Functional network dysconnectivity as a biomarker of treatment resistance in schizophrenia

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Schizophrenia may develop from disruptions in functional connectivity regulated by neurotransmitters such as dopamine and acetylcholine. The modulatory effects of these neurotransmitters might explain how antipsychotics attenuate symptoms of schizophrenia and account for the variable response to antipsychotics observed in clinical practice. Based on the putative mechanisms of antipsychotics and evidence of disrupted connectivity in schizophrenia, we hypothesised that functional network connectivity, as assessed using network-based statistics, would exhibit differences between treatment response subtypes of schizophrenia and healthy controls. Resting-state functional MRI data were obtained from 17 healthy controls as well as individuals with schizophrenia who responded well to first-line atypical antipsychotics (first-line responders; FLR, n=18), had failed at least two trials of antipsychotics but responded to clozapine (treatment-resistant schizophrenia; TRS, n=18), or failed at least two trials of antipsychotics and a trial of clozapine (ultra-treatment-resistant schizophrenia; UTRS, n=16). Data were pre-processed using the Advanced Normalisation Toolkit and BrainWavelet Toolbox. Network connectivity was assessed using the Network-Based Statistics toolbox in Matlab. ANOVA revealed a significant difference in functional connectivity between groups that extended between cerebellar and parietal regions to the frontal cortex ($p<0.05$). Post-hoc T-tests revealed weaker network connectivity in individuals with UTRS compared with healthy controls but no other differences

between groups. Results demonstrated distinct differences in functional connectivity between individuals with UTRS and healthy controls. Future work must determine whether these changes occur prior to the onset of treatment and if they can be used to predict resistance to antipsychotics during first-episode psychosis.

Keywords: schizophrenia, treatment resistance, treatment response, magnetic resonance imaging, network based statistics, clozapine

1. Introduction

Post-mortem and in vivo studies have provided overwhelming evidence of aberrant functional connectivity in schizophrenia (Friston et al., 2016; Kanaan et al., 2005; Karbasforoushan and Woodward, 2012; Lynall et al., 2010; Menon, 2011; Zhou et al., 2007), supporting a role for dysconnection in the aetiology of the disorder (Stephan et al., 2009). Evidence suggests that functional dysconnectivity in schizophrenia could arise from the abnormal regulation of synaptic plasticity (Stephan et al., 2009). In particular, disrupted synaptic plasticity could be attributed to the downstream effects of dopamine, acetylcholine and serotonin on *N*-methyl-D-aspartate (NMDA) receptor-mediated synaptic function (Stephan et al., 2009). NMDA receptors mediate long-term potentiation (LTP) and long-term depression (LTD) via their effects on the functional state and number of α-amino-3-hydroxyl-5-methyl-4-isoxazolepropionoic acid (AMPA) receptors at synaptic junctions (Lau and Zukin, 2007; Montgomery and Madison, 2004; Stephan et al., 2009). Therefore, modulating the activity or transport of NMDA receptors is likely to affect LTP and LTD by inducing downstream changes in brain connectivity (Stephan et al., 2009).

Given the large body of literature identifying disrupted resting-state networks (RSNs) in schizophrenia (Lynall et al., 2010; Menon, 2011), the modulatory effects of these neurotransmitters on synaptic plasticity and overall functional connectivity might explain how antipsychotic drugs (D_2 and 5-HT_{2A} receptor antagonists) attenuate symptoms of the disorder. However, while there is a general consensus that dysconnectivity is a hallmark of schizophrenia, several studies disagree about the nature of dysconnections within specific networks (Yu et al., 2012). Considering the heterogeneous nature of schizophrenia, it is conceivable that the discrepancies in functional dysconnectivity may be attributed to disrupted neurotransmission. If the functional network connectivity and pathophysiology of schizophrenia is different amongst individuals with the disorder, the likelihood of a single antipsychotic agent or class inducing remission in all individuals is improbable. In fact, what we observe is a division of schizophrenia into different response subtypes, with first- and second-generation antipsychotics providing relief for ~70% of individuals (Agid et al., 2011) and clozapine (the gold-standard treatment for those who fail to respond to first-line therapy) providing relief for only 30%-70% of its recipients (Elkis, 2007; Essali et al., 2009; Kane and Correll,

