Resting-state hyper- and hypo-connectivity in early schizophrenia: which tip of the iceberg should we focus on?

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Abstract

In this study, we explore the intricate landscape of brain connectivity in the early stages of schizophrenia, focusing on the patterns of hyper- and hypoconnectivity. Despite existing literature's support for altered functional connectivity (FC) in schizophrenia, inconsistencies and controversies persist regarding specific dysconnections.

Leveraging a large sample of 100 first-episode schizophrenia patients (42 females/58 males) and 90 healthy controls (50 females/40 males), we compare the functional connectivity (FC) across 90 brain regions of the Automated Anatomical Labeling atlas. We inspected the effects of medication and examined the association between FC changes and duration of illness as well as symptom severity of the disorder. Our approach also includes a comparative analysis of two denoising strategies for functional magnetic resonance imaging data.

In the group of patients, 247 region pairs exhibited greater FC, while 134 region pairs showed reduced FC compared to healthy controls. We found a significant correlation between patients' FC and symptom severity, and antipsychotic medication. However, when a more moderate denoising scheme was used, the results became significantly skewed towards hypoconnectivity in patients. Moreover, there was no relationship between FC and medication or symptom severity in the case of the moderate denoising scheme.

Altogether, we found an overall balanced picture of both hyper- and hypoconnectivity in patients with schizophrenia compared to the healthy controls and unraveled a link between FC in patients and the severity of symptoms and medication. Notably, the balanced picture of FC gets significantly disrupted when less stringent data denoising is applied.

1 Introduction

Although a neurodevelopmental hypothesis for schizophrenia complemented by neurodegenerative processes following the onset of psychosis is now well established, it is not clear how these processes are linked to the brain dysfunction underlying specific schizophrenia psychopathology [1]. The converging evidence from brain imaging studies indicates that these processes lead to disordered brain inter-regional functional connectivity (FC), and dysconnection represents the major candidate for the pathophysiological substrate of psychotic symptoms [2]. Concretely, the dysconnection concept suggests that the specific symptoms of schizophrenia can be described in terms of reduced or increased functional coupling between distinct brain regions [3, 4]. This concept is in line with earlier intuition referring to the title of the disease (i.e., "fragmented mind"), and it has been conceptualized in a neurobiological framework proposing a disruption of the anatomical and functional connectivity between brain areas

as the neurobiological correlate of altered information processing [3]. To quantify such connectivity disruption, the concept of functional connectivity formalized as temporal covariation of neural signals between spatially disparate brain areas [5] is often used.

The functional dysconnection in schizophrenia has been reported to affect connections of a multitude of brain regions, such as the frontal lobe, including language areas [6–9], sensory-motor cortex [10], temporal and limbic structures [11, 12], and thalamus [13–17]. Despite the fact that neuroimaging studies strongly support the role of altered FC in schizophrenia, these reported findings are highly inconsistent; specific regions associated with disconnection in schizophrenia still remain controversial, and a robust conclusion has not yet been obtained. Some of the probable reasons for these inconclusive results are the heterogeneity of clinical samples regarding the stage of illness [18] and low sample sizes based on up to 40 participants on average [19]. Indeed, a small sample size has been previously systematically documented to give rise to heterogeneous localized findings of white matter abnormalities in first-episode psychosis [20]. We further conjecture that variability in the data preprocessing and analysis approaches might also contribute to the variability of the observed results [21]. The majority of previous rs-fMRI studies in schizophrenia reported hypoconnectivity between brain regions [19, 22], but hyperconnectivity has also been repeatedly observed in various disease stages [18, 19, 23, 24]. Longitudinal studies of FEP subjects reported (partial) normalization in terms of increased fMRI activation after antipsychotic treatment [23]. Another factor responsible for the heterogeneity of the findings is the methodological approaches focused on FC between a priori preselected regions, as contrasted with a whole-brain analysis based on the parcellation according to suitable anatomical or functional brain atlases. Given these principal differences, the reported findings are strongly influenced by the a priori choice of the regions of interest, which in some cases may not include all the pathophysiologically relevant areas. On the other hand, whole-brain studies may suffer from the fact that the correction for multiple comparisons could bring false negative results, specifically in the case of underpowered studies. Our study aimed to remediate some of the above-mentioned inconclusiveness and to utilize the whole-brain FC analysis on a large cohort of 100 first-episode schizophrenia (FES) patients, ensuring high homogeneity in terms of chronicity and exposure to medication. The connectivity was calculated between all 90 brain regions comprising the Automated Anatomical Labeling (AAL) template image [25] and compared with a group of 90 healthy controls. On the basis of the previous evidence, we hypothesized that schizophrenia would show decreased connectivity between the candidate brain regions. Furthermore, we tested whether FC changes were moderated by the effect of medication and whether they were linked to clinical factors such as positive and negative symptoms of schizophrenia. In addition, we

evaluated two different denoising strategies of the fMRI data, as this step potentially affects the resulting FC measures [21].

2 Methods

2.1 Study overview and samples

In total, 190 subjects participated in the study: 100 FES patients (mean age=28.75, SD=6.83, 42 females/58 males) and 90 healthy volunteers serving as controls (mean age=27.81, SD=6.82, 50 females/40 males). There were no significant differences between the patient and control samples in age and sex. In the patient group, at the time of the MRI scan, the average duration of untreated psychosis was 3.23 months (SD = 4.82), and the average duration of antipsychotic treatment was 2.29 months (SD = 4.58). The study design was approved by the local Ethics Committee of the Institute of Clinical and Experimental Medicine and the Psychiatric Center Prague. All subjects provided written informed consent after receiving a complete description of the study.

The FES patients were diagnosed according to ICD-10 criteria and structured MINI International Neuropsychiatric Interview [26]. FES subjects were investigated during their first hospitalization in the Prague psychiatric hospitals with a catchment area of 1 million inhabitants. Patients were considered as FEP if they fulfilled these criteria: a) first hospitalization for schizophrenia, and b) clinical interview identified first psychotic and/or prodromal symptoms of psychosis not earlier than 24 months ago (mean=5.90 months, SD=6.16).

The resting fMRI was performed at the initial stage of second-generation antipsychotic therapy (mean 10 weeks of medication at the time of rsfMRI). The mean dose of chlorpromazine equivalents [27] was 381.7 mg (SD=231.8) per day. Psychometrics included the Positive and Negative Symptom Scale (PANSS) [28]. Ninety healthy control subjects (HC) were recruited via a local advertisement; they had a similar socio-demographic background as the FES to whom they were matched by age and sex.

