Supplementary Online Content


eAppendix. Supplemental methods, supplemental results, and supplemental references.
eFigure 1. Concurrent TMS-fMRI targeting and apparatus. (A) Frameless stereotaxy is used to align the subject to their T1 high-resolution structural image. (B, C) A custom plastic mount holds the TMS coil allowing it to rotate in all directions.
eFigure 2. Concurrent TMS-fMRI image acquisition timing. spTMS (and button press tone) stimuli occurred during a 230 ms gap in MRI acquisition (nominal TR: 2000ms; effective TR: 1170ms), ensuring that electromagnetic artifacts from discharge of the TMS coil did not corrupt acquisition of fMRI images.
eFigure 3. Group averaged spTMS-evoked response in insula. *P < .01, shaded areas: 95% CI. Inset: single-subject representation of ROI (yellow); voxels most responsive to condition (red). N=14 in each group.
eTable 1. Mean number of voxels (SEM) in each ROI.
eTable 2. Group mean correlation coefficient (r) between ROI time series.

This supplementary material has been provided by the authors to give readers additional information about their work.
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Supplemental Methods

Minimizing RF leakage
Several procedures were used to minimize RF leakage into the Faraday cage housing the scanner. First, four ferrite cores (impedance=380ohm; frequency=100MHz, Newark, Palatine, IL) were clamped to the TMS cable. Second, aluminum foil was used to cover the inner and outer openings of the waveguide. Finally, the stimulator was grounded to the waveguide panel. Extensive piloting demonstrated that these procedures permitted the acquisition of high-quality fMRI BOLD data with minimal distortion in the vicinity of the TMS coil.

Subject Exclusion Criteria
Exclusion criteria included substance abuse/dependence within the prior 6 months, diagnosed neurological disorders, insulin-dependent diabetes, seizure history, recent heart attack or cancer, diagnosed sleep disorders, night-shift work, and standard MRI contraindications (e.g. ferrous implants). Healthy subjects were additionally excluded if they were taking psychotropic medications or had first-degree relatives with a psychiatric diagnosis.

Frameless Stereotaxy and TMS/fMRI Image Acquisition
At the beginning of the second session, subjects were fitted with a snug cloth cap, earplugs, and spectacles equipped with infrared (IR) reflectors that were tracked by ceiling–mounted IR cameras. The TMS coil used for determination of motor threshold was equipped with similar reflectors, permitting real-time, three-dimensional coregistration of the coil, the subject’s head, and their own structural MRI (“frameless stereotaxy,” eFigure 1). eFigure 2 illustrates the method of interleaving TMS stimulation with fMRI acquisition. Subjects were instructed to remain awake with their eyes open. Compliance was confirmed using a closed-circuit audiovisual system. In the few cases where compliance was doubtful, the scan was re-run.

Sham TMS Methods
To minimize possible confounding factors such as the “click” or tactile sensation produced when the TMS coil is discharged, we created a sham condition (similar to Casali et al1) in which the TMS coil is fitted with a 4cm thick, hollow, plastic block. Because spTMS is only effective to a depth of 2-3 cm,2 the block prevented cortical stimulation, but preserved the tactile and acoustic components of a TMS pulse. Procedurally, subjects were removed from the scanner; the plastic block was securely attached to the coil with clear medical tape, and the “sham” coil was aligned to approximately the same position used for spTMS. Two 20-trial runs of EPI data were collected. Imaging parameters were identical to those used for spTMS and are described in the main text. Subjects were not aware of the coil manipulation. On an exploratory basis, the ROI voxels most responsive to sham were separately identified and their responses parameterized using methods identical to those used for active spTMS and BP analysis. There were no group differences in the thalamic response to sham TMS (not shown, F(1,24)=.41, n.s., η²=.02).

ROI selection and size
The thalamic ROI extended bilaterally in the ventral-dorsal direction from the slice in which the vermis of the cerebellum was no longer visible to the slice in which the frontal horn and atrium of the lateral ventricles were no longer distinct. Laterally, the ROI followed the grey-white matter intersection of thalamus and the internal capsule. Medially, the ROI followed the contour of the lateral ventricles. The thalamic ROI included all nuclei within these boundaries. The precentral gyrus ROI was limited to the left-hemisphere (in accord with the site of spTMS/sham delivery and contralateral activation during the motor task) and began dorsally with the first occurrence of grey matter in the MRI image and continued ventrally until the hand knob3 was no longer identifiable. The ROI extended from the edge of the brain to the interhemispheric fissure. Rostrally the ROI extended to the edge of the gyrus and caudally it included the central sulcus. The mSFG ROI was drawn, bilaterally, dorsoventrally from the first emergence of frontal grey matter to the final slice that did not include the cingulate sulcus. Mediodorsally, it extended from the crown of the SFG to the interhemispheric fissure. The insular ROI was drawn bilaterally, ventrodorsally from the first emergence of the circular sulcus until the sulcus was no longer distinguishable from surrounding tissue. The insular ROI extended the length of the lateral fissure in the anterior-posterior direction with care taken to exclude the superior temporal and orbital gyri. In the lateral-medial direction the insular ROI extended from the edge of the brain to the intersection with white matter with care taken to exclude the claustrum. ROI’s were drawn by the first author and ROI sizes did not significantly differ between groups (eTable 1).