2016; Kane et al., 1988). Farooq and colleagues proposed subtyping schizophrenia according to treatment response, suggesting that division into subgroups, especially within the scope of research and drug development, could help us better understand and thereby treat this often disabling disorder (Farooq et al., 2013; Lee et al., 2015). This concept is supported by work demonstrating differences in dopaminergic and glutamatergic transmission between first-line responders (FLR) and individuals who fail to respond to treatment (Demjaha et al., 2014; Goldstein et al., 2015; Howes et al., 2015).

Network-based statistics provide a useful tool for investigating the functional organisation of the human brain (Zalesky et al., 2010) and have been used to investigate differences between healthy controls and people with schizophrenia. Zalesky et al. reported a sub-network of 40 pairwise functional connections that were significantly weaker in those with schizophrenia when compared with healthy controls (Zalesky et al., 2010). This sub-network comprised fronto-temporal, occipito-temporal, supplementary motor area-temporal and -occipital connections as well as connections within the cingulum (Zalesky et al., 2010), consistent with previously reported abnormalities (Ellison-Wright et al., 2008; Fletcher et al., 1999; Fornito et al., 2009). A study by Cocchi et al. employing the same analytical technique identified three sub-networks with differing connectivity in people with schizophrenia and reported that although structure-function relationships were disrupted in one sub-network (lower correlation between functional connectivity and white matter integrity), the other two sub-networks exhibited no such disruption (Cocchi et al., 2014).

In contrast to more traditional methods for analysing resting-state brain data (such as independent components analysis (ICA)), network-based statistics consider the brain as a network, permitting investigation of the brain as an integrated system, rather than a collection of individual components (Bullmore and Sporns, 2009). By shifting away from low-dimensional ICA and seed-based correlation methods toward high-dimensional analysis, a richer examination of network connections is possible (Smith et al., 2013).

Network organisation is likely to be influenced by disturbances in structural or functional connectivity and may vary between individuals exhibiting different types of disruption. Modulation of NMDA receptor-mediated synaptic plasticity by dopamine, serotonin and acetylcholine is hypothesised to account for the functional dysconnectivity observed in individuals with schizophrenia (Stephan et al., 2009). Should the underlying mechanisms responsible for modulation differ between treatment responders and non-responders, then network connectivity will also be affected to varying degrees. Given the growing body of literature indicating disrupted network connectivity in people with schizophrenia, it was hypothesised that network connectivity, as

assessed using network-based statistics, would exhibit differences between treatment response subtypes of schizophrenia and healthy controls. We anticipated that those who failed to respond to first-line therapy and clozapine monotherapy would exhibit the greatest degree of dysconnectivity; however, disruptions in network organisation in treatment responders and those with treatment resistant schizophrenia (TRS; clozapine responders) were also expected.

2. Methods

2.1 Participants

Details about participant recruitment have been described previously (Anderson et al., 2015). Briefly, individuals with a diagnosis of schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) were recruited from mental health services in Auckland, New Zealand. Participants were enrolled into one of three study arms. Those who were responding well to first-line atypical antipsychotic monotherapy were assigned to the "first-line responder" (FLR) group; response to treatment was assessed by the treating psychiatrist, based on an improvement of positive symptoms and according to standard practice and current treatment guidelines for schizophrenia (Lehman et al., 2004; McGorry, 2005). Those who had failed at least two previous six-to-eight-week trials of atypical antipsychotics and were now receiving clozapine were assigned to the "treatment-resistant" (TRS) group and participants who had failed at least two previous six-to-eight-week trials of atypical antipsychotics and had also failed an adequate trial of clozapine monotherapy (at least 8 weeks post titration (Mouaffak et al., 2006)) were assigned to the "ultra-treatment-resistant" (UTRS) group. The study was approved by the Northern X Regional Ethics Committee and all participants gave informed written consent.

