Schizophrenia Bulletin vol. 43 no. 2 pp. 425–435, 2017
doi:10.1093/schbul/sbw100
Advance Access publication August 17, 2016

White Matter Disruptions in Schizophrenia Are Spatially Widespread and Topologically Converge on Brain Network Hubs

Paul Klauser*[,1–3], Simon T. Baker[2], Vanessa L. Cropley[1], Chad Bousman[1,4], Alex Fornito[1,2], Luca Cocchi[5], Janice M. Fullerton[6,7], Paul Rasser[8–10], Ulrich Schall[8–10], Frans Henskens[11], Patricia T. Michie[8–10,12], Carmel Loughland[13,6], Stanley V. Catts[14], Bryan Mowry[15,16], Thomas W. Weickert[6,10,17,1], Cynthia Shannon Weickert[6,10,17,1], Vaughan Carr[10,17,18], Rhoshel Lenroot[17,6,10], Christos Pantelis[1,4,10,19], and Andrew Zalesky[1]

[1]Melbourne Neuropsychiatry Centre, Department of Psychiatry, The University of Melbourne and Melbourne Health, Carlton South, Victoria, Australia; [2]Brain and Mental Health Laboratory, Monash Institute of Cognitive and Clinical Neurosciences, School of Psychological Sciences and Monash Biomedical Imaging, Monash University, Clayton, Victoria, Australia; [3]Lausanne University Hospital, Department of Psychiatry, Prilly, Switzerland; [4]Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, Victoria, Australia; [5]QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia; [6]Neuroscience Research Australia, Randwick, New South Wales, Australia; [7]School of Medical Sciences, University of New South Wales, Sydney, New South Wales, Australia; [8]Centre for Brain and Mental Health Research, University of Newcastle, Waratah, New South Wales, Australia; [9]Hunter Medical Research Institute, Newcastle, New South Wales, Australia; [10]Schizophrenia Research Institute, Randwick, New South Wales, Australia; [11]School of Electrical Engineering and Computer Science, University of Newcastle, Callaghan, New South Wales, Australia; [12]School of Psychology, University of Newcastle, Callaghan, New South Wales, Australia; [13]Faculty of Health and Medicine, University of Newcastle, Callaghan, New South Wales, Australia; [14]School of Medicine, The University of Queensland, Brisbane, Qeensland, Australia; [15]Queensland Brain Institute, The University of Queensland, Brisbane, Queensland, Australia; [16]Queensland Centre for Mental Health Research, The University of Queensland, Brisbane, Queensland, Australia; [17]School of Psychiatry, University of New South Wales, Sydney, New South Wales, Australia; [18]Department of Psychiatry, Monash University, Clayton, Victoria, Australia; [19]Centre for Neural Engineering, Department of Electrical and Electronic Engineering, University of Melbourne, Parkville, Victoria, Australia

*To whom correspondence should be addressed; Melbourne Neuropsychiatry Centre, Level 3, Alan Gilbert Building, 161 Barry Street, Carlton South 3053, Australia; tel: 61-3-9035-8628, fax: 61-3-9348-0469, e-mail: paul.klauser@unimelb.edu.au

White matter abnormalities associated with schizophrenia have been widely reported, although the consistency of findings across studies is moderate. In this study, neuroimaging was used to investigate white matter pathology and its impact on whole-brain white matter connectivity in one of the largest samples of patients with schizophrenia. Fractional anisotropy (FA) and mean diffusivity (MD) were compared between patients with schizophrenia or schizoaffective disorder (n = 326) and age-matched healthy controls (n = 197). Between-group differences in FA and MD were assessed using voxel-based analysis and permutation testing. Automated whole-brain white matter fiber tracking and the network-based statistic were used to characterize the impact of white matter pathology on the connectome and its rich club. Significant reductions in FA associated with schizophrenia were widespread, encompassing more than 40% (234 ml) of cerebral white matter by volume and involving all cerebral lobes. Significant increases in MD were also widespread and distributed similarly. The corpus callosum, cingulum, and thalamic radiations exhibited the most extensive pathology according to effect size. More than 50% of cortico-cortical and cortico-subcortical white matter fiber bundles comprising the connectome were disrupted in schizophrenia. Connections between hub regions comprising the rich club were disproportionately affected. Pathology did not differ between patients with schizophrenia and schizoaffective disorder and was not mediated by medication. In conclusion, although connectivity between cerebral hubs is most extensively disturbed in schizophrenia, white matter pathology is widespread, affecting all cerebral lobes and the cerebellum, leading to disruptions in the majority of the brain's fiber bundles.

Key words: magnetic resonance imaging/fractional anisotropy/tractography/schizophrenia/white matter/rich club/diffusion tensor imaging/mean diffusivity

Introduction

The underlying pathophysiology of mental illness is intrinsically linked to disturbances of brain connectivity.[1–5] Dysconnectivity hypotheses were formulated early in the study of schizophrenia[6] but have only been intensively studied recently.[7–9] Early neuroimaging evidence of altered fronto-temporal interactions in schizophrenia, as measured

with positron emission tomography, revived interest in dysconnectivity as a central paradigm for the disorder.[10] New pathophysiological theories emerged, integrating brain plasticity mechanisms[11] and neurodevelopmental alterations[12] in cortical but also subcortical and cerebellar structures.[13]

Recent technological advances in magnetic resonance imaging (MRI), particularly diffusion-weighted imaging (DWI), have led to a new wave of studies mapping white matter connectivity disruptions in schizophrenia. Most of this work has examined fractional anisotropy (FA), an established and widely used neuroimaging measure of overall white matter microstructure. Reductions in this composite measure are considered a marker of myelin abnormalities and/or axonal disruption,[14] but can also be caused by differences in fiber organization.[15,16]

While several region-of-interest (ROI) studies have reported schizophrenia-related reductions of FA in the white matter of all 4 cerebral lobes,[17,18] other studies have revealed more heterogeneous findings. This includes reports of decreased FA limited to frontal and/or temporal regions (see ref.[19] for a recent review), increased FA in the arcuate fasciculus,[20,21] and no difference in FA between patients with schizophrenia and controls,[22–24] suggesting that dysconnectivity in schizophrenia could be primarily mediated by synaptic changes in cortical gray matter rather than macroscopic alterations of white matter.[9]

Several explanations for these inconsistencies have been posited, including the presence of confounds such as medication[25] and insufficient statistical power due to inadequate sample sizes,[26,27] which may have obscured the identification of more widespread or diffuse effects.[19] Despite inconsistencies in the literature, a reduction of FA in frontal and temporal areas is a robust finding in systematic reviews[19] and meta-analyses.[28,29]

Studies examining specific white matter fiber bundles, as reconstructed using automated fiber tracking techniques (tractography), have confirmed the presence of white matter disruptions in schizophrenia.[30] These studies have shown widespread structural dysconnectivity between all 4 cerebral lobes,[31] with the most extensive disruptions identified between putative brain hub regions comprising the so-called rich club.[32] Accordingly, the location of these brain hubs coincides with areas of gray matter loss in schizophrenia.[5]