The HC had a slightly higher number of years of education than the FEP (15.64, SD=3.34 and 13.48, SD=2.28, t=4.466, $p \leq 0.001$). HC were evaluated with MINI and were excluded if they had a lifetime history of any psychiatric disorder or a family history of psychotic disorders. Other exclusion criteria for both groups included a history of seizures or significant head trauma, mental retardation, a history of substance dependence, and any MRI contraindications. The protocol was approved by the institutional review boards of the National Institute on Mental Health, Klecany. Written informed consent was obtained from all participants.

2.2 fMRI data acquisition

Scanning was performed with a 3T MRI scanner (Siemens Magnetom Trio) located at the Institute of Clinical and Experimental Medicine in Prague, Czech Republic. Functional images were obtained using T2-weighted echo-planar imaging (EPI) with blood oxygenation level-dependent (BOLD) contrast using SENSE imaging. GE-EPIs (TR/TE=2000/30 ms, flip angle=70°) consisted of 35 axial slices acquired continuously in sequential decreasing order covering the entire cerebrum (voxel size=3×3×3 mm, slice dimensions 48×64 voxels). The next 400 functional volumes were used for the analysis. A three-dimensional high-resolution MPRAGE T1-weighted image (TR/TE=2300/4.63 ms, flip angle 10°, voxel size=1×1×1 mm) covering the entire brain was acquired at the beginning of the scanning session and used for anatomical reference.

2.3 Data preprocessing, brain parcellation, and FC analysis

Functional MRI is a neuroimaging method that is based on measuring blood oxygen level-dependent (BOLD) signal [29]. One of the typical features of the fMRI data is the noise that is present in the raw BOLD signal [30, 31], which significantly limits the reliability of functional connectivity measures [32]. Typical artifacts, such as subject movements, arterial pulsation, respiration, and also the hardware of the MRI scanner itself, induce non-neural temporal correlations in the BOLD [33], and relatively sophisticated data preprocessing is warranted to maximize the level to which the functional connectivity estimates reflect the underlying neuronal dynamics.

The rsfMRI data were corrected for head movement and registered to MNI standard stereotactic space (Montreal Neurological Institute, MNI) with a voxel size of 2×2×2 mm using the CONN toolbox (www.nitrc.org/projects/conn) [38]. This was followed by the segmentation of the anatomical images to create subject-specific white matter (WM) and cerebrospinal fluid (CSF) masks used further. The resulting anatomical images and masks were spatially normalized to a standard stereotaxic MNI space with a voxel size of 2×2×2 mm.

The denoising steps included the regression of six head-motion parameters with their first-order temporal derivatives and five principal components of WM and CSF signals using a component-based noise correction method (CompCor) implemented in the CONN toolbox [34]. Time series from defined regions of interest were additionally linearly detrended and finally filtered by a band-pass filter with cutoff frequencies 0.004 - 0.1 Hz. We shall refer to this preprocessing setup as the stringent denoising scheme.

As an alternative denoising pipeline, closer to the practice in some studies [36, 37], we used a more moderate denoising scheme including six head-motion parameters without

their first-order derivatives and only the mean time series of WM and CSF. This pipeline was performed without explicit linear detrending. However, time series were also finally filtered by a band-pass filter with cutoff frequencies 0.004 - 0.1 Hz.

2.4 Analysis

The whole analysis was carried out using the CONN toolbox (www.nitrc.org/projects/conn) [38]. For each subject, to quantify the whole-brain pattern of functional connectivity, we performed an ROI-to-ROI connectivity analysis by computing the Pearson's correlation matrix among the regional mean time series extracted from 90 brain regions (excluding the cerebellar regions) of the Automated Anatomical Labeling (AAL) atlas [25]. The resulting connectivity matrices were represented as Fisher's z-transformed Pearson's correlation coefficients.

We used a two-sample Student's t-test to evaluate differences between groups. Linear regression was used to determine the relation between the four subscales of the PANSS scale [28], antipsychotic medication at the time of the MRI scan (measured by chlorpromazine equivalent), duration of illness, and functional connectivity in patients. To increase the statistical analysis power, these analyses were limited to ROI pairs that showed a significant effect of disease (i.e., effect in the initial between-group comparison). The resulting p-values from each analysis were corrected for multiple testing by controlling the False Discovery Rate (FDR) [39] as implemented in the CONN toolbox. Additional analysis with the moderate denoising scheme was performed using the identical pipeline.

3 Results

3.1 Difference between healthy controls and schizophrenia patients

In the group comparison using the default, *stringent*, denoising scheme, a difference was observed in a multitude of ROI pairs. 247 ROI pairs had significantly greater functional connectivity (FDR-corrected $p<0.05$) in patients, while healthy volunteers exhibited significantly greater (FDR-corrected $p<0.05$) functional connectivity in 134 ROI-pairs compared to patients (Figure 1). Despite the numerical prevalence of observed hyperconnections in patients, healthy volunteers and patients did not show a difference in the average functional connectivity (mean FC in patients=0.1315, SD=0.1758, mean FC in controls=0.1318, SD=0.1687, t=0.0484, p=0.9615).

The most increased links in patients included a range of connections involving the left and right thalamus, left and right globus pallidus, left middle cingulate gyrus, and right parietal inferior gyrus. The strongest hyperconnectivity was observed in links that involved the bilateral thalamic regions. In the opposite direction, the most decreased links in patients involved the left fusiform gyrus, left parahippocampal gyrus, and right Heschl's gyrus. See Figure 2 for a graphical overview of the group comparison of healthy controls and patients, including its stability with respect to additional control for inter-subject variability in the amount of head motion; see Discussion for details.

3.2 Association between symptom severity, medication, duration of illness, and functional connectivity

We have observed a significant correlation between symptom severity measured by PANSS and functional connectivity, see Figure 3. To increase the power by focusing on the most relevant candidate connections, we used a masking matrix of p-values from a group comparison of healthy volunteers and patients to distinguish regions with significantly greater functional connectivity in patients. PANSS total scale significantly correlated with the functional connectivity of 17 ROI pairs. The strongest correlations were observed in the right parietal inferior gyrus and left thalamus. PANSS general correlated with FC of 2 ROI pairs (both included in the above-mentioned 17).