Duration of psychosis, Positive and Negative Syndrome Scale (PANSS) scores (Kay et al., 1987) and past and present substance abuse (evaluated using the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST; *World Health Organisation*) scale) were assessed at study entry. Antipsychotic dose at the time of assessment was converted to chlorpromazine equivalents using formulae with power transformation (Andreasen et al., 2010). In the absence of a power formula, amisulpride chlorpromazine equivalents were calculated using expert consensus regarding antipsychotic dosing (Gardner et al., 2010). Participants also provided a urine sample, which was screened for the presence of amphetamine, methamphetamine, benzodiazepines, cocaine, opiates and tetrahydrocannabinol (Medix Pro-Split Integrated Cup, Multi Drug Screening Test; Sobercheck Ltd). Participant demographics were compared across cohorts using the appropriate statistical tests in IBM SPSS Statistics Version 23.

2.2. Data acquisition

Structural and resting-state fMRI scans were acquired using a Siemens Magnetom Skyra 3T scanner. All but four of the participants were imaged using a 32-channel head coil. Two FLR and two with UTRS were imaged using a 20-channel head coil. T1-weighted images were acquired using a magnetization-prepared 180-degrees radio-frequency pulses and rapid gradient-echo (MPRAGE) sequence (Brant-Zawadzki et al., 1992). Acquisition parameters were as follows: repetition time (TR) 1900 ms; echo time (TE) 2.39 ms; inversion time (TI) 900 ms; flip angle 9°; repetition 1; acceleration factor 2; field of view (FOV) 230 mm; matrix 256 x 256; voxel size 0.9 x 0.9 x 0.8 mm.

Resting-state functional images were acquired over 8 minutes using echo-planar imaging (EPI) with the following parameters: TR 3000 ms, TE 30 ms; echo spacing 0.65 ms (0.62 ms for last 7 participants, following software upgrade); phase-encode direction A>>P; 54 slices; 160 volumes; FOV 192 mm; acceleration factor 2; matrix 64 x 64; voxel size 3.0 x 3.0 x 3.0 mm. Participants were asked to lie still with eyes open and concentrate on a fixation cross. Gradient distortion images for functional data were acquired using a gradient echo pulse sequence with the following parameters: TR 655 ms; TE1 4.92 ms; TE2 7.38 ms; voxel size 3.4 x 3.4 x 2.4 mm; phase-encode direction A>>P; FOV 220 mm.

2.3. Image pre-processing

Structural data were processed with the Advanced Normalization Toolkit (Tustison et al., 2014). Processing steps included initial N4 bias correction of raw structural images; brain extraction using a hybrid segmentation/template-based strategy; construction of a study-specific template and segmentation priors based on all participants in the cohort; alternation between study-specific prior-based segmentation and "pure tissue" posterior probability weighted bias correction using Atropos and N4; DiReCT-based cortical thickness estimation; normalization to a study-specific template and cortical parcellation using the AT116 anatomical parcellation template.

Pre-processing of functional data was conducted using the BrainWavelet Toolbox (www.brainwavelet.org) (Patel et al., 2014). Pre-processing steps have previously been reported (Simas et al., 2015) and included slice time correction; rigid-body head movement correction; affine co-registration to the skull-stripped structural image using a grey matter mask; registration to the MNI152_T1_1mm template in Talairach space (TT_N27); and spatial smoothing (6 mm full width at half maximum). Secondary motion artefacts in the fMRI data were modelled and removed using unsupervised timeseries despiking in the wavelet domain (Patel et al., 2014). Default parameters were used for despiking and were equivalent to those used by Patel et al. (Patel et al., 2014).

