SUPPLEMENTARY INFORMATION

Title: Relationship between cortical glutamatergic metabolite levels and hippocampal activity in schizotypy

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1. Materials and methods

1.1. Participants

The study was approved through the King's College London (KCL) College Research Ethics Committee system. All participants gave written informed consent to the study protocol.

Participants were recruited through their response to online advertisement (Research Volunteer Recruitment Webpage of KCL). Pre-screening of the 250 responders using the unusual experiences (UE) subscale of the Oxford and Liverpool Inventory of Feelings and Experiences (O-LIFE; Mason et al., 2005) resulted in 48 participants being entered into the study. This subscale was used for screening as it is associated with severity of positive symptoms in patients with schizophrenia (Cochrane et al., 2010). Individuals scoring greater than 7 on the UE subscale were recruited in the HS group, and individuals scoring less than 2 were recruited in the LS group, following previous imaging research in schizotypy (Premkumar et al., 2012). Participants were excluded if they had a personal history of neurologic/psychiatric disorders according to the Mini International Neuropsychiatric Inventory (Sheehan et al., 1997) as administered by a trained researcher. Other exclusion criteria included contraindications to MRI scanning, having a first-degree relative with present/past history of psychotic disorder, present/past history of use of psychotropic medications, and use of recreational drugs in the two weeks prior to scanning or meeting criteria for substance abuse/dependency by self-report. Four participants out of the initial 48

were excluded due to non-compliance with the inclusion criteria.

1.2. Questionnaire assessment

IQ was estimated using the Wechsler Adult Intelligence Scale-III short version (Velthorst et al., 2013). Medication history and use of tobacco, caffeine, alcohol, marijuana and illicit drugs were assessed using a semi-structured interview from the Early Psychosis Prevention and Intervention Centre (EPPIC; http://www.eppic.org.au).

1.3. ^{1}H-MRS acquisition and preprocessing

A proton MRS (^{1}H-MRS; PRESS, Point RESolved Spectroscopy) scan was acquired from the ACC on a Discovery MR750 3T system (General Electric, Chicago, USA) at the Institute of Psychiatry, Psychology & Neuroscience, KCL. The ^{1}H-MRS acquisition protocol was described in detail in our recent published reports from this sample (Modinos et al., 2017; Modinos et al., 2018a). Briefly, the region of interest (ROI) in the ACC was prescribed from the midline sagittal localizer, placing the center of the 20×20×20 mm ROI 13mm above the anterior section of the genu of corpus callosum at 90° to the anterior commissure – posterior commissure line. For voxel prescription and image registration, anatomical images were obtained using a T1-weighted inversion recovery prepared spoiled gradient echo sequence based on the well-validated ADNI 2/ADNI GO protocols (see http://adni.loni.usc.edu/methods/documents/mri-protocols/), with the following

parameters: slices=196, TR=7.3 ms, TE=3.0 ms, field of view=270 mm, inversion time=400 ms, and voxel size=1.05×1.05×1.2 mm^3.

MRS data preprocessing is also described in detail in our recent publications in this sample (Modinos et al., 2017; Modinos et al., 2018a). In brief, the LCModel version 6.3-1M (Provencher, 1993; http://sprovencher.com/pages/lcmodel.shtml) was used to estimate the metabolite concentrations. The Gannet version 3.1 (http://www.gabamrs.com) software was used to segment and estimate the percentage of grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) of the ACC ROI. Next, the segmented T1-weighted images were used for correction of partial volume cerebrospinal fluid contamination and to correct for the varying water contents of GM, WM and CSF. Water-scaled glutamate values were corrected using the formula:

$$metabolite\ corrected = \frac{\text{metabolite concentraton} * [\text{proportion WM} + (1.283 * \text{proportion GM}) + (1.548 * \text{proportion CSF})]}{(\text{proportion WM} + \text{proportion GM})}$$

We used Cramer-Rao minimum variance bounds (CRLB) > 20% as reported by LCModel, which are estimates of fit of the metabolite peaks, and signal-to-noise ratio (SNR) < 8 to exclude poorly fitted metabolite peaks from statistical analysis (Mouchlianitis et al. 2016; Provencher, 2016); only one HS participant exceeded the CRLB threshold and the final sample thus involved 21 HS participants and 22 LS

participants.

