



Decreased interhemispheric connectivity and increased cortical excitability in unmedicated schizophrenia: A prefrontal interleaved TMS fMRI study

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ABSTRACT

Background: Prefrontal abnormalities in schizophrenia have consistently emerged from resting state and cognitive neuroimaging studies. However, these correlative findings require causal verification via combined imaging/stimulation approaches. To date, no interleaved transcranial magnetic stimulation and functional magnetic resonance imaging study (TMS fMRI) has probed putative prefrontal cortex abnormalities in schizophrenia.

Objective: Hypothesis: We hypothesized that subjects with schizophrenia would show significant hyperexcitability at the site of stimulation (BA9) and decreased interhemispheric functional connectivity.

Methods: We enrolled 19 unmedicated subjects with schizophrenia and 22 controls. All subjects underwent brain imaging using a 3T MRI scanner with a SENSE coil. They also underwent a single TMS fMRI session involving motor threshold (rMT) determination, structural imaging, and a parametric TMS fMRI protocol with 10 Hz triplet pulses at 0, 80, 100 and 120% rMT. Scanning involved a surface MR coil optimized for bilateral prefrontal cortex image acquisition.

Results: Of the original 41 enrolled subjects, 8 subjects with schizophrenia and 11 controls met full criteria for final data analyses. At equal TMS intensity, subjects with schizophrenia showed hyperexcitability in left BA9 ($p = 0.0157$; max z-score = 4.7) and neighboring BA46 ($p = 0.019$; max z-score = 4.47). Controls showed more contralateral functional connectivity between left BA9 and right BA9 through increased activation in right BA9 ($p = 0.02$; max z-score = 3.4). GM density in subjects with schizophrenia positively correlated with normalized prefrontal to motor cortex ratio of the corresponding distance from skull to cortex ratio (S-BA9/S-MC) ($r = 0.83$, $p = 0.004$).

Conclusions: Subjects with schizophrenia showed hyperexcitability in left BA9 and impaired interhemispheric functional connectivity compared to controls. Interleaved TMS fMRI is a promising tool to investigate prefrontal dysfunction in schizophrenia.

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Introduction

Schizophrenia is a severely disabling, chronic illness characterized by a heterogeneous range of positive (hallucinations, delusions, thought disturbances), negative (social, affective deficits),

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and cognitive (attention, working memory, and executive functioning deficits) symptoms [1]. Abnormal genetic, cellular, and neural circuit findings have been associated with these disorder features [2–4]. However, synthesizing these results into a unified account of the full disorder phenotype has proven extremely difficult.

Recent advances in our understanding of the pathophysiology of schizophrenia have come from studies focused more narrowly on the schizophrenia endophenotype of cognitive dysfunction [5]. Compared to fluid, heterogeneous phenotypes, endophenotypes are easier to systematically define and measure because they are relatively stable and more heritable [6]. Cognitive dysfunction has been identified in both schizophrenia patients and otherwise asymptomatic first-degree relatives compared to controls [7,8]. Additionally, unlike other schizophrenia symptoms which fluctuate over time, cognitive deficits seem largely stable [9] – although some studies provide evidence for graded remission [10].

Studies pairing cognitive batteries with neuroimaging paradigms have shed light on neural substrates potentially underlying schizophrenia related cognitive deficits. In previous work, our group linked cognitive dysfunction in schizophrenia to gray matter atrophy in BA9, an area of the dorsolateral prefrontal cortex (dlPFC) [11]. Functional magnetic resonance imaging (fMRI) meta-analyses reinforce this finding, associating disorder related differences in dlPFC volume and activation patterns with executive dysfunction [12,13]. In addition to volume and activation differences, disruptions in prefrontal connectivity have been found in schizophrenia [14]. More specifically, abnormal interhemispheric structural connectivity between the left and right dlPFC has been identified and associated with disorder related working memory deficits [15]. Collectively, these cognitive neuroimaging studies suggest that abnormalities in the prefrontal cortex may play a key functional role in schizophrenia.

Although fMRI studies have advanced our understanding of schizophrenia related cognitive dysfunction, current findings must be interpreted in light of methodological limitations. First, fMRI may imply correlations between brain activity and mental functions, but it cannot be used to infer causality (i.e. region A initiated a given mental process) or distinguish between neural excitation and inhibition. Additionally, although cognitive batteries have elucidated a schizophrenia endophenotype, task complexity and subjects' interests and skills may influence the magnitude of blood flow changes in cognitive fMRI studies [16,17]. These factors potentially account for why similar experiments in independent cohorts have reached opposite findings [18–20]. In the context of these limitations, candidate schizophrenia mechanisms emerging from fMRI and cognitive task paradigms require causal verification.