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Methodological considerations
Like the spTMS-evoked response, the BP-evoked thalamic response was also decreased in the thalamus in schizophrenia patients. However, the response to BP in the precentral gyrus also differed across groups. Because the spTMS-evoked response did not differ between groups in the region that experienced direct electromagnetic induction, differences in thalamic response are unlikely due to differences in the cortical input that it received. Rather, they are most likely due to differences in local thalamic physiology. The same reasoning cannot be applied to the thalamic response in the BP task, however, because the precentral gyrus response to the BP also differed between the two groups. Consequently, it is more difficult to rule out the possibility that the reduced response in thalamus was a downstream effect of differences at the cortical level. Concurrent spTMS-fMRI has the advantage of circumventing these uncertainties and leaves less room for alternative explanations.

Supplemental Results

Analyses of “unselected” runs
There was no difference in the proportion of subjects for whom the “unselected” run was the second run versus the first run (patients with schizophrenia: 42.9%, healthy control subjects: 57.1%, $\chi^2(1) = .14, p=.71$.) For the “unselected” run, healthy control subjects had a spTMS-evoked thalamic response that was greater than that of patients with schizophrenia ($F_{(1,26)}=7.322, P = .012, \eta^2=.22$). The responses for both groups were significantly greater than the response in the sham TMS condition in voxels identical to those used for the above analysis (healthy control subjects: $F(1,12)=73.26, P = 1.9x10^{-4}, \eta^2=.859$; schizophrenia patients: $F_{(1,12)}=29.92, P = 1.4x10^{-4}, \eta^2=.714$; group difference: $F_{(1,24)}=3.280, n.s., \eta^2=.12$). These results did not change when education was added as a covariate. Note that the run that was selected for hypothesis testing was that in which the t-statistic (corresponding to the greatest difference from baseline) was most similar to that of the BP run and was invariably the run with the stronger response. Thus, it is necessarily the case that the “unselected” run has a weaker response and this is reflected in the weaker, albeit still significant, group difference reported above.

Insula response to spTMS of precentral gyrus is reduced in schizophrenia
Analyses of the insula revealed similar results to those reported for the mSFG. We observed a decrease of the spTMS-evoked response in the insula in patients with schizophrenia compared to healthy control subjects ($F_{(1,26)}=7.456, P = .01, \eta^2=.22$, eFigure 3). Additionally, a time series correlation between precentral gyrus and insula (cortico-cortical projections) revealed no difference between groups ($F_{(1,26)}=0.839, n.s., \eta^2=.03$). However, patients with schizophrenia had reduced coupling between thalamus and insula ($F_{(1,26)}=6.805, P=.02, \eta^2=.207$, eTable 2). Further, when the data from these two groups were combined, the correlation between thalamus and insula predicted amplitude of insula ($\rho_{(26)}=.39, P=.04$), reflecting the fact that individuals who showed less thalamo-cortical coupling showed a smaller evoked response in insula.

Functional connectivity
Interestingly, healthy control subjects displayed a stronger coupling between the thalamus and mSFG compared to coupling between precentral gyrus and thalamus that was not seen in schizophrenia patients ($F_{(1,13)}=12.95, P = .003, \eta^2=.50$). This group difference may be attributed to the decreased coupling between thalamus and mSFG observed in schizophrenia patients.

Supplemental References


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**eFigure 3.** Group averaged spTMS-evoked response in insula. *P < .01, shaded areas: 95% CI. Inset: single-subject representation of ROI (yellow); voxels most responsive to condition (red). N=14 in each group.
**eTable 1.** Mean Number of Voxels (SEM) in Each ROI

<table>
<thead>
<tr>
<th></th>
<th>Thalamus</th>
<th>Precentral Gyrus</th>
<th>mSFG</th>
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<tbody>
<tr>
<td><strong>Schizophrenia Patients</strong></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>307.64 (11.34)</td>
<td>359.24 (24.58)</td>
<td>564.57 (30.04)</td>
</tr>
<tr>
<td><strong>Healthy Control Participants</strong></td>
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<tr>
<td></td>
<td>286.69 (14.33)</td>
<td>321.31 (15.3)</td>
<td>518 (43.08)</td>
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</tbody>
</table>

Voxel size: 3×3×3.6mm
Between-groups comparisons were all n.s.

**eTable 2.** Group Mean Correlation Coefficient (r) Between ROI Time Series

<table>
<thead>
<tr>
<th></th>
<th>Precentral Gyrus</th>
<th>Thalamus</th>
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<tbody>
<tr>
<td><strong>Insula</strong></td>
<td>.43 n.s.</td>
<td>.56 *</td>
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<tr>
<td></td>
<td>.36</td>
<td>.40</td>
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</tbody>
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Blue: healthy control subjects; Red: schizophrenia subjects
* P < .02