1.4. ASL acquisition and preprocessing

The ASL images were acquired during the same scanning session, after the ^{1}H-MRS scan, as described in our recently published study (Modinos et al., 2018b). In short, four pairs of control-labeled pseudo-continuous ASL images were acquired using a 3D Fast Spin Echo stack-of-spiral multi-shot readout sequence with the following parameters: TE=11.1 ms; TR=5500 ms; slices=60; slice thickness=3 mm; field of view=240; flip angle=90°. Arterial blood was labelled with a 1825ms train of Hanning shaped RF pulses, followed by a post-labelling delay of 2025ms. One non-selective and three selective inversion RF pulses were used for minimisation of the static background signal. A single proton density volume was also acquired within the same imaging series, using the same readout parameters. This volume was used to generate the quantitative CBF maps used for the study according to the formula suggested in the ASL consensus paper (Alsop et al., 2015).

ASL data preprocessing was as described in our recent study (Modinos et al., 2018b). In brief, automatic software for ASL processing toolbox version 2.0 (Mato Abad et al., 2016) and Statistical Parametric Mapping version 12 (SPM12; http://www.fil.ion.ucl.ac.uk/spm/) was used, employing the co-registration of the proton density image to the T1 anatomical scan, as an intermediate step to yield individual rCBF maps spatially normalized to the MNI T1 template. These normalised

maps were then smoothed with an 8-mm Gaussian kernel.

1.5. Statistical analysis

1.5.1. Demographic and questionnaire data

Demographic and questionnaire differences between the two groups were analyzed using independent samples t-tests for parametric data and Chi-square tests for non-parametric data in SPSS version 26 (Chicago, IL). The significance level was set at $p<0.05$, two tailed.

1.5.2. Relationship between ^{1}H-MRS glutamate and rCBF data

The relationship between glutamate levels in the ACC and rCBF in our ACC and hippocampal ROIs was examined by using the individual glutamate values as a covariate in an analysis of variance design with the rCBF maps from each group (HS, LS) using SPM12. We used small volume correction for our ROI approach, with a pre-defined anatomical mask created with the automated anatomical labeling atlas from the WFU_Pickatlas toolbox in SPM12 comprising the bilateral hippocampus and the ACC. This voxel-wise ANCOVA controlled for age, sex, cigarettes, caffeine and cannabis use. Mean global rCBF value was also included as a covariate to account for inter-individual differences in global perfusion (Modinos et al., 2018c). We used an initial cluster-defining threshold of $p<0.005$ uncorrected, then only considered significant effects which survived a voxel-wise family-wise error (FWE) correction for multiple comparisons of $p<0.05$. For completeness, exploratory voxel-wise whole

brain interactions were also assessed at a voxel-wise p<.05 FWE threshold.

1.5.3. Relationship between ^{1}H-MRS Glx and rCBF data

We used the individual Glx concentrations as a regressor in a voxel-wise ROI approach in SPM12, in the same way as reported above for glutamate, with the same covariates of no interest and significant thresholds. For completeness, exploratory voxel-wise whole brain effects were also assessed at a voxel-wise p<.05 FWE threshold.

1.5.4. Relationship between rCBF data and schizotypy severity levels

Within the HS group, we explored the relationship between rCBF in our ROIs (hippocampus and ACC, as described above) and levels of subclinical psychotic-like experiences using the O-LIFE total score as regressor in a voxel-wise multiple regression analysis, using the same covariates of no interest and correction for multiple comparisons as described above. For completeness, complementary analyses included (i) voxel-wise whole brain associations and (ii) hippocampal rCBF associations with O-LIFE subscale scores (UE, cognitive disorganization, introvertive anhedonia, and impulsive nonconformity), all reported at a voxel-wise p<.05 FWE threshold.

2. Results

2.1. Demographic and questionnaire data

These results are presented in Table S1.

Table S1. Demographic and questionnaire information for the high and low schizotypy groups.

	HS (n = 21)	LS (n = 22)	χ^2/t	*p*
Gender (% Female)	42.9%	50%	*0.22*	*0.76*
Age (years)	26.52 (6.93)	26.32 (5.20)	*0.11*	*0.91*
Ethnicity (% Caucasian)	59.1%	76.2%	*4.29*	*0.23*
Estimated IQ	119.29 (16.73)	122.45 (13.84)	*-0.68*	*0.50*
O-LIFE total	39.05 (12.32)	16.05 (8.78)	*7.08*	***<0.001*****
O-LIFE UE	12.14 (4.29)	1.00 (1.02)	*11.83*	***<0.001*****
O-LIFE CD	11.86 (6.34)	5.36 (4.02)	*4.03*	***<0.001*****
O-LIFE IA	9.19 (2.46)	4.86 (3.23)	*4.93*	***<0.001*****
O-LIFE IN	5.86 (4.26)	4.82 (4.34)	*0.79*	*0.43*
Daily tobacco use	0.30 (0.75)	0.78 (3.35)	*-0.62*	*0.54*
Daily caffeine use	2.82 (2.52)	1.82 (1.52)	*1.57*	*0.13*
Daily marijuana use (median (range))	0 (0-3)	1 (0-3)	*2.56*	*0.46*
Parental socio-economic status (% professional level)	63.6%	66.7%	*0.34*	*0.84*
Educational university level (%)	77.3%	90.5%	*1.37*	*0.24*

