Appendix 1 to Yunzhi P, Dempster K, Jeon P et al. Acute conceptual disorganization in untreated first-episode psychosis: a combined magnetic resonance spectroscopy and diffusion imaging study of the cingulum. *J Psychiatry Neurosci* 2021.

doi: 10.1503/jpn.200167

S1. Justification for the use of PANSS measure of Disorganization

In our study, we chose to use CD rather than thought and language index (TLI)[1] for the following reasons:

1. As we recruited untreated acutely psychotic patients, we did not collect the full TLI but opted for a shorter version with 3 minutes of speech sample. Neither the full not the shorter version is validated in acute FES to date while PANSS, especially P2, has been used in numerous treatment trials in samples with acute psychosis.
2. Based on a detailed analysis of elements of speech from the 3-minute speech sample using CohMetrix (a corpus-based linguistic analysis program), we have demonstrated in the same dataset that PANSS P2 score for CD is a valid marker of poorly connected speech[2].

Nevertheless, to ensure that our findings are sensitive to various instruments, we present the correlation between the TLI data from 3 pictures (short version) and AD, glutamate metabolism, in the Supplementary Table.1 below. These results reaffirm the relationship between total TLI scores, indexing formal thought disorder, and lower cingulum AD and higher GSH, in line with our findings based on PANSS P2 scores.

Appendix 1 to Yunzhi P, Dempster K, Jeon P et al. Acute conceptual disorganization in untreated first-episode psychosis: a combined magnetic resonance spectroscopy and diffusion imaging study of the cingulum. *J Psychiatry Neurosci* 2021.

doi: 10.1503/jpn.200167

Supplementary Table.1

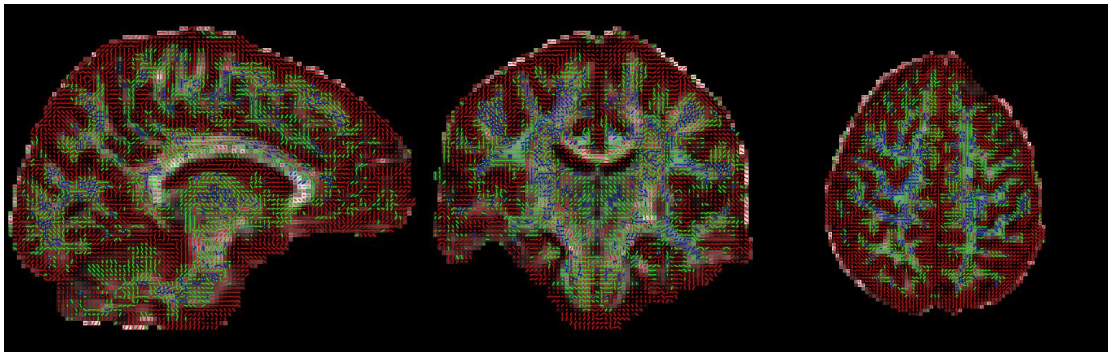
	AD		Glutamate		GSH		Glutamine	
	CC	P	CC	P	CC	P	CC	P
TLI Impoverished thinking subscale	-0.336	0.060	0.454	**0.023**	0.413	**0.040**	0.006	0.979
TLI Disorganized thinking subscale	-0.326	0.068	0.251	0.225	0.294	0.154	0.125	0.552
TLI Total score	-0.463	**0.009**	0.322	0.125	0.506	**0.012**	0.145	0.499

Note: CC: Spearman's Correlation Coefficient.