Taken together, while schizophrenia is classically associated with fronto-temporal connectivity disturbances, recent investigations of the human connectome suggest that white matter pathology is more widespread than suggested by seminal studies focusing on specific regions. Moreover, recent evidence suggests that white matter connectivity between hub regions of the cortex is particularly affected. To test these hypotheses, we examined between-group differences in the directional preference of water diffusion in white matter (FA) and the molecular diffusion rate (mean diffusivity [MD][33]) in one of the largest schizophrenia cohorts investigated to date, comprising more than 500 individuals recruited from 5 different sites in Australia.[34] We hypothesized that white matter pathology would be widespread; recapitulating the diversity of aberrant white matter structures reported in previous smaller studies (ie, uncinate, arcuate, superior/inferior longitudinal fasciculi). We also hypothesized that long association and commissural fibers would be disproportionately affected by white matter pathology because they span a larger volume of white matter and they underpin the brain's putative rich club. The main novelty of our study is its exceptional size, the clinically well-characterized nature of our sample, and the combined use of voxel- and network-based analyses, which enabled us to characterize both the widespread spatial extent of white matter pathology in schizophrenia and its disproportionate impact on hub regions.

Materials and Methods

This study was approved by the Melbourne Health Human Research Ethics Committee (Project ID: 2010.250). All participants provided written informed consent for the analysis of their data.

Participants

Participants were recruited from 5 states and territories in Australia under the auspices of the Australian Schizophrenia Research Bank (ASRB). The full description of recruitment strategies and data collection are described elsewhere.[34] Briefly, participants were recruited through medical treatment settings and national multimedia campaigns. Participants were English speaking and aged between 18 and 65 years. Exclusion criteria included any organic brain disorder, history of brain trauma followed by a long period of amnesia (>24 hours), mental retardation (full-scale IQ < 70), movement disorders, current drug or alcohol dependence, and electroconvulsive therapy in the past 6 months. Control subjects were excluded if they had a personal or family history of psychosis or bipolar I disorder. A total of 328 patients with a confirmed diagnosis of schizophrenia ($n = 275$) or schizoaffective disorder ($n = 53$), based on DSM-IV criteria, and 197 healthy controls participated in the present study. Two patients with schizophrenia were later excluded from our analyses due to the presence of artifacts in their imaging data (see below), leaving a total sample of 523. Full demographics from the ASRB dataset can be found in previous papers.[34–37]

Clinical Assessment

Clinical status was assessed using the Diagnostic Interview for Psychosis (DIP)[38] and the Scale for the Assessment of Negative Symptoms (SANS).[39] Current IQ was assessed with the Wechsler Abbreviated Scale of Intelligence.[40] Type of medication used during the past month was self-reported by patients. Regular reviews of clinical assessments as well as inter-rater and intersite reliability tests

were part of the regular procedures conducted to ensure the quality and integrity of the ASRB data.[34]

Image Acquisition

Diffusion-weighted magnetic resonance images were acquired in each participant with a Siemens Avanto 1.5 Tesla system across 5 different sites in Australia (ie, Brisbane, Melbourne, Newcastle, Perth, Sydney). After calibration using an identical Siemens phantom, the exact same MRI system and acquisition sequence was used at each of the 5 sites. A total of 64 unique gradient directions distributed on the half-sphere were acquired using a spin-echo EPI sequence with the following parameters: b value, 1000 s/mm^2; 65 consecutive axial slices of thickness 2.4 mm; 104 × 104 image matrix with an in-plane voxel resolution of 2.4 × 2.4 mm; field of view, 25 × 25 cm; repetition time, 8.4/8.5 seconds; echo time, 88 ms; and flip angle, 90°. Additionally, a separate T2-weighted (ie, b = 0) volume was acquired before acquisition of the diffusion-weighted volumes. A T1-weighted image of brain anatomy using an optimized magnetization-prepared rapid acquisition gradient echo was also acquired as part of the MRI protocol. It is important to note that the same image acquisition protocol was used at all sites and nuisance confounds were included in all statistical analyses to remove any systematic site-related variance in white matter measurements.

Preprocessing

MRtrix 0.2.12 (http://www.nitrc.org/projects/mrtrix/) was used to generate FA and MD maps. FA measures the degree to which water diffusion is oriented toward one particular direction in space and restricted along others. MD measures the average displacement of water by diffusion, with larger values indicating less coherence in white matter microstructure. Diffusion-weighted images were corrected for distortions and head movement using affine registration to the T2-weighted volume (eddy_correct command in FSL 5.0.7, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). Gradient tables were rotated according to the rotations that were applied to correct for head movement, using an in-house script for MATLAB version 2011a (MathWorks). Finally, diffusion tensors were fitted to the DWI using least squares estimation, enabling the computation of an FA and MD image for each participant.

Image Registration. Each FA map was normalized to MNI standard space using a nonlinear registration procedure (sequential use of FLIRT and FNIRT commands with default parameters for registration of FA images as implemented in FSL 5.0.7). For MD, the same warp field generated for FA was used. FA and MD maps were smoothed with a Gaussian kernel of SD = 1 mm. Quality control included careful manual inspection of each FA map for gross abnormalities and/or artifacts. No participant was excluded on the basis of poor quality or corrupted imaging data. Two participants were excluded due to artifacts resulting from registration failure of their FA images.

Diffusion Tractography. For each individual, 1 million streamlines were seeded throughout all of white matter and propagated using a deterministic white matter fiber tracking algorithm that was guided by the principal direction of diffusion inferred from the diffusion tensor. Crossing fiber models were avoided due to the relatively low field strength of the MRI system (1.5 T), which can result in excessive dispersion in the orientation distribution functions associated with higher order models, potentially leading to spurious tracking results.[41] All streamlines were registered to MNI space using standard procedures and parameters in MRtrix 0.2.12. An in-house MATLAB script was used to average FA across all voxels traversed by streamlines linking a pair of regions comprising the 116-node Automated Anatomical Labeling atlas[42] (see supplementary material and methods for details). This was repeated for all possible pairs of regions, yielding a 116 × 116 connectivity matrix for each individual, where each matrix element stored the average FA across the fiber bundle linking a particular pair of regions. Each non-zero matrix element was independently tested for a between-group difference in FA.

Rich Club Delineation. Some brain regions are connected to a remarkably large number of other regions. Moreover, some of these so-called hub regions are also more likely to be interconnected among each other than expected by chance. This densely interconnected group of hub regions is called a rich club. The rich club is thought to facilitate the integration of neural information by providing shorter and faster routes of information transfer between remote brain areas.[43,44]

The rich club was delineated for a binary network comprising connections that were consistently identified across the group of controls.[45] This yielded a group representative network with a connection density of approximately 30% and a maximal node degree of 50. The rich club coefficient was calculated as the connection density of the subnetwork defined by the set of nodes with degrees exceeding k. The degree threshold, k, was systematically varied to test rich club organization at different levels. The rich club coefficient was normalized with respect to an ensemble of 1000 surrogate networks with the same number of nodes, edges, and degree distribution as the empirical data, but which was randomized in all other respects. These networks were generated using the Maslov-Sneppen[46] rewiring procedure, as implemented in the Brain Connectivity Toolbox.[47]

Statistical Analysis

Voxel-Based Analysis. The spatial extent of any between-group differences in FA, or correlation between FA and symptom scores, was independently tested at all white matter

voxels using the general linear model. Age, gender, and site were set as nuisance factors in the model. We restricted statistical testing to white matter by using an inclusive mask (ie, FMRIB58_FA_1mm template from FSL, thresholded at 2500). All results were corrected for Type I errors with Randomise (FSL 5.0.7) using the Threshold-Free Cluster Enhancement method, a nonparametric cluster-size-based procedure.[48,49] A family-wise error corrected *P* value was calculated for each cluster (10 000 permutations). Corrected $P < .05$ values were considered significant.

