Associations Between Cognitive Function and Levels of Glutamatergic Metabolites and GABA in Antipsychotic-Naïve Patients With Schizophrenia or Psychosis

Supplemental Information

Participants and methods

The participants in this study are part of baseline examinations in an observational, multimodal follow-up study called Pan European Collaboration on Antipsychotic Naïve Schizophrenia II (PECANSII) (https://clinicaltrials.gov/ct2/show/NCT02339844?term=Glenth%C3%B8j&cond=Schizophrenia&cntry=DK&rank=5), where initially antipsychotic-naïve patients (ANPs) with schizophrenia or psychosis and matched healthy controls (HCs) are recruited.

Participants

Exclusion criteria for all participants were: substance abuse in the past three months, previous head injury with unconsciousness of >5 min, medical or neurological illness, and contraindications to MR scans.

Further exclusion criteria for patients were: treatment with an antidepressant within the last 30 days and involuntary admission or treatment; and for HCs: fulfilling the criteria for ultra high risk of psychosis as assessed with the Comprehensive Assessment of At Risk Mental States (1) or having a first-degree relative with a psychiatric disorder. Occasional use of benzodiazepines (maximum 3 times per day) was accepted in patients if deemed clinically necessary because antipsychotic treatment could not be initiated before all examinations were performed, but not later than 12 hours prior to examinations.

After the diagnostic interview was done, patients were re-assessed by a psychiatrist. Patients were only included if a consensus diagnosis within the inclusion criteria was agreed on.

MRI and 1H-MRS

Participants underwent MRI on a 3.0 Tesla scanner (Achieva; Philips Healthcare, Eindhoven, The Netherlands) using a 32-channel head coil (Invivo, Orlando, Florida, USA). Prior to the scan, the participants

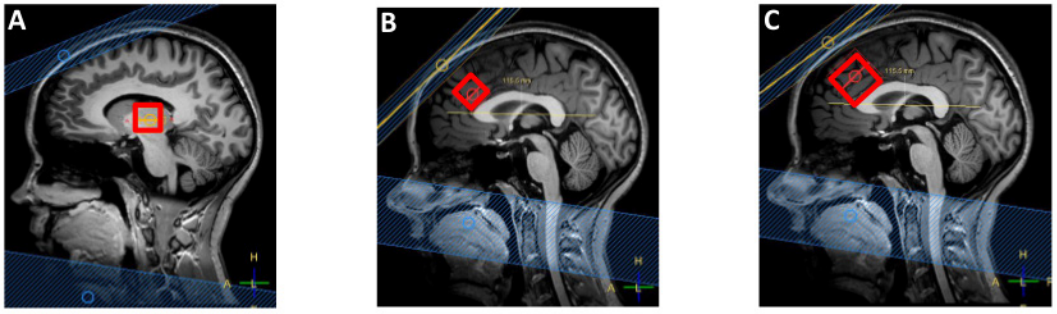
were told not to move and keep the head still during the scans. Before the magnetic resonance spectroscopy sequences were acquired, the participants had a high-resolution, three-dimensional, T1-weighted (T1w) structural image of the brain (TR 10 ms, TE 4.6 ms, flip angle = 8°, voxel size = 0.79 × 0.79 × 0.80mm). The T1w structural image was used to place the spectroscopic voxels and for segmentation of brain tissue in the spectroscopic voxels into fractions of cerebrospinal fluid (CSF), and gray- and white matter.

Spectroscopic voxel placement

For the PRESS sequences, a 2.0x1.5x2.0cm^3 voxel was prescribed in the left thalamus on the axial slice so that it only covered thalamic brain tissue and the content of cerebrospinal fluid was minimized. The left thalamus was chosen to allow for comparison with previous studies that have placed the MRS-voxel in this side (2-4). Also, a 2.0x2.0x2.0cm^3 voxel was prescribed in the dorsal ACC (dACC) (Brodmann area 24 and 33) by placing the corner at the intersection of a line drawn though the extremities of the corpus callosum on the sagittal slice and thereafter aligning the voxel to the corpus callosum. A lipid saturation band was placed to cover the scalp and a water saturation band to cover circulus willisii to avoid signal interference. Total acquisition time for both regions was 14 min. For the MEGAPRESS sequence, a 3.0x3.0x3.0cm^3 voxel was placed in the dACC as described above. Total acquisition time was 11 min.

Voxel placement is shown in supplementary Figure S1 for left thalamus in A (PRESS sequence), and for dACC in B (PRESS sequence) and C (MEGAPRESS sequence), respectively.

