



Clozapine potentiation of GABA mediated cortical inhibition in treatment resistant schizophrenia



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ABSTRACT

Background: Cortical inhibition (CI) deficits have been demonstrated in schizophrenia using transcranial magnetic stimulation (TMS). These CI deficits may be related to decreased GABA activity which may be involved in schizophrenia pathophysiology. Previous cross-sectional studies have also demonstrated greater CI in patients treated with clozapine than other typical/atypical antipsychotics. However, it is not clear if these differences in CI are a result of treatment-resistant illness which necessitates clozapine or are related to clozapine treatment. **Methods:** TMS measures of CI (i.e., cortical silent period (CSP) and short-interval cortical inhibition (SICI)) were measured over the motor cortex in 16 patients with schizophrenia before starting clozapine, then 6 weeks and 6 months after starting clozapine.

Results: CSP was significantly longer after 6 weeks of treatment with clozapine ($p = 0.014$). From 6 weeks to 6 months, there was no significant difference in CSP ($p > 0.05$). Short-interval cortical inhibition (SICI) was not significantly different at any time after treatment with clozapine ($p > 0.05$).

Conclusions: This prospective-longitudinal study demonstrates that treatment with clozapine is associated with an increase in GABA_B mediated inhibitory neurotransmission. Potentiation of GABA_B may be a novel neurotransmitter mechanism that is involved in the pathophysiology and treatment of schizophrenia.

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1. Introduction

Schizophrenia is a debilitating illness that affects 0.3–0.7% of the population, and whose pathophysiology remains poorly understood (McGrath et al., 2008). Multiple converging lines of evidence in neuropathology, neurophysiology, and pharmacology suggest that persons with schizophrenia have deficits in cortical inhibition (CI). Neuropathological studies have shown a reduced number of gamma-aminobutyric acid (GABA) interneurons, which are cells that mediate CI (Del Río and DeFelipe, 1997). Neurophysiological studies also report CI deficits in persons with schizophrenia, as demonstrated by deficits in P50 auditory gating (Freedman et al., 2000). This P50 suppression is related to presynaptic GABA_B receptors on excitatory neurons that input to pyramidal cells (Freedman et al., 2000). Pharmacologically, clozapine

improves neurophysiological measures of CI (Daskalakis et al., 2008), which may be mediated by direct action of clozapine upon the GABA_B receptor (Wu et al., 2011).

CI is thought to be mediated by two subtypes of GABA interneurons: GABA_A and GABA_B. GABA_A is the fast-acting ionotropic receptor (Macdonald and Olsen, 1994), while GABA_B is the slow-acting metabotropic receptors (Bettler et al., 2004). The independent contribution to CI of each GABA subtype can be indexed using transcranial magnetic stimulation (TMS). GABA_A inhibitory activity can be measured by short-interval cortical inhibition (SICI) (Kujirai et al., 1993), which consists of a subthreshold conditioning pulse preceding the suprathreshold pulse by 1–5 ms. In this scenario, motor evoked potential (MEP) response is inhibited by 50–90% (Kujirai et al., 1993). GABA_B inhibitory activity can be measured by the cortical silent period (CSP) (Fuhr et al., 1991; Cantello et al., 1992). CSP measurement consists of motor cortex stimulation paired with voluntary electromyographic activity, resulting in cessation of muscle movement. The duration of muscle movement cessation (in milliseconds) is a measure of CI (Fuhr et al., 1991). Several lines of evidence suggest that CSP and SICI represent GABA_B- and GABA_A-mediated inhibition, respectively. First, the GABA_B receptor-dependent IPSP peaks at 150–200 ms corresponding to the duration of the CSP while the GABA_A receptor-dependent IPSP peaks at 20 ms corresponding to the duration of SICI (McCormick, 1989; Davies et al.,

Abbreviations: CI, cortical inhibition; CSP, cortical silent period; SICI, short-interval cortical inhibition; ICF, intracortical facilitation; GABA, gamma-aminobutyric acid; TMS, transcranial magnetic stimulation; MEP, motor evoked potential; EMG, electromyography; TS, testing stimulus; CS, conditioning stimulus; ISI, inter-stimulus interval.