Interleaved transcranial magnetic stimulation (TMS fMRI) enables the simultaneous stimulation and imaging of the brain and overcomes some of the limitations inherent to cognitive fMRI paradigms [21–25]. With combined TMS fMRI, circuits can be manipulated in vivo and causal inferences may be drawn regarding functional activation and connectivity. Here, we examine putative functional differences in the prefrontal cortex, a key region implicated in cognitive fMRI studies. To our knowledge, this is the first study to use interleaved TMS fMRI to compare BA9 activation and connectivity in subjects with schizophrenia versus controls. In line with previous studies finding increased prefrontal cortical excitability and decreased prefrontal cortical inhibition [26–28], we hypothesized that subjects with schizophrenia would show hyperexcitability at the site of stimulation. In line with evidence suggestive of abnormal interhemispheric connectivity between the left and right dlPFC [15], we hypothesized that interleaved TMS fMRI would reveal schizophrenia related interhemispheric dysconnectivity between left and right BA9.

Methods and materials

Subjects

We enrolled 41 subjects (19 unmedicated subjects with schizophrenia, 22 matched controls). The majority of subjects with schizophrenia came from regional community mental health clinics and a local homeless shelter. A few subjects were recruited from the inpatient units operated by the Department of Psychiatry at the Institute of Psychiatry and Ralph H. Johnson Veterans Affairs Medical Center in Charleston, South Carolina. Subjects had met DSM-IV-TR criteria for schizophrenia determined by the Structured Clinical Interview for DSM-IV (SCID). At the time of the study, all subjects were free of psychotropic medications (antipsychotics, neuroleptics, antidepressants, mood stabilizers, anticholinergics or stimulants) for at least two weeks. Clinical and neuropsychological results pertaining to these subjects were previously reported in Bonilha et al., 2008 [11]. All subjects were judged clinically competent to give written informed consent.

Exclusion criteria for subjects with schizophrenia included a past history of neurological disorders such as epilepsy, subjects who demonstrated severe exacerbation of their psychosis or the catatonic subtype, subjects currently diagnosed with substance dependence (DSM-IV) or major depressive disorder (Calgary depression rating scale > 9), and tobacco smokers with a greater use than two packs per day.

Control subjects were recruited from the local community through advertisements, and matched with subjects with schizophrenia based on sex, race, smoking habits, handedness, and socioeconomic status including personal level of education. Control subjects did not meet any active DSM-IV-TR Axis I criteria for psychotic, anxiety, mood, substance abuse or dependence disorder, nor did they have any history of neurological disorder. Occasionally they did report a history of substance abuse similar to that of our schizophrenia subject sample.

There were no significant differences in age, sex distribution, race, handedness, smoking habits and marital status between groups (see Table 1). Although the original sample did not differ in level of education, the final cohort used for the TMS fMRI data analysis did with subjects with schizophrenia being less educated (Fisher's exact test $p = 0.025$) and less frequently employed (Fisher's exact test $p = 0.020$) compared to the control group (Table 1). A more general categorization of levels of education showed no significant difference (Fisher's exact test $p = 0.290$).

The final sample included 8 subjects with schizophrenia and 11 healthy controls. Six subjects with schizophrenia were excluded due to excessive motion during TMS fMRI and an additional five could not tolerate the structural scan. One healthy control was excluded due to excessive motion during TMS fMRI, three were excluded due to substance abuse, three could not tolerate the structural scan, and an additional five could not tolerate TMS fMRI. The final sample did not differ from the excluded sample across a range of demographic variables including: sex, race, handedness, education, marital status, employment, smoking, and age (Supplementary Table 1).

On the day of scanning, subjects were not allowed to smoke for at least 1 h before image acquisition. This study was funded by the NIMH (R21-MH065630-01A1) and approved by the Medical University of South Carolina Institutional Review Board.

MRI scanning: high resolution structural scan

All subjects underwent brain imaging using a 3T MRI scanner with a SENSE coil (Intera, Philips Medical Systems; Bothell, WA) that would later be used for morphometric analyses. T1-weighted

Table 1
Demographics for unmedicated participants with schizophrenia and healthy matched controls.

Variables	Participants with schizophrenia		Controls %		p value
	n		n		
Sex					1.000
Female	1		1		
Male	7		10		
Race					.611
African-American	4		6		
Caucasian	3		5		
American-Indian					
Asian	1				
Handedness					1.000
Left			1		
Right	7		8		
Ambidextrous	1		1		
Unknown			1		
Education					0.025*
Grade 6 or less	1				
Grade 7 to 12 without graduating	4				
High school graduate or GED	1		5		
Part college or technical school	1		4		
Graduated 2 yr college			1		
Graduated 4 yr college	1		1		
Part graduate or professional school					
Marital status					0.478
Married or cohabitating					
Divorced or annulled	1		1		
Separated			2		
Never married	6		6		
Unknown	1		2		
Employment					0.020*
Full time	1		5		
Part time					
Unemployed	7		3		
Unknown			3		
Smoking					1.000
Yes	8		10		
No	1				
	Mean	Std. Dev.	Mean	Std. Dev.	p value
Age	43.000	5.976	36.909	7.918	0.073
S-PFC	15.909	0.980	15.841	1.445	0.9073
S-MC	17.43	2.761	17.754	3.682	0.8347
S-PFC/S-MC	0.971	0.176	0.92	0.172	0.5396

MT = Motors Threshold.

