Altered thalamocortical structural connectivity in persons with schizophrenia and healthy siblings

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Abstract

Schizophrenia has long been theorized as a “misconnection syndrome” – abnormal connections in thalamocortical circuitry may be an underlying mechanism of psychotic symptomatology and cognitive impairments. There is emerging evidence for functional and structural hypoconnectivity between thalamus and prefrontal cortex in persons with schizophrenia (SZ), as well as hyperconnectivity between thalamus and sensory and motor cortices. However, it is unclear whether thalamocortical dysconnectivity is a general marker of vulnerability to schizophrenia or a specific mechanism of schizophrenia pathophysiology. This study aimed to answer this question by examining thalamocortical structural connectivity in healthy siblings of persons with schizophrenia (SIB). Twenty-two SZ, 20 SIB, and 45 healthy controls (HC) of either sex underwent diffusion tensor imaging (DTI). Probabilistic tractography was used to quantify structural connectivity between thalamus and six cortical regions of interest. Thalamocortical structural connectivity was compared among the three groups using cross-thalamic and voxel-wise approaches. Thalamo-prefrontal structural connectivity was reduced in both SZ and SIB relative to HC, while SZ and SIB did not differ from each other. Thalamo-motor structural connectivity was increased in SZ relative to SIB and HC, while SIB and HC did not differ from each other. Hemispheric differences also emerged in thalamic connectivity with motor, posterior parietal, and temporal cortices across all groups. The results support the hypothesis that altered thalamo-prefrontal structural connectivity is a general marker of vulnerability to schizophrenia, whereas altered connectivity between thalamus and motor cortex is related to illness expression or illness-related secondary factors.

Keywords: probabilistic tractography, anatomical connectivity, first-degree relatives, thalamus, thalamo-prefrontal connectivity, diffusion tensor imaging
Introduction

Schizophrenia has been framed as a disorder of altered brain connectivity, with dysfunction in thalamocortical circuitry argued to play a key role in the development of the illness phenotype (1). The thalamus relays sensory signals from subcortical regions to cortex and also actively participates in communication between different cortical areas, thus supporting basic sensorimotor coordination as well as higher-order cognitive functions like executive control and language (2, 3). Given its broad role in regulating cortical functioning, altered thalamocortical connectivity may thus provide a comprehensive neurological basis for the diverse clinical symptoms of schizophrenia, as well as the wide range of cognitive, social, and emotional impairments (4, 5) that are observed in the illness.

Increasing evidence from neuroimaging studies supports altered thalamocortical connectivity in persons with schizophrenia. The majority of this evidence comes from functional connectivity studies (see (6, 7) for review and (8) for meta-analysis), which report a consistent pattern of reduced coordination of resting endogenous activity between the thalamus and prefrontal cortex and increased coordination between thalamus and sensory and motor areas. Data from structural connectivity studies using diffusion tensor imaging (DTI), though limited, revealed a similar pattern to functional connectivity studies: reduced connectivity between thalamus and prefrontal cortex (9–12) and, in a subset of the aforementioned studies, increased connectivity with sensory and motor areas (10–12). A similar thalamocortical connectivity pattern has also been found in individuals at clinical high risk for psychosis in both functional (13) and structural (12) imaging studies, which is furthermore predictive of conversion to a full-blown psychotic disorder (13) and associated with functional decline (12). In addition, this pattern of hypoconnectivity between thalamus and prefrontal cortex and hyperconnectivity between thalamus and sensorimotor regions has been observed in persons with psychotic bipolar disorder (14, 15), intimating the possibility that thalamocortical dysconnectivity may represent a transdiagnostic marker of psychosis. Taken together, these neuroimaging findings suggest that
thalamocortical dysconnectivity may be a pathophysiological mechanism of schizophrenia, and possibly psychosis broadly.