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1990; Kang et al., 1994; Deisz, 1999). Secondly, the GABA_B agonist baclofen prolongs the CSP (Siebner et al., 1998), while the GABA_A agonist diazepam increases SICI (Ziemann et al., 1996). Finally, subthreshold conditioning stimuli are used to activate SICI, which indexes GABA_A activity, while suprathreshold stimuli are used to activate CSP, which indexes GABA_B activity. This is consistent with the finding that GABA_A-mediated IPSPs have lower activation thresholds than GABA_B-mediated IPSPs (Mody et al., 1994).

Two recent cross-sectional studies have reported that clozapine is associated with potentiation of GABA_B inhibitory neurotransmission when indexed by TMS (Daskalakis et al., 2008; Liu et al., 2009). Specifically, patients on clozapine had significantly longer CSP than unmedicated patients (Daskalakis et al., 2008) and patients on other typical/atypical antipsychotics (Liu et al., 2009). However, both of these studies were cross-sectional in nature, therefore it is possible that the increased CI observed may be due to illness severity that necessitated clozapine treatment, rather than the effect of clozapine itself.

Previous work measuring GABA_A mediated potentiation of clozapine has been less robust than that for CSP (Daskalakis et al., 2008; Liu et al., 2009). Initial work demonstrated that there was no significant difference in SICI between healthy volunteers, unmedicated patients with schizophrenia, and patients treated with clozapine (Daskalakis et al., 2008). There was only a slight non-significant increase in SICI (or smaller ratio) between unmedicated and clozapine groups. Subsequent work did not find significant differences between healthy subjects, unmedicated, typical/atypical antipsychotic, or clozapine groups (Liu et al., 2009). It was found however, that patients treated with clozapine had less SICI (or larger ratio) than healthy subjects and patients with schizophrenia receiving non-clozapine antipsychotics (Liu et al., 2009). This is consistent with the finding of clozapine's suppression of GABA_A receptor mediated inhibitory neurotransmission (Michel and Trudeau, 2000), which may explain the increased risk of seizures for patients on clozapine (Devinsky et al., 1991).

The objective of this study was to measure TMS indices of CI (i.e., CSP and SICI) in patients with schizophrenia before and after clozapine treatment. Our primary hypothesis is that GABA_B mediated CI (as measured by CSP) would be increased through clozapine and not simply associated with treatment resistance. We also explored whether any change in the CSP was associated with symptom improvement. Intracortical facilitation (ICF) was also investigated since ICF is thought to be related to NMDA mechanisms (Schwenkreis et al., 1999) – another potential neurochemical target of clozapine (Schwieler et al., 2008) – and since ICF may interact with the different inhibitory measures (Sanger et al., 2001; Daskalakis et al., 2002).

2. Materials and methods

2.1. Subjects

There were a total of 33 subjects in this study. Eighteen subjects were patients with a DSM-IV diagnosis of schizophrenia or schizoaffective disorder, recruited at the Center for Addition and Mental Health (Toronto, Ontario, Canada). Inclusion criteria for this study were: (1) age between 18 and 65, (2) documented medication resistance, defined as documented treatment failure to adequate trials of at least 2 antipsychotic medications, including at least 1 atypical antipsychotic (Suzuki et al., 2012), and (3) willingness to switch to another antipsychotic medication, either clozapine or another non-clozapine antipsychotic as decided by their treating psychiatrist, with no input or interference from the study personnel. Exclusion criteria included a self-reported comorbid medical illness, history of drug or alcohol abuse/dependence, active suicidal ideation, or traumatic brain injury. The remaining 15 subjects were age and sex matched healthy subjects who were recruited as part of a separate study and were measured once at baseline.

2.2. Study design

This study was a prospective study. Clinical diagnoses of the subjects were confirmed using the Structured Clinical Interview for DSM-IV (SCID) (First et al., 2002). Patients were treated in an open-label fashion, and the medication was selected by the patient in consultation with their treating psychiatrist. In order to minimize influence on medication selection from the study protocol, the choice of medication was not revealed to study personnel until after medication selection was finalized. Prior to starting the new antipsychotic, for baseline measurements the patient was brought to the lab for neurophysiological testing and clinical assessment using the positive and negative syndrome scale (PANSS) (Kay et al., 1987). There was no washout period prior to starting the new antipsychotic and patients were immediately transitioned to either clozapine or a new antipsychotic. After starting the new antipsychotic, the patient was brought back to the lab at 6 weeks and 6 months to repeat the neurophysiological measurements and clinical assessments. Response to clozapine was defined as 20% reduction in PANSS score from baseline (Rosenheck et al., 1999). The protocol was approved by the Ethical Review Board of Centre for Addiction and Mental Health in accordance with the Declaration of Helsinki, and all subjects gave written informed consent.