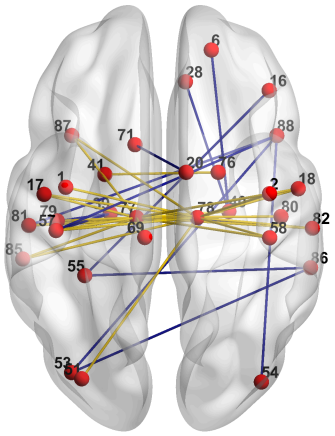

Figure 1: Difference between HC and SCH. The most significant changes in FC; higher FC in healthy controls = blue, higher FC in patients = yellow. For visualization purposes, only the top 10% of the most significant results of each analysis ($p<0.05$, FDR corrected) are shown.

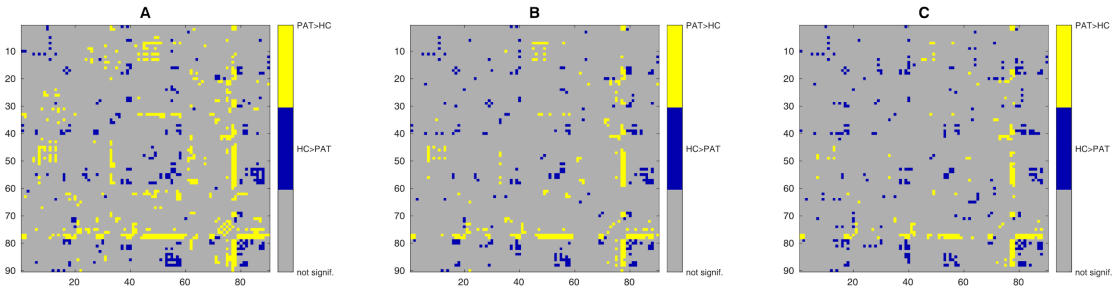

Figure 2: Difference between HC and SCH. The significant differences after the FDR<0.05 correction are shown in yellow (higher FC in patients) and blue (higher FC in healthy controls). A) Default analysis; B) Analysis including additional inter-subject correction for amount of motion; C) analysis including additional inter-subject correction for amount of motion as well as rejecting outliers. See the Discussion section for details of the additional analysis shown in the C and D panels.

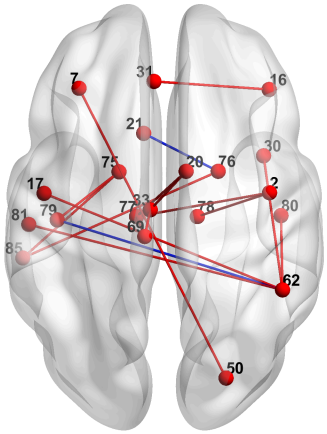

Figure 3: Association between symptom severity and functional connectivity. PANSS total scale significantly correlated with the functional connectivity of 17 ROI pairs (red + blue links), and PANSS general scale significantly correlated with the functional connectivity of two ROI pairs (blue links). Results with p<0.05, FDR corrected, are shown.

These were the functional connectivity between the left olfactory cortex (AAL region 21) and the right globus pallidus (AAL region 76) and between the left Heschl's gyrus (AAL region 79) and the right inferior parietal lobule (AAL region 62).

Further, we have observed a significant correlation between antipsychotic medication and functional connectivity. The strongest correlations were observed in the right parietal

inferior gyrus and left thalamus in the right inferior frontal gyrus (triangular part), right parietal inferior gyrus, and left anterior cingulum (see Table 1).

We have also observed a significant correlation between antipsychotic medication and symptom severity measured by PANSS ($r = 0.33$, $p < 0.05$).

Conversely, we found no relationship between the duration of illness and functional connectivity.

Region 1	Region 2	Correlation Significance
Frontal_Inf_Tri_R	Frontal_Mid_Orb_R	0.010
Frontal_Inf_Tri_R	Cingulum_Ant_R	0.016
Frontal_Inf_Tri_R	Cingulum_Post_R	0.016
Frontal_Mid_R	Amygdala_R	0.016
Parietal_Inf_R	Heschl_L	0.016
Parietal_Inf_R	Heschl_R	0.016
Olfactory_L	Caudate_R	0.022
Cingulum_Ant_L	Temporal_Mid_L	0.022
Parietal_Inf_R	Temporal_Sup_L	0.027
Frontal_Inf_Tri_R	Cingulum_Ant_L	0.038
Frontal_Inf_Orb_R	Cingulum_Ant_L	0.038
Parietal_Inf_R	Temporal_Sup_R	0.045

Table 1: Correlation between antipsychotic medication and functional connectivity; note only $p<0.05$ FDR-corrected are reported.

3.3 Effect of data-denoising on functional connectivity

To evaluate the stability of the above-presented findings with respect to the strictness of denoising, we also performed our analysis with moderate denoising settings. Healthy volunteers exhibited significantly higher (FDR-corrected $p<0.05$) functional connectivity in 1862 ROI-pairs compared to patients. Conversely, 13 ROI pairs had significantly higher (FDR-corrected $p<0.05$) functional connectivity in patients, see Figure 5. In stark contrast to the stringent denoising scheme, the average functional connectivity was different between both groups for the moderate denoising scheme. The average connectivity difference between healthy controls and patients in the moderate scheme was 0.0616 (mean FC in patients=0.2719, SD=0.2261, mean FC in controls=0.3335, SD=0.2280, $t=3.5428$, $p=0.0005$). Indeed, the disease effect on functional connectivity indices was clearly biased towards hypoconnectivity in the moderately denoised dataset, while there was a relatively balanced hypoconnectivity and hyperconnectivity observed when using the default stringent denoising scheme, see Figure 4 Right.

Despite these vast differences, the disease effect observed with the two denoising schemes were strongly correlated (r=0.74, p<0.001), see Figure 4 Left for the scatterplot across region pairs.

We observed no significant relation between functional connectivity and PANSS scales, antipsychotic medication or the duration of illness.