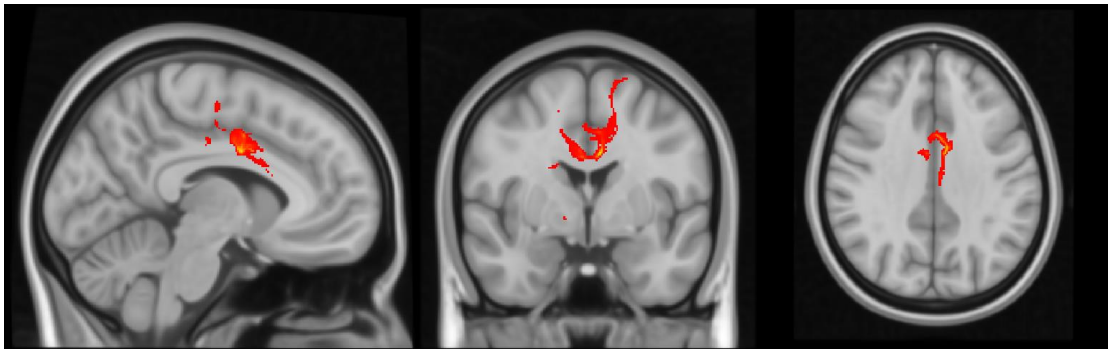
S2. Fiber Track Sketch Map

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



Appendix 1 to Yunzhi P, Dempster K, Jeon P et al. Acute conceptual disorganization in untreated first-episode psychosis: a combined magnetic resonance spectroscopy and diffusion imaging study of the cingulum. *J Psychiatry Neurosci* 2021.

doi: 10.1503/jpn.200167

S3. 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[3, 4]. 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 activity [5]. Synaptic glutamate is considered to be the source of 80-90% of the substrate pool for glutamine [6]. Thus glutamine concentration is often taken to reflect synaptic glutamatergic activity in MRS studies of psychiatric disorders [7-11]. Nevertheless, 7T MRS studies in schizophrenia fail to observe changes in glutamine, despite a reduction in glutamate signal in patients when compared to controls [7, 12-15]. 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 days [16]. Chronic ketamine exposure model of schizophrenia is associated with glutamate, but not glutamine excess in vivo [17]. In hippocampal tissue slices, synaptic glutamatergic transmission continues long after withdrawing the glutamine supply from astrocytes [18] ; thus, glutamine concentration dissociates from synaptic glutamatergic activity when the glial integrity is compromised, as suspected in schizophrenia [19]. 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 signals [20]. These observations indicate that while MRS measures of glutamine could be a valid index of

Appendix 1 to Yunzhi P, Dempster K, Jeon P et al. Acute conceptual disorganization in untreated first-episode psychosis: a combined magnetic resonance spectroscopy and diffusion imaging study of the cingulum. *J Psychiatry Neurosci* 2021.

doi: 10.1503/jpn.200167

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.2 Differences in MRS Glutamine

Measure	Group			Group Comparison	
	High P2	**Low P2**	Healthy controls	F / T / χ2	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.3 Correlation between glutamine and DTI metrics in the cingulum cluster.

	In all samples		In HC		In low P2		In high P2	
	Pearson	P value	Pearson	P value	Pearson	P value	Pearson	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 4	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)

Appendix 1 to Yunzhi P, Dempster K, Jeon P et al. Acute conceptual disorganization in untreated first-episode psychosis: a combined magnetic resonance spectroscopy and diffusion imaging study of the cingulum. *J Psychiatry Neurosci* 2021.

doi: 10.1503/jpn.200167

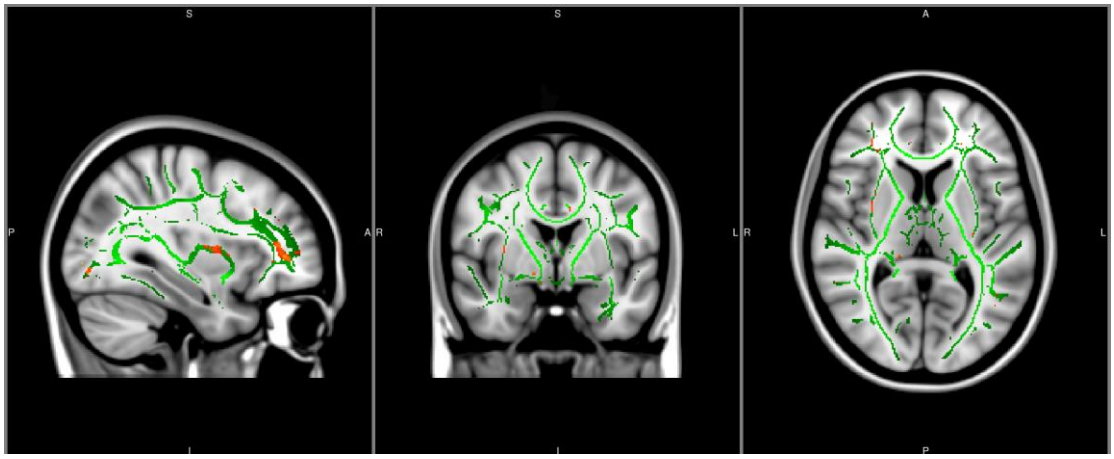
NAAG	0.03 (0.09)	9.15 (29.16)	0.07 (0.17)	17.40 (49.68)
Creatine	8.43 (1.67)	1.26 (0.28)	8.99 (1.48)	1.33 (0.37)
Choline	2.40 (0.53)	1.82 (0.50)	2.49 (0.48)	1.91 (0.58)
Myo-inositol	4.60 (1.17)	4.05 (1.17)	4.62 (1.21)	4.23 (1.38)
Glucose	0.29 (0.35)	20.33 (24.87)	0.48 (0.39)	32.26 (23.60)
Scyllo-inositol	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				