2.3. Measurement of cortical inhibition

Surface electromyography (EMG) was recorded from the right abductor pollicis brevis (APB) muscle, and the signal was amplified (Intronix Technologies, Model 2024 F, Bolton, Canada), filtered (bandpass 2–2.5 kHz), and digitalized at 5 kHz (Micro 1401, Cambridge Electronics Design, Cambridge, United Kingdom).

TMS was applied to the left motor cortex, and motor threshold was determined according to a previously outlined protocol (Rossini et al., 1994). Briefly, focal TMS was administered with a 7-cm figure-of-eight coil using two Magstim 200 magnetic stimulators connected via a Bistim module (Magstim, Whitland, Dyfed, United Kingdom). The site of optimal motor response in the APB muscle was located by moving the coil to find the position that produced the largest MEP. The coil was placed on the spot and held tangential to the scalp with the handle pointing back and away from the midline at 45°. The current induced in the cortex was posterior–anterior, perpendicular to the line of the central sulcus.

The resting motor threshold (RMT), expressed as a percentage of maximum stimulator output, was determined as the lowest stimulation intensity that evoked peak-to-peak MEPs of 50 µV in at least 5 of 10 consecutive trials in the relaxed APB muscle. Measurement of the CSP was performed on an actively contracted APB muscle. The subjects were instructed to pinch a dynamometer with thumb and index finger to determine the magnitude of maximal APB contraction. Subjects were then asked to pinch with a force that kept the readings of the dynamometer at 20% of maximal contraction force. When TMS stimuli were delivered at 140% RMT at the site of optimal motor response, there was a pause of all muscle activities at the actively contracted APB. The absolute CSP duration was defined as the time from the MEP onset to the return of any voluntary muscular activity detected by EMG (Tergau et al., 1999). Ten TMS stimuli were delivered with an interstimulus interval (ISI) of 5 s, and mean CSP duration was calculated by averaging 10 CSP durations. The CSP duration was manually measured using Signal (v2.09, Cambridge Electronics Design, Cambridge, United Kingdom).

SICI and ICF were determined using paired-pulse TMS with recordings in the relaxed APB muscle according to published protocols (Kujirai et al., 1993). A subthreshold conditioning stimulus (CS) at 80% of RMT was followed by a suprathreshold test stimulus (TS) that was adjusted to produce mean peak-to-peak MEP amplitude of 1 mV. The inter-stimulus interval (ISI) determines whether the paired-pulse stimulus is assessing SICI or ICF. Specifically, an ISI of 2 or 4 ms measures SICI, while an ISI of 10, 15, or 20 ms measures ICF. The MEP amplitudes for

the paired-pulse data were measured using an automated script in Signal. The degree of SICI and ICF was indexed by calculating a ratio of amplitudes of the conditioned MEP to the mean unconditioned MEP amplitude. For statistical calculations, the ISI was averaged across the corresponding ISIs for SICI (2 ms, and 4 ms) and ICF (10 ms, 15 ms, and 20 ms).

2.4. Statistical analysis

TMS indices (CSP, SICI, and ICF) were compared before and during treatment with clozapine as a group using analysis of variance (ANOVA) models with post hoc analysis using Tukey's honest significant difference test, which corrects for multiple comparisons. Spearman's rho was calculated between change in severity of psychotic symptoms (PANSS may not represent a true linear scale) and change in any TMS indices that were significantly different with clozapine treatment. Linear regression was used to determine correlations between clozapine dose and TMS indices that were impacted by clozapine treatment. As well, TMS indices that were significantly different with clozapine treatment were also compared with age-sex matched healthy subjects. All statistical procedures were two-tailed, and significance was set at an alpha level of .05. All analyses were computed using SPSS 20.0 (IBM Corporation, Armonk New York, USA).

