Acute conceptual disorganization in untreated first-episode psychosis: A combined magnetic resonance spectroscopy and diffusion imaging study of the cingulum

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Abstract:

Background:

Disorganized thinking is a core feature of acute psychotic episodes that is linked to social and vocational functioning. Based on the close association between cingulum and disorganized thinking, we examine three candidate mechanistic markers in relation to acute conceptual disorganization (CD) in first episode psychosis: glutamate excess; cortical antioxidant (glutathione) status and the integrity of the cingulum bundle.

Methods:

We used fractional anisotropy (FA) maps from 7T diffusion-weighted imaging to investigate bilateral cingulum based on a probabilistic white-matter atlas. We compared the high-CD, low-CD and healthy control groups and performed probabilistic fiber tracking from the identified clusters (ROI within cingulum) to the rest of the brain. We quantified glutamate and glutathione with magnetic-resonance-spectroscopy (MRS) in the dorsal anterior cingulate cortex.

Results:

There was a significant FA reduction ($F=9.04$; $p=0.036$) in a cluster in left cingulum in high-CD compared to low-CD (Cohen's $d=1.39$; $p=0.003$) and controls (Cohen's $d=0.86$; $p=0.009$). Glutamate levels did not vary among the groups, but glutathione levels were higher in high-CD compared to the low-CD group. Higher glutathione related to lower FA in the high-CD group in the cingulum cluster.

Discussion:

Acute CD relates to indicators of oxidative stress as well as reduced white matter integrity of the cingulum but not to MRI-based glutamatergic excess. We propose that both oxidative imbalance and structural dysconnectivity underlie acute disorganization.

Limitation: MRS measures of glutamine were highly uncertain and MRs was acquired from a single voxel only.

1. Introduction

Conceptual disorganization (CD) refers to difficulties in the goal-directed sequencing of thoughts that manifest as circumstantial, illogical or tangential speech or weakened goal of thinking (loose associations) in patients with psychotic disorders such as schizophrenia¹. CD is an important contributor to the reduced real world functioning among patients with schizophrenia². CD is closely linked to the construct of Formal Thought Disorder (FTD), but unlike FTD, CD is a clinical interpretation that is not restricted to speech domain alone and refers specifically to the positive, rather than the negative dimension of disorganized thinking.

Based on the idea that conceptual disorganization relates to the aberrant shunting of cognitive processes^{3, 4}, structural disconnection among brain regions has been investigated as the neural basis of CD using Diffusion Tensor Imaging (DTI). Viher and colleagues⁵ and Cavelti and colleagues⁶ did not find any association between white matter integrity and the severity of disorganization or FTD, while Boppa and colleagues noted more severe FTD in patients with reduced integrity of the cingulum tract⁷. More recently, de Boer and colleagues⁸ found an association between computational linguistic indices relating to negative symptoms and white matter integrity in schizophrenia. This effect was stronger for the entire white matter structure rather than language-related tracts⁸. These prior DTI studies were limited to patients with established illness (>2 years), with long exposure to antipsychotic medications that affects both DTI indices and symptom severity scores^{6, 8, 9}. At present, the structural basis of acute CD in minimally treated, first episode patients is unknown^{10, 11}.

The structural integrity of the cingulum has been repeatedly observed to be abnormal in psychosis^{5, 12}. Recently, aberrant myelination of frontal fasciculi, especially, cingulum has been proposed to underlie delayed corollary discharges that can affect sense of agency and contribute to disorganization. Studies that specifically report on radial diffusivity, a proxy measure of myelin, suggest that most of the observed structural deficit of cingulum is likely to be a result of aberrant myelination in schizophrenia^{13, 14}. Thus, we selected cingulum as target region to examine glutamate excess, cortical antioxidant status and the integrity of the cingulum bundle in relation to acute conceptual disorganization (CD) in first episode psychosis.

An experimental approach that provokes symptoms of acute CD is the subanaesthetic ketamine infusion in both healthy volunteers and patients with schizophrenia¹⁵. Ketamine

disrupts inhibitory interneuron function, resulting in the disinhibition of the excitatory glutamatergic outputs of the prefrontal cortex. Sustained disinhibition increases extracellular glutamate, resulting in oxidative stress¹⁶. Glutathione (GSH), a neuronal antioxidant, is the primary defence against excitotoxicity in the human brain¹⁷. Subgroups of psychotic patients with higher levels of CD show elevated peripheral markers of oxidative stress¹⁸⁻²⁰. Despite the relevance of ketamine model to CD¹⁰, the role of brain glutamate and glutathione in CD remains unstudied to date.