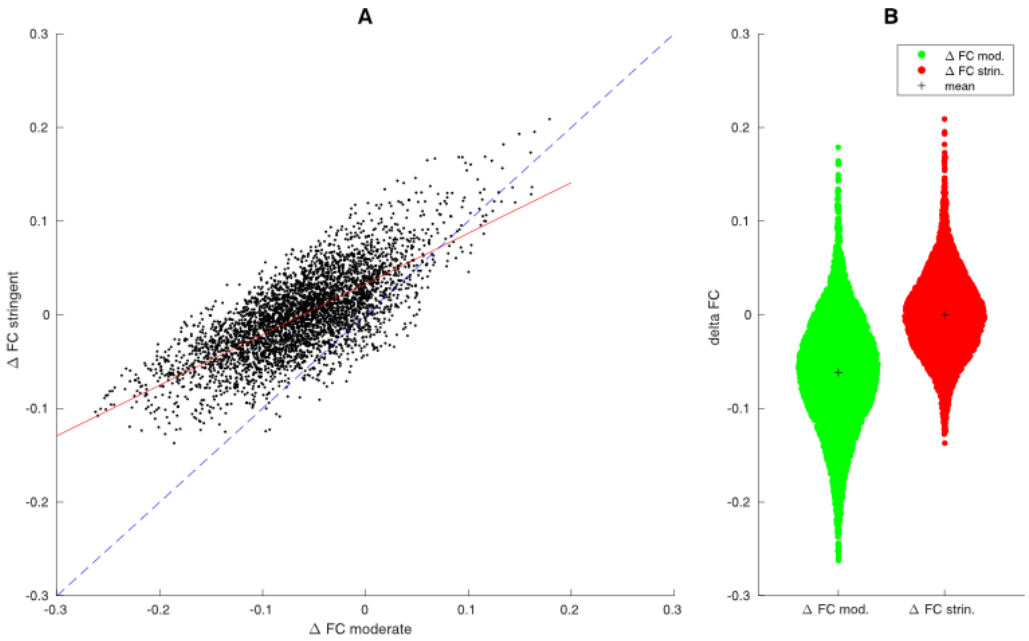

Figure 4: Comparison of the effect of disease on functional connectivity observed with the stringent and moderate denoising. A) Scatterplot of the mean FC difference between patients and healthy controls in the stringent and moderate denoising scheme. Blue dashed lines mark identity, red line corresponds to the best linear fit. Each dot corresponds to one pair of regions, showing the difference in their means in the two denoising schemes. B) histogram of the FC differences between healthy controls and patients in the stringent and moderate denoising schemes.

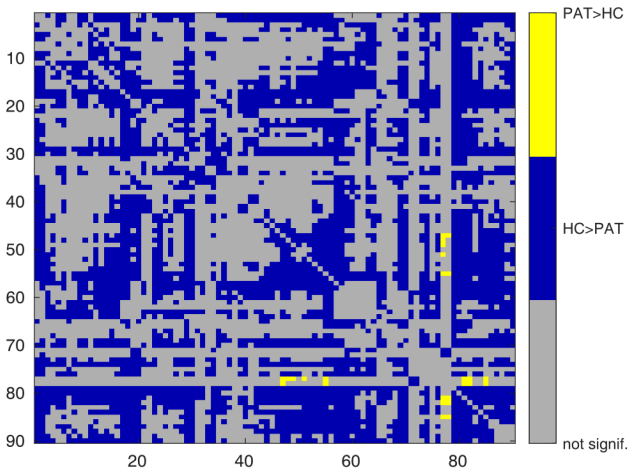

Figure 5: Difference between HC and SCH for the moderate denoising variant. The differences significant after the FDR<0.05 correction are shown in yellow (higher FC in patients) and blue (higher FC in healthy controls).

4 Discussion

Using the stringent denoising scheme, we observed prevailing brain hyperconnectivity, which correlated with both symptom severity as well as antipsychotic medication in subjects at their first episode of psychosis. The most increased links in patients involved the bilateral thalamus, globus pallidus, left middle cingulate gyrus, and right parietal inferior gyrus. The most decreased links in patients involved the left fusiform gyrus, left parahippocampal gyrus, and right Heschl's gyrus. There was a considerable difference between the stringent and moderate denoising schemes in the number of significant ROI pairs in each group comparison. The moderate denoising scheme resulted in a higher number of ROI pairs that showed significantly higher FC in healthy controls compared to patients, while the stringent denoising scheme balanced the proportions of significant FC between the two groups. Note that the use of a substantially stricter denoising scheme than used in a range of studies in the literature may explain much of the deviation of our results with respect to the field. Despite the differences between denoising strategies, mean connectivity matrices are strongly correlated within each group (for healthy controls, the correlation of FC in moderate denoising and FC in the stringent denoising was 0.9402; for the patient group, it was 0.9533; both $p<0.001$). Also, the difference between the groups, i.e., the disease effect, is strongly correlated (across region pairs) between the denoising strategies ($r=0.74$, $p<0.001$, see Fig 4 left).

The localization of the most prominent disease effects (the tips of the iceberg), are generally in line with previous findings. which however reported mostly the hypoconnectivity, or hypoactivation in task-activation studies [19, 23, 24]. In terms of hypoconnectivity, two recent meta-analyses provide overall context. Dong et al. [19], observed hypoconnectivity within various brain networks such as the default network, affective network, ventral attention network, thalamus network, and somatosensory network. Hyperconnectivity was only observed between the affective network and the ventral attention network. Li et al. [22] observed significant hypoconnectivities in schizophrenia patients between the seed regions and the areas in the auditory network (left insula), core network (right superior temporal cortex), default mode network (right medial prefrontal cortex, and left precuneus and anterior cingulate cortices), self-referential network (right superior temporal cortex), and somatomotor network (right precentral gyrus).

In terms of hyperconnectivity, Avram et al. [40] found increased intrinsic FC between thalamic nuclei and primary-sensorimotor cortices. The increased thalamic FC to somatosensory was observed in both early stages [16] and chronic patients [13, 17]. Ikuta et al. [41] implemented cognitive control paradigms and reported hyperactivation in the basal ganglia and thalamus. Our findings of hyperconnectivity in FEP dominantly in the thalamus (secondary in basal ganglia) correspond with the above-mentioned studies. Our data show increased thalamic connectivity to the lateral temporal cortex (including operculum and Heschl gyrus), parahippocampal, fusiform and lingual gyri, the somatosensory cortex (postcentral gyrus), and occipital cortex. Li et al. [18] described an increase of thalamo-temporal and thalamo-sensorimotor connectivity towards the later stages of schizophrenia, where thalamic changes became prominent.

