medication dose (in chlorpromazine equivalents). The group means were reported in the form of mean ± standard deviation, and a \( P \)-value less than 0.05 was considered statistically significant. All statistical analyses were performed using SAS 9.1.3 (SAS Inc. Cary, NC, USA) and SPSS 15.0 (SPSS Inc. Chicago, Illinois, USA).

**Results**

**Inhibitory effect of long interval CI on cortical oscillations**

To compare the modulatory effect of LICI100 on oscillations across cortical regions (motor cortex versus DLPFC) and groups (healthy subjects, bipolar disorder, schizophrenia), a mixed model was fit with the main effects of Group and Region, and an interaction effect of Group \( \times \) Region. The results indicated that CI\(_\gamma\) was significantly different across groups, as the group \( \times \) region interaction effect was significant (\( F_{2,39} = 4.9, \ P = 0.01 \)). Post hoc analyses revealed that patients with schizophrenia had significantly lower
CI in the DLPFC (CI = 10.7% ± 37.0%) compared to patients with bipolar disorder (P < 0.01; CI = 48.2% ± 28.1%, Cohen d = 1.1) and healthy subjects with a large effect size (P < 0.01; CI = 52.2% ± 12.4%, Cohen d = 1.5) (Figs 1–4). That is, in the DLPFC of patients with schizophrenia, the area under the curve for mean rectified oscillations following LICI 100 (paired pulse TMS) was not reduced compared to the area under the curve for mean rectified oscillations following test stimulus alone (t = 1.3, df = 13, P = 0.2), while it was significantly reduced in healthy subjects (t = 9.5, df = 13, P < 0.001) and patients with bipolar disorder (t = 4.9, df = 13, P < 0.001) (Fig 1). Finally, in the motor cortex, CI was not significantly different compared to patients with bipolar disorder (P = 0.9) (Figs 2 and 3). We found no differences between groups for the remaining EEG rhythms (Fig 3).

When EEG waveforms were analysed without frequency decomposition, there was a significant suppression in the area under the mean rectified EEG curve through LICI100 as compared to test stimulus alone in DLPFC across all three groups (CI total: healthy controls = 31.8 ± 47.2%, P = 0.003; bipolar disorder = 46.1 ± 52.9%, P = 0.002; schizophrenia = 35.1 ± 24.5%, P < 0.001). Similarly, in the motor cortex, the mean area under rectified EEG curve was significantly suppressed through LICI100 as compared to test stimulus alone (CI total: healthy controls = 48.4 ± 27.5%, P = 0.002; bipolar disorder = 44.8 ± 35.8%, P = 0.007; schizophrenia = 35.6 ± 24.9%, P < 0.001).

Figure 1 TMS evoked γ oscillations following single and paired pulse TMS applied to the left DLPFC. Data obtained from 42 subjects: 14 patients with schizophrenia (SCZ), 14 healthy subjects (HLT) and 14 patients with bipolar disorder (BD). In all figures, γ oscillations are recorded from the AF3 electrode, which optimally represents the overlap of Brodmann areas 9 and 46 of the DLPFC. (A) and (B) represent data from individual subjects and (C) represents averaged data. (A) The y-axes illustrate cortical evoked potentials (µV) for γ oscillations following single (left panel) and paired pulse TMS (right panel), and the x-axes represent time (ms) following the delivery of test stimulus (TS). In these figures, γ oscillations are plotted for individual subjects in each group (healthy, bipolar disorder, and schizophrenia). (B) Figures illustrate the area under the rectified curve (µV ms) for γ oscillations following single and paired pulse TMS for individual subjects in each group. For each subject, a line connects the area under the curve in single pulse to area under the curve in paired pulse condition to illustrate the change in area within individuals. (C) Histograms represent the mean area under the rectified curve for γ oscillations, averaged across 14 subjects per group, in paired pulse compared to single pulse condition. Error bars indicate standard error. As illustrated, in schizophrenia, area under the curve following paired pulse condition is not significantly reduced compared to single pulse.
Excitatory effect of single pulse of TMS

In order to examine the effect of single pulse of TMS on cortical oscillations across cortical regions and groups, similar analyses were performed and a mixed model was fit for the area under the curve following single pulse of TMS for each frequency band. We found no between group differences, as the main effect of Group and the interaction effect of Group \times Region were not significant for any of the frequency bands, including the \(\gamma\) oscillations (Group: \(F_{1,39} = 1.1, P = 0.3\); Group \times Region: \(F_{2,39} = 0.1, P = 0.9\)).