In the current study, we bring together 2 candidate mechanistic markers in relation to acute CD in first episode psychosis: (1) cortical glutamate excess and antioxidant (glutathione) status in the anterior cingulate cortex (ACC) and (2) the structural integrity of the cingulum that connects anterior cingulate cortex to other brain regions. Abnormalities in the structural integrity of the cingulum has been repeatedly observed in psychosis^{7, 21, 22}, and linked to CD by Boppa and colleagues⁷. We studied the effect of the severity of CD on (1) fractional anisotropy (FA) indicating white matter integrity across the cingulum tract, (2) MRS (magnetic resonance spectroscopy) derived glutamate and GSH concentrations. We also investigated the relationship among these mechanistic features in patients with untreated first episode psychosis, with median antipsychotic medication exposure of less than three days.

2. Methods

2.1 Participants

Patients with first episode psychosis (31 with schizophrenia; 9 with schizoaffective/schizophreniform psychosis) between the aged of 15-39 years were recruited from PEPP (Prevention and Early Intervention for Psychosis Program) at London Health Sciences Centre, London, Ontario, Canada between April 2017 and July 2019. The PEPP program uses an assertive case-management model to provide assessment and treatment to individuals experiencing FEP.

In total, all patients and 25 healthy controls had scans of adequate quality (without obvious artifact and visible structural damage) for DTI analysis. Inclusion criteria for study participation were as follows: individuals experiencing a first episode of psychosis and having received fewer

14 days of antipsychotic treatment. Both inpatients and outpatients were eligible to participate if they were able provide informed consent and safely participate in the magnetic resonance imaging (MRI) protocol. All participants received a consensus diagnosis from 3 psychiatrists (LP/KD and the primary treatment provider) after approximately 6 months on the basis of the best estimate procedure (as described by Leckman²³) and the Structured Clinical Interview for DSM-5²⁴. Following the 6-month consensus diagnosis, participants meeting criteria for bipolar disorder with psychotic features, major depressive disorder with psychotic features, or suspected drug-induced psychoses were excluded from further analyses. Care was provided as usual to the study participants through their psychiatrist and other allied health members with the PEPP program. Antipsychotic medications were chosen by the treating psychiatrist in collaboration with the patient and/or their substitute decision-maker. Individuals were offered the option of treatment with a long-acting injectable at the earliest opportunity²⁵ in accordance with current national guidelines for the treatment of FEP.

Recruitment of healthy control subjects (n = 25) was carried out through posters which advertised the opportunity to participate in a neuroimaging study to track the outcomes of FEP. Healthy control (HC) subjects had no personal history of mental illness and no first degree relatives with a history of psychotic disorders. Group matching with the FEP cohort for age, gender and parental education was maintained.

Exclusion criteria for both the FEP and healthy control groups involved meeting criteria for a substance use disorder in the past year according to DSM-5 criteria²⁴, having a history of a major head injury (leading to a significant period of unconsciousness or seizures), having a significant, uncontrolled medical illness, or having any MRI scan contraindications.

All potential study participants provided written, informed consent prior to participation as outlined by the Western University Health Sciences Research Ethics Board, London, Ontario. The sample reported here partly overlaps with 2 of our prior studies^{26, 27}.

2.2 Symptom assessment

The severity of symptoms in patients was assessed using the 8-item Positive and Negative Syndrome Scale (PANSS-8) which measures the severity of positive and negative symptoms. We also used the Social and Occupational Functioning Assessment Scale (SOFAS) to assess the

overall level of functioning²⁸. We also used a modified digit symbol substitution task (DSST) to quantify processing speed, a cognitive function that shows the most prominent reduction among patients with psychosis²⁹. The written and oral items on the DSST were scored separately, and the mean scores from these items were used for assessment as in our prior study³⁰.

We used ultra-high resolution (7-Tesla) diffusion weighted imaging in 40 patients with first-episode psychosis and 25 controls, to investigate bilateral cingulum, defined according to JHU white matter atlas. Based on the conceptual disorganization scores of PANSS-8, we divided patients to two groups, low (P2=1,2,3) and high (P2=4,5,6) groups. We chose this cut-off to distinguish those who have clinically severe versus minimal symptoms recommended by the Remission Working Group³¹. This cut-off has also been recently employed to distinguish patients with or without formal thought disorder and language dysfunction³². These two groups were not different in their severity of other positive and negative symptoms (Table 1). Tract-Based Spatial Statistics (TBSS) was applied to compare fractional anisotropy (FA) values using FSL software with 5000 permutation correction was used within the selected tract. F tests were used for group comparisons. For the clusters showing significant FA abnormalities, we undertook post-hoc comparisons of radial diffusivity (reflecting myelin content) and axial diffusivity (reflecting axonal integrity) and mode of anisotropy (reflecting fiber organization) to determine the possible nature of the microstructural alteration contributing to aberrant FA in patients.