Motion-related events across different frequencies were detected as chains of maximal and minimal wavelet coefficients. All coefficients belonging to a maximal or minimal chain were set to zero in the wavelet domain and the timeseries was recomposed. Due to the manner in which this algorithm detects these events, it is able to remove both slower, prolonged motion artefacts (such as spin history type effects) and higher frequency artefacts (such as spikes) (Patel et al., 2014). This method has been shown to out-perform standard despiking algorithms and achieve superior removal of motion artefacts in high-motion cohorts compared to scrubbing and regression-only models (Patel et al., 2014).

Following wavelet despiking, further motion correction was performed using signal regression of the six motion parameters estimated during rigid-body head movement correction, their first order temporal derivatives and the cerebrospinal fluid (CSF) signal. High pass frequency filtering above 0.02 Hz was then performed, followed by spatial smoothing (6 mm full width at half maximum Gaussian kernel) to minimise the influence of border placement during parcellation.

Difference in head motion between groups was assessed using DVARS, the root mean square variance of frame-to-frame difference in percent signal change across all voxels of the brain (Smyser et al., 2010). Mean DVARS were compared between groups using a one-way ANOVA.

Motion-corrected fMRI data were subjected to parcellation and divided into 116 parcels using the AFNI TT N27 EZ ML atlas. For each individual, the mean timeseries was extracted from each of the 116 anatomically parcellated regions (nodes). The extracted signals were decomposed into four frequency bands by wavelet transform (Salvador et al., 2005): scale 1, 0.125–0.25 Hz; scale 2, 0.06–0.125 Hz; scale 3, 0.03–0.06 Hz; scale 4, 0.02–0.03 Hz (Achard et al., 2006). Based on evidence from previous resting-state fMRI studies demonstrating that most salient differences between healthy controls and people with schizophrenia occur at frequencies in the range of 0.06 to 0.125 Hz (Achard et al., 2006; Lynall et al., 2010), the scale 2 wavelet was selected for comparisons in the current study.

The strength of a connection between two nodes was the Pearson's correlation coefficient of the wavelet coefficients. Positively and negatively weighted, undirected correlation matrices were derived and further analysis was undertaken using Matlab 2015a (MathWorks, U.S.A). All self-connections were removed from correlation matrices prior to analysis.

2.4. Network-based statistics

Comparisons of functional network organisation were performed in Matlab 2015a using the Network Based Statistic (NBS) Toolbox (Zalesky et al., 2010). NBS seeks to identify arrangements of node-to-

node connections (structures) formed by links that surpass a given threshold (Zalesky et al., 2010). The topological extent of each structure is then used to determine its significance (Zalesky et al., 2010). Permutation testing (using random assignment of each subject to a group) ascribes a p value (controlled for the family-wise error; FWE) to each structure based on its size (Zalesky et al., 2010). The total number of permutations for which the size of the permuted structure is greater than the size of the actual structure determines the p value for that arrangement of connections (Zalesky et al., 2010). Using NBS, a one-way ANOVA (FWE-corrected $\alpha \leq 0.05$) applying equal weighting to all groups was performed to establish whether there was a difference between the groups. Data were permuted 5000 times using the network-based statistics method, applying a range of test statistic thresholds. The test statistic represents the relative weighting of an edge in a network. The size of the threshold affects the extent (and thereby visualisation) of the network but does not affect the statistical significance of connections within the network (Zalesky et al., 2010). This is illustrated in the visual depiction of network edges shown in Figure 1 (edges shown in light blue will be eliminated when viewed at a higher threshold but still contribute to the significant difference in connectivity between the groups). For optimal visualisation of sub-networks identified in the post-hoc analysis, a threshold of 4.9 was chosen, as it showed a substantial degree of dysconnection while still granting partition of dysconnections into meaningful subnetworks; lower thresholds produced networks that were too dense to enable inference and larger thresholds produced networks that were too sparse. Networks were determined based on their extent (i.e. the number of connections they comprised). Post-hoc T-tests ($\alpha \leq 0.05$, corrected for multiple comparisons using the Bonferroni method) were performed to reveal the directionality of any differences established during the ANOVA (again, the network-based statistic method with 5000 permutations was employed). As only 1-tailed T-tests are permitted by the NBS software, all comparisons were run in both directions and corrected for using the Bonferroni method. Brain networks were visualized with the BrainNet Viewer (http://www.nitrc.org/projects/bnv/) (Xia et al., 2013).