Connectivity Analysis. The impact of white matter pathology on whole-brain white matter connectivity was tested with the network-based statistic (NBS).[50] The NBS is a procedure for controlling the family-wise error rate across the set of all connections that were tested for a between-group difference in FA (4230 in total). Age, gender, and site were included as nuisance factors in the model. The NBS identified between-group differences at the level of connected subnetworks of fiber bundles. Connected subnetworks were considered significant if their corrected *P* values were <.05 at the whole-network level using a preliminary *t*-statistic threshold of 3 ($P = .001$) and 10 000 permutations.

Results

Demographics

The patient and control groups are compared demographically in table 1. Notably, the patient group comprised a significantly higher proportion of males. Current IQ was significantly lower in the patient group. Demographic comparisons across sites are available in supplementary material.

Table 1. Demographics of Control and Patient Groups

	Controls	Patients	*P* value
	n = 197	*n* = 326	
Age	41 (18–65)	38 (20–65)	.214
Sex (males; females)	99/98	225/101	<.001
WASI	119 (80–138)	104 (58–133)	<.001
Positive symptoms	—	8 (0–18)	—
Negative symptoms	—	25.5 (0–85)	—
Illness duration	—	14 (1–47)	—
Diagnostic (SCZA; SCZP)	—	53; 275	—
Taking typical antipsychotics	—	31 (10%)	—
Taking atypical antipsychotics	—	246 (82%)	—
Taking antidepressants	—	89 (30%)	—

Note: Negative symptoms score, total score from the Scale for the Assessment of Negative Symptoms (data were unavailable for 14 cases); positive symptoms score, sum of lifetime hallucinations and lifetime delusions from the Diagnostic Interview for Psychosis (data were unavailable for 34 cases); SCZA, schizoaffective disorder; SCZP, schizophrenia; WASI, Wechsler Abbreviated Scale of Intelligence. If not otherwise specified, the values represent the median and the range is given in brackets. Age and illness duration are given in years. Medication data were unavailable for 25 cases; percentage was calculated on reported cases (*n* = 301).

White Matter Pathology

Spatial clusters of significantly reduced FA (233 746 voxels or 234 ml by total volume) were found in the patient group (*n* = 326) compared to the control group (*n* = 197). These clusters were widespread, encompassing 44% of white matter and involving all cerebral lobes and the cerebellum (figure 1; supplementary movie S1). No white matter location was found to show significantly increased FA in the patient group. Significant increases in MD were also widespread and distributed similarly. The corpus callosum, cingulum, and thalamic radiations exhibited the most extensive reductions in FA according to effect size, as quantified with the *t*-statistic image (figure 2). Between-group comparisons of FA across sites (supplementary figure S3 and table S1) and analyses involving axial and radial diffusivities (supplementary figure S4) are available in supplementary material.

FA in the patient group was not significantly associated with any lifetime positive symptoms (ie, olfactory, auditory, and visual hallucinations or delusions, as derived from the DIP) or current negative symptoms (ie, SANS). We found no significant group differences in FA between patients with schizophrenia and schizoaffective disorder. The between-group differences in FA were not mediated by antipsychotic medication; namely, FA averaged across all voxels associated with a significant reduction in the patient group showed no difference between medicated (*n* = 259) and unmedicated (*n* = 42) patients.

Connectivity Disruptions

A total of 4230 cortico-cortical and cortico-subcortical white matter fiber bundles were consistently delineated across all individuals. A subnetwork comprising 52% (2190 connections) of these connections was disrupted in the patient group (supplementary figure S1). No white matter fiber bundle was found to show significantly increased connectivity in the patient group. Differences in white matter connectivity between the patient and control groups were tested individually for each of the 5 scanning sites (supplementary figure S3 and table S1).

A connectogram was used to visualize the network of disrupted white matter fiber bundles (figure 3). It can be seen that white matter connectivity is disrupted between virtually all gray matter regions. The most severe disruptions according to effect size, as quantified with the *t*-statistic, were between the frontal lobes, subcortical structures, and the cerebellum. The summary matrix in supplementary figure S2 shows that disruptions were

more extensive in the right hemisphere and that disrupted intrahemispheric fiber bundles were greater in number than interhemispheric disruptions.

The rich club coefficient was plotted as a function of k for the group-representative network and an ensemble of 1000 degree-preserving random networks (figure 4A). It can be seen that the rich club coefficient significantly exceeded that of the random networks over a broad range of rich club levels (degrees), thereby indicating significant rich club organization. The proportion of connections comprising the rich club that were also significantly disrupted in the patient group was plotted as a function of the rich club level (figure 4B). Approximately 77%–90% of hub-to-hub connections comprising the rich club were disrupted in the patient group. To determine whether this proportion was unexpectedly high, 5000 random subnetworks matched in size to the rich club were defined on the group-representative network and the extent to which each of these subnetworks overlapped the disrupted schizophrenia network was quantified. Each random subnetwork comprised the same number of connections as the rich club. The overlap expected due to chance was approximately 73%. Not one of the 5000 random subnetworks comprised more disrupted connections than the rich club (ie, $P = 0$). Hub nodes comprising the rich club included the precuneus, cingulate cortex, superior frontal cortex, and superior parietal cortex (figure 4C).

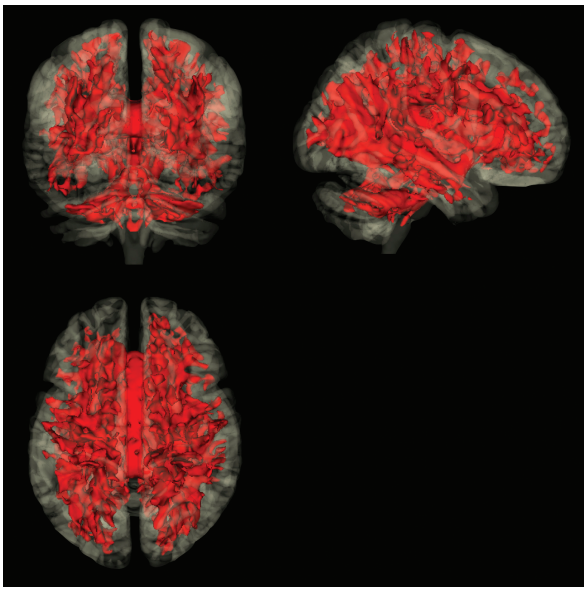

Fig. 1. White matter pathology in schizophrenia is widespread, encompassing all cerebral lobes and the cerebellum. White matter showing reduced fractional anisotropy in patients with schizophrenia is colored red (corrected $P < .05$). Light gray regions denote unaffected white matter. See also supplementary movie.