2.3 MRI data acquisition

All magnetic resonance (MR) images were acquired on a 7.0 T Siemens (Erlangen, Germany) Magnetom Terra magnetic resonance imaging (MRI) scanner using a 8-channel Transmit/32-channel receive head coil at the Centre for Functional and Metabolic Mapping (CFMM), Robarts Research Institute, Western University. Diffusion tensor imaging was acquired using 64 diffusion-weighted volumes (b-value = 1000 s/mm²) and an unweighted volume, with a 2D spin-echo EPI sequence (matrix size=104x104, 72 slices, voxel resolution 2x2x2 mm, TR=5100ms, TE=50.2ms, GRAPPA=3, multi-band=3, phase partial Fourier=6/8).

2.4 MRI preprocessing

Diffusion-weighted images were processed using an open-source and containerized application, “prepdwi” (<http://www.github.com/khanlab/prepdwi>), which uses the BIDS³³ and BIDS Apps³⁴ standards to perform standardized pre-processing and fitting. Diffusion-weighted MRI data were first pre-processed with denoising using a local PCA method with (“dwdenoise” from *mrtrix*³⁵), and correction of ringing artifacts with the “unring” tool³⁶. Eddy current and susceptibility-based distortions were corrected using “topup”³⁷ and “eddy”³⁸ based on FSL, with the “--repol” option enabled for outlier replacement³⁹. Within-subject rigid registration of the corrected DWI volume and the T1w volume was performed using block-matching⁴⁰, to bring the DWI images in the same space as the T1w. Gradient non-linearities were corrected using vendor-provided spherical harmonic coefficients for the gradient system (AC84), applied with the “gradient_unwarp” tool⁴¹ to generate a non-linear transformation, which was composed with the T1w linear transformation to resample the DWI images into the corrected T1w space in a single step. Modulation with the determinant of the Jacobian of the unwarping was used to correct for intensity differences in the magnitude images due to gradient non-linearities. Pre-processed DWI images in the T1w space were then used to estimate diffusion tensor metrics using “dtifit”⁴². All subjects' FA images were first non-linearly aligned to FMRIB58_FA standard brain image using a nonlinear registration and then used to create a mean FA skeleton representing the centers of all tracts common to all subjects (threshold of 0.2). After that, each subject's aligned FA was projected onto this skeleton and fed into voxel-wise comparison. The voxels with significant group differences were labeled by using the “JHU ICBM-DTI-81 White-Matter Atlas”, and enlarged using the TBSS_fill tool for illustrative purposes. Besides FA, we undertook post-hoc comparisons of radial diffusivity (RD, reflecting myelin content), axial diffusivity (AD, reflecting axonal integrity) and mode of anisotropy (MO, reflecting fiber organization) for the regions showing significant FA abnormalities.

Fiber tracking analysis was performed using the Bedpostx (based on FSL) for local modeling of diffusion parameters and the Probtrackx for tractography and connectivity-based segmentation (eFigure.1). Probabilistic fiber tracking is performed from the seed region showing difference in group comparison to whole brain of fibers in native space with parameters as follow: the way total number = 70000, the number of samples (the number of individual pathways that are drawn through the probability distributions on principle fiber direction) = 5000, curvature threshold = 0.2, maximum number of steps = 2000, step length = 0.5mm, fibthresh

(subsidiary fiber volume threshold) = 0.01. We performed two-stage registration; initially with FLIRT for linear registration, and then FNIRT for nonlinear registration, both using FSL. Mean FA value and the volume of the traced fibers were extracted from each subject for further analysis.

