Comparison of schizophrenia and methamphetamine-induced psychosis: a proton magnetic resonance spectroscopy and cytokine study.

Antoinette Burger[1], Michael Lindner[2], Frances Robertson[1], Lauren Blake[1], Kimberley Williams[1], Petrus Naude[1], Henk Temmingh[1], and dan stein[1]

[1]University of Cape Town Faculty of Health Sciences
[2]University of Reading

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Abstract

Background: There is similarity in schizophrenia and methamphetamine-induced psychosis neurobiology. Few studies have directly compared neurometabolites in thalamo-cortical circuitry across these disorders or assessed the relationship with peripheral cytokines. This study compared neurometabolites and neuronal integrity in thalamo-cortical circuitry, and investigated associations with peripheral cytokine levels in both disorders. Methods: Ninety-five participants were recruited – 36 with schizophrenia, 27 with methamphetamine-induced psychosis, and 32 healthy controls. All participants underwent a magnetic resonance imaging scan, which included magnetic resonance spectroscopy. Glutamatergic and neuroinflammatory neurometabolites were examined. Serum cytokine concentrations included Interleukin 1-beta, Interleukin-8, Interleukin-10, Tumor Necrosis Factor alpha and Interferon gamma. Parametric data were analyzed with one-way analysis of variance and non-parametric data were analyzed with Kruskal Wallis tests. Associations were determined using Spearman's rank-order coefficient. Results: The methamphetamine-induced psychosis group had lower n-acetyl aspartate with n-acetyl-aspartyl glutamate in left dorsolateral prefrontal cortex and left frontal white matter, compared to controls. In schizophrenia, positive associations were found between glutamate and n-acetyl aspartate and n-acetyl aspartate with n-acetyl-aspartyl glutamate in the anterior cingulate cortex. In the methamphetamine-induced psychosis group, positive relationships were found between myo-inositol in the left thalamus and bilateral anterior cingulate cortex. Conclusion: In schizophrenia, there is suggestion of dysfunction in neuronal tissues in the glutamate-glutamine cycle within the thalamo-cortical circuit. In methamphetamine-induced psychosis, there is evidence of compromised neuronal integrity associated with chronic disease progression, and suggestion of aberrant neuroinflammatory regulation in the thalamus-ACC circuit. This study highlights similarities and differences in the psychobiology of the two disorders

Introduction

There are similarities in the phenomenology and psychobiology of schizophrenia (SZ) and methamphetamine (MA)-induced psychosis (MAP) , with evidence of alterations in glutamatergic function in both conditions , and of involvement of neuroinflammatory pathways in SZ and MA abuse . In SZ, positron emission tomography (PET) and single-photon emission computed tomography (SPECT) studies suggest hypofunction of N-methyl-D-aspartate receptors (NMDAR) , while ^{1}H-MRS studies of myo-inositol (mI), which may indicate neuroinflammation , are inconsistent . Studies of prefrontal glutamatergic function in MA abuse and MAP are inconsistent, with some reporting higher glutamate (Glu) or glutamate with glutamine (Glx) , others reporting lower Glx , and several reporting no glutamatergic metabolite changes . In MAP, higher concentration of prefrontal mI , suggestive of neuroinflammation have been reported. Cytokines have been proposed to be involved during psychotic states as IL-1β, TNF-α, IFN-γ, IL-8 and IL-10 are all reported to

be elevated during acute . IL-1β however is reported to return to normal concentrations outside of psychotic state, whereas TNF-α, IFN-γ, IL-8 remain elevated even in remission . It is proposed that IL-1β, TNF-α, IFN-γ can therefore reflect neuroinflammation, and subsequent disruption in thalamo-cortical circuitry. Pre-clinical studies and animal models of MA abuse, and post-mortem studies of SZ have shown that higher concentrations of IL-1β and IFN-γ have been associated with lower concentrations of NAA , while higher concentration of IL-8 has been associated with increased Glu .