Discussion

Reports of white matter pathology in schizophrenia are numerous, but a precise understanding of the extent of these changes and their effect on whole-brain white matter connectivity remains elusive. In the largest diffusion-imaging sample of patients with schizophrenia to date, we identified a widespread and diffuse reduction in FA, involving white matter in all cerebral lobes. These findings were identified spatially using voxel-based analysis and topologically using tractography-delineated white matter fiber bundles.

The few previous studies of schizophrenia patients that have suggested global effects of the disease on white matter microstructure were based on substantially smaller sample sizes and typically relied on the comparison of FA averaged across selected ROI: cerebral lobes[17,18] or white matter tracts.[51] Interpretation and comparison of their findings is challenging due to the poor spatial

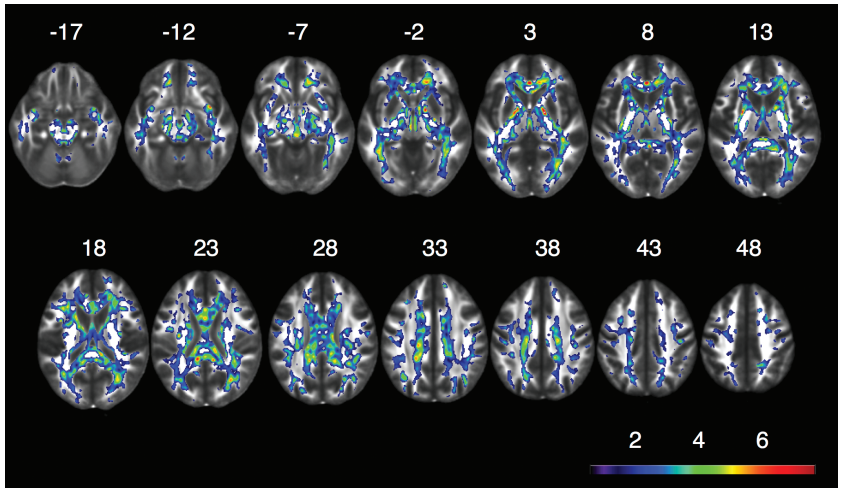

Fig. 2. Effect size of white matter pathology in schizophrenia. Effect size of the significant reduction in fractional anisotropy in patients with schizophrenia, as quantified with the t-statistic and represented as a series of axial brain slices. Z coordinates are shown at the top of each slice. The color bar indicates the t-statistic. Right side of the brain is on the right side of the figure. See supplementary figure S5 for Cohen's d.

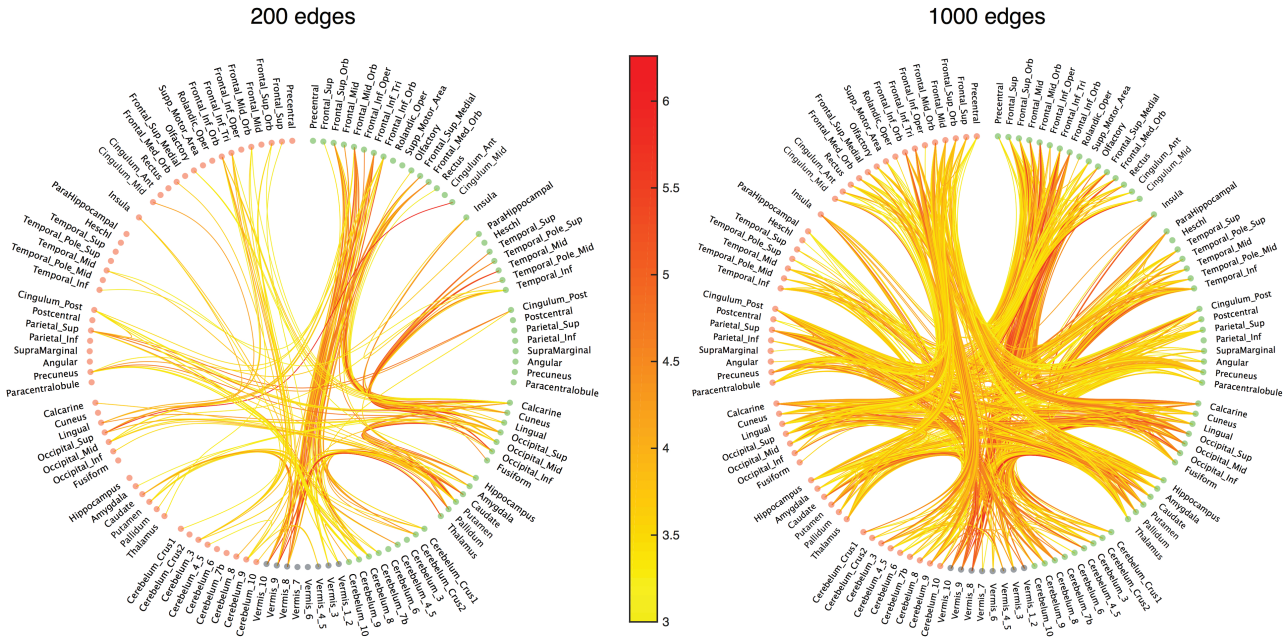

Fig. 3. Connectograms of disrupted white matter connectivity in schizophrenia. The network has been thresholded to show 200 and 1000 edges (left and right circles, respectively), showing the largest group differences, based on their individual *t*-statistic values (scale bar in the middle). Within each circle, left hemisphere structures are represented on the left (indicated by orange dots in the inner circle), with right hemisphere structures indicated by green dots in the inner circle.

resolution and the different ROI choices across the studies. Independent of any ROI choice and with the largest sample size to date, our study is the first to demonstrate that abnormalities in white matter microstructure and disruptions to white matter connectivity are widespread in schizophrenia, encompassing more than 40% of cerebral white matter and disrupting 52% of the connections comprising the human connectome. These findings are in agreement with functional imaging evidence for a widespread and generalized connectivity deficit in patients with schizophrenia.[8,52,53]

This widespread deterioration of white matter in schizophrenia is consistent with the substantial heterogeneity in clinical presentations seen between patients. If distinct clinical subtypes are indeed associated with distinct patterns of white matter disruption, a cohort of patients that includes multiple clinical subtypes is likely to show a widespread distribution of deficits that represent a superposition of the pathology associated with each constituent subtype. Our study therefore highlights the importance of future work aimed at disaggregating schizophrenia into biologically delineated subtypes and shows that measures of brain network topology (eg, rich club) may provide greater specificity in delineating these subtypes. Furthermore, our study points to the limitations of analyses that focus on circumscribed regions or tracts of interest. While significant deterioration may be found in these circumscribed structures, this belies the possibility of widespread deterioration spanning the entire brain.