It is still unclear, however, whether thalamocortical dysconnectivity in schizophrenia is a proximal illness mechanism or reflects (genetic) vulnerability to develop the illness. Unaffected relatives of individuals with schizophrenia are at higher risk of the disorder due to their high proportion of shared genes (16) and common environment, and thus may provide insights into the significance of thalamocortical dysconnectivity in schizophrenia. Data from unaffected relatives are consistent with altered thalamic function, structure, and/or connectivity being a marker of vulnerability; however, interpretation of these findings is complicated somewhat by inclusion criteria. In some studies, relatives were screened only for history of psychotic disorder, whereas in others, relatives were screened for any psychiatric illness. Compared to healthy controls, previous studies have found altered fMRI activation in prefrontal cortex and bilateral thalamus during working memory tasks (see (17) for a meta-analysis) in healthy first-degree relatives with no psychiatric disorder, as well as increased thalamic glutamate levels (18), reduced thalamic volume (see (19) for review), and reduced white matter integrity in the anterior limb of the internal capsules (20), superior longitudinal fasciculus (21), and the thalamus (22) in relatives screened for a history of psychotic disorder. Several studies reported reduced thalamo-frontal functional connectivity in healthy siblings with no psychiatric illness (23) and in relatives with no psychotic disorder (24, 25), but see (26). To date, however, only one study has examined tractography-defined thalamocortical structural connectivity in first-degree relatives unaffected by psychosis and major mood disorders (27). Compared to healthy controls, relatives had reduced white matter integrity in the left thalamo-orbitofrontal tract. However, the interpretation of this finding is limited by the fact that the study did not examine the connectivity between thalamus and other cortical regions, nor were SZ included as a comparison group.

In the current study, we sought to determine whether thalamocortical structural dysconnectivity, and thalamo-prefrontal hypoconnectivity more specifically, was a marker of
familial risk towards schizophrenia. To this end, we investigated structural connectivity patterns between the thalamus and 6 cortical regions using probabilistic diffusion tensor tractography in individuals with schizophrenia, healthy siblings of individuals with schizophrenia, and healthy controls. We first expected to replicate previous findings of hypoconnectivity between thalamus and prefrontal cortex and hyperconnectivity between thalamus and sensorimotor regions in schizophrenia. Our primary research question was whether healthy siblings would show similar patterns of thalamocortical connectivity to individuals with schizophrenia, suggesting that these connectivity patterns are related to illness vulnerability, or whether they would look more similar to healthy controls, which would suggest that altered thalamocortical connectivity was related to illness expression or to other secondary factors related to schizophrenia (e.g. psychosocial consequences, antipsychotic medication use, etc.). Results of the current study will add to our understanding of the etiological significance of thalamocortical dysconnectivity in schizophrenia and in turn help the development of more targeted treatment and intervention strategies.

**Methods and Materials**

**Participants**

Eighty-seven participants between the ages of 18 and 55 completed this study. Twenty-two antipsychotic-medicated persons with schizophrenia or schizoaffective disorder (SZ) were recruited from a longitudinal study (28) and an outpatient psychiatric facility in the Netherlands. 20 healthy siblings of persons with schizophrenia or schizoaffective disorder (SIB) were recruited from the same longitudinal study. Through community advertisements, 45 healthy individuals (HC) were recruited to serve as a control group. Diagnoses in SZ and SIB were established using Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria and verified with the Comprehensive Assessment of Symptoms and History interview (29) or Schedules for Clinical Assessment for Neuropsychiatry, version 2.1 (30). Participants in the HC group were excluded if they had a family history of schizophrenia. Participants in the SIB and HC groups were excluded if they had any current Axis I disorder. Participants in all groups
were excluded if they had a history of head trauma or neurological illness, or substance abuse or dependence within 6 months before the study. The participants in the SIB and SZ groups were not biologically related.

Table 1. Demographic information.

|                         | SZ (N = 22) | SIB (N = 20) | HC (N = 45) | Statistic | p   |
|-------------------------|-------------|--------------|-------------|-----------|-----|
| Age (Years)             | 37.4 (7.8)  | 31.7 (5.6)   | 30.2 (8.3)  | F = 6.6   | 0.002|
| Sex (Female/Male)       | 5/17        | 7/13         | 22/23       | χ² = 4.4  | 0.11 |
| IQ⁵                     | 96.0 (12.8) | 104.2 (12.2) | 99.0 (13.3) | F = 2.2   | 0.12 |
| Education⁶              | 4.8 (1.7)   | 6.4 (1.8)    | 6.9 (1.4)   | χ² = 33.6 | 0.001|
| Handedness⁷             | 0.85 (0.45) | 0.88 (0.30)  | 0.67 (0.69) | F = 1.3   | 0.27 |
| Illness Duration (Years)| 14.2 (5.2)  |              |             |           |      |
| PANSS Positive          | 11.6 (5.2)  |              |             |           |      |
| PANSS Negative          | 13.1 (6.3)  |              |             |           |      |
| PANSS General           | 25.0 (7.9)  |              |             |           |      |
| CPZ Equivalent (mg)     | 269.6 (247.9)|           |             |           |      |

Notes: CPZ, chlorpromazine; HC, healthy control participants; PANSS, Positive and Negative Syndrome Scale; SIB, healthy siblings; SZ, persons with schizophrenia.