MT TMS fMRI = Motors Threshold during the TMS fMRI session.

S-PFC/S-MC = Skull to Prefrontal Cortex over Skull to Motor Cortex ratio.

* When, instead of dividing the subjects into seven groups, you divide them into four (grouping *Grade 7 to 12 without graduating* with *High school graduate or GED*, and *Part college or technical school* with *Graduated 2 yr college*, and *Graduated 4 yr college* with *Part graduate or professional school*) and then perform a Fisher's exact test, the significance is 0.290.

structural images encompassing the whole brain were collected from all subjects using the following parameters, TR = 11.23 ms, TE = 5.7 ms, slice thickness = 1 mm, gap = 0 mm, field of view (FOV) = 256 mm, number of slices = 160, matrix = 256 × 256.

TMS fMRI session

In a single session, we performed motor threshold (rMT), structural imaging for targeted TMS placement and intensity adjustment, and interleaved TMS fMRI. Scanning was conducted using a 1.5T MRI scanner with a surface MR coil (Intera, Philips Medical Systems, Bothell, WA, USA) optimized for bilateral prefrontal cortex image acquisition, including the area under TMS. This also helped improve the signal-to-noise ratio observed from earlier studies—an artifact caused by the TMS coil [21].

Motor threshold

Subjects laid on the gantry of the scanner and we recorded surface EMG from the right abductor pollicis brevis (APB) muscle at

rest using 9 mm Ag–AgCl electrodes in a belly-tendon montage. Using the TMS fMRI equipment, we ascertained individual rMT, based on muscle evoked potentials (MEPs) of more than 50 μ V and BEST-PEST, a parametric estimate method [29]. Vitamin E capsules were placed at the ends of the MRI compatible TMS coil (Magstim Inc), behind it, and at its center to help identification on structural images.

Targeted stimulation

A survey scan ascertained head location for subsequent anatomical and functional scans. A set of T1-weighted sagittal structural images encompassing the whole brain were acquired using the following parameters: TR 1/4625 ms, TE 1/4 20 ms, slice thickness 1/4 5 mm, gap 1/4 1 mm, FOV 1/4256 mm, number of slices 1/4 27, matrix 1/4256 × 256. The T1 weighted structural scan was then imported into a semi-automated target TMS software via a local area network (LAN) and automatically transformed into a common brain space. The reverse transformation was applied to a Talairach model, co-registered and displayed using modified

Register software (McGill University). The coordinates for left BA9 were then translated into a custom-built coil-holder 6° of freedom positioner.

The shortest distance from tag to skull was outputted and the intensity for stimulation was corrected for target-skull/M1-skull distance ratio taking into account the magnetic fields logarithmic drop-off. Distances from skull to motor cortex (MC) and BA9 were used to adjust for any regional atrophy and insure that prefrontal stimulation intensities were comparable across subjects. The whole process of determining the optimal target and adjusting BA9 stimulation intensity took less than 10 min; the setup of the TMS coil took another five.

TMS fMRI single event paradigm

The interleaved stimulation and fMRI consisted of 35 triplet TMS pulses at 100 ms apart (10 Hz) over 18 min and 48 s. T2*-weighted echo-planar images (EPI) were acquired with the following parameters: 557 vol, repetition time 2000 ms, echo time 35 ms, flip angle 90°, FOV 256 × 256, voxel size 3 × 3 × 3 mm, 32 slices. The intensity of each triplet was randomized across 0%, 80%, 100% and 120% of the adjusted rMT, using a randomization algorithm with five blocks. Each of the five blocks comprised 7 triplets that fired randomly with an 8 or 10 s interval at 0%, 80%, 100% or 120% of rMT. Simultaneously, subjects were instructed to perform a very simple auditory continuous performance task (CPT) that was previously validated in our lab and used similarly in other interleaved TMS fMRI investigations [21]. The CPT was designed to ensure engagement of all subjects and also helped to distract from the noise of the TMS pulses and the mild discomfort.

Imaging preprocessing and analyses

Skull to cortex distance and gray matter volumes

Detailed 3T MRI image analyses has been reported elsewhere [11]. In brief, T1 images were transformed into Analyze format using the software MRICro [30]. Optimized voxel-based morphometry (VBM) analysis was performed using SPM5 [30]. Images were spatially normalized and “modulated” in order to preserve the total amount of signal in the images [31]; therefore, areas that were expanded during warping were correspondingly reduced in intensity. Spatially normalized images were then resliced to an isotropic 1 mm and underwent segmentation of gray and white matter using SPM5, estimating the probability of each voxel being gray matter (GM). Images were smoothed with an isotropic Gaussian kernel of 10 mm to minimize inter-individual gyral variability. We performed a region of interest (ROI) analysis to derive GM volumes in BA9 and 46, and their relationship with cortical excitability. First, the areas corresponding to BA9 and BA46 were selected and extracted from the BA map inbuilt in MRICro [30]. Then, the mean GM volume of BA9 and of BA46 were extracted from each individual using the software Marsbar [32].