3. Results

3.1. Demographics

Included in this study were a total of 18 patients with schizophrenia, and 15 healthy subjects. Sixteen patients received clozapine, while only two patients were treated with non-clozapine antipsychotics. These two patients were excluded from subsequent analysis due to insufficient sample size. Of the 16 patients treated with clozapine, 11 were diagnosed with schizophrenia, while 5 were diagnosed with schizoaffective disorder. The clozapine group included 11 males and 5 females, with an average (\pm standard deviation) age of 33.3 (\pm 10.9), with an average length of illness of 9.4 (\pm 7.4) years. In terms of medications at baseline, data was not available for one patient. For the remaining patients 93% (14/15) were on antipsychotics, 40% (6/15) were on antidepressants, 33% (5/15) were on benzodiazepines, and 13% (2/15) were on mood stabilizers (Table 1). The mean RMT for patients measured at baseline

(47.3 [% maximal output]), 6 weeks (49.1), and 6 months (56.6) was not significantly different (ANOVA: $F = 1.03$, $p = 0.374$). Complete neurophysiology data was not available for all patients due to some patients being unable to tolerate the TMS stimulus. Due to the nature of their illness, many patients refused bloodwork for clozapine monitoring: 66% at 6 weeks, and 40% at 6 months. Therefore, clozapine dosage was used to estimate blood levels. At 6 weeks the average clozapine dose was 258 ± 86 mg (minimum of 62.5 mg and maximum of 350 mg), and at 6 months was 263 ± 77 mg (minimum of 225 mg and maximum of 350 mg). The mean total PANSS at baseline was 67, at 6 weeks was 66, and at 6 months was 68. There was a 23% treatment response rate to clozapine by 6 weeks, with no further patients responding at 6 months. Five patients were lost to follow-up at six weeks, and a further five patients were lost to follow-up at six months. For the healthy subjects, the mean age was 34.9 (\pm 10.7) with 10 males and 4 females.

3.2. Cortical silent period

There were significant differences in mean CSP durations between groups (ANOVA: $F = 10.46$, $p < 0.001$, $df = 42$). Post-hoc comparisons were then performed between healthy subjects and patients with schizophrenia. The CSP of healthy subjects and patients with schizophrenia at baseline did not significantly differ ($p = 0.14$). However, compared to healthy subjects, CSP was significantly longer after 6 weeks of clozapine treatment ($p < 0.001$), and 6 months of clozapine treatment ($p = 0.006$). For patients with schizophrenia, the mean CSP after 6 weeks of treatment with clozapine was significantly longer than baseline ($p = 0.014$, Cohen's $d = 1.33$) (Cohen, 1988). After 6 months of treatment with clozapine the CSP was still longer compared with baseline, but trended towards significance (Fig. 1, $p = 0.23$, Cohen's $d = 0.86$). The mean CSP at 6 weeks compared to 6 months was not significant ($p = 0.92$). When the analysis was repeated including only patients with at least one follow-up after baseline measurements, the conclusions were unchanged (ANOVA: $F = 12.160$, $p < 0.001$, $df = 28$).

There was no significant correlation between change in CSP (baseline to 6 weeks) with change in clinical symptoms as measured by total PANSS ($\rho = 0.13$, $p = 0.79$), positive PANSS ($\rho = 0.08$, $p = 0.87$), or negative PANSS ($\rho = 0.29$, $p = 0.53$) (Fig. 2). There was no significant difference in CSP change between clozapine responders compared with clozapine non-responders at 6 weeks ($p = 0.86$). Furthermore, there

Table 1

Medications at baseline for study participants. Data was not available for one patient. IM: intramuscular injection.

Patient	Antipsychotics	Antidepressants	Benzodiazepines	Mood stabilizers	Other
01				Divalproex sodium 1500 mg daily Lithium 900 mg daily	Metformin 1000 mg daily Atorvastatin 40 mg daily
02	Olanzapine IM 60 mg biweekly		Clonazepam 1 mg daily		Benzotropine 1 mg daily
03	Olanzapine 40 mg daily Haloperidol 5 mg daily	Nortriptyline 125 mg daily Citalopram 60 mg daily	Bromazepam 3 mg three times daily	Lamotrigine 300 mg daily	Atorvastatin 10 mg daily Synthroid daily Docusate sodium 100 mg twice daily
04	Risperidone 12.5 mg daily				
05	Olanzapine 20 mg daily	Venlafaxine 37.5 mg daily			Vitamin D 400 units daily
06	Quetiapine 900 mg daily	Citalopram 50 mg daily			
07	Quetiapine 100 mg daily		Clonazepam 0.75 mg daily		
08	Paliperidone IM 75 mg monthly Quetiapine 150 mg daily	Escitalopram 20 mg daily			Levonorgestrel and ethinyl estradiol contraceptive
09	Risperidone IM 25 mg biweekly				
10	Risperidone 12.5 mg daily				
11	Zuclopenthixol IM 250 mg biweekly				
12	Olanzapine 5 mg daily				
13	Fluphenazine IM 100 mg biweekly	Sertraline 50 mg daily	Lorazepam 1 mg daily		Benzotropine 1 mg daily Atorvastatin 10 mg daily
14	Risperidone 1 mg daily				Metformin 2500 mg daily Sitagliptin 5 mg daily Unknown lipid lowering agent
15	Quetiapine 600 mg daily	Venlafaxine 225 mg daily			