It has been suggested that the inconsistent results of previous studies of FA may be driven by inaccurate intersubject registration of brain scans. Subtle variations in gross brain anatomy in patients could have led to the misregistration of their brain scans to the template and driven differences in FA relative to controls.[54] Studies using tract-based spatial statistics[55] that involve a skeletonization step of FA maps may improve residual misalignment errors remaining after intersubject registration; however, several methodological studies have demonstrated that the skeletonization process can itself introduce artifacts and confines inference to a limited "skeleton" of white matter regions.[56–58] In the present study, these concerns motivated the use of a more conventional voxel-based analysis involving nonlinear intersubject registration followed by application of a smoothing kernel to reduce residual misalignment errors. We also performed tractography to ensure our findings were not driven by misalignment errors. Crucially, the tractography-based analysis was performed such that FA was averaged across white matter bundles delineated in each individual's native space. This approach is less prone to subtle intersubject variations in brain anatomy that were poorly accounted for by the nonlinear registration algorithm. The same widespread effects in the patient group were observed with both analyses, thereby confirming the robustness of our findings and suggesting that residual registration inaccuracies were inconsequential.

Additional analyses performed for each site individually (see supplementary material) showed that a minimum number of participants was required to observe a comparable widespread reduction in FA in any particular ASRB subsample (ie, Melbourne, n = 163 or Brisbane,

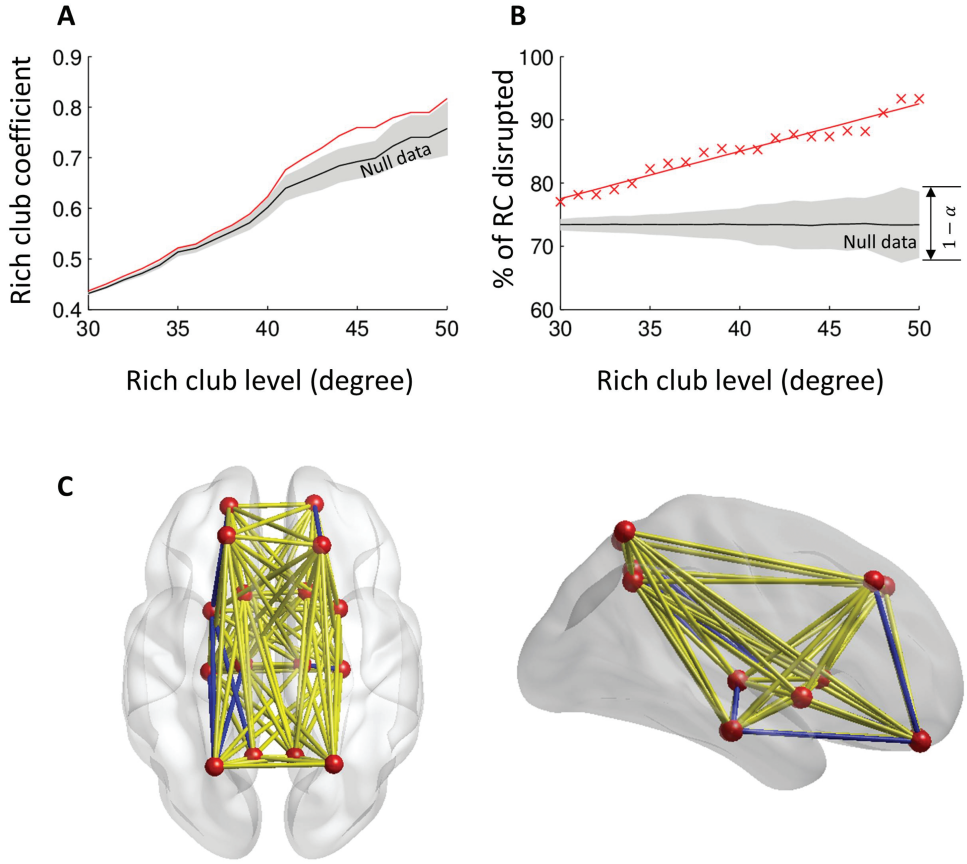

Fig. 4. Hub-to-hub connections forming the rich club are disproportionately affected by white matter pathology in schizophrenia. (A) Rich club coefficient plotted as a function of the rich club level (degree) in a network representative of the control group (red line). Null data are an ensemble of degree-preserving random networks (black line). The shaded area comprises regions where the null hypothesis of absent rich club structure cannot be rejected ($\alpha = .05$). It can be seen that rich club organization is particularly evident in the range of degrees from 40 to 50. (B) Proportion of connections comprising the rich club that also form part of the disrupted schizophrenia network (red crosses). The line of best fit is shown in red. An ensemble of random subnetworks comprising the same number of edges as the rich club was generated and the proportion to which each of them overlapped with the disrupted schizophrenia network was quantified (black line). None of the random subnetworks comprised more disrupted connections than the rich club. (C) Visualization of the rich club for a nodal degree threshold of 50. Rich club connections that overlap with the schizophrenia network are colored yellow. Rich club connections that are not disrupted in the patients with schizophrenia are colored blue.

$n = 154$). This is not surprising given the modest effect sizes observed in most white matter structures (figure 2) and confirms that sample size is an important limiting factor to detect these subtle differences with the current methodology employed.

As illustrated in supplementary figure S3, ordering the sites by sample size shows an increase in both the number of significant clusters and their size. We have also reproduced at the single-site level some of these previous findings, including no change in FA (ie, Perth, $n = 68$), one small and isolated cluster (ie, Newcastle, $n = 35$), or several clusters mostly limited to one hemisphere (ie, Sydney, $n = 103$). Analyses of smaller sample sizes employing ROI approaches might therefore be prone to concluding that white matter deficits in schizophrenia are focal and confined to specific white matter structures. These analyses might identify ROI associated with the greatest effect sizes, but white matter pathology is in fact substantially more widespread than indicated by previous analyses, albeit with a relatively modest effect size. Spatial variations in effect size can indicate heterogeneity in the sample and/or differences in the severity of white matter disruptions (eg, demyelination). In the latter case, it is imperative for future work to investigate the functional consequences of these significant but relatively modest effect sizes, and to elucidate specific subdisorders, clinical symptoms, and specific genetic factors or backgrounds associated with each disruption. In this study, white matter pathology did not differ between patients with schizophrenia and schizoaffective disorder, and it was not mediated by medication status.

A significant association between FA and measures of lifetime positive and negative symptoms was not found. This suggests that enduring expression of symptoms may not vary as a simple function of regional diffusion properties but may be due to complex interactions that could involve the timing of the pathophysiological process as well as individual genetic vulnerabilities.[59] Findings from

previous studies reporting associations between FA and positive symptoms in chronic patients are difficult to compare, first because they focused on the current expression of symptoms (in contrast to lifetime) and second because they found either positive[20,60–62] or negative correlations.[63] Nakamura and colleagues reported a negative correlation between FA from a small cluster in the anterior corpus callosum and the severity of avolition in patients with established schizophrenia.[64]

Our negative findings could also be related to the high clinical heterogeneity of schizophrenia. Identification of patients with a deficit syndrome, characterized by persistent, trait-like negative symptoms[65] could represent a more homogeneous population that can be identified using the Schedule for the Deficit Syndrome or a "proxy" extracted from the Positive And Negative Syndrome Scale.[66] Multidimensional analyses that may emerge within the Research Domain Criteria framework[67–69] should be considered in future work with respect to functional consequences.