TMS fMRI

All TMS fMRI scans were preprocessed using FSL version 4.1.9 (FMRIB's Software Library). The following preprocessing steps were applied using FEAT (FMRI Expert Analysis Tool) version 5.98: non-brain removal, motion correction using MCFLIRT [33], slice timing correction using sinc interpolation, spatial smoothing with a Gaussian kernel (FWHM = 8 mm) and grand mean intensity normalization of the entire 4D dataset by a single multiplicative factor (see Fig. 1). In addition, the images were high pass filtered to remove frequencies below 0.01 Hz. Signals from white matter (WM), cerebrospinal fluid (CSF) and the global signal were used as nuisance regressors to control for non-neuronal activation. The six motion parameters, three translations and three rotations

estimated by MCFLIRT, were also regressed to account for any remaining effects of motion. The images were also temporally bandpass filtered (0.01–0.08 Hz) to limit the analysis to the low frequency fluctuations. Subjects with excessive motion of 2 mm maximum translation or 2-degree rotation were excluded. We also generated individual time series from each of the ROIs using MRI-Cron software [34].

Statistical analysis

Primary hypothesis

We conducted 4 s-level unpaired t-tests with ROIs for bilateral BA9 and 46. Our primary hypothesis outlined in our R21-NIMH grant proposal was to compare BOLD signal in left BA9 (area stimulated with TMS) between unmedicated subjects with schizophrenia and healthy controls. Secondary analyses investigated differences in activation in adjacent cortical regions (ipsilateral BA46) and contralateral BA9 and BA46.

Exploratory hypotheses

Time series, peak BOLD signals and GM ROIs were exported to JMP statistical software (version 9.0.2; 2010 SAS Institute Inc, Cary, North Carolina). We transformed the peak BOLD signals and the mean BA9 and BA46 GM volumes into Z scores, i.e., standardized scores that express the number of standard deviations away from the mean of the control group.

We performed descriptive, Chi-squares, Fisher's exact tests, t-tests and a simple regression analysis to examine the relationship between the structural and functional data. The level of statistical significance was set at $p < 0.05$ for primary analyses. Secondary analyses' Bonferroni corrected significance was set at $p < 0.0125$.

Results

Motor thresholds and skull-to-cortex distances

The distance from skull to the BA9 was not significantly different between the two groups ($p = 0.907$), neither was the distance from skull to MC ($p = 0.835$) nor the BA9/MC ratio ($p = 0.540$). rMT positively correlated with MC distance from skull ($r^2 = 0.49$, $p = 0.01$) [see Table 1]. Left BA9 GM volume significantly negatively correlated with the distance from skull to MC ($r = 0.38$, $p = 0.018$) but not with skull to BA9 ($r = 0.03$, $p = 0.54$) [see Fig. 2]. Note that distance from skull to MC on average tended to be larger than distance from skull to BA9 (see Fig. 3).

Prefrontal cortical excitability

At equal intensity of stimulation, subjects with schizophrenia showed increased activation in the stimulation target (left BA9) and ipsilateral regions adjacent to the stimulation target (left BA46), whereas healthy controls showed better functional connectivity with increased contralateral BA9 activation (see Fig. 3). Specifically, 100% rMT TMS showed significantly increased activation in left BA9 ($p = 0.0157$; max z-score = 4.7) in subjects with schizophrenia. Most importantly, the ‘spread of activation’ to adjacent BA46 was also significantly increased in subjects with schizophrenia ($p = 0.019$; max z-score = 4.47). Healthy controls showed increased activation in right BA9 ($p = 0.02$; max z-score = 3.4). Bilateral BA8 and BA10 and right BA46 showed no significant differences between the two groups (see Table 2 and Fig. 4). GM density in subjects with schizophrenia positively correlated with normalized BA9 to motor cortex ratio of the corresponding distance from skull to cortex ratio (S-BA9/S-MC) ($r = 0.83$, $p = 0.004$).