O-LIFE, Oxford-Liverpool Inventory of Feelings and Experiences; UE, unusual experiences; CD, cognitive disorganization; IA, introvertive anhedonia; IN, impulsive nonconformity; HS, high schizotypy; LS, low schizotypy; L, left; R, right; data are presented as means (standard deviation).

2.2. ^{1}H-MRS spectral quality

As reported in our recent publications in this sample (Modinos et al., 2017; Modinos et al., 2018a), spectra were of good quality: mean and SD signal to noise

ratio was 25.56±4.69, line width was 4.06±4.04 Hz and FWHM was 0.04±0.01. There were no group differences in any of the spectral parameters (Table S2). An example of the spectrum and voxel placement is also shown in the Supplement (Figure S1).

Table S2. ^{1}H-MRS spectra information for the high and low schizotypy groups.

	HS (n = 21)	LS (n = 22)	χ^2/t	p
Linewidth	4.07 (3.91)	4.05 (4.16)	0.14	0.89
FWHM	0.04 (0.01)	0.04 (0.01)	-1.09	0.28
SNR	25.62 (4.85)	25.50 (4.53)	0.08	0.93
Voxel GM (percentage)	0.63 (0.04)	0.63 (0.04)	0.04	0.97
Voxel WM (percentage)	0.07 (0.02)	0.08 (0.02)	-0.70	0.49
Voxel CSF (percentage)	0.30 (0.04)	0.30 (0.04)	0.25	0.81
Glutamate	17.31 (1.91)	17.75 (2.27)	-0.42	0.52
Glutamate %CRLB	5.67 (0.97)	5.91 (0.97)	-0.73	0.40
Glx (glutamate + glutamine)	22.90 (2.96)	22.98 (2.94)	-0.02	0.90
Glx %CRLB	6.19 (1.17)	6.36 (1.18)	-0.19	0.66

^{1}H-MRS, proton magnetic resonance spectroscopy; SNR, signal to noise ratio; CRLB, Cramer–Rao Lower Bounds; Glx, glutamate+glutamine; GM, grey matter; WM, white matter; CSF, cerebrospinal fluid; HS, high schizotypy; LS, low schizotypy; data are presented as means (standard deviation).

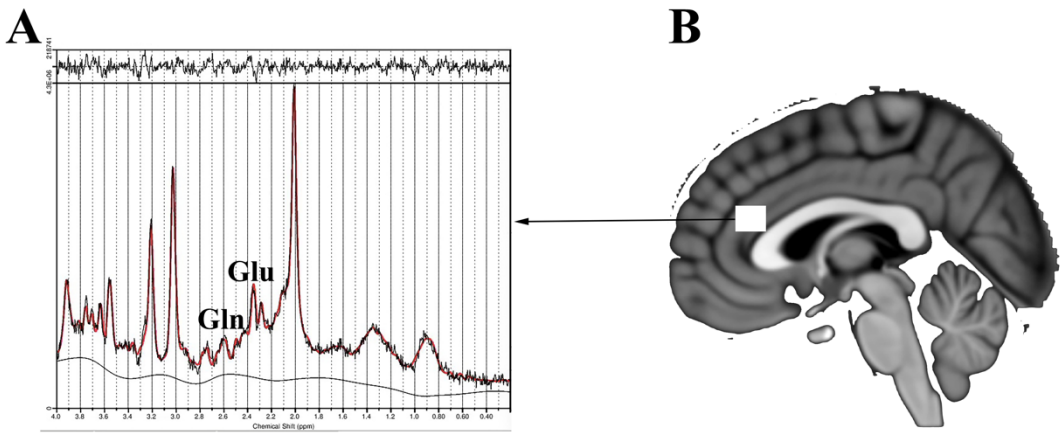

Figure S1. Magnetic resonance spectroscopy voxel placement in the anterior cingulate cortex (B), and representative spectra (A). Gln, glutamine; Glu, glutamate.

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