⁵Based on the Nederlandse Leestest voor Volwassenen.

⁶Education category: 0 = <6 years of primary education; 1 = finished 6 years of primary education; 2 = 6 years of primary education and low-level secondary education; 3 = 4 years of low-level secondary education; 4 = 4 years of average-level secondary education; 5 = 5 years of average-level secondary education; 6 = 4 years of secondary vocational training; 7 = 4 years of high-level professional education; 8 = university degree.

⁷Based on the Edinburgh Handedness Inventory; scores range from 0 indicating complete left-handedness to 1 indicating complete right-handedness.

Demographic and clinical data are presented in Table 1. All SZ were taking antipsychotic medications, and chlorpromazine (CPZ) equivalent antipsychotic dosages were calculated (31). Clinical symptoms were assessed in SZ only with the Positive and Negative Syndrome Scale.
(PANSS) (32). Premorbid IQ was assessed with the Dutch version of the National Adult Reading Test (Nederlandse Leestest voor Volwassenen (33)). The Edinburgh Handedness Scale was used to measure handedness (34). The three groups were matched on sex, handedness, and IQ. SZ were significantly older than SIB and HC, so age was included as a covariate in between-group analyses.

All participants gave written informed consent and were compensated for participation. The study was approved by the Medical Ethical Committee of the University Medical Center, Utrecht, The Netherlands.

DTI

**Image acquisition.** All DTI data were acquired at the University Medical Center Utrecht on an Achieva 3T scanner (Philips Medical Systems) equipped with an eight-channel head coil allowing parallel imaging. Two diffusion images were acquired using single-shot echoplanar imaging sequences, consisting of 30 diffusion-weighted scans \((b = 1000 \text{s/mm}^2)\) with noncollinear gradient directions and one image without diffusion weighting \((b = 0 \text{s/mm}^2)\), covering the entire brain [repetition time (TR) = 7057 ms; echo time (TE) = 68 ms; field of view = 240 × 240 × 150 mm; in-plane resolution = 1.875 × 1.875 mm; slice thickness = 2 mm; no slice gap; 75 axial slices; matrix size, 128 × 99 mm]. The diffusion-weighted scans were measured twice, once with phase-encoding direction reversed (first scan, posterior-anterior; second scan, anterior-posterior), to correct for susceptibility-induced spatial distortions (35). For registration purposes, a whole-brain three-dimensional T1-weighted scan (200 slices; TR = 10 ms; TE = 4.6 ms; flip angle = 8°; field of view, 240 × 240 × 160 mm; voxel size: 0.75 × 0.8 × 0.75 mm) was acquired.

**Preprocessing.** The diffusion-weighted scans were preprocessed and analyzed using FSL 5.0 (FMRIB Software Library; www.fmrib.ox.ac.uk/fsl). As DTI scans suffer from spatial distortions along the phase-encoding direction, two diffusion-weighted scans were acquired with
reversed phase-encoding blips, resulting in pairs of images with distortions going in opposite directions. From these two images, the off-resonance field was estimated using a method similar to that described by (35) as implemented in FSL (36). Next, the 30 diffusion-weighted images from each phase-encoding direction were realigned to the \( b_0 \) image using affine registration, and eddy current correction was applied. The eddy-corrected scans with opposite phase-encoding blips were then combined into a single corrected image using the previously estimated off-resonance field. A brain mask was created for the mean \( b_0 \) image and applied to all diffusion-weighted images.

