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Functional network dysconnectivity as a biomarker of treatment resistance in schizophrenia

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ABSTRACT

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 Normalization 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.

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

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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 among 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 3 T 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 × 256; voxel size 0.9 × 0.9 × 0.8 mm.

Resting-state functional images were acquired over 8 min 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 × 64; voxel size 3.0 × 3.0 × 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 × 3.4 × 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

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