Effect of TMS click-induced auditory activation

In order to rule out the possibility that suppression of cortical evoked activity measured through LI CI could be due to the suppression of auditory evoked potentials (e.g. N100 suppression following presentation of paired auditory clicks separated by 100 ms), sham long interval CI was applied for both the motor cortex and DLPFC. In this way, sham stimulation preserves the auditory stimulation produced by TMS clicks, without eliciting direct brain stimulation (Figs 5 and 6). To control for the effects of TMS induced auditory activation, for the subset of subjects who had received both active and sham stimulation (DLPFC: \(n_{\text{schizophrenia}} = 14, n_{\text{bipolar}} = 14, n_{\text{healthy}} = 11\); motor cortex: \(n_{\text{schizophrenia}} = 12, n_{\text{bipolar}} = 14, n_{\text{healthy}} = 11\)), the cortical evoked potential following single and paired pulses of TMS in sham stimulation was subtracted from the TMS evoked cortical potential in the active stimulation of both the motor cortex and the DLPFC (Daskalakis et al., 2008). In patients with schizophrenia, similar to healthy subjects and patients with bipolar disorder (data not shown), suppression of cortical evoked potential did not change after controlling for the effect of auditory evoked responses in the DLPFC (CI\text{total}: Active = 45.5 \pm 28.5\%, Active-Sham = 42.8 \pm 28.9\%, \(P = 0.4\); CI\text{sub}: Active = 10.7 \pm 37.0\%, Active-Sham = 1.1 \pm 47.3\%, \(P = 0.3\)) or the motor cortex (CI\text{total}: Active = 35.1 \pm 26.9\%, Active-Sham = 27.2 \pm 31.6\%, \(P = 0.2\); CI\text{sub}: Active = 6.3 \pm 41.0\%, Active-Sham = 11.6 \pm 45.8\%, \(P = 0.6\)).

Effect of antipsychotic treatments

As a further measure to ensure that the observed deficit in CI\text{sub} is not related to antipsychotic treatment we evaluated the relationship between CI\text{sub} and antipsychotic medications (converted chlorpromazine equivalents) (Woods, 2003) in patients with bipolar disorder and schizophrenia who were treated with antipsychotic medications (\(n = 19\)). This analysis revealed no significant correlation between CI\text{sub} and chlorpromazine equivalents (\(r = -0.1, P = 0.5\)). Furthermore, this analysis was re-evaluated across all patients (\(n = 28\), with the chlorpromazine equivalents set to zero for patients who were not treated with antipsychotics. This analysis also revealed no significant relationship between CI\text{sub} and chlorpromazine equivalents (\(r = -0.2, P = 0.3\)).
Finally, the fact that these deficits were specific to schizophrenia and demonstrated only in the DLPFC suggests that such findings are not part of a generalized deficit that is simply related to psychopathology or psychotropic medications.

Deficits in CI\textsubscript{\textgamma} probably lead to an imbalance between excitatory and inhibitory circuits in schizophrenia. This imbalanced circuitry is also evident from previous studies. For example, both increased (Lee et al., 2003) and reduced \textgamma oscillations (Cho et al., 2006; Ferrarelli et al., 2008) have been reported in patients with schizophrenia. However, our findings suggest that it is the modulation rather than the generation of \textgamma oscillations that is deficient. The mechanism that leads to this deficiency can be reconciled by understanding the interaction between the GABA\textsubscript{\textbeta} and GABA\textsubscript{\textalpha} receptor mediated inhibitory post-synaptic potentials in the cortex. As previously mentioned, generation of \textgamma oscillations is related to GABA\textsubscript{\textalpha} receptor neurotransmission (Whittington et al., 1995; Wang and Buzsaki, 1996; Bartos et al., 2007). GABA\textsubscript{\textbeta} receptors typically discharge at 30–50 Hz resulting in a high frequency on–off oscillatory pattern of pyramidal cell discharge that is recorded on EEG as \textgamma frequency oscillations. By contrast, GABA\textsubscript{\textalpha} receptors, which have longer lasting inhibitory effect in the cortex (i.e. 250–500 ms), have been shown to inhibit GABA\textsubscript{\textbeta} receptors (Sanger et al., 2001; Daskalakis et al., 2002b) and as such, represent important modulators of \textgamma oscillatory discharge. It is through this modulatory effect of GABA\textsubscript{\textbeta} receptors that GABA\textsubscript{\textalpha} receptor mediated inhibitory post-synaptic potentials suppress \textgamma oscillations. This modulatory effect of GABA\textsubscript{\textbeta} receptors on \textgamma oscillatory discharge is also consistent with related animal experiments (Whittington et al., 1995; Brown et al., 2007b; Leung and Shen, 2007). For example, it has been shown that administration of GABA\textsubscript{\textalpha} receptor agonists such as baclofen eliminates \textgamma oscillations in rat hippocampus slices (Brown et al., 2007b), while the blockade of GABA\textsubscript{\textbeta} receptors enhances the \textgamma oscillations in the hippocampus of behaving bats (Leung and Shen, 2007). Through optogenetic studies, Sohal et al. (2009) have also shown that inhibition of fast spiking parvalbumin interneurons, whose currents have been closely related to the generation of \textgamma oscillations, results in suppression of such oscillatory activity. In schizophrenia, decreased parvalbumin and glutamate decarboxylase-67 expression was found in parvalbumin positive GABAergic interneurons (Hashimoto et al., 2003). Therefore, a selective impairment in CI\textsubscript{\textgamma} in the DLPFC in schizophrenia represents in vivo evidence which confirms these post-mortem findings.