*Regions of interest.* Each participant’s structural T1-weighted image was automatically segmented and labeled into known cortical and subcortical structures using Freesurfer (http://surfer.nmr.mgh.harvard.edu). Selected cortical parcellations (Table S1) were combined to form six cortical regions of interest (ROIs) in each hemisphere: prefrontal cortex, motor cortex, somatosensory cortex, posterior parietal cortex, temporal cortex, and occipital cortex (Figure 1). To increase comparability, we defined our ROIs as close to previous studies as possible (11). The thalamus segmentation was then manually edited to include the lateral and medial geniculate nuclei, following an established approach (37) (Figure S1). Transformation matrices were derived for each participant by registering their mean \( b_0 \) images to the Freesurfer conformed space (38). Each registration was visually inspected and adjusted for quality control. The inverse of these registration matrices was then applied to transform the ROIs to diffusion space with trilinear interpolation.

*Probabilistic tractography.* Thalamocortical connectivity was assessed using probabilistic tractography, an analysis technique that reconstructs anatomical pathways between brain regions based on a distribution profile of probable fiber orientations in each voxel (39, 40). In each hemisphere, probabilistic tractography was performed six times, using thalamus as the seed (start point) and one of the six cortical ROIs as a target (end point), using the protrackx2 command. The mid-sagittal plane was used as an exclusion mask (41–43), along with the other
Figure 1. Cortical regions of interest (ROIs) and thalamus for a representative participant, displayed on the participant’s T1 image in horizontal view on multiple slices from superior to inferior. Masks based on Freesurfer’s automatic parcellation were combined to create an initial thalamus mask and six cortical ROI masks: prefrontal cortex (blue), motor cortex (red), somatosensory cortex (cyan), posterior parietal cortex (yellow), temporal cortex (green), and occipital cortex (magenta). The initial thalamus mask was then manually edited to include the lateral and medial geniculate nuclei to form the final thalamus mask (orange). These masks were then used in probabilistic tractography analyses to quantify thalamocortical connectivity five cortical regions that were not the target at a given analysis. The distribution profile of probabilistic connectivity was computed by sending out 5000 streamlines from each voxel within the thalamus, going in a direction drawn from a distribution around the principal diffusion direction until it was determined structurally impossible for a white matter tract to continue. Only
streamlines that reached the target were preserved, and streamlines were not allowed to continue after reaching the target. Two crossing fibers per voxel were modeled. From this analysis, six seed-to-target images were generated within both hemispheres where each voxel in the thalamus contains a value representing the number of streamlines originating from that voxel that reached the corresponding cortical ROI target.

*Cross-thalamic cortical connectivity analysis.* To increase comparability, we calculated our main measures of interest the same way as previous studies did (10–12). To examine group differences in the connectivity pattern between thalamus and each cortical ROI within each hemisphere, we computed percent connectivity for each thalamus-ROI pair as follows:

\[
\text{Percent Connectivity to ROI}_j = \frac{\sum_{\text{thalamus} - \text{to} - \text{ROI}_j} \text{streamlines}}{\sum_{i=1}^{6} (\sum_{\text{thalamus} - \text{to} - \text{ROI}_i} \text{streamlines})}
\]

The absolute connectivity between thalamus and each cortical ROI was quantified as the sum of streamlines originating across all voxels in the thalamus that reached the corresponding ROI (i.e. the sum of values for each seed-to-target image). Total thalamocortical connectivity for a particular hemisphere was calculated as the sum of all streamlines from the thalamus that reached any of the six cortical ROIs (i.e. the sum of values for each seed-to-target image summed across all six seed-to-target images). To control for individual differences in total thalamocortical connectivity, percent connectivity was calculated for each cortical ROI by dividing its absolute connectivity with thalamus by total thalamocortical connectivity for that hemisphere (10). These tractography-defined connectivity values were measures of relative connectivity that were less influenced by differences in brain size and global white matter integrity. They were also independent of where the tract originated from inside the thalamus and are closer to a summary measure of structural connectivity patterns rather than an integrity measure of any specific white matter tract. These percent connectivity measures were used as the dependent variables in main group comparisons. To further rule out the possibility of any potential connectivity differences due to ROI size differences, we conducted a supplementary
analysis on comparing total volumes of thalamus and cortical ROI masks among the groups with ANOVAs.

Group comparisons of percent connectivity between each cortical ROI and the thalamus was evaluated with a repeated-measures ANCOVA (i.e., six comparisons in total), conducted in SPSS Statistics version 25.0 (IBM). For percent connectivity in each cortical ROI, diagnostic group was included as a between-subject variable and age as a covariate. Hemisphere was included as a within-subject variable due to its understudied potential significance in thalamocortical connectivity (44). To examine the potential confounding effect of antipsychotic use, CPZ equivalent dose (31) was correlated with total percent connectivity using Spearman's rank correlation ($r_s$).