2.5 Metabolic assessment

As described in our previous study⁴³, metabolites (glutamate, GSH, glutamine) were measured using a Siemens MAGNETOM 7.0T MRI (Agilent, Walnut Creek, **Bavaria**, USA) using an 8-channel transmit/32-channel receive, head-only, radiofrequency coil at the Centre for Metabolic Mapping at Western University in London, Ontario. A 2.0 x 2.0 x 2.0 cm (8 cm³) 1H-MRS (proton magnetic resonance spectroscopy) voxel was placed in the bilateral dorsal ACC using a two-dimensional anatomical imaging sequence in the sagittal direction (37 slices, TR=8000ms, TE=70ms, flip-angle (α) = 120 °, thickness = 3.5mm, field of view = 240×191mm). A total of 32 channel-combined, water-suppressed spectral averages were acquired using the semi-LASER 1H-MRS pulse sequence (TR=7500ms, TE=100ms) during each scan session, while participants were at rest and asked to stare at a white cross on a screen for 4 minutes. Water suppression was achieved using the VAPOR preparation sequence, and water-unsuppressed spectra were acquired for spectral post-processing (lineshape deconvolution) and quantification (water amplitude as concentration reference). Each of the 32 spectral acquisitions was corrected for frequency and phase drifts as described in Near et al ⁴⁴ prior to averaging. Metabolite concentrations were corrected for gray and white matter volumes using previously described methods⁴⁵. All spectra were inspected visually for quality and only those metabolites with Cramer-Rao lower bounds (CRLB) of less than 10% were included in the analyses. An example of representation of fit was presented below (Figure.1). For glutamine, the mean CRLB of over 20% for both first-episode psychosis (FEP) and healthy control (HC) groups did not meet our rigorous CRLB cut-off of < 10%, indicating higher uncertainty in its quantification compared to glutamate and GSH spectra. We report the results for glutamine in the Supplement.

2.6 Statistical analyses

Statistical analyses of participant demographic, clinical characteristics and parameters of imaging were carried out using SPSS 22.0. Group comparisons of the variables were made using

independent-samples t-tests for continuous variables and Kruskal-Wallis H test for dichotomous variables. Voxel-wise group comparison and within group comparison on the mean FA skeleton were performed in the framework of the general linear model using FSL's Randomise tool⁴⁶. All analyses were conducted with 5000 permutations. The significance level was set at $p < 0.05$ with threshold-free cluster enhancement (TFCE) correction⁴⁷ and family-wise error rate correction for multiple comparisons.

3. Results

3.1. Demographic and Clinical Characteristics

All patients were acutely psychotic, minimally treated and had significant social and occupational dysfunction at the time of scanning. The demographic and clinical characteristics of the sample are presented in Table 1. The two patient groups did not differ in terms of age, gender, socio-economic status, duration of untreated illness in days, exposure to antipsychotic medication in days, total daily dose of antipsychotic medication and the number of patients with schizophrenia in either group (diagnosed 6 months after the initial presentation). Across the three groups, we observed a significant difference for the SOFAS and DSST scores, confirmed by ANOVA.

3.2 Group Differences in White Matter Integrity

In the voxel-wise F test, we observed a cluster extending cingulum at MNI coordinate: X=-8, Y=-1, Z=34, with significant difference in FA ($p=0.036$, corrected) among the 3 groups within the cingulum region (Figure.2A). Mean FA, AD, RD, and MO value were extracted in this cluster and depicted in Figure 1B. High P2 group showed lower FA than low P2 (Cohen's $d=1.39$, $p=0.0003$) and healthy controls (Cohen's $d=0.86$, $p=0.0091$) (Figure.2B). Moreover, high P2 group had lower AD values compared to low P2 group (Cohen's $d=0.84$, $p=0.022$) (Figure.2B). Interestingly, we also noted that low P2 group had significantly higher AD values (Cohen's $d=0.63$, $p=0.025$) than healthy controls, indicating an aberrant increase in the axonal integrity of the cingulum in patients with lower levels of disorganization. We did not find any significant difference in RD and MO value among the three groups.

Upon fiber tracking from the seed region to whole brain, high P2 group showed significantly less volume of all fibers than low P2 (Cohen's $d=0.69$, $p=0.030$) and HC (Cohen's

$d=0.81$, $p=0.019$). Moreover, volume of all fibers was negatively correlated with P2 value ($r=-0.264$, $p=0.034$).

Kruskal-Wallis H test found no group difference ($p=0.452$) in the mean FA of the tracked fibers, though mean FA of the tracked fibers was lower in patients with lower DSST scores ($r = 0.381$, $P=0.015$); no significant relationship between FA and DSST was seen in the HC group ($r = -0.152$, $P = 0.468$).

3.2 Group Differences of MRS metabolite measures

F-test showed no significant difference in glutamate levels among three groups, but a significant in GSH levels ($F=3.894$, $P=0.027$). Patients with High P2 had higher GSH levels than healthy controls (Cohen's $d= 1.096$, $p=0.008$). Given the suspected differences in the neuronal vs. astrocytic origin of the Glu (glutamate) and GSH signal respectively (see Duarte⁴⁸ and Harris⁴⁹ for a review), we calculated Glu/GSH ratio as a proxy indicator of relative neuronal density. We found that high-P2 group had significantly low Glu/GSH ratio (driven by higher GSH concentration) compared to low-P2 (Cohen's $d= 0.912$, $p=0.011$) and healthy subjects (Cohen's $d=1.663$, $p=0.001$) indicating the possible increase in metabolite signals of astrocytic origin (GSH) compared to neuronal origin (Glu) in relation to disorganization.