2.5. Influence of antipsychotic dose, symptom severity and drug-use on dysconnectivity

As antipsychotic dose and symptom severity may have influenced dysconnectivity outcomes, associations between chlorpromazine equivalents/PANSS subscale scores and the strength of network connectivity in the ANOVA sub-network were assessed. Healthy controls were not interviewed using the PANSS but were given a contrived score of 30 for the purpose of this analysis. Likewise, all controls were given a chlorpromazine equivalent score of zero. The potential influence of recreational drug-use (measured using the ASSIST) was assessed only for those participants that completed the ASSIST questionnaire.

To assess the effect of each covariate on the strength of connectivity in the ANOVA sub-network, first, the binary sub-network matrix (with ones representing edges included in the sub-network) was linearised and multiplied by the edge strength information contained in each participant's scale 2 wavelet correlation matrix; this produced a matrix containing connection strength information for all edges contained in the sub-network for every participant. As correlation matrices were positively and negatively weighted, the absolute value of connection strength was used for further analysis (to retain weight information without positive and negative values cancelling one another out). The mean absolute connection strength across all edges of the sub-network was then compared between groups, adding chlorpromazine equivalents, PANSS sub-scale scores and ASSIST scores as covariates. Limited degrees of freedom required that each covariate be assessed in a separate analysis of covariance (ANCOVA). Pairwise comparisons of main effects were conducted, using the Bonferroni correction for multiple comparisons.

2.6. Validation of results using alternative parcellation scheme

To ensure that the results obtained with the AFNI 116 parcellation scheme were robust, findings were validated using an alternative parcellation scheme. To ensure full coverage of the cerebrum and cerebellum, brain data was parcellated using a custom-made 1 mm MNI atlas combining parcels from the Human Brainnetome Atlas (Fan et al., 2016) (modified from the original Desikan–Killiany (DK) atlas (Desikan et al., 2006)) and the probabilistic MR atlas of the human cerebellum (MNIflirt-maxprob-thr50-1mm) (Diedrichsen et al., 2009). The final atlas contained 272 parcels; any overlapping voxels were removed from the atlas to prevent bias toward one parcellation scheme or the other. As before, motion-corrected fMRI data from participants were subjected to parcellation and the mean timeseries was extracted from each of the 272 regions. Scale 2 wavelet data were used for further analysis in NBS.

3. Results

3.1. Participant demographics

Data from 17 healthy controls, 18 FLR, 18 individuals with TRS and 16 individuals with UTRS were included in the analysis. Demographic data are presented in Table 1.

3.2. Between-groups comparison of head motion

Mean (standard deviation) DVARS were 11.1 (1.6), 12.7 (2.8), 12.6 (3.2) and 13.1 (4.3) for controls, FLR, those with TRS and those with UTRS, respectively; ANOVA revealed no significant differences in head motion between groups ($F=1.205$; $p=0.315$).

3.3. Network-based statistics

Network organisation across groups was compared using network-based statistics (Zalesky et al., 2010). ANOVA revealed a significant difference in connectivity between groups that extended primarily between cerebellar and parietal regions to the frontal cortex ($p<0.05$; Figure 1). Post-hoc T-tests revealed significantly weaker network connectivity in individuals with UTRS compared to healthy controls ($p<0.05$, Bonferroni corrected) but no differences in connectivity between controls and FLR or those with TRS or between any of the schizophrenia cohorts. Differences observed in the post-hoc T-tests mirrored those identified in the ANOVA.

