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*Case Control Study*

**Altered thalamic subregion functional networks in patients with treatment-resistant schizophrenia**

Thalamic subregion functional networks in TRS

Woo-Sung Kim, Jie Shen, Uyanga Tsogt, SOYOLSAIKHAN ODKHUU, Young-Chul Chung

**Abstract**

BACKGROUND

The thalamus plays a key role in filtering information and has extensive interconnectivity with other brain regions. A large body of evidence points to impaired functional connectivity (FC) of the thalamocortical pathway in schizophrenia (SZ). However, the functional network of the thalamic subregions has not been investigated in patients with treatment-resistant schizophrenia (TRS).

AIM

We hypothesized that the FC of thalamic sub-regions with cortical networks and voxels would differ between TRS patients and HCs. To identify the neural mechanisms underlying TRS, we investigated FC of thalamic sub-regions with cortical networks and voxels, and the associations of this FC with clinical symptoms.

METHODS

In total, 50 patients with TRS and 61 healthy controls (HCs) matched for age, sex, and education underwent resting-state functional magnetic resonance imaging (rs-fMRI) and clinical evaluation. Based on the rs-fMRI data, we conducted a FC analysis between thalamic subregions and cortical functional networks and voxels, and within thalamic subregions and cortical functional networks, in the patients with TRS. A functional parcellation atlas was used to segment the thalamus into nine subregions. Correlations between altered FC and TRS symptoms were explored.

RESULTS

We found differences in FC within thalamic subregions and cortical functional networks between patients with TRS and HCs. In addition, increased FC was observed between thalamic subregions and the sensorimotor cortex, frontal medial cortex, and lingual gyrus. These abnormalities were associated with the pathophysiology of TRS.

CONCLUSION

Our findings suggest that disrupted FC within thalamic subregions and cortical functional networks, and within the thalamocortical pathway, has potential as a marker for TRS. Our findings also improve our understanding of the relationship between the thalamocortical pathway and TRS symptoms.

**Key Words:** Treatment-resistant schizophrenia; Thalamus; Rs-fMRI; Functional connectivity; Thalamocortical pathway.

Kim WS, Shen J, Tsogt U, ODKHUU S, Chung YC. Altered thalamic subregion functional networks in patients with treatment-resistant schizophrenia. *World J Psychiatr* 2021; In press

**Core Tip:** The thalamus represents the interface between the sensory and motor systems, and is a major hub for cognitive processes. A large body of evidence has demonstrated involvement of the thalamus in the pathophysiology of schizophrenia. Most previous studies employing resting state functional magnetic resonance imaging used the whole thalamus as a seed region to identify abnormalities in thalamic connectivity. To identify more specific disturbances, we conducted functional connectivity analysis of thalamic subregions with cortical networks and voxels in patients with treatment resistant schizophrenia. Important novel findings regarding the pathophysiology of treatment resistant schizophrenia were obtained.

## INTRODUCTION

The thalamus is an important deep gray matter structure that transmits sensory information from the peripheral sensory nervous system to the cortex, and serves as a major hub for cognitive processes[1]. Given its 'central roles' in perception and cognition, disturbances of which are major symptoms of schizophrenia (SZ), the thalamus has been focused on in brain imaging studies of SZ. Structural magnetic resonance imaging

(sMRI) studies revealed thalamic surface deformation in patients with first-episode and chronic SZ,[2,3,4] as well as reduced thalamus volume in a cohort of early onset psychosis patients[5]. Moreover, resting state functional magnetic resonance imaging (rs-fMRI) studies have consistently revealed decreased thalamic connectivity with the prefrontal cortex (PFC) in SZ, as well as increased connectivity with motor and somatosensory cortical areas[6]. Furthermore, these aberrant connectivities were also observed in clinical high-risk (CHR) individuals[7,8], suggesting that thalamic dysconnectivity onsets prior to the disease itself.