Few studies have, however, directly compared glutamatergic (Glu, Gln and Glx) and neuroinflammatory (mI) neurometabolites and neuronal integrity markers (NAA and NAA+NAAG) in thalamo-cortical circuitry across SZ and MA abuse and none have assessed the relationship between these neurometabolites and peripheral inflammatory markers in either disorder aside from preclinical and post-mortem studies and animal models. The small literature on associations between neurometabolites and peripheral inflammatory markers has found an association between higher concentrations of TNF-α and IL-1β and lower NAA concentrations in active psychosis in SZ as well as MA abuse . Increased IL-8 concentrations have been associated with increased NMDAR in SZ and with withdrawal symptoms in MA abuse . Lower concentrations of interferon gamma (IFN-γ) and IL-10 were associated with decreased neuronal integrity in the prefrontal cortex and thalamus in SZ , and MA abuse . However, these associations have not been explored using a combination of neuroimaging and peripheral cytokine measures in living patients with SZ and MAP.

This study had two aims. First, to compare glutamatergic and neuroinflammatory neurometabolites and neuronal integrity in thalamo-cortical circuitry in SZ and MAP. It was hypothesized that glutamatergic neurometabolites would be increased in SZ compared to healthy controls, and that mI would be higher in SZ and MAP than in healthy controls, consistent with neuroinflammation. It was also hypothesized that NAA/NAA+NAAG concentrations would be lower in both SZ and MAP groups. Second, this study aimed to investigate associations between glutamatergic and neuroinflammatory neurometabolites, neuronal integrity markers and peripheral cytokine levels in both disorders. It was hypothesized that associations between neurometabolites, brain areas and peripheral cytokines would differ in SZ compared with MAP.

Materials and methods

Ninety-five (95) participants were recruited – 36 with schizophrenia, 27 with methamphetamine-induced psychosis, and 32 healthy controls (Table 1). All participants underwent the Diagnostic and Statistical Manual IV – Text Revision (DSM-IV-TR) Structured Clinical Interview For DSM-IV-TR Axis I Disorders (SCID) to assess eligibility. Additional clinical questionnaires were completed with all participants, which include the Positive and Negative Syndrome Scale (PANSS), Clinical Global Impressions scale (GGI), Global Assessment of Functioning scale (GAF) and a subjective questionnaire on drug abuse, the Kreek-McHugh-Schluger-Kellogg scale (KMSK). Participants were excluded during screening for chronic medical illnesses known to affect metabolic processes (e.g., hyper/hypo thyroidism, diabetes type I or II, etc.), illnesses where the immune system is dysfunctional or compromised (e.g., HIV, lupus), major brain trauma, and intellectual disability. Additionally, female participants were excluded if there was current or recent pregnancy, or if they were breastfeeding. The study was approved by the Human Research Ethics Committee, Faculty of Health Sciences of the University of Cape Town – HREC Reference Number: 062/2017 and was conducted in accordance with the Declaration of Helsinki .

Additional screening for MRI brain imaging for all participants included ensuring that participants were not claustrophobic or had any form of foreign material in their bodies which could interfere with the MRI scanning process.

Table 1

All participants underwent magnetic resonance imaging on a Siemens Skyra 3 Tesla scanner with a 32-channel head coil. A high-resolution Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) structural image was acquired and was used for placement of single-voxel spectroscopy (SVS) voxels located in the anterior cingulate cortex (ACC) (Figure 1) and left thalamus (Figure 1) as well as chemical-shift imaging (CSI) 2-dimensional voxel grid (Figure 2). Single voxel spectroscopy (SVS) of the ACC and left thalamus