Voxel-wise thalamocortical connectivity analysis. To localize putative group differences in thalamocortical connectivity patterns, we computed a probability value at each thalamic voxel for each cortical ROI as follows:

\[
P_{\text{voxel}} = \frac{\sum_{i=1}^{\text{voxel}} - \text{ROI}_i \text{ streamlines}}{\sum_{i=1}^{\text{voxel}} (\sum_{i=1}^{\text{voxel}} - \text{ROI}_i \text{ streamlines})}
\]

From each seed-to-target image, probability maps were calculated by dividing the value at each voxel (representing the number of streamlines that arrived at a particular cortical ROI) by the sum of all six images at each voxel. These probability maps thus represent the probability of a given voxel in the thalamus connecting with a particular cortical ROI, relative to general thalamocortical connectivity.

In order to examine voxel-wise thalamocortical connectivity differences across groups, we normalized individual probability maps. Specifically, each participant’s anatomical T1-weighted volume was realigned to their mean $b_0$-weighted image and subsequently segmented into gray matter, white matter, and CSF, and normalized to MNI space using the unified segmentation algorithm as implemented in SPM8 (45). Then all probability maps were transformed into MNI space using each individual’s normalization matrix, and then averaged within each group. To
exclude voxels that don’t contain reliable projections to cortex, we created a within-group mask for each group to include all voxels in the thalamus that have a >10% value for any of the cortical ROI targets. The union of these three group masks formed the final inclusion mask. Normalized individual probability maps were smoothed using a 4mm kernel before the inclusion mask was applied. These normalized, smoothed, and masked probability maps were then analyzed in voxel-wise group comparisons using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12).

For each cortical ROI within each hemisphere, ANCOVAs were conducted at each voxel in the seed-to-target probability maps, with diagnostic group as a between-subject variable and age as a covariate. Statistical maps were tested for a significant effect of group using cluster-level inference (cluster-defining threshold, p<0.001; cluster probability of p<0.05, family wise error-corrected for multiple comparisons). Then independent groups t tests were performed to follow up on significant main effects of group. To examine the potential confounding effect of antipsychotic use, we averaged probability values within significant clusters and correlated those values with CPZ equivalent dose (31) using Spearman’s rank correlation ($r_s$).

**Results**

**Cross-thalamic cortical connectivity**

Percent connectivity between each cortical ROI and the thalamus in all three groups are presented in Figure 2. For connections between thalamus and prefrontal cortex, there was a significant main effect of group ($F(2,83) = 6.00$, $p = 0.004$, partial $\eta^2 = 0.13$). Post Hoc analyses based on Tukey’s HSD revealed that, compared to HC, both SIB ($p = 0.043$) and SZ ($p = 0.028$) had significantly lower thalamo-prefrontal connectivity. SIB and SZ did not differ from each other ($p = 0.997$). There was no significant effect of hemisphere ($F(1,84) = 0.47$, $p = 0.50$, partial $\eta^2 = 0.01$), nor any group by hemisphere interaction ($F(2,84) = 1.53$, $p = 0.22$, partial $\eta^2 = 0.04$). For connections between thalamus and motor cortex, there was also a significant effect of group
Figure 2. Structural connectivity between cortical regions and thalamus for each group collapsed across hemispheres. Insets show hemisphere effects collapsed across groups. HC, healthy control participants; L, left hemisphere; SIB, healthy siblings; SZ, persons with schizophrenia; R, right hemisphere. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. 
Post-hoc tests revealed significantly higher connectivity in the SZ than HC group (\(p = 0.027\)), while SIB did not differ significantly from either HC (\(p = 1.0\)) or SZ (\(p = 0.074\)). There was also a significant main effect of hemisphere (\(F(1,84) = 9.23, p = 0.003, \text{partial } \eta^2 = 0.11\)), with thalamo-motor connectivity being higher in the left hemisphere, but no significant group-by-hemisphere interaction effect (\(F(2,84) = 0.95, p = 0.39, \text{partial } \eta^2 = 0.02\)).