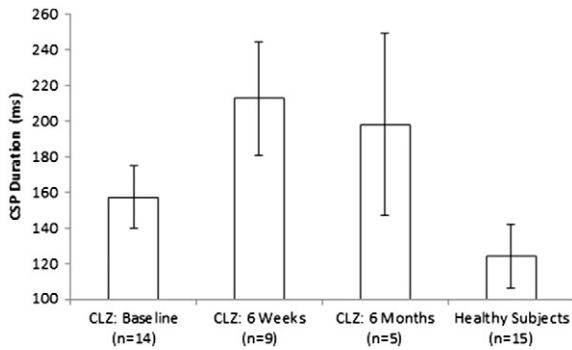


Fig. 1. Cortical silent period (CSP) in patients with schizophrenia at various stages of treatment with clozapine. The x-axis indicates length of treatment with clozapine (baseline vs 6 weeks vs 6 months) and healthy subjects. The y-axis represents CSP duration. Error bars are 95% confidence intervals. There was a significant effect of group on CSP duration ($F = 10.46$, $p < 0.001$). Post-hoc testing found that CSP duration was significantly longer after 6 weeks of clozapine treatment compared to baseline ($p = 0.014$), while the CSP duration after 6 months of treatment was still longer but not significant compared to baseline ($p = 0.23$). CSP duration at 6 months compared to 6 weeks of clozapine treatment was not significantly different ($p = 0.92$). As well, CSP duration of healthy subjects was significantly less than patients with schizophrenia after 6 weeks ($p < 0.001$) and 6 months ($p = 0.006$) of treatment with clozapine. However, CSP duration of patients with schizophrenia at baseline compared to healthy subjects was not significantly different ($p = 0.14$).

was no significant correlation between change in CSP (baseline to 6 weeks) with clozapine dose at 6 weeks ($r = 0.25$, $p = 0.54$) (Fig. 3). At 6 months, follow-up data was only available for 3 patients and therefore this correlation was not performed due to insufficient sample size.

3.3. Short-interval cortical inhibition and intracortical facilitation

There were no significant differences in mean SICI between groups (ANOVA: $F = 1.77$, $p = 0.17$, $df = 42$). However, there is a trend towards less SICI (larger ratio) after 6 months of clozapine treatment compared to either baseline (Cohen's $d = 0.72$) or 6 weeks of treatment (Cohen's $d = 1.10$, Fig. 4). Including only patients with at least one follow-up after baseline measurements, the conclusions were unchanged (ANOVA: $F = 1.60$, $p = 0.22$, $df = 23$). Similarly, there were no significant differences in mean ICF between groups ($F = 0.43$, $p = 0.73$, $df = 42$). Including only patients with at least one follow-up after baseline measurements, the conclusions were unchanged (ANOVA: $F = 0.96$, $p = 0.43$, $df = 23$). As well, the unconditioned MEP was not significantly different between groups (ANOVA: $F = 0.76$, $p = 0.53$, $df = 46$). Including only patients with at least one follow-up after baseline measurements, the conclusions were unchanged (ANOVA: $F = 1.12$, $p = 0.37$, $df = 23$). As there were no significant differences in SICI or ICF after treatment with clozapine, both a post-hoc analysis and correlation with clinical symptoms were not performed.

4. Discussion

These results demonstrate that treatment with clozapine resulted in significant potentiation of the CSP in patients with schizophrenia. Using a prospective-longitudinal study design, we have demonstrated a significant increase in CSP after treatment with clozapine as early as 6 weeks. This supports our primary hypothesis that clozapine prolongs the CSP, rather than it being a function of treatment resistance. The effect size, as measured by Cohen's d , of clozapine on CSP (compared to an unmedicated state) has ranged from 1.41 (Daskalakis et al., 2008) to 3.17 (Liu et al., 2009). The effect size of clozapine on CSP (compared to non-clozapine antipsychotic) has been found to range from 1.22 (risperidone/typical antipsychotic) to 1.41 (olanzapine/quetiapine) (Liu et al., 2009). In the current study, the effect size transitioning from a non-clozapine antipsychotic to clozapine was found to be 1.33, which is in line with previous findings. We have also