were acquired for standard metabolites (PRESS, TE = 30 ms, TR = 2000 ms, 128 averages, delta = -2.6 ppm delta frequency, VOI 20 x 20 mm with a thickness of 15mm, scan time 4:40, with unsuppressed water MRS spectra for the same volume, two averages were acquired). An additional SVS sequence was acquired for the ACC, with parameters optimized for glutamate / glutamine separation (Schubert et al., 2004). The parameters used for this sequence were similar to that of the sequence for standard metabolites, except for the echo time, which was increased to 80 milliseconds (PRESS, TE = 80 ms, TR = 2000 ms, 128 averages, delta = -2.6 ppm delta frequency, VOI 20 x 20 mm with a thickness of 15mm, scan time 8:56, with unsuppressed water MRS spectra for the same volume, two averages were acquired). Partial volume correction was applied to SVS voxels to obtain absolute neurometabolite concentrations. This was achieved with combination of LCModel and MRSParVolCo software. The 2D CSI 1H-MRS slice was acquired (PRESS, TE = 30 ms, TR=2000 ms, Hamming filter, 2 averages, delta = -2.7 ppm delta frequency, weighted phase encoding, FOV = 256 × 256 mm, voxel size 10 × 8 mm, thickness 15 mm, automated CHESS water suppression, scan time 10:52). The slice was positioned to include, bilaterally, the dorsolateral prefrontal cortex (DLPFC), anterior cingulate cortex (ACC), frontal white matter (FWM) located between the DLPFC and ACC. Neurometabolites examined were glutamatergic neurometabolites (glutamate (Glu), glutamine (Gln) and glutamate with glutamine (Glx)), neuroinflammatory neurometabolite (myo-inositol (mI)) and neuronal integrity markers (n-acetyl-aspartate (NAA), and n-acetyl aspartate with n-acetyl-aspartyl glutamate (NAA+NAAG)). CSI neurometabolites are reported in relation to creatine with phosphocreatine (Cr+PCr).

Figure 1

Figure 1 – Placement of the anterior cingulate cortex and left thalamus single voxel spectroscopy voxels on the sagittal (A & D), coronal (B & E) and axial (C & F) planes.

Figure 2

Figure 2 - Representative 1H-MRS chemical shift imaging brain slice orientation on 3 planes (A – axial, B – coronal, C – sagittal) and LCModel spectra of VOIs which included: (D & G) bilateral dorsolateral prefrontal cortices (DLPFC), (E & H) bilateral frontal white matter (FWM) and (F & I) bilateral anterior cingulate cortices (ACC).

Bloods were collected via venepuncture into serum tubes that were kept a room temperature for 30 minutes to allow for clotting. The tubes were centrifuged, serum samples were collected into cryovials and immediately stored at -80 °C. The immune markers IL-1β, IL-8, IL-10, TNF-α and IFN-γ, were analyzed with a Milliplex® Luminex human cytokine magnetic bead panel (HSTCMAG28SK07; Merck) according to the manufacturer's instructions. Plates were assayed on a Luminex system (Bio-Plex 200 System: Bio-Rad). All samples were blinded and assayed in duplicate. The intra-assay coefficients of variation for each cytokine marker were < 14%.

For group differences, parametric data were analyzed with one-way analysis of variance and non-parametric data analyzed with Kruskal Wallis and Chi Square tests, with post-hoc testing using Fisher's Least Significant Difference for parametric data. Associations were determined using Spearman's rank-order coefficient. Significant associations were followed by correcting for multiple comparisons.

Results

No significant difference in neurometabolite concentrations was found between SZ and the healthy control groups. The MAP group had lower NAA+NAAG/Cr+PCr in left DLPFC ($p<0.05$) and left FWM ($p<0.05$), compared to healthy controls (Figure 3). There were no significant associations between neurometabolites, neuronal integrity markers and peripheral cytokines. In the SZ group positive associations were found between absolute glutamatergic and inflammatory neurometabolites in the anterior cingulate cortex (NAA and Glu ($P<0.0001$), NAA+NAAG and Glu ($P<0.001$), mI and Glu ($p<0.0001$), mI and Glx ($p<0.01$)) (Figure 4). The positive associations found between mI in the left thalamus and mI/Cr+PCr in bilateral ACC ($p<0.01$) in the MAP group, were significantly greater than these associations in healthy controls (Figure 4).

Figure 3

Figure 3 - The methamphetamine-induced psychosis group had lower absolute n-acetyl-aspartate with n-acetyl-aspartyl-glutamate (NAA+NAAG) in the A) left dorsolateral prefrontal cortex (DLPFC) ($p<0.05$) and B) left frontal white matter (FWM) ($p<0.05$) compared to the control group. Neurometabolite concentrations are reported as ratios to total creatine.