Previous studies treated the thalamus as a homogeneous structure, averaging blood oxygen level-dependent (BOLD) signals across the entire thalamus. However, this approach may fail to capture disturbances in specific networks, so it is necessary to investigate functional connectivity (FC) between sub-regions of the thalamus and cortex to better understand altered neural circuits in SZ. Several atlases segment the thalamus, including histological-[9,10], structural-[11,12] and functional-based atlases[13]. In this study, we used a functional parcellation atlas to identify nine thalamic sub-regions having strong FC with various cortical functional networks[13]. The atlas allows the topological roles of thalamic nuclei in functional brain networks to be elucidated through graph-theoretic network analysis of rs-fMRI data. Few studies have examined altered FC in thalamic sub-regions in SZ. Three studies reported findings similar to those of investigations using average BOLD signals for the thalamus, i.e., weaker PFC–thalamic network connectivity and stronger motor–thalamic and somatosensory–thalamic network connectivity compared to healthy controls (HCs)[14,15,16]. On the other hand, Gong *et al* (2019)[17] observed loss of connectivity between several thalamic sub-regions and the sensorimotor system, anterior cingulate cortex, and cerebellum in patients with SZ. To the best of our knowledge, no study has examined the FC of thalamic sub-regions in patients with treatment-resistant schizophrenia (TRS).

We hypothesized that the FC of thalamic sub-regions with cortical networks and voxels would differ between TRS patients and HCs. To identify the neural mechanisms

underlying TRS, we investigated FC of thalamic sub-regions with cortical networks and voxels, and the associations of this FC with clinical symptoms.

## MATERIALS AND METHODS

### Participants

This study included 111 subjects (50 patients with TRS and 61 HCs). SZ was diagnosed based on the DSM-IV[18] criteria by a board-certified psychiatrist and psychiatric residents. The exclusion criteria were as follows: alcohol or substance use disorder; intellectual disability (IQ ≤ 70); current or past neurological disease, serious medical illness, or pregnancy; and claustrophobia. Treatment resistance was defined as follows: failure to respond to at least two different antipsychotic medications administered in adequate doses (equivalent to ≥ 600 mg/day of chlorpromazine [CPZ]) for at least 6 wk; and persistence of clinically relevant positive or negative symptoms (at least one positive or negative symptom and a Positive and Negative Syndrome Scale (PANSS) score of ≥ 4)[19]. The second criterion was not applied to patients on clozapine. The severity of symptoms was evaluated within a week of fMRI using the PANSS[20,21]. HCs were recruited *via* advertisements and interviewed using the Structured Clinical Interview for DSM, Non-Patient Edition (SCID-NP).[22] A requirement for study inclusion was no previous or current psychiatric disorders, neurological disorders, or significant medical conditions. Controls having a first-degree relative with a psychiatric disorder were also excluded. All participants were aged between 19 and 60 years, and all were confirmed as right-handed by the Edinburgh Handedness Inventory[23]. They all participated voluntarily and provided written informed consent. The study was approved by the Ethics Committee of Jeonbuk National University Hospital (approval number: CUH 2012-08-001).

### Image acquisition and preprocessing

The rs-fMRI and sMRI data were obtained at the Jeonbuk National University Hospital using a 3T Verio scanner (Magnetom Verio; Siemens, Erlangen, Germany) with a 12-