3.3 Relationship between cingulum microstructure and MRS glutamate and glutathione

AD of the cingulum cluster was negatively related to glutamate ($df=16$, $r= -0.586$, $P=0.009$) and GSH ($df=16$, $r=-0.523$, $P=0.026$) among healthy controls, but only to GSH ($df=9$, $r=-0.797$, $P=0.003$) among high-P2 patients. No DTI-MRS correlations were observed in the low-P2 group (all $p>0.1$).

To determine if the observed relationship between DTI metrics, GSH and P2 was driven by other symptom differences or antipsychotic exposure status, we related GSH concentration, AD, and fiber volume to negative symptom burden, positive symptom burden, Total DDD and Medication_days. None of these correlations were significant ($r = -0.16$ to 0.2 ; $p = 0.27$ to 0.98). Besides, to determine if the observed relationship between DTI metrics, GSH and P2 was an

artifact of binarisation, we ran a Spearman's correlation between GSH concentration, AD, and fiber volume and P2 scores in all participants. We noted P2 scores was negatively correlated to the volume of fibers from seed-region to whole brain ($r=-0.264$, $p=0.034$), but there was no significant correlation between P2, AD and GSH ($p>0.1$).

4. Discussion:

To our knowledge, this is the first study linking conceptual disorganization, to markers of excitotoxicity (glutamate), oxidative stress (glutathione) as well as white matter integrity within the cingulum in first episode psychosis. We report three major observations: the presence of severe acute conceptual disorganization in first-episode psychosis is associated with (1) reduced white matter integrity of the cingulum characterised by reduced axial diffusivity and decreased volume of fibers in the cingulum tract (2) increased glutathione concentration in the dorsal anterior cingulate cortex (3) but not with glutamate concentration. In addition, higher cingulum integrity related to lower glutamate and glutathione levels among healthy controls. Our findings suggest that the severity of conceptual disorganization in untreated first-episode of psychosis may relate to markers of oxidative stress and white matter damage though not with glutamate deficits as indexed using MRS.

Our observation of reduced white matter integrity of cingulum in patients with high conceptual disorganization is consistent with Bopp and colleagues who systematically studied white matter integrity in relation to persistent disorganization⁷. Reduced white matter integrity in the cingulum is a well replicated finding in psychotic disorders^{22, 50 51, 52}, though heterogeneous changes are often reported at the whole brain level⁵³ (ref). . Cingulum is a large body of fiber that connects multiple midlines brain regions that play a crucial role in self-related processing (precuneus, ventromedial prefrontal cortex, medial temporal cortex)⁵⁴. Individual differences in cingulum microstructure have been linked to cognitive control measures among healthy individuals⁵⁵ as well as in patients with schizophrenia^{56, 57}. Disrupted connectivity of the cingulum reported here supports the notion that the severity of CD relates to disrupted domain-general control aspects of information processing in schizophrenia⁵⁸.

We found increased GSH level in patients with high degree of conceptual disorganization in acute FEP, despite the normal levels of glutamate in the anterior cingulate cortex. This association was specific to conceptual disorganization as neither negative symptom burden, nor the other positive symptom severity related to GSH concentration in this sample. In an overlapping sample we have previously noted that FEP patients with higher GSH are likely to respond faster to treatment, despite the higher symptom burden. Interestingly, among adolescents at risk for psychosis, increased GSH levels in the anterior cingulate cortex has been linked to higher degree of disorganization⁵⁹. Taken together these results suggest that an acute oxidative stress response associated with disrupted WM integrity may contribute to CD in early stages of psychosis, but higher GSH may indicate an intact antioxidant response mounted to this neuter the oxidative stress. Patients who mount this GSH response may fare better in terms of treatment response in due course, while those with persistent illness profile may show a significant, albeit small effect-size reduction in GSH^{60,61}. An alternative possibility is that higher GSH itself may be an index of excitotoxicity, as GSH is known to act as a reservoir for glutamate when normal routes of glutamate synthesis are impaired⁶². A reduction in Glu/GSH ratio may indicate acute astrogliosis and/or rarefication of glutamatergic synapses. While these conflicting hypotheses cannot be resolved with our current data, pharmaco-fMRI studies manipulating GSH levels will be critical for future insights.