NAA – n-acetyl-aspartate; mI – myo-inositol; ACC – anterior cingulate cortex

Figure 4

Figure 4 - Positive associations were seen between glutamate in the anterior cingulate cortex (ACC) and A) n-acetyl-aspartate (NAA) ($p<0.0001$), B) n-acetyl-aspartate with n-acetyl-aspartyl-glutamate (NAA+NAAG) ($p<0.001$), and C) myo-inositol (mI) ($p<0.0001$) in the anterior cingulate cortex (ACC). D) Glutamate with glutamine (Glx) was positively associated with myo-inositol (mI) in the anterior cingulate cortex in the schizophrenia group ($p<0.01$). Positive associations were found in the methamphetamine (MA)-psychosis group between E) absolute thalamic myo-inositol (mI) and myo-inositol (mI) in the left anterior cingulate cortex ($p<0.01$), and F) myo-inositol (mI) in the right anterior cingulate cortex ($p<0.01$).

Discussion

The main findings of this study were that 1) there were no significant differences in neurometabolites in SZ compared to healthy controls, 2) the MAP group had lower NAA+NAAG/Cr+PCr than healthy controls in the left DLPFC and left FWM, 3) There were significant positive associations between glutamatergic neurometabolites and neuronal integrity markers in the ACC in the SZ group, and 4) there were significant positive associations between mI in the left thalamus and mI in bilateral ACC in the MAP group.

The lack of a significant differences between ^{1}H-MRS neurometabolite findings in SZ group and healthy controls could be attributed to the chronic use of antipsychotic medicine in these patients. Antipsychotic medicine has been shown to normalize neurometabolites over time and thus increase metabolic activity in the thalamus, reduce neuroinflammation and partially reverse NMDAR dysfunction in patients with SZ .

The finding of altered lower NAA+NAAG in left DLPFC and left FWM in MAP is consistent with previous studies of MA abuse and one study of MAP and could indicate compromised neuronal health, density and metabolism . Previous studies found lower NAA and NAA+NAAG in frontal brain areas in MA abuse , and the present work extends this finding by showing lower NAA+NAAG/Cr+PCr in the left DLPFC and left FWM in the MAP group, suggesting that neuronal integrity remains compromised over time.

The associations between glutamatergic neurometabolites and neuronal integrity markers in the ACC in SZ, but not in healthy controls, may suggest disruptions in the glutamate-glutamine cycle. Few studies on these associations have been done and much further work is needed to understand the relevant underlying causal mechanisms. The brain areas investigated in the present study form part of the thalamo-cortical circuitry and previous studies, limited to post-mortem , structural magnetic resonance imaging and imaging techniques to assess neurocognitive functioning (PET/SPECT) and fMRI, did not specifically investigate the thalamo-cortical circuit) . Two previous studies report positive relationships between NAA and Glu concentrations in healthy controls, but not in SZ patients . Considering that no individual neurometabolite differences for the SZ were found, it could be considered that the mechanism of dysfunction in SZ lies within associations between neurometabolites and not changes in specific standalone neurometabolites.

Stronger associations of mI between the left thalamus and bilateral ACC were also found in the MAP group, compared to healthy controls. Higher thalamic mI was positively associated with higher mI in the left and right ACC, suggesting neuroinflammation in the thalamus-ACC circuit in chronic MAP. MA has been shown to disrupt the thalamo-cortical circuit at the ventral striatum, within the ACC-thalamus circuit . This is supported by previous studies in MA abstinence where higher concentrations of mI have been reported . These are the first data on higher mI concentrations, suggestive of neuroinflammation, within the thalamus-ACC circuit in chronic MAP.

Several limitations deserve emphasis. First, use of antipsychotic medication use was obtained through re-

trospective self-report and may not be accurate. Second, studies of substance abuse are almost invariably confounded by selective disclosure and polysubstance use. Third, the effect of alcohol and nicotine could potentially have influenced neurometabolite concentrations, as participants did not cease their alcohol or nicotine use during the study.

Conclusion

In summary, this study is the first to report on neuroinflammatory neurometabolites in the thalamo-cortical circuitry in MAP, and also suggests disruption of the glutamate-glutamine cycle within brain areas in the thalamo-cortical circuitry in SZ. This study highlights similarities as well as differences in the psychobiology of SZ and MAP.