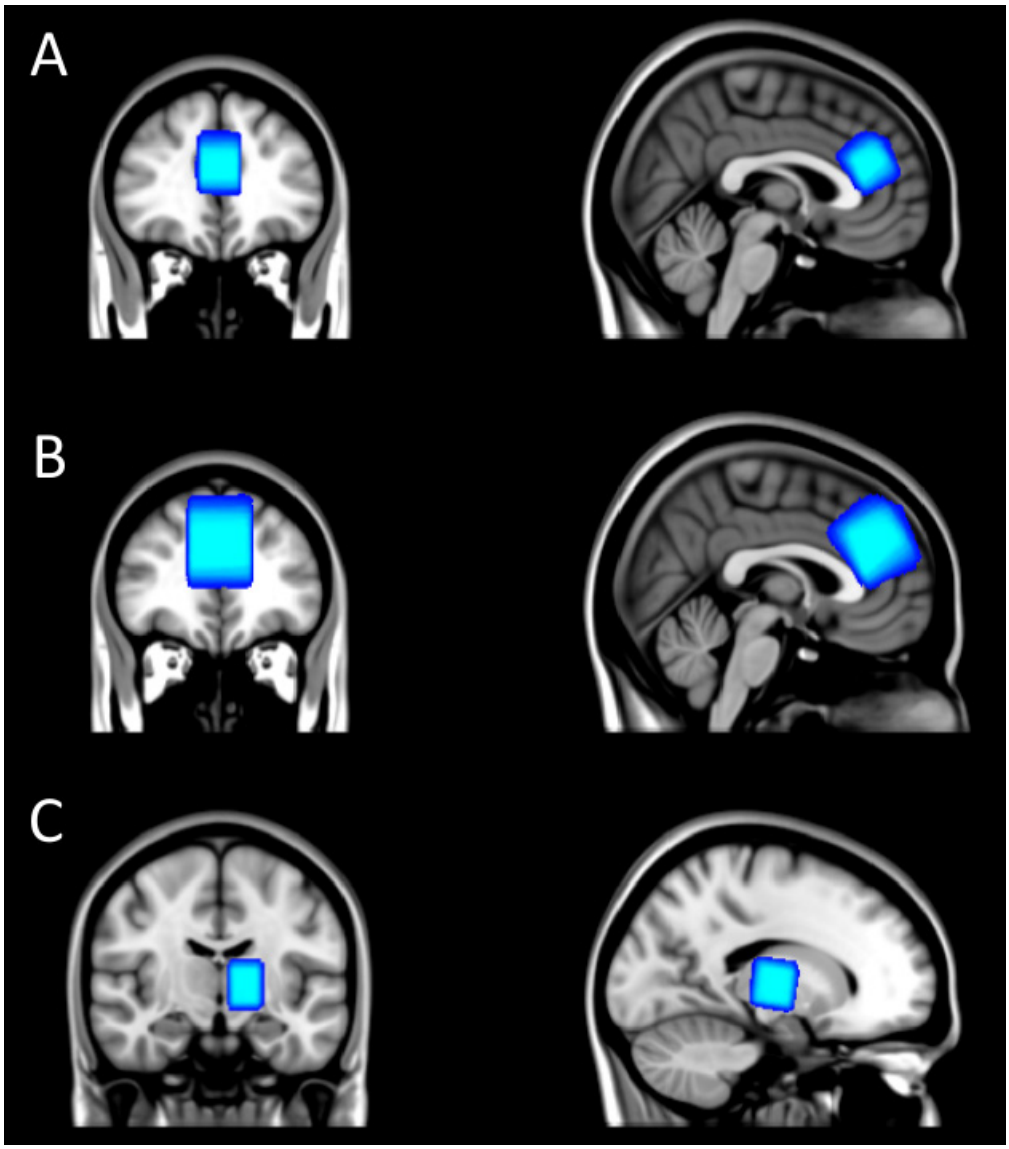
Supplementary figure S1: Location of 1H-MRS voxels in left thalamus and dorsal ACC



The thalamic voxel location is shown in A (PRESS sequence), and the voxel located in dorsal anterior cingulate cortex in B (PRESS-sequence) and in C (MEGAPRESS-sequence).

The between subject overlap of voxel placement was assessed by creating maps of mean voxel placement for all conducted MRS scans in model space. As shown in supplementary figure S2, the overlap of voxel placement between subjects were good for both the PRESS voxel in dACC (A), the MEGAPRESS voxel in dACC (B), and for the voxel placed in left thalamus (C).

Supplementary figure S2. Mean voxel placement in dorsal ACC and left thalamus



Mean voxel position in the coronal and sagittal plane is shown for all subjects in model space for the dorsal ACC voxel (PRESS sequence) in A, the dorsal ACC voxel (MEGAPRESS sequence) in B, and the left thalamus voxel (PRESS sequence) in C. A high overlap is indicated by a lighter blue area.
Abbreviation: ACC: Anterior cingulate cortex; PRESS: Point-resolved spectroscopy; MEGAPRESS: Mescher–Garwood Point-resolved spectroscopy.