At least two factors could potentially contribute to observed connectivity changes: the neurodevelopmental process, which at this state manifests as psychosis, and the effect of antipsychotic medication. Interestingly, a review of longitudinal FEP fMRI studies [23] revealed a pattern of predominantly hypoactivation in several brain areas at baseline that may normalize to a certain extent after antipsychotic treatment. Chopra et al. recently revealed an association of antipsychotic exposure with increased FC from the thalamus to the rest of the brain [42], in line with previous longitudinal studies reporting FC normalization after antipsychotic exposure [23], particularly within fronto-striatal-thalamic circuits [43– 45] and corticolimbic [44, 46, 47] and corticocortical systems [24, 48]. Our findings of antipsychotic dosage association with increased FC in the parietal inferior gyrus, thalamus, and inferior frontal gyrus correspond with the previous studies. We observed a significant correlation between symptom severity measured by PANSS and functional connectivity, with the strongest correlations in the

right parietal inferior gyrus and left thalamus. Similar findings were reported by Anticevic et al. [13]: an association between sensorimotor cortico-thalamic hyperconnectivity and general psychopathology but no association with decreased FC of PFC-thalamic pathways [13]. Due to the correlation between PANSS and chlorpromazine equivalent (medication might reflect observed symptom levels) it is challenging to statistically distinguish their respective contributions to hyperconnectivity.

Other regions with significantly higher FC in FEP compared to HC in our study were the pallidum and left middle cingulate gyrus (part of the ventral attention network). This is partially in line with the meta-analysis of [19], which described hyperconnectivity in schizophrenia between the affective network and the ventral attention network. Hyperconnectivity of the auditory-sensorimotor network covering primary sensorimotor cortices with the thalamus, striatum, and pallidum was described by [40].

For other functional connections, we observed a decrease in FEP compared to HC, especially in the parahippocampal gyrus, transverse temporal gyrus (Heschl's gyrus), and the fusiform gyrus. The former is in accordance with the meta-analysis of Li et al. [22]. The transverse temporal gyrus findings are also consistent with previous fMRI studies in which individuals with schizophrenia exhibited abnormal connectivity involving the superior temporal plane [22], which may underlie the vulnerability to psychopathology [54–57]. Interestingly, the fusiform gyrus (FG) has been previously related to schizophrenia symptoms [59], albeit more commonly discussed in relation to the social impairments seen in autistic spectrum disorders (ASD) [58]. Interestingly, we did not find any decrease in thalamic connectivity, in contrast with the majority of previous studies documenting the pattern of thalamic under-connectivity with PFC and over-connectivity with somatosensory cortices [13, 16, 17, 60–64]. As some of these studies found decreased thalamo-PFC connectivity in early-stage patients [16] or even in high-risk individuals [24], it is unlikely that the absence of thalamo-PFC connectivity decrease in our sample is caused by us using the FEP sample. We can speculate that this discrepancy could be caused by the "normalization" of connectivity due to medication or by the methodological differences in parcellation (previous studies typically used either a voxel-based approach [13] or computed thalamic FC for the entire PFC together with a few larger ROIs only [16].

It is worth mentioning some technical considerations of the present study. We decided to exclude slice-timing correction since some studies suggest that this step has a negligible effect on resting-state data [65], and spatial smoothing: region-based analysis introduces noise suppression by spatial averaging without artificially introducing correlations between regions [66]. Several denoising strategies were discussed in recent papers. While a unique consensus has not been reached in the field, the implementation of

a more stringent denoising scheme has become a standard practice [67, 68], motivating our default choice. To quantify functional connectivity, in line with common practice, we use Pearson's linear correlation coefficient, as it was shown to be sufficient under standard conditions as with the current dataset [69]. A possibly fruitful further directions would indeed lie in extension to mapping the consequences or analogies of our findings to other network characteristics previously shown to be altered in clinical high risk and early-illness schizophrenia individuals, in particular to dynamic functional connectivity, [70], and amplitude of low frequency fluctuations or graph-theoretical functional dysconnectivity characterization [71].

While the groups were matched for age, sex, as well as head-motion, we also carried out a control analysis where these three variables were included as covariates of no interest. The results again remained qualitatively equivalent, suggesting that the effect of these variables on FC is relatively small compared to the effect of disease; see Figure 2 Middle. The results remained largely unchanged after the additional removal of the 23 subjects who showed maximum head motion larger than 1.5×IQR (interquartile range), the Tukey criterion for detection of outliers [72]; see Figure 2 Right. The difference in the number of ROI pairs with significantly changed FC could be due to lower degrees of freedom in the additional corrected analyses.

In conclusion, our study using a whole-brain functional connectivity approach on a relatively large sample has shown a balanced picture of hyperconnectivity and hypoconnectivity in first-episode psychosis while demonstrating a significant relation of the affected connection strengths to clinical symptoms and cumulative dosage of antipsychotic medication. Importantly, we have shown that this balanced picture gets substantially skewed in the direction of dominantly observed hypo- or dysconnectivity when applying more moderate data-denoising schemes, while the relation to symptoms and medication is rendered insignificant. While the results of both approaches are in overall qualitative agreement, including with localization of most commonly reported disease connectivity effects in the literature, taking into account the results obtained with the current stringent denoising may provide a stronger relation to clinical variables and contribute to the understanding of the diversity of previously reported results, as well as the interpretation of schizophrenia as a dysconnection disease in general.

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FS, JH, and JHl are responsible for conceptualization; DT and JHl are responsible for methodology; DT is responsible for software; DT a JHl performed the validation; DT, JH, and JHl are responsible for the formal analysis; DT, MK, BRB, FS, JH, and JHl are responsible for investigation; JT, FS, and JHl provided the resources; DT, JT, FS, JH, and JHl are responsible for data curation; DT, MK, BRB, JH, and JHl wrote the original draft; DT, MK, BRB, JT, FS, JH, and JHl reviewed and edited the manuscript; DT is responsible for the visualization; JT, FS, JH, and JHl performed the supervision; JT, FS, and JHl administered the project; FS, JH and JHl are responsible for funding acquisition.

References

[1] M. R. Dauvermann, G. Lee, and N. Dawson, "Glutamatergic regulation of cognition and functional brain connectivity: Insights from pharmacological, genetic and translational schizophrenia research," *British Journal of Pharmacology*, 2017.

[2] K. Friston, "Dysfunctional connectivity in schizophrenia," *World Psychiatry*, vol. 1, pp. 66–71, 2002.

[3] K. Friston and C. Frith, "Schizophrenia: A disconnection syndrome?" *Clin Neurosci*, vol. 3, pp. 89–97, 1995.