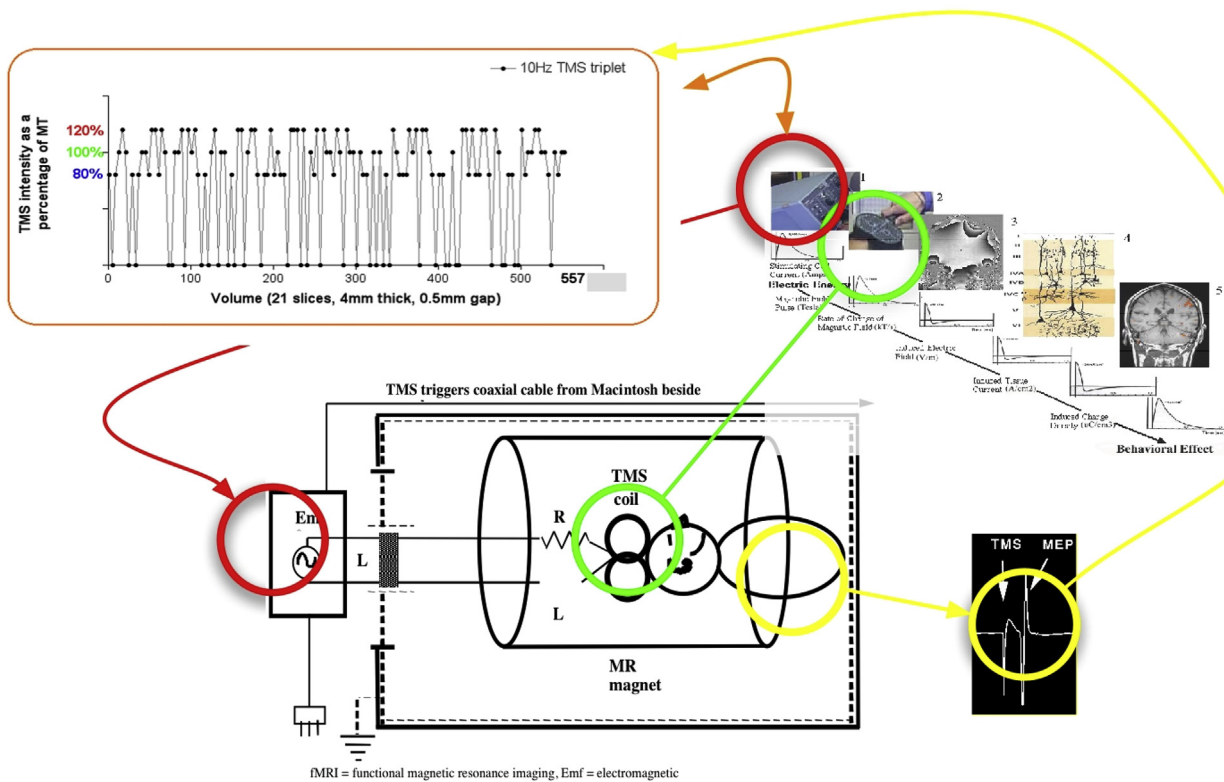


Fig. 1. Schematic for the MT determination using EMG and for the Interleaved TMS fMRI single event paradigm at 3 different intensities (80, 100 and 120%MT).

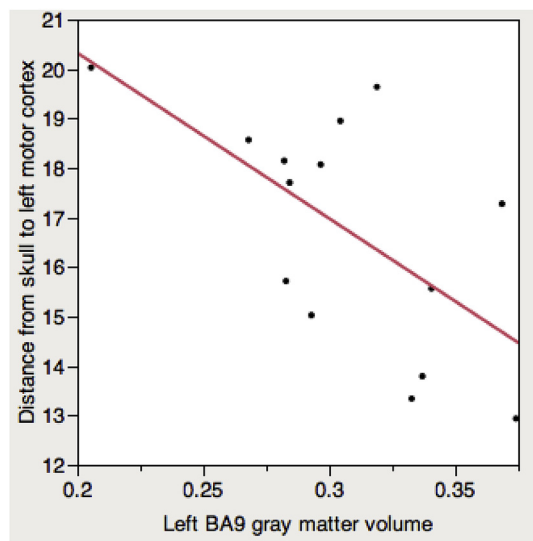


Fig. 2. Positive correlations between gray matter (GM) volume in left BA9 and the skull-to-cortex ratio of left prefrontal over left motor cortex (S-BA9/S-MC). The red line tracks a linear relationship; the black dots denote values for individual subjects. Distance is measured in (); volume is measured in (). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Exploratory hypotheses

Normalized peak activations generated from time series at 100% rMT TMS for subjects with schizophrenia relative to healthy controls showed significant increases in left BA46 (estimate = 1.04 ± 1.49 ; $z = 1.97$, $p = 0.04$), and decreases in right BA9 (estimate = -0.7 ± 0.57 ; $z = -3.51$; $p = 0.0004$) and right BA46

(estimate = -0.77 ; $z = -2.91$; $p = 0.0035$). Not accounting for cluster size as in primary analyses, there was no difference in left BA9 activation between groups (estimate = 0.081 ± 0.94 ; $z = 0.25$; $p = 0.79$).

There were no significant correlations between any of the four ROI's normalized peak activations generated from time series at 100% rMT TMS for subjects with schizophrenia relative to healthy controls and normalized BA9 GM density ($p > 0.05$).

Discussion

To our knowledge, this is the first interleaved TMS fMRI study to investigate BA9 excitability and functional connectivity in unmedicated subjects with schizophrenia and matched controls. In line with previous work, subjects with schizophrenia exhibited increased prefrontal cortical excitability, whereby stimulation with an activating TMS sequence (triplet at 10 Hz) over the left BA9 resulted in a local “spill-over” in activation to neighboring ipsilateral regions. In addition to increased local signal propagation, our stimulation paradigm revealed *decreased* interhemispheric signal propagation from left BA9 to right BA9 in subjects with schizophrenia, suggestive of prefrontal dysconnectivity in an important executive control region. Interestingly, while subjects demonstrated positive correlations between rMT and skull-to-cortex distance over MC and a positive correlation between left BA9 GM volume and S-BA9/S-MC (a proxy of prefrontal atrophy), there was no relationship between left BA9 GM volume and relative cortical excitability, nor a distinct difference between groups on rMT.