References

Table 1 - Demographic, drug use and clinical questionnaire data

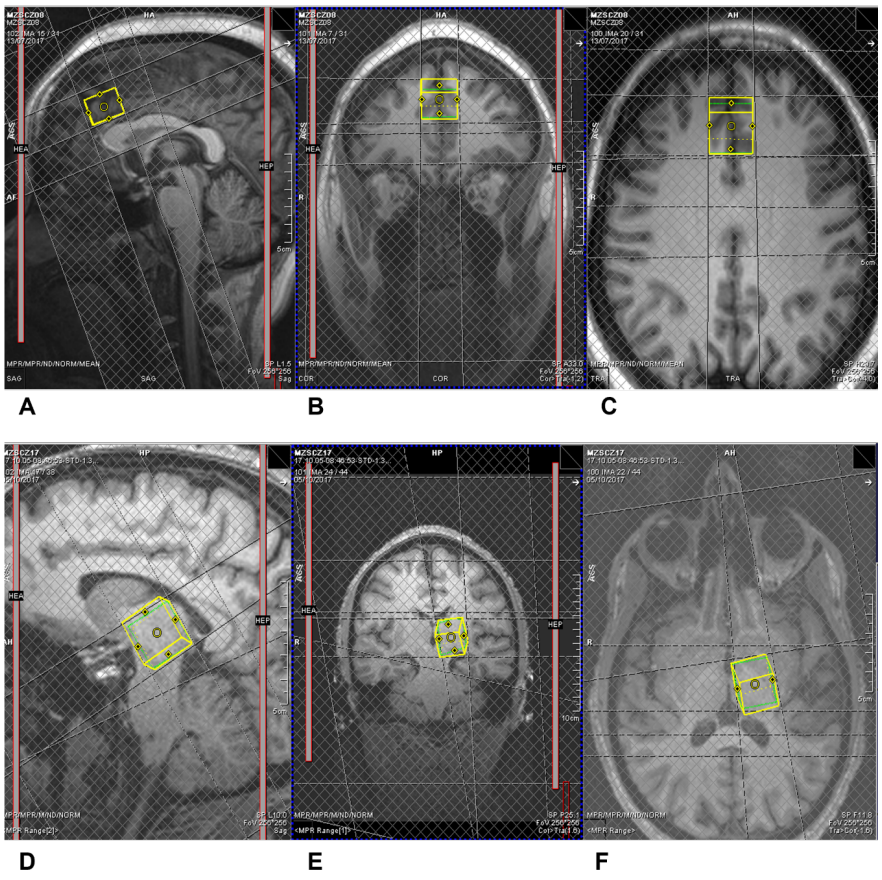
	Schizophrenia (SZ)	**Schizophrenia (SZ)**	**Methamphet**
	n=36	**n=36**	**n=27**
	Male n=28	**Female n=8**	**Male n=21**
	Mean	**StDev**	**Mean**
Demographic information			
Age	30.44	5.89	27.91
Years of education - school	10.04	1.99	9.18
Years of education - post school	0.27	0.90	0.53
Diagnosis and drug use information			
Duration of current diagnosis (years)	8.44	4.72	5.04
Number of psychotic episodes	3.65	1.62	2.32
Has used methamphetamine	14 of 39	14 of 39	31 of 31
Onset of methamphetamine use (age in years)	23.93	6.96	18.13*
Duration of methamphetamine use (years)	4.86	3.95	8.72#
Duration of abstinence from methamphetamine (months)	40.41	46.79	8.01*
Clinical scale scores			
PANSS positive scale score	12.36#	5.55	10.33#
PANSS negative scale score	14.82#	6.35	13.76#
PANSS general psychopathology scale score	22.27#	6.92	20.48#
PANSS total score	49.44#	15.85	44.94#
CGI-S score	3.19#	1.10	2.67#
GAF score	60.69	14.24	67.30
Kreek-McHugh-Schluger-Kellogg scale scores			
Alcohol lifetime - Total score	5.18	4.07	7.41
Tobacco lifetime - Total score	8.14	4.99	9.56
Cocaine lifetime - Total score	0.70	2.18	2.63*
Heroin lifetime score - Total score	0.05	0.32	1.59@
Cannabis lifetime - Total score	5.57	5.91	8.42**
Methamphetamine - Total score	3.46	4.47	9.82#