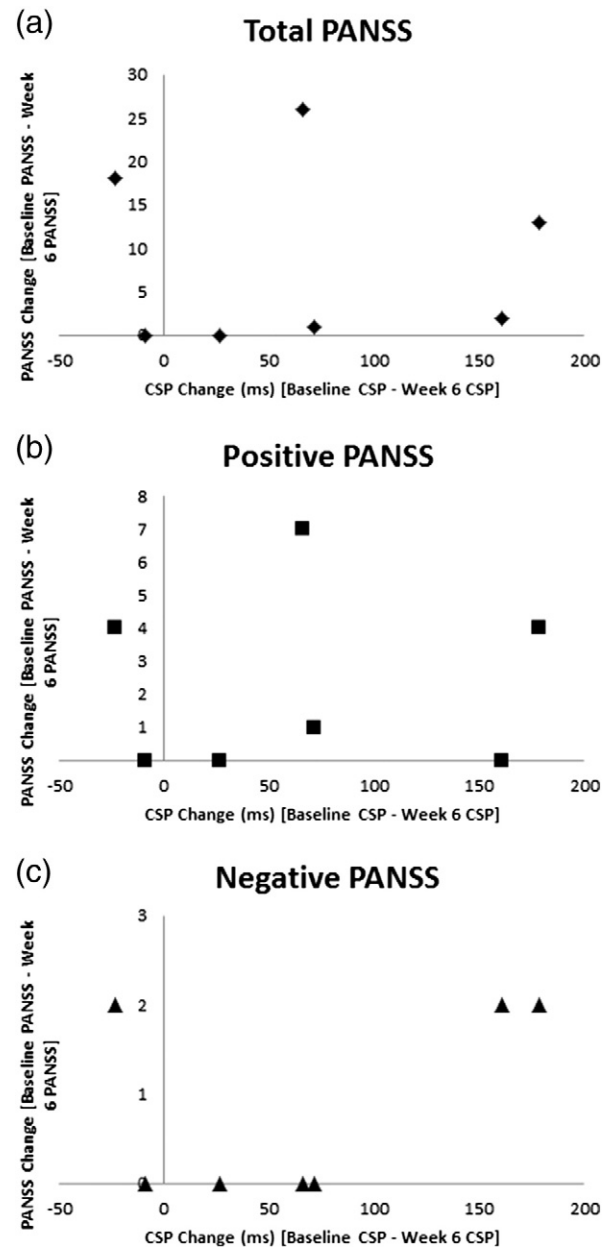


Fig. 2. Change in (a) total, (b) positive, and (c) negative PANSS score and change in cortical silent period (CSP) in patients with schizophrenia. The x-axis is change in CSP, while the y-axis is the change in PANSS score. Change in CSP and PANSS is the difference between measures at baseline and measures at 6 weeks. There was no significant correlation between CSP and total PANSS ($\rho = 0.13$, $p = 0.79$), positive PANSS ($\rho = 0.08$, $p = 0.87$), or negative PANSS ($\rho = 0.29$, $p = 0.53$).

shown that this prolongation of CSP from 6 weeks onwards continues for up to 6 months of clozapine treatment. Our results did not demonstrate a significant correlation between CSP and clinical symptoms as measured by the PANSS. In relation to SICI, there was no significant difference between treatment duration with clozapine. However, there does appear to be a trend towards less SICI (greater ratio) after 6 months of treatment with clozapine compared to 6 weeks or prior to clozapine treatment. These results suggest that there may be an interaction between GABA_A mediated CI and clozapine that required an extended period of treatment (at least 6 months). With respect to ICF, there was no significant difference between baseline, 6 weeks, and 6 months of treatment with clozapine suggesting that there was no effect of clozapine on NMDA receptor mediated neurotransmission.