Dysconnections in those with UTRS divided into three sub-networks, representing cerebellar-frontal dysconnections (Figure 2, sub-network 1; $p<0.012$ corrected), cingulo-frontal-temporal dysconnections (Figure 2, sub-network 2; $p=0.036$ corrected) and fronto-parietal dysconnections (Figure 2, sub-network 3; $p=0.036$ corrected). Mean absolute connection strengths for each sub-network were compared between groups and are illustrated in Figure S1 of the supplementary material. In all cases, healthy controls had the greatest mean connection strength and those with UTRS the weakest connection strength. FLR generally had weaker mean connection strength than those with TRS; however, as mentioned above, no significant differences were observed between these groups.

3.4. Influence of antipsychotic dose and symptom scores on dysconnectivity

Details of the effects of chlorpromazine equivalents, PANSS sub-scores and ASSIST score on the relationship between treatment group and sub-network connection strength are provided in supplementary table S1. Briefly, no effect of any baseline characteristic was observed in the ANCOVA ($p>0.05$ for all covariates). Post-hoc pairwise comparisons revealed some additional effects of treatment group (see supplementary table S1); however these effects should be interpreted with care, given that healthy controls were assigned contrived values for chlorpromazine equivalents and PANSS scores and not all participants completed the ASSIST questionnaire.

3.5. Validation of results using alternative parcellation scheme

To validate findings obtained with the AFNI 116 parcellation scheme, data were analysed using an alternative 272-parcellation method. Network-based statistics revealed a statistically significant

effect between groups (ANOVA: $p<0.05$), attributable to a significant reduction in connectivity in those with UTRS compared with healthy controls (post-hoc T-test: $p<0.05$, Bonferroni corrected). Two sub-networks were identified, which most closely resembled sub-networks 1 and 3 from the original analysis (details provided in supplementary Figure S2).

4. Discussion

Here, we investigated whether disruptions in resting-state functional connectivity are associated with resistance to antipsychotic treatment in people with schizophrenia. Network-based statistics revealed large disruptions in functional connectivity across three sub-networks in those with UTRS compared to healthy controls, but no significant differences between any other groups. A key distinction between this study and previous network-based statistics studies in schizophrenia was the identification of a large sub-network (sub-network 1) consisting primarily of interhemispheric dysconnections between cerebellar and prefrontal nodes. Prior exclusion of cerebellar nodes had prevented identification of any potential cerebellar dysconnections in previous work, though other nodes identified as dysconnected in the current study are in agreement with previous findings (Cocchi et al., 2014; Zalesky et al., 2010).

The identification of a dysconnected cerebellar network in UTRS follows results from voxel-based morphometry analysis in the same cohort of individuals that identified, among other disruptions, a reduction in grey matter density in the left cerebellum of individuals with UTRS in contrast to healthy controls (Anderson et al., 2015). Likewise, regions of sub-network 2, including middle temporal gyri, anterior cingulate gyrus and ventromedial prefrontal cortices, exhibited decreased grey matter density in people with UTRS compared with healthy controls (Anderson et al., 2015). Post-mortem studies indicate that grey matter reductions observed in schizophrenia are attributable to a decrease in the cortical neuropil, comprised of axons, dendrites and pre- and post-synaptic terminals of cortical neurons (Glantz et al., 2006). Functional dysconnections in sub-networks 1 and 2 of those with UTRS may therefore arise from disruptions to synaptic communication at the cellular level. Unlike nodes within the first two sub-networks, regions of sub-network 3 (consisting of prefrontal and medial parietal cortices) were not associated with areas of grey matter loss in those with UTRS (Anderson et al., 2015). Cocchi et al. previously showed that functional dysconnections identified using network-based statistics correlate with decreases in structural integrity in only some cases; functional and structural dysconnections may not always occur concurrently (Cocchi et al., 2014).

Consequently, the underlying pathophysiology of sub-network 3 may differ from that of sub-networks 1 and 2.