There was no group difference in percent connectivity between thalamus and any other cortical ROI (Somatosensory: \(F(2,83) = 1.89, p = 0.16, \text{partial } \eta^2 = 0.00\); Posterior Parietal: \(F(2,83) = 2.75, p = 0.07, \text{partial } \eta^2 = 0.06\); Temporal: \(F(2,83) = 0.84, p = 0.44, \text{partial } \eta^2 = 0.01\); Occipital: \(F(2,83) = 2.75, p = 0.07, \text{partial } \eta^2 = 0.00\)). There was, however, a significant effect of hemisphere on thalamo-posterior parietal (\(F(1,84) = 27.08, p < 0.001, \text{partial } \eta^2 = 0.24\)) and thalamo-temporal (\(F(1,84) = 12.11, p = 0.001, \text{partial } \eta^2 = 0.13\)) connectivity. Thalamo-posterior parietal connectivity was higher in the right hemisphere, while thalamo-temporal connectivity was higher in the left hemisphere. There was no hemisphere difference in percent connectivity between thalamus and somatosensory cortex (\(F(1,84) = 0.00, p = 0.95, \text{partial } \eta^2 = 0.00\)) and between thalamus and occipital cortex (\(F(1,84) = 1.13, p = 0.29, \text{partial } \eta^2 = 0.00\)). Lastly, there was no significant group \(\times\) hemisphere interaction effect on connectivity between thalamus and either somatosensory (\(F(2,84) = 2.53, p = 0.086, \text{partial } \eta^2 = 0.00\)), posterior parietal (\(F(2,84) = 0.32, p = 0.73, \text{partial } \eta^2 = 0.007\)), temporal (\(F(2,84) = 2.48, p = 0.09, \text{partial } \eta^2 = 0.05\)), or occipital (\(F(2,84) = 0.72, p = 0.49, \text{partial } \eta^2 = 0.00\)) cortex.

There was also no effect of age on connectivity between thalamus and any of the cortical ROIs (\(0.13 \leq p \leq 0.78\)). Additionally, there were no correlations between antipsychotic dosage and thalamocortical connectivity for any of the cortical ROIs (-0.27 \(\leq r_s \leq 0.32\), 0.20 \(\leq p \leq 0.99\)). Of note, there were no group differences in total volumes of thalamus and cortical ROI masks used in the probabilistic tractography analysis (Table S2).
Figure 3. Thalamocortical connectivity voxel-wise analysis results. The left three columns displayed voxel-wise probability maps indicating probability of connectivity of thalamus voxels with each cortical region of interest in each group. Maps for motor, somatosensory, and occipital cortex were thresholded at 5%, and maps for prefrontal, temporal, and posterior parietal cortex were thresholded at 10%. The middle column displayed group differences across all groups, and the right three columns displayed pair-wise group comparisons. All group comparisons were thresholded at uncorrected \( p < 0.01 \). HC, healthy control participants; SIB, healthy siblings; SZ, persons with schizophrenia.
Voxel-wise thalamocortical connectivity

Voxel-wise connectivity patterns were qualitatively similar across all groups (Figure 3). The between-group analyses revealed two clusters where thalamocortical connectivity differed significantly across groups after correcting for multiple comparisons (Table 2). Specifically, follow-up pair-wise comparisons revealed that left thalamo-motor connectivity was significantly higher in SIB than in HC group in a cluster located at MNI coordinates -24, -32, 10 (cluster size = 531 voxels, \( p_{\text{FWE-corrected}} = 0.018 \)). Left thalamo-temporal connectivity in the SZ group was significantly higher than in the HC group in a cluster located at MNI coordinates -24, -24, -4 (cluster size = 541 voxels, \( p_{\text{FWE-corrected}} = 0.007 \)). Finally, there were no correlations between antipsychotic dosage and mean connectivity values in either cluster (thalamo-motor cluster: \( r_s = -0.34, p = 0.17 \); thalamo-temporal cluster: \( r_s = 0.30, p = 0.24 \)).

Discussion

In this study, we used probabilistic tractography analyses of DTI data to examine thalamocortical structural connectivity patterns in persons with schizophrenia, healthy siblings of persons with schizophrenia, and healthy controls. We found reduced connectivity between thalamus and prefrontal cortex in both persons with schizophrenia and healthy siblings, compared to healthy controls. In addition, compared to healthy controls, persons with schizophrenia had increased structural connectivity between thalamus and motor cortex; healthy siblings did not. Moreover, we observed hemispheric differences in several thalamocortical connectivity patterns across all groups. Taken together, these findings are consistent with prior reports in schizophrenia, and results from healthy siblings suggest that hypo-connectivity between thalamus and prefrontal cortex may represent a marker of vulnerability related to familial risk. In this way, these data thus provide novel insights into the etiology and functional significance of thalamocortical dysconnectivity in the illness.