Table 1 - The control group had higher levels of school ($p<0.05$) and post school ($p<0.01$) education than the schizophrenia and methamphetamine-induced psychosis groups; the methamphetamine-induced psychosis group had earlier onset of methamphetamine use compared to the schizophrenia group ($p<0.05$), longer duration of methamphetamine use ($p<0.0001$) and shorter duration of abstinence from methamphetamine ($p<0.05$) than the schizophrenia and control groups; the schizophrenia and methamphetamine-induced psychosis groups scored higher than the control group on the PANSS positive scale score ($p<0.0001$), PANSS

negative scale score (p<0.0001), PANSS general psychopathology scale score (p<0.0001); PANSS total score (p<0.0001) and CGI-S score (p<0.0001); the control group scored higher than the methamphetamine-induced psychosis and schizophrenia groups on the GAF (p<0.0001); the methamphetamine-induced psychosis group scored higher than the schizophrenia and control groups on the KMSK Cocaine lifetime total score (p<0.05), Heroin lifetime total score (p<0.001), Cannabis lifetime total score (p<0.01), KMSK Methamphetamine total score (p<0.0001).

CGI-S - Clinical global impression of illness severity scale; KMSK - Kreek-McHugh-Schluger-Kellogg scale

** - p<0.05; ** - p<0.01; @ - p<0.001; # - p<0.0001*



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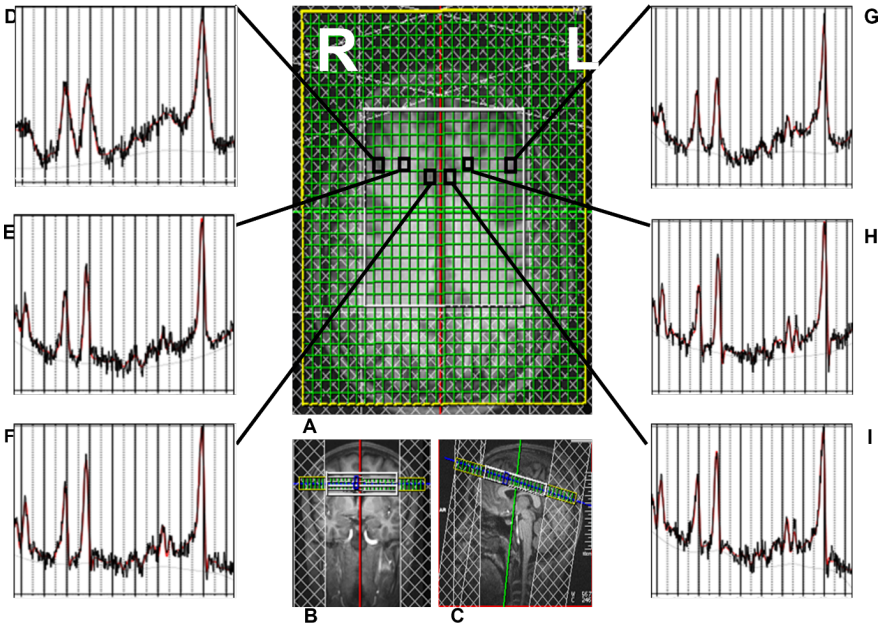
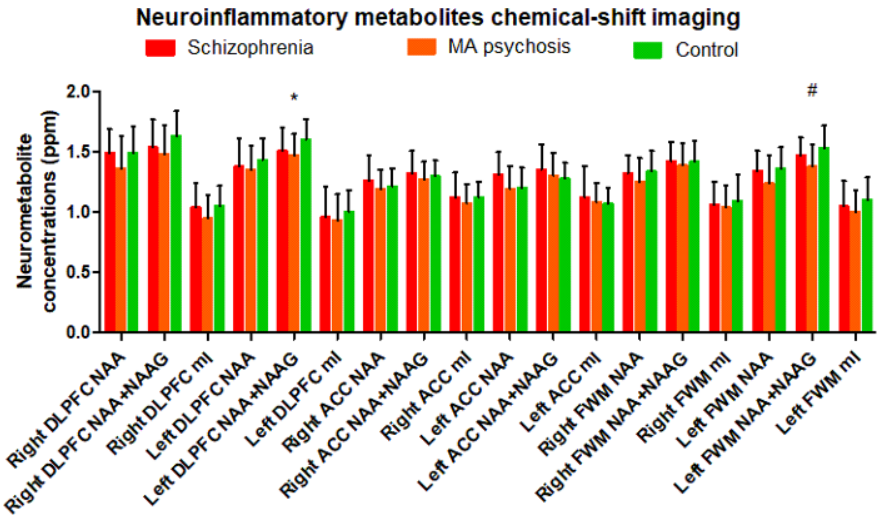