This is the first study to employ network-based statistics to identify functional biomarkers of treatment resistance in people with schizophrenia. Although individuals with this disorder have been experiencing variable responses to antipsychotic medication for decades (Kane and Correll, 2016), until recently, few studies had sought to investigate structural, functional or neurochemical differences between FLR, those with TRS and those with UTRS (Gillespie et al., 2017). With regard to functional differences, Molina Rodriguez et al. identified lower perfusion in the thalamus, left basal ganglia and right prefrontal regions in those who developed UTRS (Rodriguez et al., 1996) and found that individuals with high metabolic activity in the dorsolateral prefrontal cortex were more likely to experience improvements in negative symptoms following administration of clozapine (Molina et al., 2003). Although no statistically significant differences in functional connectivity were observed between those with TRS and UTRS in the current study, connection strength across all three sub-networks was lower in those with UTRS compared with TRS and a lack of significance in this case may stem from insufficient statistical power.

More recent research has mainly focused on white matter disruptions in treatment-resistant or clozapine-eligible individuals rather than those with UTRS but may still provide context in which to consider the current findings. Of specific interest to the current study, Reis Marques et al. conducted an investigation in first-episode psychosis to determine whether pre-treatment fractional anisotropy (FA) could distinguish responders from non-responders to a 12 week course of antipsychotics (Reis Marques et al., 2014). They identified lower FA in non-responders compared with responders in several white matter tracts, including the uncinate, stria terminali, superior frontal-occipital tract, CC, internal and external capsule and corona radiata (Reis Marques et al., 2014). Unpublished work from our lab has revealed lower FA in people with TRS compared with healthy controls (and those with UTRS) but no significant differences between healthy controls and FLR or people with UTRS. These findings in combination with those of the current study suggest that the disruptions in functional connectivity observed in those with UTRS are unlikely to be due to abnormalities in white matter structure, despite reports of white matter disruption in those eligible for and responding to clozapine.

This study benefits from a well characterised cohort of participants, demonstrating similar degrees of symptom severity and duration of illness, as well as similar ratios of male to female participants. Statistical comparison of participants who completed the ASSIST questionnaire revealed higher rates of drug-taking behaviour in FLR and those with UTRS compared with healthy controls. However, no

statistically significant differences in positive drug screen on the day of testing and no effect of ASSIST score on connection strength were observed, suggesting that exposure to recreational drugs was unlikely to account for the differences observed between groups in the current study. Similarly, although prescribed antipsychotic dose (measured in chlorpromazine equivalents) was higher in those with UTRS compared with FLR and those with TRS, no effect of chlorpromazine equivalents on network connection strength was observed. The same was true for symptom severity.

A common criticism of fMRI (and particularly connectomics) studies is a lack of reproducibility, both within and between study cohorts (Thirion et al., 2014). To evaluate the robustness of the current findings, data were subjected to two parcellation schemes (116 AFNI parcellation and 272 custom-made parcellation). Results of the second parcellation corroborated those of the first, demonstrating statistically significant dysconnections in the UTRS group compared with healthy controls. Sub-networks were also similar across parcellation schemes. Although the 272 parcellation gave only two sub-networks where the 116 parcellation gave three, regions of the missing sub-network were incorporated into the two that remained.

The study's cross–sectional design imparts a degree of uncertainty about the underlying cause of observed differences. Future work is required to establish whether differences between controls and individuals with UTRS exist at treatment onset. If so, functional connectivity may become a useful predictive biomarker of treatment resistance in schizophrenia.

Results of the current analysis extend on those of earlier studies utilising network-based statistics to reveal functional dysconnectivity in people with schizophrenia (Cocchi et al., 2014; Zalesky et al., 2010). Here we have demonstrated that ultra-treatment-resistance is associated with large disruptions to network connectivity, in particular cerebellar-frontal networks, in people with schizophrenia. Although we did not observe any significant differences between FLR, those with TRS and those with UTRS in the current study, investigation of a larger cohort of participants studied longitudinally may reveal some relationship between degree of dysconnection and treatment resistance.

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