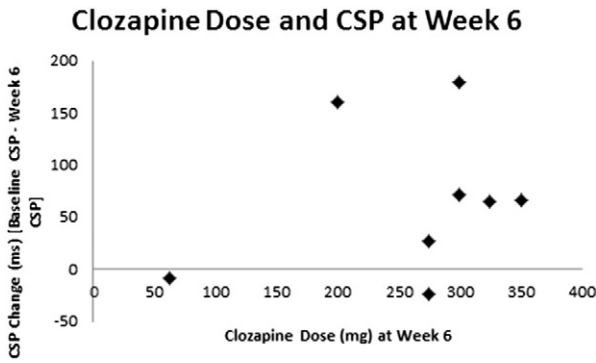


Fig. 3. Change in duration of cortical silent period (CSP) after 6 weeks of clozapine treatment and clozapine dose at 6 weeks. The x-axis indicates the dose of clozapine at 6 weeks in milligrams. The y-axis is the change in CSP from baseline to 6 weeks. There was no significant correlation between change in CSP with clozapine dose at 6 weeks ($r = 0.25, p = 0.54$).

Our results did not demonstrate a correlation between CSP and clinical symptoms (Liu et al., 2009) or between CSP and clozapine dosage. However, Spearman's rho between CSP and negative PANSS in the present work (0.29) is similar to the Spearman's rho from previous work (0.34) (Liu et al., 2009). This indicates that if a significant correlation is present between CSP and clinical symptoms, it explains only a relatively small amount of variation. Another possibility for this lack of significance is that a 6-week trial is an insufficient period of time to observe peak efficacy of clozapine, as previous work has suggested that patients on clozapine will continue to clinically improve for up to six months, after which they plateau (Breier et al., 1993).

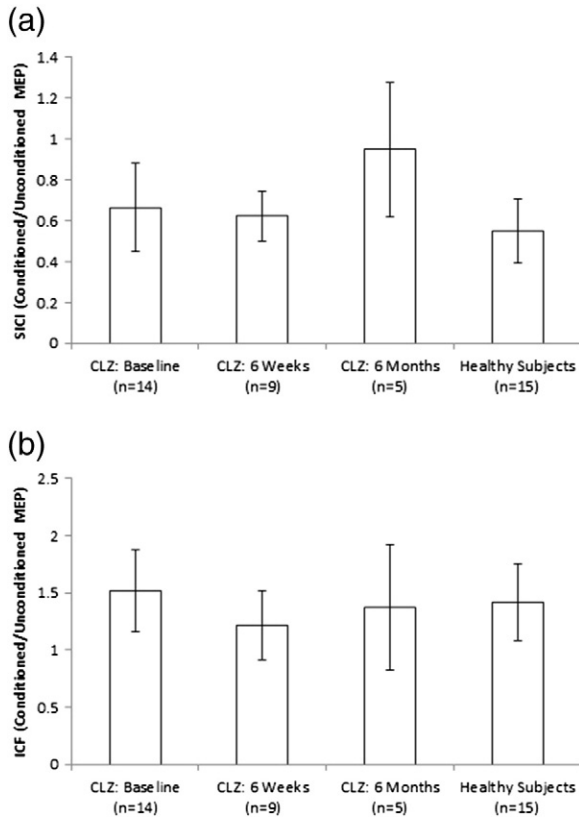


Fig. 4. (a) Short interval cortical inhibition (SICI) and (b) intracortical facilitation (ICF) in patients with schizophrenia. The x-axis indicates length of treatment with clozapine (baseline vs 6 weeks vs 6 months) and healthy subjects. The y-axis is the ratio of conditioned MEP to unconditioned MEP for (a) inhibitory or (b) facilitatory ISIs. Error bars are the 95% confidence interval. There was no significant effect of treatment length with clozapine for SICI ($F = 1.77, p = 0.17$) or ICF ($F = 0.43, p = 0.73$).

Unfortunately due to dropouts, we were not able to draw any conclusions about CSP and clinical symptoms with six months of treatment.

Recent work from a separate group that used a similar pre- and post-treatment longitudinal design examined the impact of quetiapine on CSP (Frank et al., 2014). In this study, the researchers measured CSP of 24 first-episode psychosis patients before and after 3 weeks of treatment with quetiapine. They found that after quetiapine treatment the CSP increased from 105 to 117 ms ($p = 0.028$). However, the effect sizes (compared to unmedicated state) of CSP prolongation were only 0.42, as compared to effect sizes of approximately 1.4 that are consistently seen with clozapine (Daskalakis et al., 2008; Liu et al., 2009). This implies that clozapine has an effect approximately 3.5 times greater on the CSP than quetiapine. Additionally, the fact that patients in our study were on antipsychotics at baseline suggests that clozapine's effect on CSP was additive to any CSP prolongation from other antipsychotic treatment.