Our results provide causal evidence for interhemispheric dysconnectivity within the prefrontal cortex in subjects with schizophrenia. Previous work suggests that interhemispheric dysconnectivity may be central to the etiology of schizophrenia [35]. Decreased resting state homotopic connectivity has been

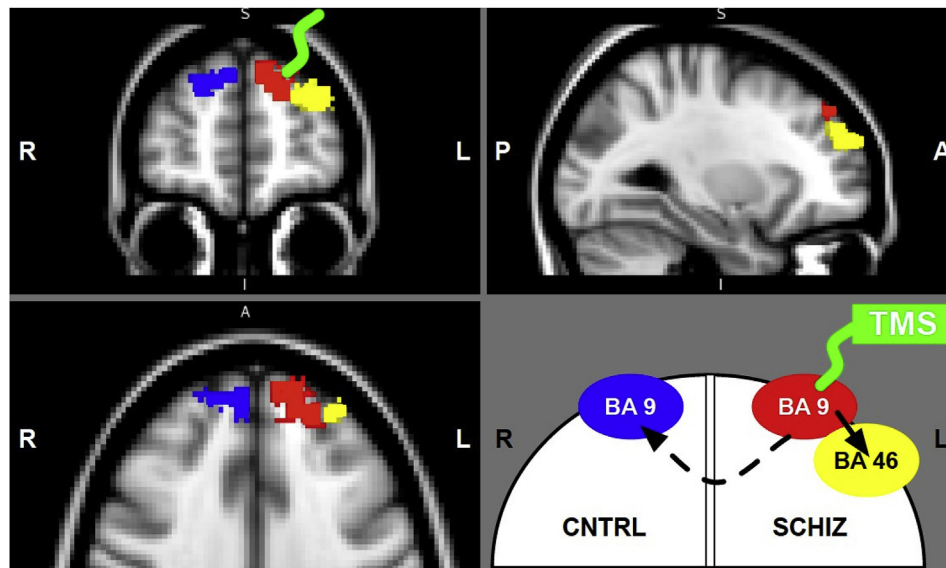


Fig. 3. Local inhibition deficits and poor transcallosal functional connectivity in schizophrenia. Direct TMS over the left BA9 is associated with significant increases in BOLD signal in adjacent BA46 in schizophrenia patients. Conversely, healthy controls show more effective transcallosal activation.

Table 2
Regional activation group comparisons following stimulation.

	Region	% MT	Coordinates x, y, z	Z value
<i>Schizophrenia > Controls</i>	lBA9	80	(-22, 52, 34)	4.73
		100	(-20, 54, 36)	4.73
		120	(-22, 50, 38)	4.83
	lBA46	80	(-26, 50, 32)	4.57
		100	(-28, 52, 32)	4.47
		120	(-26, 52, 32)	4.66
<i>Controls > Schizophrenia</i>	rBA9	80	(14, 52, 30)	3.51
		100	(14, 50, 30)	3.21
		120	(16, 50, 30)	3.28

lBA9 = left Brodmann area 9 lBA46 = left Brodmann area 46 rBA9 = right Brodmann area 9.

%MT = percentage of Motor Threshold used for the stimulation after adjusting for PFC/MFC ratio.

Increased activations following various prefrontal cortical regions of interest. Results are presented for left BA9 TMS triplets at 100 ms apart at 80, 100, and 120% intensities. Note that all prefrontal stimulation intensities were adjusted by S-BA9/S-MC ratio.

identified in several regions, including the occipital lobe, thalamus, cerebellum, precuneus, precentral gyrus, postcentral gyrus, superior temporal gyrus, middle occipital gyrus, and the fusiform gyrus [36–38]. In addition to dysfunctional regional interhemispheric connectivity, dysfunctional interhemispheric connectivity within larger networks such as the sensorimotor and default mode networks have also been found and linked with neurocognitive deficits and auditory verbal hallucinations, respectively [39,40]. Decreased interhemispheric connectivity within regions and larger networks may lead to increased hemispheric autonomy and decreased interhemispheric cooperation [35]. Our findings are consistent with this notion; stimulation of the left BA9 revealed decreased contralateral activation and increased local ipsilateral activation in subjects with schizophrenia relative to controls. How interhemispheric dysconnectivity in the right and left BA9 circuit identified in the current study relates to interhemispheric dysconnectivity within functionally associated regions and larger networks should be the subject of future study.