[4] K. Stephan, T. Baldeweg, and K. Friston, "Synaptic plasticity and dysconnection in schizophrenia," *Biological Psychiatry*, vol. 59, no. 10, pp. 929–939, 2006. doi: 10.1016/j.biopsych.2005.10.005.

[5] K. J. Friston, C. D. Frith, P. F. Liddle, and R. S. Frackowiak, "Functional connectivity: The principal-component analysis of large (pet) data sets," *Journal of Cerebral Blood Flow & Metabolism*, vol. 13, no. 1, pp. 5–14, 1993.

[6] B. Backasch, I. Sommer, F. Klohn-Saghatolislam, M. J. Muller, T. T. Kircher, and D. T. Leube, "Dysconnectivity of the inferior frontal gyrus: Implications for an impaired self-other distinction in patients with schizophrenia," *Psychiatry Research*, vol. 223, no. 3, pp. 202–209, 2014.

[7] S. Benetti, A. Mechelli, M. Picchioni, M. Broome, S. Williams, and P. McGuire, "Functional integration between the posterior hippocampus and prefrontal cortex is impaired in both first episode schizophrenia and the at risk mental state," *Brain*, vol. 132, no. 9, pp. 2426–2436, 2009.

[8] M. W. Cole, A. Anticevic, G. Repovs, and D. Barch, "Variable global dysconnectivity and individual differences in schizophrenia," *Biological Psychiatry*, vol. 70, no. 1, pp. 43–50, 2011.

[9] D. Weinberger, M. Aloia, T. Goldberg, and K. Berman, "The frontal lobes and schizophrenia," *Journal of Neuropsychiatry and Clinical Neurosciences*, vol. 6, no. 4, pp. 419–427, 1994. doi: 10.1176/jnp.6.4.419.

[10] T. Kaufmann *et al.*, "Disintegration of sensorimotor brain networks in schizophrenia," *Schizophrenia Bulletin*, vol. 41, no. 5, pp. 1326–1335, 2015. doi: 10.1093/ schbul/sbv074.

[11] N. A. Crossley *et al.*, "Superior temporal lobe dysfunction and frontotemporal dysconnectivity in subjects at risk of psychosis and in first-episode psychosis," *Human Brain Mapping*, vol. 30, no. 12, pp. 4129–4137, 2009.

[12] A. Meyer-Lindenberg *et al.*, "Regionally specific disturbance of dorsolateral prefrontal-hippocampal functional connectivity in schizophrenia," *Archives of General Psychiatry*, vol. 62, no. 4, pp. 379–386, 2005. doi: 10.1001/archpsyc. 62.4.379.

[13] A. Anticevic *et al.*, "Characterizing thalamo-cortical disturbances in schizophrenia and bipolar illness," *Cerebral Cortex*, vol. 24, no. 12, pp. 3116–3130, 2014.

[14] M. Giraldo-Chica and N. Woodward, "Review of thalamocortical resting-state fmri studies in schizophrenia," *Schizophrenia Research*, vol. 180, pp. 58–63, 2017. doi: 10.1016/j.schres.2016.10.017.

[15] Y. Guller *et al.*, "Probing thalamic integrity in schizophrenia using concurrent transcranial magnetic stimulation and functional magnetic resonance imaging," *Archives of General Psychiatry*, vol. 69, no. 7, pp. 662–671, 2012. doi: 10.1001/ archgenpsychiatry.2011.1522.

[16] N. Woodward and S. Heckers, "Mapping thalamocortical functional connectivity in chronic and early stages of psychotic disorders," *Biological Psychiatry*, vol. 79, no. 12, pp. 1016–1025, 2016. doi: 10.1016/j.biopsych.2015.05.024.

[17] N. Woodward, H. Karbasforoushan, and S. Heckers, "Thalamocortical dysconnectivity in schizophrenia," *American Journal of Psychiatry*, vol. 169, no. 10, pp. 1092–1099, 2012. doi: 10.1176/appi.ajp.2012.11091447.

[18] T. Li *et al.*, "Brain-wide analysis of functional connectivity in first-episode and chronic stages of schizophrenia," *Schizophrenia Bulletin*, vol. 43, no. 2, pp. 436– 448, 2017. doi: 10.1093/schbul/sbw075.

[19] D. Dong, Y. Wang, X. Chang, C. Luo, and D. Yao, “Dysfunction of large-scale brain networks in schizophrenia: A meta-analysis of resting-state functional connectivity,” *Schizophrenia bulletin*, vol. 44, no. 1, pp. 168–181, 2018.

[20] T. Melicher *et al.*, “White matter changes in first episode psychosis and their relation to the size of sample studied: A dti study,” *Schizophrenia Research*, vol. 162, no. 1-3, pp. 22–28, 2015. doi: 10.1016/j.schres.2014.11.007.

[21] G. C. Burgess *et al.*, “Evaluation of denoising strategies to address motioncorrelated artifacts in resting-state functional magnetic resonance imaging data from the human connectome project,” *Brain Connectivity*, vol. 6, no. 9, pp. 669– 680, 2016.

[22] S. Li, N. Hu, and W. Zhang, “Dysconnectivity of multiple brain networks in schizophrenia: A meta-analysis of resting-state functional connectivity,” *Frontiers in Psychiatry*, vol. 10, p. 482, 2019. doi: 10.3389/fpsyt.2019.00482.

[23] C. Gonza´lez-Vivas, P. Soldevila-Mat´ıas, and O. Sparano, “Longitudinal studies of functional magnetic resonance imaging in first-episode psychosis: A systematic review,” *European Psychiatry*, vol. 59, pp. 60–69, 2019. doi: 10.1016/j. eurpsy.2019.04.009.

[24] A. Anticevic *et al.*, “Association of thalamic dysconnectivity and conversion to psychosis in youth and young adults at elevated clinical risk,” *JAMA Psychiatry*, vol. 72, no. 9, pp. 882–891, 2015.

[25] N. Tzourio-Mazoyer *et al.*, “Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the mni MRI single-subject brain,” *NeuroImage*, vol. 15, no. 1, pp. 273–289, 2002. doi: 10.1006/nimg. 2001.0978. [Online]. Available: https://doi.org/10.1006/nimg.2001.0978.