The GABA_B receptor potentially represents a novel treatment target for schizophrenia. Clozapine has unique efficacy for the treatment of refractory schizophrenia (Lieberman et al., 2005) which is likely unrelated to D2-receptor antagonism given its low D2 receptor occupancy (Kapur et al., 1999). Further support for the theory that clozapine's antipsychotic mechanism is independent of dopamine antagonism is that clozapine effectively treats drug-induced psychosis in Parkinson's disease without significant worsening of motor function (Pollak et al., 2004). Our results suggest that clozapine's unique efficacy may be related to the magnitude of GABA_B potentiation not seen with any other typical or atypical antipsychotic (Daskalakis et al., 2008; Liu et al., 2009; Frank et al., 2014). To clarify the role of GABA_B in schizophrenia pathophysiology, future work may use GABA_B receptor agonists as an adjunct to non-clozapine antipsychotics in order to determine if targeting of GABA_B enhances treatment response. If this were to demonstrate increased CSP (i.e. GABA_B potentiation) without an improved response rate, then an alternate mechanism likely underlies clozapine's unique efficacy.

There are several limitations to this work. Firstly, the sample size is small with 16 patients in the study at baseline, 11 at 6 weeks, and 6 at 6 months. A major reason for the small sample size was the severity of patients' symptoms, with an average PANSS of 67 for all patients at all treatment periods, which resulted in numerous patients being unable to complete testing, or not returning for follow-up. However, despite this, our results are consistent with previous cross-sectional studies demonstrating an increased CSP in patients treated with clozapine (Daskalakis et al., 2008; Liu et al., 2009). As well, the response rate in this study is similar to previous studies, which indicate that approximately 30% of patients respond to clozapine by 6 weeks (Meltzer, 1992; Rosenheck et al., 1999). For example, in a similar sized study assuming a 30% response rate to clozapine we would expect to see 4 patients respond, which is comparable to the 3 patients responding in this study. With respect to clozapine treatment response there is the concern that change in CSP is a measure of treatment response. However, this is not supported by our results which indicate that the CSP change is not different between clozapine responders and non-responders. Our results did not demonstrate a significant relationship between SICI and length of treatment with clozapine, however, this was likely due to the small sample size at 6 months. A second limitation of this study is that we assessed CI peripherally through TMS-EMG, while CI assessed centrally using TMS-EEG may be able to more closely reveal the effects of clozapine on the central nervous system (Farzan et al., 2013). While EEG correlates of CI would likely provide stronger evidence for clozapine mechanisms of action, we have previously shown a strong correlation between the peripheral and central indices of CI in the motor cortex (Farzan et al., 2010). Therefore, while current methods only indexed motor areas, TMS-EMG measures provide a reliable estimate of CI in non-motor regions. Lastly, the lack of a longitudinal control group was a limitation of this study. We used a healthy control group with one measurement of TMS indices because previous

measures of test–retest reliability for TMS–EMG have demonstrated high reproducibility and consistency with a Cronbach's alpha of 0.88 (Farzan et al., 2010). A placebo control group of patients with schizophrenia followed longitudinally without antipsychotic treatment would have been unethical due to the potential for worsening symptoms and would have limited recruitment. The non-clozapine antipsychotic group had intended to be the active control unfortunately was extremely small and not included in statistical analysis. This study was designed to recruit equal numbers of patients on clozapine and non-clozapine antipsychotics, however, recruiting patients who were being switched from one non-clozapine antipsychotic to another non-clozapine antipsychotic proved to be a significant challenge. However, given that patients were measured over time (i.e. before and after starting clozapine), we were still able to compare a non-clozapine treatment state, with a clozapine treated state.

This work demonstrates that clozapine treatment in patients with schizophrenia potentiates GABA_B mediated CI as early as 6 weeks. It used a longitudinal study to demonstrate that clozapine lengthens CSP and increases GABA_B mediated CI. The results also suggest that there may be decreased SICI with long-term clozapine treatment. This work strengthens the hypothesis that the GABA_B receptor may be a novel neurotransmitter target for refractory schizophrenia.

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Contributors

Author TK performed the statistical analysis and prepared the manuscript. Author DJ performed the study recruitment and study testing. ZJD designed the study and wrote the protocol. All authors contributed to and have approved the final manuscript.

Conflict of interest

In the last 5 years, ZJD received research and equipment in-kind support for an investigator-initiated study through Brainsway Inc. ZJD has also served on the advisory board for Hoffmann-La Roche Limited and Merck and received speaker support from Sepracor and Eli Lilly.

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