Figure 2 - Representative 1H-MRS chemical shift imaging brain slice orientation on 3 planes (A – axial, B – coronal, C – sagittal) and LCModel spectra of VOIs which included: (D & G) bilateral dorsolateral prefrontal cortices (DLPFC), (E & H) bilateral frontal white matter (FWM) and (F & I) bilateral anterior cingulate cortices (ACC).



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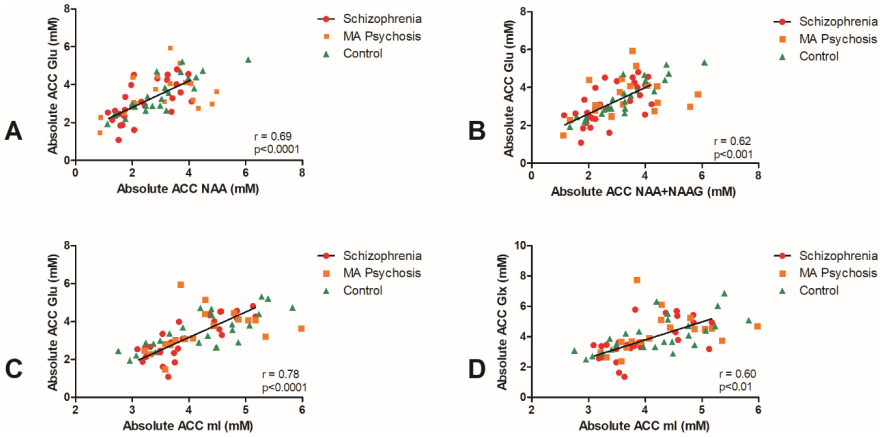

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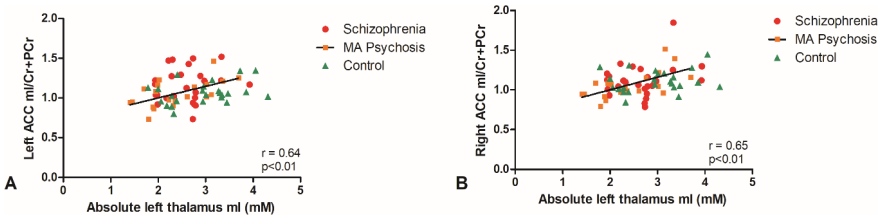

Figure 5 - Positive associations were found in the methamphetamine (MA)-psychosis group between 1) absolute thalamic myo-inositol (mI) and myo-inositol (mI) in the left anterior cingulate cortex ($p<0.01$), and b) myo-inositol (mI) in the right anterior cingulate cortex ($p<0.01$).

Graphical abstract

Recent research report glutamatergic dysfunction and involvement of inflammatory pathways in schizophrenia and methamphetamine-induced psychosis. Comparison between neurometabolites in thalamo-cortical circuitry across schizophrenia and methamphetamine-induced psychosis is limited, and no studies have assessed the relationship between neurometabolites and peripheral cytokines in living humans. Glutamatergic and neuroinflammatory neurometabolites and neuronal integrity markers in thalamo-cortical circuitry were measured and relationships were determined with peripheral cytokine levels in both disorders. Important differences in the neurobiology of schizophrenia and methamphetamine-induced psychosis were highlighted.

Data Accessibility Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.