Deficits in CI\textsubscript{\textgamma} in DLPFC are consistent with neurophysiological evidence of frontal deficits in schizophrenia. For example, deficits in cognitive functions, such as working memory, constitute the major feature of schizophrenia (Weinberger et al., 1986), while the functional role of \textgamma oscillations has been closely linked with processing of higher order cognitive functions including working memory (Tallon-Baudry and Bertrand, 1999; Lewis et al., 2005). By contrast, deficits in \textgamma oscillations have not been consistently reported in bipolar disorder (for review, see Basar and Gunterekin, 2008), and working memory performance is preserved in this illness (Brown et al., 2007a). The modulation of \textgamma oscillatory activity in the DLPFC has been closely related to several cognitive and behavioural processes. For example, the successful encoding of information may depend on its arrival time relative to the \textgamma

Discussion

Our findings suggest that patients with schizophrenia demonstrated selective deficits in CI\textsubscript{\textgamma} in the DLPFC compared to bipolar disorder and healthy subjects. By contrast, there were no differences in inhibition of other oscillatory frequencies in the DLPFC or in the motor cortex between groups. Our results also suggest that it is the inhibition rather than the generation of \textgamma oscillatory activity that is dysfunctional in schizophrenia, as there were no differences in \textgamma oscillatory activity in response to single pulse TMS.

Figure 3 Modulation of cortical oscillations in response to application of long interval CI to the left motor cortex and left DLPFC. Histograms represent inhibition of cortical oscillations, obtained through Equation (1) (see Materials and methods section), in response to activation of GABA\textsubscript{\textbeta} mediated inhibitory neurotransmission, induced by LICI\textsubscript{100}, and averaged across 14 healthy subjects (HLT), 14 patients with bipolar disorder (BD) and 14 patients with schizophrenia (SCZ). Motor Cortex (left panel): across all groups, long interval CI resulted in significant suppression of \textdelta, \texttheta and \textalpha oscillations but \textbeta and \textgamma oscillations were not inhibited. DLPFC (right panel): in healthy subjects and patients with bipolar disorder, long interval CI resulted in significant suppression of all frequency bands, but patients with schizophrenia showed a selective deficit in inhibition of \textgamma oscillations compared to patients with bipolar disorder and healthy subjects.
cycle (Fries et al., 2007). Information arriving during the fading phase of inhibition would be potentiated, while information arriving at the beginning of the inhibitory period will be blocked from further propagation (Fries et al., 2007). Furthermore, recordings from the DLPFC of monkeys engaged in a spatial working memory task demonstrated that the activity of inhibitory neurons were maximal at different time intervals during the task performance, suggesting that inhibitory interneurons shape the time course for the prefrontal pyramidal neuron activation (Constantinidis et al., 2002). That is, pyramidal neurons were maximally active when the fast spiking interneurons were not firing (Wilson et al., 1994), suggesting that the functional role of inhibition may be to shape the temporal profile of incoming information during different phases of cognitive tasks such as