Our study has notable strengths including the use of highly resolved 7T spectroscopy that allowed concurrent GSH and glutamate quantification, combined DTI-MRS protocol, as well as the recruitment of minimally medicated FEP subjects. Several limitations must be considered as well. Firstly, as we optimized our acquisition for concurrent measurement of glutamate and glutathione, glutamine spectra were estimated with higher uncertainty (See Supplement).. Secondly, we relied on a single voxel placed at the dorsal anterior cingulate cortex, in line with other 7T MRS studies in psychosis^{63,64}; thus, the status of GSH and glutamate in relation to CD may differ in other brain regions. We also restricted our DTI analysis to cingulum given the prior work of Bopp and colleagues et al⁷. A TBSS analysis across the entire white matter skeleton (reported in the supplement) did not reveal any significant group differences. While we had sufficient power to detect a group difference between high and low-P2 groups in a hypothesis driven search restricted to a single tract, our sample is likely of insufficient size for a whole brain mass univariate search with necessary corrections for multiple testing.

In summary, the neural correlates of acute conceptual disorganization likely relate to cortical oxidative stress and disrupted white matter integrity. Given the functional importance of persistent disorganization, we call for clinical trials of interventions to restore redox imbalance focused on addressing conceptual disorganization in psychosis.

Author contributions

PY performed MRI processing, statistical analyses and took the lead in writing the first draft of the manuscript. AK and JT developed imaging protocol and assisted in data acquisition. MM and KD collected the clinical data. PJ assisted in MRS data acquisition and analysis, supervised by JT. LP designed and coordinated the project; supervised PY, KD, PJ and MM. All authors provided critical feedback and helped shape the interpretation and approved the final version of this manuscript.

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Conflict of Interest

The authors have declared that there are no conflicts of interest in relation to the subject of this study.

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<u>S1.SUPPLEMENTARY FIGURE</u>	<u>1</u>
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S1.Supplementary Figure

Fiber tracking: The figure below displays the DTI images after preprocessing of one subject in our data and the result of probabilistic fiber tracking. This process was performed by bedpost and probtrack based on FSL. Panel A: Overlay of fitted tensor image on a single subject's native space. Panel B: Probabilistic fiber tracking from the seed region to the rest of the brain in a single subject; the fibers tracked in the native space were transformed to a standard normal space for further group comparisons. The fibers are overlaid on a T1 weighted image template using FSL.

A

B

S2.Interpretation of MRS glutamate and glutamine concentration

Glutamate MRS signal is a composite of both neuronal metabolic as well as synaptic transmitter pool; synaptic activity triggers a redistribution from metabolic pool to transmitter pool, but this redistribution is not expected to change the measured glutamate concentration in MRS, as MRS cannot resolve cellular compartmental differences^{1, 2}. Thus, in the absence of an activation paradigm (task or pharmacological), the individual differences in static MRS glutamate signal may reflect the variations in intracellular glutamate synthesis (i.e. neuronal metabolism via glycolysis or glutamine cycle) or the amount of synaptic glutamate relative to vesicular glutamate (i.e. both synaptic release and the integrity of glial clearance).

Glutamine content in astrocytes is tightly linked to the synaptic glutamate concentration that triggers glutaminase activity³. Synaptic glutamate is considered to be the source of 80-90% of the substrate pool for glutamine⁴. Thus glutamine concentration is often taken to reflect synaptic glutamatergic activity in MRS studies of psychiatric disorders⁵⁻⁹. Nevertheless, 7T MRS studies in schizophrenia fail to observe changes in glutamine, despite a reduction in glutamate signal in patients when compared to controls^{5, 10-13}. Thus, the appropriateness of MRS glutamine as an index for measuring the glutamatergic aberration in schizophrenia is unclear.

Experimental cortical injury in rodents results in a rapid increase in the MRS measures of glutamine but a decrease in glutamate. But the increase in glutamine is limited to immediate post-injury phase, despite the persistent reduction in glutamate for many subsequent days¹⁴. Chronic ketamine exposure model of schizophrenia is associated with glutamate, but not glutamine excess in vivo¹⁵. In hippocampal tissue slices, synaptic glutamatergic transmission continues long after withdrawing the glutamine supply from astrocytes¹⁶; thus, glutamine concentration dissociates from synaptic glutamatergic activity when the glial integrity is compromised, as suspected in schizophrenia¹⁷. Furthermore, glutamine has notable blood-brain barrier permeability; MRS glutamine signal correlates highly with plasma glutamine, adding a further source of variability that does not affect MRS glutamate signals¹⁸. These

observations indicate that while MRS measures of glutamine could be a valid index of acute synaptic glutamatergic surge, with sustained disruption in glutamatergic tone expected in pathological states, concomitant changes in glutamine cannot be expected. In the context of issues stated above, we primarily focused on MRS glutamate signal to test our hypothesis of glutamatergic excess relating to CD and cingulum integrity in schizophrenia. Nevertheless we report the results for MRS glutamine as well in this supplement.