[26] D. Sheehan *et al.*, “The mini-international neuropsychiatric interview (m.i.n.i.): The development and validation of a structured diagnostic psychiatric interview for dsm-iv and icd-10,” *Journal of Clinical Psychiatry*, vol. 59, no. 20, pp. 22–33, 1998, Suppl. doi: 10.1016/j.biotechadv.2011.08.021.Secreted.

[27] S. Woods, “Chlorpromazine equivalent doses for the newer atypical antipsychotics,” *Journal of Clinical Psychiatry*, vol. 64, no. 6, pp. 663–667, 2003. doi: 10.4088/jcp.v64n0607.

[28] S. R. Kay, A. Fiszbein, and L. A. Opler, “The positive and negative syndrome scale (panss) for schizophrenia,” *Schizophrenia Bulletin*, vol. 13, no. 2, pp. 261– 276, 1987.

[29] S. Ulmer and O. Jansen, *FMRI*. Berlin: Springer, 2013.

[30] B. B. Biswal, F. Z. Yetkin, V. M. Haughton, and J. S. Hyde, "Functional connectivity in the motor cortex of resting human brain using echo-planar mri," *Magnetic Resonance in Medicine*, vol. 34, no. 4, pp. 537–541, 1995.

[31] M. Raichle, "The restless brain: How intrinsic activity organizes brain function," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 370, no. 1668, p. 20140172, 2015.

[32] W. Shirer, H. Jiang, C. Price, B. Ng, and M. Greicius, "Optimization of rs-fmri pre-processing for enhanced signal-noise separation, test-retest reliability, and group discrimination," *NeuroImage*, vol. 117, pp. 67–79, 2015.

[33] S. Pamilo, S. Malinen, J. Hotta, and M. Sepp¨a, "A correlation-based method for extracting subject-specific components and artifacts from group-fmri data," *European Journal of Neuroscience*, vol. 42, no. 9, pp. 2726–2741, 2015.

[34] Y. Behzadi, K. Restom, J. Liau, and T. T. Liu, "A component based noise correction method (compcor) for bold and perfusion based fmri," *NeuroImage*, vol. 37, no. 1, pp. 90–101, 2007.

[35] X. Chai, A. N. Castañón, D. Ongür, and S. Whitfield-Gabrieli, "Anticorrelations in resting state networks without global signal regression," *NeuroImage*, vol. 59, no. 2, pp. 1420–1428, 2012.

[36] M. Argyelan *et al.*, "Resting-state fmri connectivity impairment in schizophrenia and bipolar disorder," *Schizophrenia Bulletin*, vol. 40, no. 1, pp. 100–110, 2013.

[37] H. Wang, C.-H. Rau, Y.-C. Li, Y.-J. Chen, and R.-Y. Yu, "Disrupted thalamic resting-state functional networks in schizophrenia," *Frontiers in Behavioral Neuroscience*, vol. 9, 2015.

[38] S. Whitfield-Gabrieli and A. Nieto-Castanon, "Conn: A functional connectivity toolbox for correlated and anticorrelated brain networks," *Brain Connectivity*, vol. 2, no. 3, pp. 125–141, 2012.

[39] Y. Benjamini and Y. Hochberg, "Controlling the false discovery rate: A practical and powerful approach to multiple testing," *Journal of the Royal Statistical Society: Series B (Methodological)*, vol. 57, no. 1, pp. 289–300, 1995. doi: 10. 1111/j.2517-6161.1995.tb02031.x.

[40] M. Avram, F. Brandl, J. Bäuml, and C. Sorg, "Cortico-thalamic hypo-and hyperconnectivity extend consistently to basal ganglia in schizophrenia," *Neuropsychopharmacology*, vol. 43, no. 11, pp. 2239–2248, 2018.

[41] T. Ikuta *et al.*, "Subcortical modulation of attentional control by second-generation antipsychotics in first-episode psychosis," *Psychiatry Research: Neuroimaging*, vol. 221, no. 2, pp. 127–134, 2014.

[42] S. Chopra *et al.*, "Functional connectivity in antipsychotic-treated and antipsychoticnaive patients with first-episode psychosis and low risk of self-harm or aggression: A secondary analysis of a randomized clinical trial," *JAMA psychiatry*, vol. 78, no. 9, pp. 994–1004, 2021.

[43] X. Duan, M. Hu, and X. Huang, "Effects of risperidone monotherapy on the default-mode network in antipsychotic-na¨ıve first-episode schizophrenia: Posteromedial cortex heterogeneity and relationship with the symptom improvements," *Schizophrenia Research*, vol. 218, pp. 201–208, 2020. doi: 10.1016/j. schres.2020.01.001.

[44] D. Sarpal, D. Robinson, and T. Lencz, "Antipsychotic treatment and functional connectivity of the striatum in first-episode schizophrenia," *JAMA Psychiatry*, vol. 72, no. 1, pp. 5–13, 2015. doi: 10.1001/jamapsychiatry.2014.1734.

[45] M. Hu, X. Zong, and J. Zheng, "Short-term effects of risperidone monotherapy on spontaneous brain activity in first-episode treatment-na¨ıve schizophrenia patients: A longitudinal fmri study," *Scientific Reports*, vol. 6, p. 34287, 2016. doi: 10.1038/srep34287.

[46] D. Berge, S. Carmona, P. Salgado, M. Rovira, A. Bulbena, and O. Vilarroya, "Limbic activity in antipsychotic na¨ıve first-episode psychotic subjects during facial emotion discrimination," *European Archives of Psychiatry and Clinical Neuroscience*, vol. 264, no. 4, pp. 271–283, 2014. doi: 10.1007/s00406-0130465-5.

[47] M. Reske, T. Kellermann, and U. Habel, "Stability of emotional dysfunctions? a long-term fmri study in first-episode schizophrenia," *Journal of Psychiatric Research*, vol. 41, no. 11, pp. 918–927, 2007. doi: 10.1016/j.jpsychires. 2007.02.009.

[48] F. Li, S. Lui, and L. Yao, "Longitudinal changes in resting-state cerebral activity in patients with first-episode schizophrenia: A 1-year follow-up functional mr imaging study," *Radiology*, vol. 279, no. 3, pp. 867–875, 2016. doi: 10.1148/ radiol.2015151334.

[49] C. Abbott, A. Jaramillo, and C. Wilcox, "Antipsychotic drug effects in schizophrenia: A review of longitudinal fmri investigations and neural interpretations," *Current Medicinal Chemistry*, vol. 20, pp. 428–437, 2013. doi: 10.1016/j. biotechadv.2011.08.021.Secreted.