Using largely identical analytic methods to previous studies, we replicated findings of hypoconnectivity between thalamus and prefrontal cortex in persons with schizophrenia. These
**Table 2.** Thalamocortical connectivity voxel-wise analysis results (including all voxel-wise uncorrected $p < 0.001$ results).

| Cortical Target | Hemisphere | Cluster-level | Peak-level | MNI Coordinates |
|-----------------|------------|---------------|------------|-----------------|
|                 |            | $p_{\text{FWE-corr}}$ | $\text{Cluster Size (voxels)}$ | $p_{\text{uncorr}}$ | $F$ | $p_{\text{uncorr}}$ | mm | mm | mm |
| Prefrontal      | Left       | 0.119 | 113 | 0.152 | 0.144 | 7.4 | 0.001 | -20 | -26 | 4 |
|                 | Right      | 0.29 | 35 | 0.38 | 0.255 | 6.57 | 0.002 | 26 | -26 | -8 |
| Motor           | *Left      | 0.04 | 253 | 0.053 | 0.084 | 8.09 | 0.001 | -24 | -24 | 12 |
|                 | Right      | 0.071 | 144 | 0.082 | 0.163 | 7.34 | 0.001 | 16 | -14 | 4 |
| Somatosensory   | Left       | 0.649 | 2 | 0.829 | 0.507 | 5.78 | 0.004 | -26 | -32 | 6 |
|                 | Right      | 0.333 | 23 | 0.543 | 0.016 | 10.53 | 0 | 28 | -22 | -6 |
| Posterior       | Left       | no significant clusters |
| Parietal        | Right      | 0.464 | 10 | 0.634 | 0.143 | 7.67 | 0.001 | 28 | -32 | 0 |
| Temporal        | *Left      | 0.047 | 188 | 0.05 | 0.045 | 9.4 | 0 | -24 | -24 | -4 |
|                 | Right      | no significant clusters |
| Occipital       | Left       | 0.412 | 13 | 0.644 | 0.089 | 8.14 | 0.001 | -4 | -20 | 16 |
|                 | Right      | 0.304 | 32 | 0.385 | 0.104 | 8.11 | 0.001 | 8 | -18 | 18 |

Notes: FWE-corr, Family-wise error corrected $p = 0.05$ for voxel-wise uncorrected $p = 0.001$; uncorr, uncorrected.

*Significant clusters after familywise error correction.
findings converge with other reports of reduced structural (10–12) and functional (see (6) for review) thalamo-frontal connectivity. We also identified a hyperconnectivity between thalamus and motor cortex in persons with schizophrenia, and a specific cluster in the left thalamus with increased connectivity with the temporal cortex. Findings of structural and functional connectivity between thalamus and cortical regions other than prefrontal cortex are less consistent. While Marenco and colleagues (10) found elevated connectivity between thalamus and somato-motor cortex - consistent with our own findings - other studies did not, instead reporting increased thalamic connectivity with somatosensory (11), occipital (11), and parietal (12) cortices. The hyperconnectivity between thalamus and motor cortex is, however, in line with functional connectivity findings (see (6) for review). The inconsistent findings regarding hyper-thalamocortical connectivity may be due to differences in imaging parameters, methods of defining cortical ROIs, clinical heterogeneity, and statistical power across studies.

More importantly, our study showed reduced thalamo-prefrontal connectivity in healthy siblings of persons with schizophrenia, compared to healthy controls, corroborating recent findings of reduced white matter integrity between thalamus and orbitofrontal cortex in first-degree relatives unaffected by psychosis and major mood disorders (27). The degree of thalamo-prefrontal hypoconnectivity did not differ between healthy siblings and persons with schizophrenia. Given that siblings were screened for any history of mental illness and had, by and large, passed the age by which a psychotic disorder typically emerges, these results suggest that thalamo-prefrontal hypoconnectivity is neither a proximal mechanism of schizophrenia symptomatology nor secondary to chronic antipsychotic use or the psychosocial consequences of having a mental illness. Instead, our results suggest that it is a correlate of familial risk towards schizophrenia.