Intrahemispheric fibers extensively featured in the network of disrupted connections. Disruption to interhemispheric fibers, particularly transcallosal pathways, is perhaps one of the most replicated finding in schizophrenia,[19] and this has been linked to the reduced functional and structural asymmetry observed in patients.[70] However, it has been suggested that callosal abnormalities could be secondary to intrahemispheric dysfunctions.[71] This is in line with more recent work highlighting the dependence of distant, interregional connectivity on the integrity of closer and more local interconnections.[72] The more extensive disruption of the right hemisphere is difficult to interpret. One previous meta-analysis reported frontal and temporal FA reductions in the left hemisphere only,[28] while 2 others did not report any laterality effect.[29,54]

White matter fiber bundles between hub regions comprising the rich club were disproportionately affected by white matter pathology. This result is consistent with evidence that hub-to-hub connections extend across long anatomical distances to connect functionally diverse neural systems.[73] Given that these connections comprise a greater proportion of white matter by volume, it is more likely that they traverse an abnormal white matter region than a shorter or more circumscribed fiber. Cerebral hubs are thought to play an important role in integrating neuronal signals and their dysfunction is most likely associated with brain disorders.[43] Hub disruptions have been reported in schizophrenia in structural[31,74] and functional[32,75] connectivity studies (see also ref.[76]).

Interpretation of our findings is limited to the spatial and topological localization of differences in white matter diffusion properties. As previously mentioned in the methods, FA is a broad index, sensitive to the directionality of white matter diffusion, and under the influence of not only cellular compartments (ie, axons, myelin) but also the surrounding extracellular matrix. In the context of cumulative evidence for the role of neuroinflammation in the pathogenesis of schizophrenia,[77] free water imaging techniques[78] may help to differentiate between alterations of the extracellular compartment, most likely related to inflammation, from alterations of white matter fibers themselves.[79]

A limitation of the current study is the absence of data on antipsychotic medication dosage. Indeed, it has been suggested that antipsychotics could affect white matter diffusion properties. However, disentangling the possible effects of medication from the consequences of illness progression remains challenging. Therefore, results are very heterogeneous and even contradictory, reporting either decreases,[80,81] increases,[82] or no changes in FA[83] following the first months of treatment. In any case, decreased FA has been reported during the prodromal and early stages of the disease, before the introduction of any antipsychotic medication, suggesting that alterations of FA in schizophrenia may represent a core feature of the disease and not only an epiphenomenon secondary to pharmacological interventions.[84]

Finally, the findings reported in this study may potentially be interpreted as effects arising from the significantly lower IQ of the patient group. However, disassociating low IQ from the disorder per se is generally difficult, given the pervasive cognitive difficulties shown by most patients[85] and controlling for IQ in our statistical model might be considered inappropriate.[86] Indeed, our findings are consistent with a widespread cognitive impairment.

In conclusion, we have reported on the largest neuroimaging study to date of white matter pathology and white matter connectivity in patients with schizophrenia. Our findings indicate that white matter pathology associated with schizophrenia is substantially more widespread than suggested by previous studies, extending to all cerebral lobes as well as the cerebellum and disrupting a majority of cortico-cortical and cortico-subcortical connections, with long association fibers interconnecting hub regions disproportionately affected.

Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

Funding

This study used samples and data from the Australian Schizophrenia Research Bank (ASRB), funded by a National Health and Medical Research Council (NHMRC) Enabling Grant (386500) held by V. Carr, U. Schall, R. Scott, A. Jablensky, B. Mowry, P. Michie, S. Catts, F. Henskens, and C. Pantelis (Chief Investigators), and the Pratt Foundation, Ramsay Health Care, the Viertel Charitable Foundation, and the Schizophrenia Research Institute, using an infrastructure grant from the NSW Ministry of Health. P.K. was supported by the Swiss National Science Foundation (SNSF) and the Swiss

Society for Medicine and Biology Scholarships (148384) and the National Center for Competence in Research—SYNAPSY—funded by the SNSF. V.C. was supported by a NHMRC Early Career Fellowship (628880) and a Brain and Behavior Research Foundation (NARSAD) Young Investigator Award (21660). C.B. was supported by a Brain and Behavior Research Foundation (NARSAD) Young Investigator Grant (20526) and University of Melbourne Ronald Phillip Griffith Fellowship. A.F. was supported by an Australian Research Council Future Fellowship (FT130100589) and NHMRC Project Grants (1050504, 1066779). L.C. was supported by a NHMRC Project Grant (APP1099082). J.M.F. was supported by the Janette Mary O'Neil Fellowship and NHMRC (1063960). C.S.W. was supported by Schizophrenia Research Institute (utilizing infrastructure funding from the NSW Ministry of Health and the Macquarie Group Foundation), the University of New South Wales, Neuroscience Research Australia, and by a NHMRC Senior Research Fellowship (1021970). C.P. was supported by a NHMRC Senior Principal Research Fellowship (628386, 1105825), and a Brain and Behavior Research Foundation (NARSAD) Distinguished Investigator Award (18722). A.Z. was supported by a NHMRC Career Development Fellowship (GNT1047648).

Acknowledgments

We would like to acknowledge Jason Bridge for the management of data from the Australian Schizophrenia Research Bank. The authors have declared that there are no conflicts of interest in relation to the subject of this study.