S5. The methods and results of whole brain TBSS

After registration, a mean FA skeleton representing the centers of all tracts was created and applied to all subjects (threshold of 0.2) [21]. 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 [22], implemented in FSL randomize [23]. P-values were determined using 5000 random permutations [23]. The results were 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 ($p<0.05$); regions with a difference among three groups are shown in red. Supplementary Table 5 provides the anatomical details.

Appendix 1 to Yunzhi P, Dempster K, Jeon P et al. Acute conceptual disorganization in untreated first-episode psychosis: a combined magnetic resonance spectroscopy and diffusion imaging study of the cingulum. *J Psychiatry Neurosci* 2021.

doi: 10.1503/jpn.200167



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

Supplementary Table 5	The labels of clusters with significant difference in				
Regions	Voxels	P	COG X	COG Y	COG Z
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	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

Appendix 1 to Yunzhi P, Dempster K, Jeon P et al. Acute conceptual disorganization in untreated first-episode psychosis: a combined magnetic resonance spectroscopy and diffusion imaging study of the cingulum. *J Psychiatry Neurosci* 2021.

doi: 10.1503/jpn.200167

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 shows the labels of clusters with voxels no less than 10					

Appendix 1 to Yunzhi P, Dempster K, Jeon P et al. Acute conceptual disorganization in untreated first-episode psychosis: a combined magnetic resonance spectroscopy and diffusion imaging study of the cingulum. *J Psychiatry Neurosci* 2021.

doi: 10.1503/jpn.200167

S6. Principal component analyses for PANSS-8 items

To ensure the links with FA and GSH are specific to CD and not other symptoms, we have now carried out further analyses. Firstly, we derived principal components from PANSS-8 items (P1, P2, P3, G5, G9, N1, N4 and N6) with varimax rotation from all patients. The result showed 2 components. The first component (explaining 33.12% of variance) loaded on N1, N4 and N6, P3 and G5. The second component (explaining 30.23% of variance) loaded on P1, P2, G5 and G9. We conducted a series of ANOVA based on component 2 (patients split into 2 groups using the median loading coefficient value as the cut-off) as well as individual median values of P1, P2, G5 and G9. The results are shown in Supplementary Table 6 below.

All of the differences due to the composite symptom burden were driven by P2 scores, and not by any other symptom that co-varied with P2 in this sample. Finally, 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).

Supplementary Table 6

			Glutamate		GSH		Glutamine	
Group by	N of Low Burden Group	N of High Burden Group	F-value	P-value	F-value	P-value	F-value	P-value
PCA composite symptom factor*	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

*This factor explained 30.2% of total symptom variance and loaded on P1, P2, G5 and G9.

Appendix 1 to Yunzhi P, Dempster K, Jeon P et al. Acute conceptual disorganization in untreated first-episode psychosis: a combined magnetic resonance spectroscopy and diffusion imaging study of the cingulum. *J Psychiatry Neurosci* 2021.

doi: 10.1503/jpn.200167

S7. Rationale for Dichotomous Approach to CD

Rather than simply correlating patients' symptom severity scores with the MR measures, we split the patient group on the basis of the presence or absence of the key symptom at a clinically meaningful level (CD). The correlational approach involves assuming that the symptom severity is a ratio variable (i.e., the distance, in 'symptom space', between a score of 0 (no CD) and 1 (definite CD) is the same as the difference between a score of, say, 3 and 4. Symptom severity is also more influenced by short-term medication exposure than the dichotomous approach.