Supplementary Table. 1 Differences in MRS Glutamine

Measure	Group			Group Comparison	
	High P2	Low P2	Healthy controls	F / T / χ^2	p – value
	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)		
Number of subjects	16	24	25		
Glutamine	1.17(0.38)	1.03(0.37)	1.12(0.28)	0.657	0.523
Glutamine/Glutamate	0.18(0.06)	0.15(0.05)	0.17(0.03)	1.211	0.307
CRLB Glutamine	23.01(22.54)	25.10(13.32)	18.07(5.83)	1.251	0.296

*p<0.05 uncorrected

Supplementary Table.2 Correlation between glutamine and DTI metrics in the cingulum cluster.

	In all samples		In HC		In low P2		In high P2	
	Pearson correlation	P value	Pearson correlation	P value	Pearson correlation	P value	Pearson correlation	P value
FA	-0.163	0.198	0.082	0.696	-0.241	0.257	-0.178	0.525
RD	-0.029	0.822	-0.202	0.333	0.121	0.572	-0.170	0.544

AD	-0.293	0.019*	-0.196	0.347	-0.283	0.180	-0.410	0.129
MO	-0.160	0.206	0.073	0.731	-0.217	0.308	-0.301	0.138

S4. Other metabolites measured using 7T-MRS

Supplementary Table 3		Mean Metabolite Concentration and CRLB			
		[HC]	CRLB _{HC}	[FES]	CRLB _{FES}
NAA		10.29 (2.23)	1.01 (0.48)	10.69 (1.61)	1.23 (0.89)
NAAG		0.03 (0.09)	9.15 (29.16)	0.07 (0.17)	17.40 (49.68)
Creatine		8.43 (1.67)	1.26 (0.28)	8.99 (1.48)	1.33 (0.37)
Choline		2.40 (0.53)	1.82 (0.50)	2.49 (0.48)	1.91 (0.58)
Myo-inositol		4.60 (1.17)	4.05 (1.17)	4.62 (1.21)	4.23 (1.38)
Glucose		0.29 (0.35)	20.33 (24.87)	0.48 (0.39)	32.26 (23.60)
Scyllo-inositol		0.26 (0.12)	23.83 (12.55)	0.38 (0.15)	17.51 (7.09)
Taurine		1.06 (0.41)	26.06 (9.92)	1.31 (0.57)	24.94 (10.92)
Glutamate		6.53 (1.46)	3.30 (0.96)	6.85 (1.22)	3.55 (1.08)
Glutamine		1.02 (0.39)	23.08 (16.28)	1.09 (0.40)	23.97 (16.70)
Glutathione		1.49 (0.34)	10.25 (3.87)	1.68 (0.35)	10.43 (3.70)
CRLB Cramer-Rao Lower Bound, FUP follow-up, NAA N-acetyl aspartate, NAAG N-acetyl aspartyl glutamate. Note: Mean (SD) concentration and CRLB units are measured in mM					

and %, respectively.

S4.The methods and results of whole brain TBSS

After registration, a mean FA skeleton representing the centers of all tracts was created, which is commonly applied to all subjects (threshold of 0.2) ¹⁹. After that, each subject's aligned FA was projected onto this skeleton and fed into voxel-wise comparison. The statistical analyses employed the voxel-wise general linear model (GLM) and significant clusters were formed by employing the TFCE method to correct for multiple comparisons ²⁰, implemented in FSL randomize ²¹. P-values were determined using 5000 random permutations ²¹. The results are considered significant at $p < 0.05$, corrected for multiple comparisons using TFCE. A TBSS analysis across the entire white matter skeleton did not reveal any significant group differences. Figure below is a display of the uncorrected results ($p < 0.05$), in which there was a difference among three groups in the regions shown in red.

Supplementary Figure.2 The F-test results of analysis across the entire white matter skeleton among three groups.

Supplementary Table 4		The labels of clusters with significant difference in whole white matter skeleton voxel-wised analysis			
Regions	Voxels	P	COG X (mm)	COG Y (mm)	COG Z (mm)
Corticospinal tract R	264	0.008	12.9	-20.9	61.6
Forceps minor	202	0.008	-14.3	41.8	30
Forceps major	159	0.006	22.4	-48.7	0.488
Inferior fronto-occipital fasciculus R	141	0.014	39.3	-31.8	-2.79
Superior longitudinal fasciculus R	115	0.026	31.9	1.95	8.87
Inferior fronto-occipital fasciculus R	107	0.018	32.2	40.7	6.74
Uncinate fasciculus R	85	0.018	14.9	16.7	-8.06
Anterior thalamic radiation R	78	0.024	23.7	33.4	12.5
No label	75	0.008	41.1	-69.6	-5.39
Inferior longitudinal fasciculus R	60	0.01	34.7	-81.3	-6.56