Naturally, this finding in healthy siblings draws into question the functional significance of altered thalamo-prefrontal connectivity. Siblings had equivalent reductions in connectivity between the thalamus and prefrontal cortex, but did not exhibit clinically significant symptoms.
One simple explanation is that thalamo-prefrontal hypoconnectivity does not contribute to the emergence of schizophrenia symptoms. However, this seems unlikely given previous findings in persons at clinical high risk of developing a psychotic disorder: thalamocortical functional dysconnectivity is predictive of conversion to a full-blown psychotic disorder (13) and reduced thalamo-orbitofrontal structural connectivity is associated with functional decline (12).

Another explanation is the presence of protective factors in healthy siblings. In considering this question, we revisit the function of thalamocortical connections and the implications of dysfunction in these circuits. The thalamus is a central hub in the brain that not only relays basic sensory information from subcortical structures to the cortex, but also participates in almost all intracortical communications (2). The functional specialization and organization of thalamic nuclei and the formation of thalamocortical circuits involve complicated interactions between the thalamus and the cortex through gene expressions and neuronal signaling (46, 47). Misexpression of certain genes can lead to thalamic rearrangements through rewiring of thalamocortical axons or changes in thalamic structures (46). Consequently, alterations in this neurodevelopmental process could lead to atypical connection patterns between thalamus and different cortical regions. Indeed, in functional connectivity studies, thalamo-prefrontal hypoconnectivity and thalamo-sensory/motor hyperconnectivity in persons with schizophrenia are inversely correlated (see (8) for a meta-analysis), suggesting a potential common underlying mechanism between the two. It is possible that altered neurodevelopmental processes in thalamocortical circuit formation exists in individuals with a familial liability towards schizophrenia, but to a lesser degree in healthy siblings. That is, the wiring of thalamocortical circuits may be more imbalanced in persons with schizophrenia and give rise to both hypoconnectivity and hyperconnectivity, but only hypoconnectivity in healthy siblings. The lack of thalamo-motor hyperconnectivity may in turn serve as a protective factor for healthy siblings such that the weak top-down control (as evidenced by thalamo-prefrontal hypoconnectivity) was not further exacerbated by aberrant sensorimotor control. This possibility fits neatly with recent
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Computational accounts of psychosis that posit abnormal weighting of top-down expectations and incoming sensory information as a potential disease mechanism (48, 49), where thalamus potentially plays a crucial role in relaying expectations and sensory afferents (2, 50).

Interpretation of the current findings is limited by several factors. First, our DTI scans were acquired with 30 different diffusional gradient directions. Using probabilistic tractography, previous studies with more gradient directions were able to render sub-thalamic divisions close to histological segmentation results (51, 52). Though 30 gradient directions has been found to yield reliable tractography results for robust anatomical pathways (53–55), our results from the voxel-wise thalamocortical analysis (Figure 3) were spatially diffusive due to limited resolution and did not reflect the precise functional subdivisions of the thalamus. Therefore, the voxel-wise results should be interpreted cautiously. Nonetheless, we were still able to replicate previous findings using cross-thalamic cortical connectivity measures, attesting to the robustness of the thalamocortical dysconnectivity findings in schizophrenia. Second, the percent connectivity values likely describe an average over multiple white matter routes between the thalamus and cortical ROIs, particularly for the larger cortical regions. Accordingly, group differences in more localized tracts may be washed out in the current analyses. Future studies with larger samples and more diffusion directions may reveal potential group differences in structural connectivity between thalamus and smaller cortical subdivisions. Third, we were not able to separate contributions of familial environment versus genes in conferring risk towards schizophrenia. Future studies on healthy twins of persons with schizophrenia are needed to tease out the unique contribution of genes to thalamocortical structural connectivity abnormalities.

In conclusion, we identified significant decreases in thalamo-prefrontal structural connectivity in healthy siblings of persons with schizophrenia relative to healthy controls, which was not distinguishable from that in persons with schizophrenia. This novel finding suggests that thalamo-prefrontal hypoconnectivity may be a marker of familial vulnerability to schizophrenia.
and has important implications for understanding disease mechanisms and, thus, treatment development.
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