channel standard quadrature head coil. Three-dimensional T1-weighted images were acquired using a magnetization-prepared rapid gradient echo sequence (repetition time [TR]: 1,900 ms; echo time [TE]: 2.5 ms; flip angle: 9°; field of view [FOV]: 250 mm; image matrix: 256 × 246 mm; voxel size: 1.0 × 1.0 × 1.0 mm³; 176 slices). A 5-minute resting-state scan consisting of 150 contiguous echo-planar imaging functional images (TR: 2,000 ms; TE: 30 ms; flip angle: 90°; FOV: 220 mm; image matrix: 64 × 64 mm; voxel size: 3.4 × 3.4 × 5.0 mm³; 26 slices) was also obtained. During resting-state image acquisition, participants were asked to relax with their eyes closed, but not to sleep. MRI data processing was conducted using the Statistical Parametric Mapping software package, version 12 (SPM12; Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB (MathWorks, Natick, MA, USA). The first three volumes were discarded to adjust for magnetization equilibrium. Functional images were slice-time corrected, realigned to the first image of each series, and co-registered with each participant's structural image. Then, the co-registered functional data were transformed into standard anatomical space through spatial normalization of each T1 image to the Montreal Neurological Institute (MNI) template. Normalized images were smoothed using an 8-mm full-width at half-maximum isotropic Gaussian kernel. The voxels were resampled (2.0 × 2.0 × 2.0 mm). Head motion was considered excessive when framewise displacement (FD) was > 0.5 mm. FD values were computed using the CONN toolbox (version 14f; http://www.nitrc.org/projects/conn). Participants for whom more than 10% of the volumes showed excessive head motion were excluded from the analysis[24]. The component correction (CompCor)[25] function of the CONN toolbox was used to increase the accuracy of grey matter (GM) signals by removing physiological noise, such as heart rate and breathing signals, followed by removal of the components of interest from the global, white matter (WM), and cerebrospinal fluid (CSF) signals. The linear trend was then removed and a band-pass filter (0.008 < f < 0.09 Hz) was applied.

**Functional connectivity analysis**

The functional parcellation atlas[13] was used to segment the thalamus into nine subregions (Figure S1). Nine thalamic subregions showing strong FC with nine cortical functional networks were identified, i.e., the default mode (DM), cingulo-opercular occipital (CO), somatomotor (SM), frontal parietal (FP), lateral occipital (LO), medial occipital (MO), medial temporal (MT), temporal, and superior FP networks. The components of each cortical functional network are described in Supplementary Table S1. For each region of interest (ROI) of the thalamus and cortical functional networks, the BOLD signal was averaged to generate the BOLD time series. FC analysis was performed between thalamic ROIs and cortical functional network ROIs, within the nine thalamic and nine cortical functional network ROIs, and between the thalamic ROIs and all cortical voxels (using the CONN toolbox). The patient and HC groups were compared using one-way analysis of variance (ANOVA). We used a voxel-level height threshold of $p < 0.01$ (uncorrected) and a cluster-level extent threshold of $p < 0.05$, corrected for multiple comparisons using the family wise error (FWE). We performed 10,000 permutation tests using the CONN toolbox (www.nitrc.org/projects/conn, RRID:SCR_009550).

**Statistical analysis**

Demographic and clinical data were compared between the two groups using a two-sample $t$-test or Chi-square test. For partial correlation analysis, a ROI extraction tool (http://software.incf.org/software/rex) in the CONN toolbox was used to extract Fisher's Z-transformed signal intensity values for brain regions with significant group differences at an uncorrected p-value of $< 0.01$. Relationships between the extracted Z-scores and PANSS scores were analyzed using age, sex, and FD as covariates. The analyses were performed using SPSS software (ver. 20.0; SPSS Inc., Chicago, IL, USA).

**RESULTS**

**Functional connectivity between and within thalamic subregions and cortical functional networks in TRS**

There were no significant differences in age, sex, or education level between the two groups (Table 1). The TRS group showed significantly increased FC between thalamic subregion 2 and the MO network (t = 2.78, $p < 0.05$) compared to the HC group. The TRS group also exhibited significantly increased FC of the CO network with the MO (t = 3.29, p < 0.05) and superior MT networks, and between the MT network and superior FP network (t = 2.63, p < 0.05) ($t = 4.31$, $p < 0.05$) compared to the HC group. On the other hand, the TRS group exhibited decreased FC of thalamic subregion 1 with thalamic subregions 2 and 9 ($t = -2.95$, $p < 0.05$) and of thalamic subregion 2 with thalamic subregions 3 and 4 ($t = -4.58$, $p < 0.05$), compared to the HC group. Also, in the TRS group, decreased FC of the FP network was observed with the MT ($t = -2.69$, $p < 0.05$) and superior FP networks ($t = -2.73$, $p < 0.05$), between the DM and CO networks ($t = -2.90$, $p < 0.05$), and between the MO and MT networks ($t = -2.90$, $p < 0.05$), compared to the HC group (Table 2, Figure 1).