Patients who scored 1, 2 or 3 points on individual PANSS items were categorized to have low severity of the specific symptom (e.g. low P2), compared to those who scored 4, 5, 6 or 7 (high P2). We chose this cut-off to distinguish those who have clinically severe vs. minimal symptoms recommended by the Remission Working Group[24]. According to Andreasen et al., "with regard to severity, the working group consensus defined a score of mild or less (Positive and Negative Syndrome Scale item scores of $\leq$3) as representative of an impairment level consistent with symptomatic remission of illness". While the remission criterion, strictly speaking, is not applicable in during the acute episode, we use this cut-off to identify the scores that are likely to be of low clinical intensity "where such absent, borderline, or mild symptoms do not influence an individual's behavior"[24]. With respect to P2 item, this cut-off has also been recently employed to distinguish patients with or without formal thought disorder and language dysfunction[25]. Elsewhere, we have shown this cut-off to meaningfully map on to linguistic analysis of speech samples as well as brief assessment using Thought and Language Index[2].

Note that Andreasen et al., do not explicitly advocate for dichotomizing P2 scores. But they offer a principled approach to convert the unequal, ordinal intervals of PANSS to the categories of high and low clinical burden, which we employ in this study.

Reference

Appendix 1 to Yunzhi P, Dempster K, Jeon P et al. Acute conceptual disorganization in untreated first-episode psychosis: a combined magnetic resonance spectroscopy and diffusion imaging study of the cingulum. *J Psychiatry Neurosci* 2021.

doi: 10.1503/jpn.200167

---

1. Liddle P, Ngan E, Caissie S, Anderson C, Bates A, Quested D, White R, Weg R. Thought and Language Index: an instrument for assessing thought and language in schizophrenia. *The British journal of psychiatry : the journal of mental science* 2002;181:326-330.

2. Mackinley M, Chan J, Ke H, Dempster K, Palaniyappan L. Linguistic determinants of formal thought disorder in first episode psychosis. *Early intervention in psychiatry* Mar 3 2020.

3. Schousboe A, Scafidi S, Bak LK, Waagepetersen HS, McKenna MC. Glutamate metabolism in the brain focusing on astrocytes. *Advances in neurobiology* 2014;11:13-30.

4. Bak LK, Schousboe A, Waagepetersen HS. The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. *J Neurochem* Aug 2006;98(3):641-653.

5. Rothman DL, Behar KL, Hyder F, Shulman RG. In vivo NMR studies of the glutamate neurotransmitter flux and neuroenergetics: Implications for brain function. *Annu Rev Physiol* 2003;65:401-427.

6. Kanamori K, Ross BD, Kondrat RW. Glial uptake of neurotransmitter glutamate from the extracellular fluid studied in vivo by microdialysis and C-13 NMR. *J Neurochem* Nov 2002;83(3):682-695.

7. Wang AM, Pradhan S, Coughlin JM, et al. Assessing Brain Metabolism With 7-T Proton Magnetic Resonance Spectroscopy in Patients With First-Episode Psychosis. *Jama Psychiat* Mar 2019;76(3):314-323.

8. Dempster K, Norman R, Theberge J, Densmore M, Schaefer B, Williamson P. Glutamatergic metabolite correlations with neuropsychological tests in first episode schizophrenia. *Psychiat Res-Neuroim* Aug 30 2015;233(2):180-185.

9. Bustillo JR, Chen HJ, Jones T, Lemke N, Abbott C, Qualls C, Canive J, Gasparovic C. Increased Glutamine in Patients Undergoing Long-term Treatment for Schizophrenia A Proton Magnetic Resonance Spectroscopy Study at 3 T. *Jama Psychiat* Mar 2014;71(3):265-272.

10. Theberge J, Al-Semaan Y, Williamson PC, et al. Glutamate and glutamine in the anterior cingulate and thalamus of medicated patients with chronic schizophrenia and healthy comparison subjects measured with 4.0-T proton MRS. *Am J Psychiat* Dec 2003;160(12):2231-2233.