References

1. Fornito A, Bullmore ET. Connectomics: a new paradigm for understanding brain disease. *Eur Neuropsychopharmacol*. 2015;25:733–748.
2. Fornito A, Zalesky A, Breakspear M. The connectomics of brain disorders. *Nat Rev Neurosci*. 2015;16:159–172.
3. Griffa A, Baumann PS, Thiran JP, Hagmann P. Structural connectomics in brain diseases. *Neuroimage*. 2013;80:515–526.
4. Menon V. Large-scale brain networks and psychopathology: a unifying triple network model. *Trends Cogn Sci*. 2011;15:483–506.
5. Crossley NA, Mechelli A, Scott J, et al. The hubs of the human connectome are generally implicated in the anatomy of brain disorders. *Brain*. 2014;137:2382–2395.
6. Bleuler E. *Dementia Praecox or the Group of Schizophrenias*. Oxford, UK: International Universities Press; 1950.
7. van den Heuvel MP, Fornito A. Brain networks in schizophrenia. *Neuropsychol Rev*. 2014;24:32–48.
8. Fornito A, Zalesky A, Pantelis C, Bullmore ET. Schizophrenia, neuroimaging and connectomics. *Neuroimage*. 2012;62:2296–2314.
9. Stephan KE, Baldeweg T, Friston KJ. Synaptic plasticity and dysconnection in schizophrenia. *Biol Psychiatry*. 2006;59:929–939.
10. Friston KJ, Frith CD. Schizophrenia: a disconnection syndrome? *Clin Neurosci*. 1995;3:89–97.
11. Friston KJ. The disconnection hypothesis. *Schizophr Res*. 1998;30:115–125.
12. Bullmore ET, Frangou S, Murray RM. The dysplastic net hypothesis: an integration of developmental and dysconnectivity theories of schizophrenia. *Schizophr Res*. 1997;28:143–156.
13. Andreasen NC. A unitary model of schizophrenia: Bleuler's "fragmented phrene" as schizencephaly. *Arch Gen Psychiatry*. 1999;56:781–787.
14. Gulani V, Webb AG, Duncan ID, Lauterbur PC. Apparent diffusion tensor measurements in myelin-deficient rat spinal cords. *Magn Reson Med*. 2001;45:191–195.
15. Kubicki M, Park H, Westin CF, et al. DTI and MTR abnormalities in schizophrenia: analysis of white matter integrity. *Neuroimage*. 2005;26:1109–1118.
16. Jones DK, Knösche TR, Turner R. White matter integrity, fiber count, and other fallacies: the do's and don'ts of diffusion MRI. *Neuroimage*. 2013;73:239–254.
17. Minami T, Nobuhara K, Okugawa G, et al. Diffusion tensor magnetic resonance imaging of disruption of regional white matter in schizophrenia. *Neuropsychobiology*. 2003;47:141–145.
18. Lim KO, Hedehus M, Moseley M, de Crespigny A, Sullivan EV, Pfefferbaum A. Compromised white matter tract integrity in schizophrenia inferred from diffusion tensor imaging. *Arch Gen Psychiatry*. 1999;56:367–374.
19. Pettersson-Yeo W, Allen P, Benetti S, McGuire P, Mechelli A. Dysconnectivity in schizophrenia: where are we now? *Neurosci Biobehav Rev*. 2011;35:1110–1124.
20. Hubl D, Koenig T, Strik W, et al. Pathways that make voices: white matter changes in auditory hallucinations. *Arch Gen Psychiatry*. 2004;61:658–668.
21. Rotarska-Jagiela A, Oertel-Knoechel V, DeMartino F, et al. Anatomical brain connectivity and positive symptoms of schizophrenia: a diffusion tensor imaging study. *Psychiatry Res*. 2009;174:9–16.
22. Steel RM, Bastin ME, McConnell S, et al. Diffusion tensor imaging (DTI) and proton magnetic resonance spectroscopy (1H MRS) in schizophrenic subjects and normal controls. *Psychiatry Res*. 2001;106:161–170.
23. Foong J, Symms MR, Barker GJ, Maier M, Miller DH, Ron MA. Investigating regional white matter in schizophrenia using diffusion tensor imaging. *Neuroreport*. 2002;13:333–336.
24. Jones DK, Catani M, Pierpaoli C, et al. A diffusion tensor magnetic resonance imaging study of frontal cortex connections in very-late-onset schizophrenia-like psychosis. *Am J Geriatr Psychiatry*. 2005;13:1092–1099.
25. Begré S, Koenig T. Cerebral disconnectivity: an early event in schizophrenia. *Neuroscientist*. 2008;14:19–45.
26. Konrad A, Winterer G. Disturbed structural connectivity in schizophrenia primary factor in pathology or epiphenomenon? *Schizophr Bull*. 2008;34:72–92.
27. Kanaan RA, Kim JS, Kaufmann WE, Pearlson GD, Barker GJ, McGuire PK. Diffusion tensor imaging in schizophrenia. *Biol Psychiatry*. 2005;58:921–929.
28. Ellison-Wright I, Bullmore E. Meta-analysis of diffusion tensor imaging studies in schizophrenia. *Schizophr Res*. 2009;108:3–10.
29. Bora E, Fornito A, Radua J, et al. Neuroanatomical abnormalities in schizophrenia: a multimodal voxelwise

meta-analysis and meta-regression analysis. *Schizophr Res*. 2011;127:46–57.
30. Fitzsimmons J, Kubicki M, Shenton ME. Review of functional and anatomical brain connectivity findings in schizophrenia. *Curr Opin Psychiatry*. 2013;26:172–187.
31. Zalesky A, Fornito A, Seal ML, et al. Disrupted axonal fiber connectivity in schizophrenia. *Biol Psychiatry*. 2011;69:80–89.
32. van den Heuvel MP, Sporns O, Collin G, et al. Abnormal rich club organization and functional brain dynamics in schizophrenia. *JAMA Psychiatry*. 2013;70:783–792.
33. Beaulieu C. The basis of anisotropic water diffusion in the nervous system—a technical review. *NMR Biomed*. 2002;15:435–455.
34. Loughland C, Draganic D, McCabe K, et al. Australian Schizophrenia Research Bank: a database of comprehensive clinical, endophenotypic and genetic data for aetiological studies of schizophrenia. *Aust N Z J Psychiatry*. 2010;44:1029–1035.
35. Moore EA, Green MJ, Carr VJ. Comorbid personality traits in schizophrenia: prevalence and clinical characteristics. *J Psychiatr Res*. 2012;46:353–359.
36. McCabe KL, Maloney EA, Stain HJ, Loughland CM, Carr VJ. Relationship between childhood adversity and clinical and cognitive features in schizophrenia. *J Psychiatr Res*. 2012;46:600–607.
37. Green MJ, Chia TY, Cairns MJ, et al. Catechol-O-methyltransferase (COMT) genotype moderates the effects of childhood trauma on cognition and symptoms in schizophrenia. *J Psychiatr Res*. 2014;49:43–50.
38. Castle DJ, Jablensky A, McGrath JJ, et al. The diagnostic interview for psychoses (DIP): development, reliability and applications. *Psychol Med*. 2006;36:69–80.
39. Andreasen NC. Negative symptoms in schizophrenia. Definition and reliability. *Arch Gen Psychiatry*. 1982;39:784–788.
40. Wechsler D. *Manual for the Wechsler Abbreviated Scale of Intelligence*. San Antonio, TX: The Psychological Corporation; 1999.
41. Zalesky A, Fornito A, Cocchi L, Gollo LL, van den Heuvel MP, Breakspear M. Connectome sensitivity or specificity: which is more important. *Neuroimage*. 2016. doi:10.1016/j.neuroimage.2016.06.035.
42. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage*. 2002;15:273–289.
43. van den Heuvel MP, Sporns O. Network hubs in the human brain. *Trends Cogn Sci*. 2013;17:683–696.
44. van den Heuvel MP, Sporns O. Rich-club organization of the human connectome. *J Neurosci*. 2011;31:15775–15786.
45. de Reus MA, van den Heuvel MP. Estimating false positives and negatives in brain networks. *Neuroimage*. 2013;70:402–409.
46. Maslov S, Sneppen K. Specificity and stability in topology of protein networks. *Science*. 2002;296:910–913.
47. Rubinov M, Sporns O. Complex network measures of brain connectivity: uses and interpretations. *Neuroimage*. 2010;52:1059–1069.
48. Friston KJ, Worsley KJ, Frackowiak RSJ, Mazziotta JC, Evans AC. Assessing the significance of focal activations using their spatial extent. *Human Brain Mapping Hum. Brain Mapp*. 1993;1:210–220.
49. Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage*. 2009;44:83–98.
50. Zalesky A, Fornito A, Bullmore ET. Network-based statistic: identifying differences in brain networks. *Neuroimage*. 2010;53:1197–1207.
51. Roalf DR, Gur RE, Verma R, et al. White matter microstructure in schizophrenia: associations to neurocognition and clinical symptomatology. *Schizophr Res*. 2015;161:42–49.
52. Fornito A, Yoon J, Zalesky A, Bullmore ET, Carter CS. General and specific functional connectivity disturbances in first-episode schizophrenia during cognitive control performance. *Biol Psychiatry*. 2011;70:64–72.
53. Cocchi L, Harding IH, Lord A, Pantelis C, Yucel M, Zalesky A. Disruption of structure-function coupling in the schizophrenia connectome. *Neuroimage Clin*. 2014;4:779–787.
54. Melonakos ED, Shenton ME, Rathi Y, Terry DP, Bouix S, Kubicki M. Voxel-based morphometry (VBM) studies in schizophrenia-can white matter changes be reliably detected with VBM. *Psychiatry Res*. 2011;193:65–70.
55. Smith SM, Jenkinson M, Johansen-Berg H, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *Neuroimage*. 2006;31:1487–1505.
56. Bach M, Laun FB, Leemans A, et al. Methodological considerations on tract-based spatial statistics (TBSS). *Neuroimage*. 2014;100:358–369.
57. Schwarz CG, Reid RI, Gunter JL, et al. Improved DTI registration allows voxel-based analysis that outperforms tract-based spatial statistics. *Neuroimage*. 2014;94:65–78.
58. Zalesky A. Moderating registration misalignment in voxelwise comparisons of DTI data: a performance evaluation of skeleton projection. *Magn Reson Imaging*. 2011;29:111–125.
59. Steullet P, Cabungcal JH, Monin A, et al. Redox dysregulation, neuroinflammation, and NMDA receptor hypofunction: A "central hub" in schizophrenia pathophysiology. *Schizophr Res*. 2016;176:41–51.
60. Shergill SS, Kanaan RA, Chitnis XA, et al. A diffusion tensor imaging study of fasciculi in schizophrenia. *Am J Psychiatry*. 2007;164:467–473.
61. Seok JH, Park HJ, Chun JW, et al. White matter abnormalities associated with auditory hallucinations in schizophrenia: a combined study of voxel-based analyses of diffusion tensor imaging and structural magnetic resonance imaging. *Psychiatry Res*. 2007;156:93–104.
62. Caprihan A, Jones T, Chen H, et al. The paradoxical relationship between white matter, psychopathology and cognition in schizophrenia: a diffusion tensor and proton spectroscopic imaging study. *Neuropsychopharmacology*. 2015;40:2248–2257.
63. Fujiwara H, Namiki C, Hirao K, et al. Anterior and posterior cingulum abnormalities and their association with psychopathology in schizophrenia: a diffusion tensor imaging study. *Schizophr Res*. 2007;95:215–222.
64. Nakamura K, Kawasaki Y, Takahashi T, et al. Reduced white matter fractional anisotropy and clinical symptoms in schizophrenia: a voxel-based diffusion tensor imaging study. *Psychiatry Res*. 2012;202:233–238.
65. Carpenter WT Jr, Heinrichs DW, Wagman AM. Deficit and nondeficit forms of schizophrenia: the concept. *Am J Psychiatry*. 1988;145:578–583.
66. Goetz RR, Corcoran C, Yale S, et al. Validity of a 'proxy' for the deficit syndrome derived from the Positive And Negative Syndrome Scale (PANSS). *Schizophr Res*. 2007;93:169–177.