**Functional connectivity between thalamic subregions and cortical voxels in TRS**

Compared to the HC group, the TRS group exhibited significantly increased FC between thalamic subregion 1 and the left lingual gyrus ($t = 5.22$, $p < 0.05$), thalamic subregion 2 and the left precentral gyrus ($t = 5.22$, $p < 0.05$), thalamic subregion 3 and the right supplementary motor cortex ($t = 5.26$, $p < 0.05$), thalamic subregion 6 and the frontal medial cortex ($t = 7.05$, $p < 0.05$), the left postcentral gyrus ($t = 5.26$, $p < 0.05$) and right precentral gyrus ($t = 4.79$, $p < 0.05$), and thalamic subregion 9 and the left precentral gyrus ($t = 5.26$, $p < 0.05$). On the other hand, the TRS group exhibited significantly decreased FC between thalamic subregion 3 and the left intracalcarine cortex ($t = -4.18$, $p < 0.05$) compared to the HC group (Table 3, Figure 2).

**Correlations between altered ROI-to-ROI functional connectivity and PANSS scores**

The Z-values of FP and MT network connectivity were negatively correlated with positive symptoms, negative symptoms, general pathophysiology, and PANSS total scores ($r = -0.411$, $p = 0.005$; $r = -0.414$, $p = 0.004$; $r = -0.427$, $p = 0.003$; and $r = -0.472$, $p =$

0.001, respectively) in the TRS group. Also, negative correlations were observed between the Z-value of the DM and MT networks and negative symptoms, general pathophysiology, and the PANSS total score ($r = -0.316$, $p = 0.032$; $r = -0.322$, $p = 0.029$; and $r = -0.298$, $p = 0.044$, respectively) (Table 4, Figure 3).

**Correlations between altered seed-to-voxel functional connectivity and PANSS scores**

No significant relationship was found between altered FC and PANSS scores at an uncorrected p-value of < 0.01 (Supplementary Table S2). Therefore, the analysis was performed against an uncorrected p-value of < 0.05 (Supplementary Table S3). In the TRS group, the Z-value of thalamic subregion 3 and the right lingual gyrus FC was negatively correlated with positive symptoms, negative symptoms, general pathophysiology, and the PANSS total score ($r = -0.342$, $p = 0.020$; $r = -0.355$, $p = 0.015$; $r = -0.350$, $p = 0.017$; and $r = -0.396$, $p = 0.007$, respectively). However, there was a positive correlation between the Z-value of thalamic subregion 2 and the left precentral gyrus and the score for general pathophysiology ($r = 0.292$, $p = 0.049$) (Table 5, Figure 4).

**DISCUSSION**

The thalamus represents the interface between the sensory and motor systems, and is a major hub for cognitive processes. A large body of evidence has demonstrated involvement of the thalamus in the pathophysiology of SZ. Most previous studies employing rs-fMRI used the whole thalamus as a seed region to identify abnormalities in thalamic connectivity. To identify more specific disturbances, we conducted FC analysis of thalamic subregions with cortical networks and voxels in patients with TRS. Important novel findings regarding the pathophysiology of TRS were obtained, and are discussed below.

The network analysis revealed significant FC only between thalamic subregion 2 and the MO network. By mapping the coordinates of 333 cortical ROIs, identified using the Gordon atlas[26], to the masks of cortical functional networks[13], we were able to identify