In addition to decreased functional connectivity between left and right BA9, we found increased excitability adjacent to the site of

stimulation in subjects with schizophrenia. Increased cortical excitability has been found in previous schizophrenia TMS studies and linked to inhibitory-process dysfunction [26,41]. However, because the BOLD signal represents the sum of excitatory and inhibitory postsynaptic inputs, we are unable to determine whether this finding implicates aberrant excitation or inhibition at the local level. The prevailing pathophysiological model of schizophrenia postulates that glutamate synaptic abnormalities, particularly those that reduce the N-methyl-D-aspartate (NMDA) receptor function, lead to a dysregulation of GABA fast-spiking interneurons, consequently disinhibiting pyramidal glutamatergic output and disturbing the signal-to-noise ratio in cortical/subcortical networks [42,43]. Elucidating the relative contribution of dysfunctional excitatory versus inhibitory neurotransmission to the local prefrontal signal ‘spill-over’ noted in the current study is a critical next step given the established link between local cellular excitability and long-range functional connectivity. Indeed, our past work indicates that interhemispheric signal propagation may ultimately depend on an optimal level of local cellular excitability, synaptic efficacy, and the proper balance of excitation and inhibition. In two previous studies [44,45] we have shown that modulating local activation with lamotrigine, an anti-epileptic drug known to inhibit voltage-dependent sodium channels and decrease glutamate release [46–48], reduces BA9 TMS-induced BOLD signals at the site of stimulation and enhances activation of connected cortical and sub-cortical areas in healthy subjects. Future interleaved TMS fMRI studies that introduce pharmacologic challenges to modulate excitation or inhibition may be applied in schizophrenia research to parcel out the contribution of local prefrontal inhibition and excitation to dysfunctional local activation and interhemispheric functional connectivity within the executive control network.

Our specific focus on the prefrontal cortex was motivated by findings from previous resting state and cognitive fMRI studies suggestive of dysfunctional prefrontal cortical activation and connectivity in schizophrenia [13,49,50]. Recent work links cognitive remediation therapy improvements to changes in prefrontal function [51,52]. Interestingly, putative post-treatment changes in interhemispheric information transfer facilitated by increased prefrontal corpus callosum volume may play a particularly key role [53]. Current cognitive remediation therapies augmented with

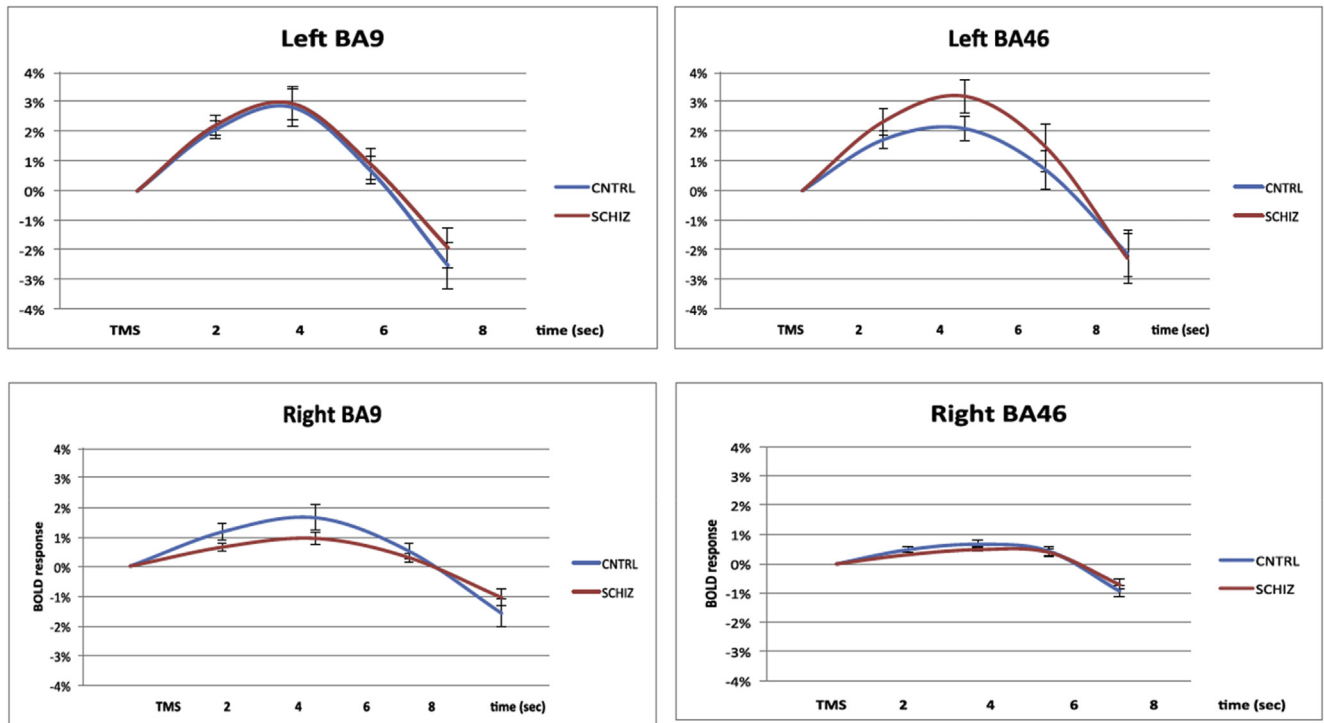


Fig. 4. Time series from 4 regions of interests, left BA9 and 46 and right BA9 and 46, across patients with schizophrenia and matched controls. Note the prominent BOLD response in left BA9 across both groups. Most interesting is the distinct greater response in BOLD signals in left BA46 and distinct lower response in right BA9 for patients with schizophrenia compared to controls.

medication have small-to-medium effects on global function and cognition [54]. Whether the concurrent stimulation of prefrontal regions such as BA9 with non-invasive brain stimulation paradigms like repetitive TMS or transcranial direct current stimulation (tDCS) might augment cognitive remediation gains by inducing changes in local excitation and inhibition and subsequently increasing inter-hemispheric signal transmission is an open and exciting question with clear clinical import.