11. Theberge J, Bartha R, Drost DJ, et al. Glutamate and glutamine measured with 4.0 T proton MRS in never-treated patients with schizophrenia and healthy volunteers. *Am J Psychiat* Nov 2002;159(11):1944-1946.

Appendix 1 to Yunzhi P, Dempster K, Jeon P et al. Acute conceptual disorganization in untreated first-episode psychosis: a combined magnetic resonance spectroscopy and diffusion imaging study of the cingulum. *J Psychiatry Neurosci* 2021.

doi: 10.1503/jpn.200167

---

12. Kumar J, Liddle EB, Fernandes CC, et al. Glutathione and glutamate in schizophrenia: a 7T MRS study. *Molecular Psychiatry* Apr 2020;25(4):873-882.

13. Reid MA, Salibi N, White DM, Gawne TJ, Denney TS, Lahti AC. 7T Proton Magnetic Resonance Spectroscopy of the Anterior Cingulate Cortex in First-Episode Schizophrenia. *Schizophrenia Bull* Jan 2019;45(1):180-189.

14. Thakkar KN, Roler L, Wijnen JP, Boer VO, Klomp DWJ, Cahn W, Kahn RS, Neggers SFW. 7T Proton Magnetic Resonance Spectroscopy of Gamma-Aminobutyric Acid,Glutamate,and Glutamine Reveals Altered Concentrations in Patients With Schizophrenia and Healthy Siblings. *Biol Psychiat* Mar 15 2017;81(6):525-535.

15. Brandt AS, Unschuld PG, Pradhan S, et al. Age-related changes in anterior cingulate cortex glutamate in schizophrenia: A H-1 MRS Study at 7 Tesla. *Schizophrenia research* Apr 2016;172(1-3):101-105.

16. Harris JL, Yeh HW, Choi IY, Lee P, Berman NE, Swerdlow RH, Craciunas SC, Brooks WM. Altered neurochemical profile after traumatic brain injury: H-1-MRS biomarkers of pathological mechanisms. *J Cerebr Blood F Met* Dec 2012;32(12):2122-2134.

17. Kim SY, Lee H, Kim HJ, et al. In vivo and ex vivo evidence for ketamine-induced hyperglutamatergic activity in the cerebral cortex of the rat: Potential relevance to schizophrenia. *Nmr Biomed* Dec 2011;24(10):1235-1242.

18. Kam K, Nicoll R. Excitatory synaptic transmission persists independently of the glutamate-glutamine cycle. *J Neurosci* Aug 22 2007;27(34):9192-9200.

19. Mei YY, Wu DC, Zhou N. Astrocytic Regulation of Glutamate Transmission in Schizophrenia. *Front Psychiatry* Nov 6 2018;9.

20. Takado Y, Sato N, Kanbe Y, et al. Association between Brain and Plasma Glutamine Levels in Healthy Young Subjects Investigated by MRS and LC/MS. *Nutrients* Jul 2019;11(7).

21. Liu YW, Spulber G, Lehtimaki KK, et al. Diffusion tensor imaging and Tract-Based Spatial Statistics in Alzheimer's disease and mild cognitive impairment. *Neurobiol Aging* Sep 2011;32(9):1558-1571.

22. Smith SM, Nichols TE. Threshold-free cluster enhancement: Addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage* Jan 1 2009;44(1):83-98.

23. Nichols TE, Holmes AP. Nonparametric permutation tests for functional neuroimaging: A primer with examples. *Human Brain Mapping* Jan 2002;15(1):1-25.

Appendix 1 to Yunzhi P, Dempster K, Jeon P et al. Acute conceptual disorganization in untreated first-episode psychosis: a combined magnetic resonance spectroscopy and diffusion imaging study of the cingulum. *J Psychiatry Neurosci* 2021.

doi: 10.1503/jpn.200167

---

24. Andreasen N, Carpenter W, Kane J, Lasser R, Marder S, Weinberger D. Remission in schizophrenia: proposed criteria and rationale for consensus. *The American journal of psychiatry* 2005;162(3):441-449.

25. Çokal D, Sevilla G, Jones W, et al. The language profile of formal thought disorder. *NPJ schizophrenia* 2018;4(1):18.