subcomponents of the MO network, including the superior frontal [13] gyrus, superior parietal gyrus, inferior parietal gyrus, precentral gyrus, postcentral gyrus, supplementary motor area, insula, precuneus, Rolandic operculum, and paracentral lobule. As the MO network consists of many different regions, it is difficult to determine which of them have the most clinical importance. However, thalamic subregion 2 has a high participation coefficient (PC), indicating that it serves as a connector hub[13]; as such, altered functioning of thalamic subregion 2 may mediate cortical-to-cortical communication in patients with TRS. The other significant FC results were related to connectivity between thalamic subregions and cortical functional networks. Among these, decreased FC between several thalamic subregions in TRS patients was of particular interest, because previous studies mainly focused on the thalamocortical or corticothalamic pathway. In line with our results, Gong *et al* (2019)[17] reported deceased within-thalamic FC in an SZ group compared to controls. Furthermore, a decreased thalamic volume in chronic SZ patients was seen[27,28,29] as well as reduced regional glucose metabolism in the medial dorsal nucleus and posterior thalamus of antipsychotic-naïve patients with SZ[30]. It may be that reduced intrathalamic connectivity mediates the key role of the thalamus in perception, motor function, and cognitive integration[1], in turn contributing to the development of pathophysiology in TRS. More commonly described abnormalities in TRS include hyperconnectivity and hypoconnectivity between cortical functional networks. In this study, each cortical functional network consisted of many heterogeneous areas, and the results were significant even when using the BOLD time series for large cortical areas. Therefore, these findings may be considered as functional biomarkers for TRS. However, our expectation that the role of the thalamus as a connector hub would be disrupted in TRS was not confirmed.

With respect to thalamic connectivity with cortical voxels, we identified significant hyperconnectivity of five thalamic subregions with various cortical regions in patients with TRS compared to HCs. Interestingly, most of these involved increased connectivity to the precentral and postcentral gyri and supplementary motor cortex. Considering the

role of these areas in integrating sensorimotor information and coordinating physical movements[31,32], these findings may be relevant to the sensory and motor abnormalities found in TRS, such as hallucinations and neurological soft signs. Many studies have reported increased functional coupling between the thalamus and sensorimotor cortices in SZ[33,34,35,36,37]. However, this is the first study to report hyperconnectivity between thalamic subregions and the sensorimotor cortex in TRS. We also found increased connectivity to the frontal medial cortex and lingual gyrus. This is in contrast to previous research showing a reduction in FC of the thalamus with the prefrontal and cingulate cortices in SZ, based on signals averaged across the entire thalamus[33,34,36,38,39,40,41] as well as separate signals for individual thalamic subregions[15,16,17]. However, the subjects in previous studies were not patients with TRS. The medial PFC has been shown to play a fundamental role in a wide range of social cognitive abilities, such as self-reflection, person perception, and theory of mind/mentalizing[42]. Also, the lingual gyrus has been implicated in visual memory[43] and divergent thinking[44]. Therefore, increased thalamo–frontal or thalamo–lingual connectivity might serve as a marker for TRS. The potential associations of FC with social cognition and divergent thinking warrant further investigation. Finally, decreased connectivity of thalamic subregion 3 with the intracalcarine cortex was observed in our TRS patients. A similar result was reported in chronic SZ[17]. Given that the calcarine cortex is known to be involved in the processing of visual mental imagery[45], an fMRI study using a visual mental imagery task could be useful for exploring the pathophysiological mechanisms of TRS.

With regard to our analyses of altered ROI-to-ROI FCs, FC between the FP and MT networks, and between the DM and MT networks, showed negative relationships with positive symptoms, negative symptoms, general pathophysiology, and PANSS total scores. These findings suggest that altered connectivity between intracortical functional networks is associated with overall pathophysiology rather than specific symptom domains. This is intuitive considering that each cortical functional network consists of many heterogenous cortical regions. Regarding our analyses of cortical voxels, FC

between thalamic subregion 3 and the right lingual gyrus showed negative relationships with positive symptoms, negative symptoms, general pathophysiology, and PANSS total scores. Even after considering the role of the lingual gyrus in visual memory and divergent thinking, it is difficult to determine how this altered FC is associated with overall pathophysiology. However, it may be that impairment of thalamic subregion 3, in terms of its role as a connector hub, has significant effects on the integration of cortical information. Finally, the positive association of FC between thalamic subregion 2 and the precentral gyrus with general pathophysiology accords with the findings of prior studies[17,33]. Even though patients with SZ often show abnormal involuntary movements or neurological soft signs[46] related to the function of the precentral gyrus, it is difficult to identify relationships between motor functions and general pathophysiology.