A negative correlation was only found between BA9 GM volume and skull-to-cortex distance over the left motor but not the prefrontal cortex. Interestingly, in those with schizophrenia, skull-to-cortex distance over the left motor cortex tended to be somewhat larger than the prefrontal cortex. Note that we had previously reported decreased BA9 GM volume in this schizophrenia cohort compared to controls [11], a proxy for atrophy in this population. Others have identified correlations between cortical and GM reductions, at the time of patients' first episode of schizophrenia. Cortical atrophy has been most pronounced in the frontal and temporal cortices, but also in the parietal cortex and cerebellum [55]. Speculatively, the reduction in GM could play a role in the increase in excitatory intersynaptic Glu observed in schizophrenia patients as it may be related to oligodendroglial dysfunction [56]. Finally, the general lack of correlation between GM and excitation, although exploratory, is at odds with existing literature. Positive correlations between reduced GM thickness and left-hemispheric prefrontal/frontal and bilateral parietal BOLD activation have been reported when performing a Tower of London (TOL) task [57]. The fact that we used TMS instead of a cognitive test, and with controls who were matched on ethnicity, socio-economic status, education, and previous comorbidities including substance abuse, could account for this discrepancy.

These results should be interpreted with caution given several key limitations. First, despite being very well characterized and

matched, the small sample size restricts the generalizability of our results. This may also explain in part why our findings showed no differences with rMT across both groups although low rMTs have been found in a population of drug-naïve but also first-episode subjects with schizophrenia [41,58]. Second, although we ensured that subjects with schizophrenia were free from antipsychotic medications for at least two weeks, medication-free cannot be equated to drug-naïve. With medication-free subjects with schizophrenia, cortical excitability may be influenced by long-term use of neuroleptics rather than by schizophrenia-related neurophysiological processes [59]. Third, we did not control for duration of the disease or number of psychotic episodes. These factors may influence cortical excitability through progressive metabolic and neuropathological changes [60,61]. Fourth, we used surface MRI coils because our primary hypothesis was focused on BA9 activity; thus, we could not investigate activity in other brain regions. Finally, inherently the BOLD response as such cannot distinguish between excitatory (glutamate) or inhibitory (GABA) activity. However, the use of concurrent rTMS conferred methodological advantages in that it is independent of a subject's effort and skills and consequently induces direct stimulation of the cortex. We also ensured that the stimulation was equal for each subject by adjusting the doses of stimulation to prefrontal atrophy. As for the matching of the controls, we went to great length to obtain a control group that did not differ in past drug abuse and social economic status. Thus, the identified neurobiological differences between groups are likely related to disorder factors.

Despite these limitations, the unique experimental design controlling for past comorbidities across both groups, structural (S-BA9/S-MC and GM volumes) and functional covariates (percent rMT), lend credibility to our results. Taken together, our findings suggest that left BA9 in subjects with schizophrenia, when probed by TMS, exhibits hyper-excitability in adjacent ipsilateral regions

and dysfunctional connectivity with the contralateral hemisphere. In light of previous findings implicating convergent prefrontal abnormalities in cognitive dysfunction and its remediation, future studies might investigate whether cognitive remediation augmented with NBS can improve cognition more rapidly or to a greater degree in schizophrenia.

Conclusions

Taken together, our findings suggest that left BA9 in subjects with schizophrenia, when probed by TMS, exhibits a local hyperexcitability and a dysfunctional connectivity to the contralateral hemisphere. TMS fMRI, if paired with psychological or pharmacological interventions geared for treatment development in schizophrenia, could yield promising results and help expedite treatment discovery.

CRedit authorship contribution statement

Ryan D. Webler: Writing - review & editing. **Carmen Hamady:** Formal analysis, Data curation. **Chris Molnar:** Funding acquisition, Data curation. **Kevin Johnson:** Funding acquisition, Data curation. **Leo Bonilha:** Formal analysis, Data curation. **Berry S. Anderson:** Data curation, Funding acquisition. **Claartje Bruin:** Formal analysis, Data curation. **Daryl E. Bohning:** Software, Conceptualization. **Mark S. George:** Conceptualization. **Ziad Nahas:** Conceptualization, Methodology, Formal analysis, Data curation, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.brs.2020.06.017>.

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Dr. Nahas and Dr. George report no conflicts of interest related to this publication.

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