[50] H. R. Powell *et al.*, "Noninvasive in vivo demonstration of the connections of the human parahippocampal gyrus," *Neuroimage*, vol. 22, no. 2, pp. 740–747, 2004.

[51] A. J. Blood, R. J. Zatorre, P. Bermudez, and A. C. Evans, “Emotional responses to pleasant and unpleasant music correlate with activity in paralimbic brain regions,” *Nature neuroscience*, vol. 2, no. 4, pp. 382–387, 1999.

[52] L. R. Squire and S. Zola-Morgan, “The medial temporal lobe memory system,” *Science*, vol. 253, no. 5026, pp. 1380–1386, 1991.

[53] R. A. Epstein, “Parahippocampal and retrosplenial contributions to human spatial navigation,” *Trends in cognitive sciences*, vol. 12, no. 10, pp. 388–396, 2008.

[54] V. Oertel-Knöchel, C. Knöchel, and S. Matura, “Association between symptoms of psychosis and reduced functional connectivity of auditory cortex,” *Schizophrenia Research*, vol. 160, pp. 35–42, 2014. doi: 10.1016/j.schres. 2014.10.036.

[55] A. Shinn, J. Baker, and B. Cohen, “Functional connectivity of left heschl’s gyrus in vulnerability to auditory hallucinations in schizophrenia,” *Schizophrenia Research*, vol. 143, pp. 260–268, 2013. doi: 10.1016/j.schres.2012.11.037.

[56] T. Takahashi, D. Sasabayashi, and Y. Takayanagi, “Increased heschl’s gyrus duplication in schizophrenia spectrum disorders: A cross-sectional mri study,” *Journal of Personalized Medicine*, vol. 11, no. 1, p. 40, 2021. doi: 10.3390/ jpm11010040.

[57] M. Gavrilescu *et al.*, “Reduced connectivity of the auditory cortex in patients with auditory hallucinations: A resting state functional magnetic resonance imaging study,” *Psychological Medicine*, vol. 40, no. 7, pp. 1149–1158, 2010.

[58] N. Uppal and P. R. Hof, “A stereologic perspective on autism neuropathology,” *Neurostereology: Unbiased Stereology of Neural Systems*, pp. 237–256, 2014.

[59] K. Kunzelmann *et al.*, “Am i hallucinating or is my fusiform cortex activated? functional activation differences in schizophrenia patients with and without hallucinations,” *The European Journal of Psychiatry*, vol. 33, no. 1, pp. 1–7, 2019.

[60] G. Atluri, M. Steinbach, K. O. Lim, V. Kumar, A. MacDonald, *et al.*, “Connectivity cluster analysis for discovering discriminative subnetworks in schizophrenia,” *Human Brain Mapping*, vol. 36, no. 2, pp. 756–767, 2015.

[61] W. Cheng *et al.*, “Voxel-based, brain-wide association study of aberrant functional connectivity in schizophrenia implicates thalamocortical circuitry,” *NPJ Schizophrenia*, vol. 1, p. 15016, 2015. doi: 10.1038/npjschz.2015.16.

[62] C. Klingner *et al.*, “Thalamocortical connectivity during resting state in schizophrenia,” *European Archives of Psychiatry and Clinical Neuroscience*, vol. 264, no. 2, pp. 111–119, 2014. doi: 10.1007/s00406-013-0445-9.

[63] D. Lerman-Sinkoff and D. Barch, “Network community structure alterations in adult schizophrenia: Identification and localization of alterations,” *NeuroImage: Clinical*, vol. 10, pp. 96–106, 2016. doi: 10.1016/j.nicl.2015.11.010.

[64] P. Tu, Y. Lee, Y. Chen, J. Hsu, C. Li, and T. Su, “Network-specific corticothalamic dysconnection in schizophrenia revealed by intrinsic functional connectivity analyses,” *Schizophrenia Research*, vol. 166, pp. 137–143, 2015. doi: 10.1016/j.schres.2015.05.022.

[65] C. Wu, C.-P. Chen, P.-H. Liu, Y.-P. Chao, B. Biswal, and C.-P. Lin, “Empirical evaluations of slice-timing, smoothing, and normalization effects in seed-based, resting-state functional magnetic resonance imaging analyses,” *Brain Connectivity*, vol. 1, no. 5, pp. 401–410, 2011.

[66] N. K. Aurich, J. P. Alves Filho, A. M. Marques da Silva, and A. R. Franco, “Evaluating the reliability of different preprocessing steps to estimate graph theoretical measures in resting state fmri data,” *Frontiers in Neuroscience*, vol. 9, 2015.

[67] J. D. Power, A. Mitra, T. O. Laumann, A. Z. Snyder, B. L. Schlaggar, and S. E. Petersen, “Methods to detect, characterize, and remove motion artifact in resting state fmri,” *NeuroImage*, vol. 84, pp. 320–341, 2014.

[68] A. Patel *et al.*, “A wavelet method for modeling and despiking motion artifacts from resting-state fmri time series,” *NeuroImage*, vol. 95, pp. 287–304, 2014.

[69] J. Hlinka, M. Palus, M. Vejmelka, D. Mantini, and M. Corbetta, “Functional connectivity in resting-state fmri: Is linear correlation sufficient?” *Neuroimage*, vol. 54, no. 3, pp. 2218–2225, 2011.

[70] E. Mennigen, S. L. Fryer, B. Rashid, E. Damaraju, Y. Du, R. L. Loewy, B. K. Stuart, V. D. Calhoun, and D. H. Mathalon, "Transient patterns of functional dysconnectivity in clinical high risk and early illness schizophrenia individuals compared with healthy controls," *Brain Connectivity*, vol. 9, no. 1, pp. 60–76, 2019.

[71] Y. Tang, Q. Zhou, M. Chang, A. Chekroud, R. Gueorguieva, X. Jiang, Y. Zhou, G. He, M. Rowland, D. Wang, S. Fu, Z. Yin, H. Leng, S. Wei, K. Xu, F. Wang, J. H. Krystal, and N. R. Driesen, "Altered functional connectivity and low-frequency signal fluctuations in early psychosis and genetic high risk," *Schizophrenia Research*, vol. 210, pp. 172–179, 2019.

[72] J. W. Tukey, *Exploratory Data Analysis*. Addison-Wesley Publishing Company, 1977.