Cingulum (hippocampus) L	50	0.02	-23.4	-26.5	-19.7
Superior longitudinal fasciculus (temporal part) R	48	0.016	48.6	-52.2	13
No label	47	0.012	16.8	25.8	-18.6
Inferior longitudinal fasciculus R	42	0.024	13.1	-72.9	4.29
Cingulum (hippocampus) L	42	0.012	-24.6	-13.9	-30.2
Inferior fronto-occipital fasciculus R	41	0.016	41.7	24.1	15.9
Inferior longitudinal fasciculus L	41	0.02	-37.4	-62.3	-10.9
No label	39	0.018	-16.8	-63.7	-37.9
No label	37	0.028	19.6	-8.09	-9.81
Inferior fronto-occipital fasciculus R	30	0.038	30.6	34.7	4
Inferior longitudinal fasciculus R	25	0.012	29.1	-4.23	-17.2
No label	21	0.038	26.7	-20.4	2.33
Cingulum (cingulate gyrus) L	20	0.036	-7.8	-2.45	33.3
Corticospinal tract R	18	0.008	17.3	-14.6	64.2
No label	18	0.034	30.9	22.1	38.2
Inferior longitudinal fasciculus L	18	0.024	-29	5.39	-34.4
Inferior longitudinal fasciculus R	15	0.032	25.6	-23.4	-0.201
Inferior longitudinal fasciculus R	15	0.028	36.6	-79.1	-1.47
No label	14	0.022	-43.1	-61.9	13.9
No label	13	0.036	11.4	-51.3	50.7
Forceps major	13	0.038	17.8	-94.9	-1.08

Superior longitudinal fasciculus R	13	0.04	51.5	-52.6	-7.08
Uncinate fasciculus R	13	0.034	23.2	17.7	-11.6
Inferior longitudinal fasciculus R	12	0.014	40.8	-59.7	-4.08
No label	12	0.032	-7.5	38.6	-18.5
No label	11	0.018	27.1	-2.55	-14
Cingulum (hippocampus) L	11	0.044	-25.1	-21.9	-25
No label	11	0.034	-15.8	-65.8	-32.3
Anterior thalamic radiation R	10	0.026	15.9	-31.4	6.9
Inferior fronto-occipital fasciculus R	10	0.042	33.8	-27	3.1
Note: This table only showed the labels of clusters with voxels no less than 10					

S5.Principal component analyses for PANSS-8 items

According to reviewer's suggestion, we did principal component analyses for PANSS-8 items (P1, P2, P3, G5, G9, N1, N4 and N6) with Varimax rotation in all patients. The result showed 2 components. The first component (take 33.12% of variance) included P3, G5, N1, N4 and N6, the second one (take 30.23% of variance) contained P1, P2, G5 and G9.

According to component 2, we split patients into 2 groups using median value as cut-off. After that, F-test was conducted to find the difference in metabolic values between low burden group, high burden group and healthy controls. And we also split patients into 2 groups separately according to the median values of P1, P2, G5 and G9 value, and then did the F-test. The results were exhibited in Table.1.

Table.1

Group by	N of Low Burden Group	N of High Burden Group	Glutamate		GSH		Glutathione	
			F value	P value	F value	P value	F value	P value
Component 2 Split	20	20	0.161	0.852	40.341	0.019	0.259	0.773
P2 Split	24	16	0.267	0.767	30.894	0.027	0.657	0.523
P1 Split	24	16	10.478	0.238	30.053	0.057	0.218	0.805
G5 Split	29	11	0.285	0.754	20.129	0.130	0.108	0.898
G9 Split	20	20	0.133	0.876	20.714	0.077	0.830	0.442

S6.The association between thought and language index (TLI) and AD, glutamate metabolism

We assessed thought and language index (TLI)²². Then we did the spearman correlation between TLI and AD, glutamate metabolism, as below table. The results showed TLI was significant correlated with AD and GSH.

	AD		Glutamate		GSH		Glutamine	
	CC	P	CC	P	CC	P	CC	P
TLI_IMPOV	-0.336	0.060	0.454	0.023	0.413	0.040	0.006	0.979
TLI_DISORGO	-0.326	0.068	0.251	0.225	0.294	0.154	0.125	0.552
TLI_Total	-0.463	0.009	0.322	0.125	0.506	0.012	0.145	0.499

Note: CC: Correlation Coefficient.

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