## CONCLUSION

Several limitations of this study need to be considered. First, although the use of a functional parcellation atlas to segment the thalamus into subregions was a strength of this study, the roles and precise locations of thalamic subregions are still largely unknown, making it difficult to determine which specific regions had the largest effect on the results. Second, we did not recruit all subtypes of TRS patients, as recommended by the Treatment Response and Resistance in Psychosis Working Group[19]. The proportions of positive, negative, and positive and negative subtypes were 22%, 14%, and 32%, respectively. The remaining 32% of patients did not meet the criteria for the positive or negative subtype, because we applied a rating of moderate severity to just one symptom item. This should be addressed in future studies. Third, the TRS patients were all heavily medicated, so it is unclear whether the significant changes in FC reported above were a consequence of the disease process or medication. In this context, it will be important to determine the relative importance of illness duration, the number of psychotic episodes, and medication. Despite these weaknesses, this is the first report on altered FC of the thalamocortical pathway in TRS using thalamic

subregions as seeds. In summary, in our TRS patients, we found altered FC between various thalamic subregions, between various cortical functional networks, and between thalamic subregions and various cortical regions. These abnormalities were associated with overall pathophysiology. Collectively, these results suggest that disrupted FC within thalamic and cortical functional networks, and within the thalamocortical pathway, could serve as markers for TRS. This study improves our understanding of the relationships between the thalamocortical pathway and symptoms of TRS.

## ARTICLE HIGHLIGHTS

### Research background

The thalamus is an important deep gray matter structure that transmits sensory information from the peripheral sensory nervous system to the cortex, and serves as a major hub for cognitive processes. Previous studies treated the thalamus as a homogeneous structure, averaging blood oxygen level-dependent (BOLD) signals across the entire thalamus. However, this approach may fail to capture disturbances in specific networks, so it is necessary to investigate functional connectivity (FC) between sub-regions of the thalamus and cortex to better understand altered neural circuits in SZ (Schizophrenia).

### Research motivation

To the best of our knowledge, no study has examined the FC of thalamic sub-regions in patients with treatment-resistant schizophrenia (TRS).

### Research objectives

We hypothesized that the FC of thalamic sub-regions with cortical networks and voxels would differ between TRS patients and HCs (Healthy Controls). To identify the neural mechanisms underlying TRS.

*Research methods*

This study included 111 subjects (50 patients with TRS and 61 HCs). The rs-fMRI and sMRI data were obtained at the Jeonbuk National University Hospital using a 3T Verio scanner (Magnetom Verio; Siemens, Erlangen, Germany) with a 12-channel standard quadrature head coil. The functional parcellation atlas was used to segment the thalamus into nine subregions. FC analysis was performed between thalamic ROIs and cortical functional network ROIs, within the nine thalamic and nine cortical functional network ROIs, and between the thalamic ROIs and all cortical voxels. Demographic and clinical data were compared between the two groups using a two-sample $t$-test or Chi-square test. For partial correlation analysis. Relationships between the extracted Z-scores and PANSS scores were analyzed using age, sex, and FD as covariates.

*Research results*

There were no significant differences in age, sex, or education level between the two groups. We found differences in FC within thalamic subregions and cortical functional networks between patients with TRS and HCs. In addition, increased FC was observed between thalamic subregions and the sensorimotor cortex, frontal medial cortex, and lingual gyrus. These abnormalities were associated with the pathophysiology of TRS.

*Research conclusions*

The thalamus represents the interface between the sensory and motor systems, and is a major hub for cognitive processes. A large body of evidence has demonstrated the involvement of the thalamus in the pathophysiology of SZ. we found altered FC between various thalamic subregions, between various cortical functional networks, and between thalamic subregions and various cortical regions. These abnormalities were associated with overall pathophysiology. Collectively, these results suggest that disrupted FC within thalamic and cortical functional networks, and within the thalamocortical pathway, could serve as markers for TRS.

*Research perspectives*

This study improves our understanding of the relationships between the thalamocortical pathway and symptoms of TRS.

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