67. Cuthbert BN, Insel TR. Toward the future of psychiatric diagnosis: the seven pillars of RDoC. *BMC Med*. 2013;11:126.
68. Ford JM, Morris SE, Hoffman RE, et al. Studying hallucinations within the NIMH RDoC framework. *Schizophr Bull*. 2014;40(suppl 4):S295–S304.
69. Anderson A, Wilcox M, Savitz A, et al. Sparse factors for the positive and negative syndrome scale: which symptoms and stage of illness? *Psychiatry Res*. 2015;225:283–290.
70. Ribolsi M, Daskalakis ZJ, Siracusano A, Koch G. Abnormal asymmetry of brain connectivity in schizophrenia. *Front Hum Neurosci*. 2014;8:1010.
71. Innocenti GM, Ansermet F, Parnas J. Schizophrenia, neurodevelopment and corpus callosum. *Mol Psychiatry*. 2003;8:261–274.
72. Zalesky A, Fornito A, Egan GF, Pantelis C, Bullmore ET. The relationship between regional and inter-regional functional connectivity deficits in schizophrenia. *Hum Brain Mapp*. 2012;33:2535–2549.
73. van den Heuvel MP, Kahn RS, Goñi J, Sporns O. High-cost, high-capacity backbone for global brain communication. *Proc Natl Acad Sci USA*. 2012;109:11372–11377.
74. van den Heuvel MP, Mandl RC, Stam CJ, Kahn RS, Hulshoff Pol HE. Aberrant frontal and temporal complex network structure in schizophrenia: a graph theoretical analysis. *J Neurosci*. 2010;30:15915–15926.
75. Lynall ME, Bassett DS, Kerwin R, et al. Functional connectivity and brain networks in schizophrenia. *J Neurosci*. 2010;30:9477–9487.
76. Fornito A, Bullmore ET. Reconciling abnormalities of brain network structure and function in schizophrenia. *Curr Opin Neurobiol*. 2015;30:44–50.
77. Hardingham GE, Do KQ. Linking early-life NMDAR hypofunction and oxidative stress in schizophrenia pathogenesis. *Nat Rev Neurosci*. 2016;17:125–134.
78. Pasternak O, Sochen N, Gur Y, Intrator N, Assaf Y. Free water elimination and mapping from diffusion MRI. *Magn Reson Med*. 2009;62:717–730.
79. Pasternak O, Westin CF, Dahlben B, Bouix S, Kubicki M. The extent of diffusion MRI markers of neuroinflammation and white matter deterioration in chronic schizophrenia. *Schizophr Res*. 2015;161:113–118.
80. Szeszko PR, Robinson DG, Ikuta T, et al. White matter changes associated with antipsychotic treatment in first-episode psychosis. *Neuropsychopharmacology*. 2014;39:1324–1331.
81. Wang Q, Cheung C, Deng W, et al. White-matter microstructure in previously drug-naive patients with schizophrenia after 6 weeks of treatment. *Psychol Med*. 2013;43:2301–2309.
82. Reis Marques T, Taylor H, Chaddock C, et al. White matter integrity as a predictor of response to treatment in first episode psychosis. *Brain*. 2014;137:172–182.
83. Zeng B, Ardekani BA, Tang Y, et al. Abnormal white matter microstructure in drug-naive first episode schizophrenia patients before and after eight weeks of antipsychotic treatment. *Schizophr Res*. 2016;172:1–8.
84. Samartzis L, Dima D, Fusar-Poli P, Kyriakopoulos M. White matter alterations in early stages of schizophrenia: a systematic review of diffusion tensor imaging studies. *J Neuroimaging*. 2014;24:101–110.
85. Heinrichs RW, Zakzanis KK. Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. *Neuropsychology*. 1998;12:426–445.
86. Miller GA, Chapman JP. Misunderstanding analysis of covariance. *J Abnorm Psychol*. 2001;110:40–48.