**Figure 4** Topographic illustration of inhibition of $\gamma$ oscillations in the DLPFC. Topographic plots illustrate the inhibition of $\gamma$ oscillations (CI$_\gamma$) following the application of LCl$_{100}100$ to the left DLPFC averaged across 14 healthy subjects (HLT), 14 patients with bipolar disorder (BD) and 14 patients with schizophrenia (SCZ). Inhibition is obtained through Equation (1) (see Materials and methods section), and the hot colours indicated the area of maximum inhibition. These plots suggest that patients with schizophrenia have low CI$_\gamma$ in the DLPFC compared to patients with bipolar disorder and healthy subjects. Topographic head plots were obtained by EEGLAB toolbox (Delorme and Makeig, 2004).

**Figure 5** Effect of TMS click-induced auditory activation in the DLPFC. Rectified EEG potentials in each frequency band recorded following the delivery of single pulse of active (solid waveform) and sham (dash waveform) stimulation to the left DLPFC averaged across 14 patients with schizophrenia. In all figures, the x-axis represents time after delivery of test stimulus (TS), and the y-axis represents evoked potentials recorded from AF3 electrode, which optimally represents the overlap of Brodmann area 9 and 46 of the DLPFC.

**Figure 6** Effect of TMS click-induced auditory activation in the motor cortex. Mean rectified EEG oscillations recorded following the delivery of single pulse of active (solid waveforms) and sham (dash waveforms) stimulation to the left motor cortex averaged across 12 patients with schizophrenia. In all figures, the x-axis represents time after delivery of test stimulus (TS), and the y-axis represents evoked potentials recorded from C3 electrode, which lies nearest to the motor cortex.
working memory. Therefore, our finding that CI, was not present in the DLPCF of patients with schizophrenia suggests that such aberrant neurophysiological circuitry may be related to a disruption in synchronized firing of fast oscillating interneurons, which is essential for optimal cognitive and behavioural performance.

There are several reasons why the present findings are related specifically to schizophrenia and are not part of a generalized deficit that is common to other psychiatric illness or medications. First, deficient CI, in the DLPCF was specific to patients with schizophrenia despite the fact that half of patients with bipolar disorder were also treated with antipsychotic medications and nearly all (12 out of 14) had a history of psychosis. Second, no correlation was found between the extent of CI, and the dose of antipsychotic treatments among patients with schizophrenia and bipolar disorder. Additionally, if medications were solely responsible for the observed deficit then it would be anticipated that both the motor cortex and DLPCF would have been affected as a regionally specific antipsychotic effect is low. Therefore, deficient CI, in the DLPCF may represent a key neurophysiological deficit that is specific to schizophrenia and independent of antipsychotic treatment, making it possible that this neurophysiological finding represents a candidate endophenotype for this illness. However, to ascertain this further, future experiments evaluating its heritability (e.g. how much of the variance of this trait is accounted for by genetic variance) and trait stability (i.e. evaluating the persistence of such deficits prior to and after treatment with antipsychotics) are needed (Braff et al., 2008).

The results of this study are limited in some important ways. First, although pharmacological findings suggest that long interval CI is mediated through GABA, receptors (McDonnell et al., 2006), the effect of other neurotransmitter systems (i.e. N-methyl d-aspartate) cannot be completely ruled out. Future work, therefore, should examine the effect of administration of a variety of agents including GABA, agonists (i.e. baclofen), as well as other neurotransmitter systems such as N-methyl d-aspartate, dopamine and serotonin on CI,. Furthermore, as sham stimulation does not produce sensory evoked potentials identical to active stimulation, subtracting waveforms following sham stimulation from the waveforms generated following active stimulation may not completely control for all potential sources of artifact (e.g. somatosensory artefact) of TMS on EEG waveforms. Finally, though we have made inferences for the role of CI, vis à vis cognition, there is currently no direct evidence to support these findings. Comparing dimensions of cognitive performance subserved by frontal brain regions with CI, may help to elucidate further the complex role these processes play in the higher order cognitive processing.

In summary, our findings suggest that CI, in the DLPCF is selectively impaired in patients with schizophrenia. Future studies are needed to explore the potential of this finding as a neurophysiological endophenotype for this illness. These results also suggest that in schizophrenia, the lack of inhibition may translate into deficient modulation of γ oscillations in the DLPCF that could be responsible for the spectrum of frontal deficits commonly found in this disorder.

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