Shimming, field homogeneity, and eddy-current correction

Shimming was done automatically using the second order pencil beam. Shimming was evaluated by measuring spectral linewidth, and the MRS sequence was repeated in cases where the linewidth was > 7Hz for the dACC voxel and > 10Hz for the voxel in left thalamus.

Field homogeneity was evaluated by full-width half-maximum values that are reported for the PRESS-sequences in supplementary Table S1 and for the MEGAPRESS sequence in supplementary Table S2.

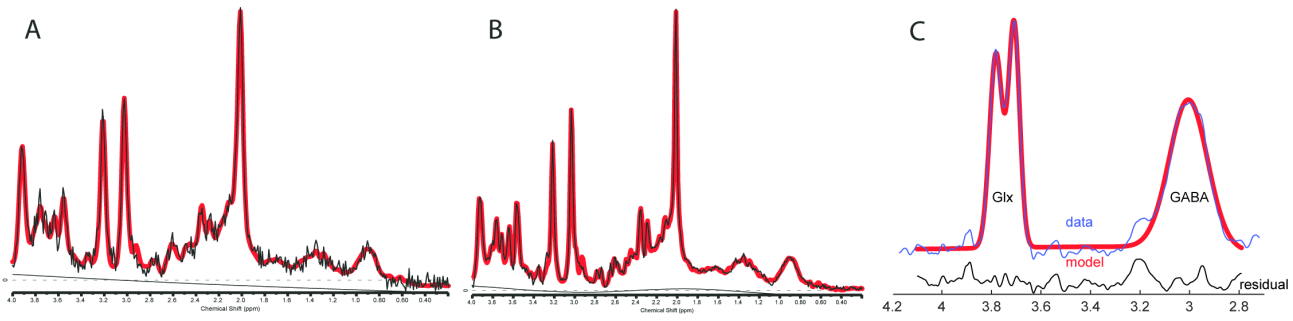
Eddy-current correction was automatically performed on the metabolite data by the scanner.

1H-MRS analysis and quality assessment

PRESS spectra were analyzed with the LCModel version 6.3-1J (5), and water scaling was used to estimate the concentrations of the following metabolites in a standard basis set: alanine, aspartate, creatine (Cr), phosphocreatine (PCr), GABA, glucose, glutamine, glutamate, glycerophosphocholine (GPC), phosphocholine (PCh), glutathione (GSH), *myo*-inositol (Ins), lactate, NAA, N-acetyl aspartate glutamate (NAAG), *scyllo*-inositol, and taurine.

Representative spectra from LCModel and Gannet are shown in supplementary figure S3.

Supplementary figure S3. Representative spectra from LCModel and Gannet



Spectra from LCModel are shown for left thalamus in A and dorsal ACC in B, and a spectrum from Gannet is shown in C. Abbreviations: Glx: Glutamate + glutamine; GABA: Gamma-aminobutyric acid.

Spectral quality was assessed by visual inspection by following the procedure described in the LCModel & LCMgui User's Manual (http://s-provencher.com/pub/LCModel/manual/manual.pdf) in chapter 3.5: 'Criteria for rejecting analyses'. Individual metabolic peaks were excluded if Cramér-Rao lower bound (CRLB) >20% for PRESS and Gannet signal fit error >15% for MEGAPRESS spectra.

Segmentation of the spectroscopic voxels

The T1-weighted structural image was initially segmented using SPM 8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) running under MATLAB® (The MathWorks Inc., Massachusetts, USA). The segmentation was then combined with the spectroscopic voxel masks from the PRESS and MEGAPRESS scans to extract the fractions of CSF, and gray- and white matter. The following formula was then used to calculate metabolite concentrations in institutional units (IU) by correcting for partial volume CSF contamination as described in detail elsewhere (6):

$$M_{IU} = M*(WM+GM+1.55*CSF)/(WM+GM)$$

M: In vivo water-scaled values of metabolites
WM: The fraction of white matter in the spectroscopic voxels
GM: The fraction of gray matter in the spectroscopic voxels
CSF: The content of cerebrospinal fluid in the spectroscopic voxels calculated as 1-(WM+GM)

Moreover, M_{IU} values were adjusted for the fraction of gray matter in the following formula:

$$M_{IU\_adjusted} = M_{IU}*(GM/(GM+WM))$$

$M_{IU\_Adjusted}$ = Metabolite concentrations in institutional units corrected for CSF content and the fraction of gray matter
M_{IU} = Metabolite concentrations in institutional units corrected for CSF content
WM: The fraction of white matter in the spectroscopic voxels
GM: The fraction of gray matter in the spectroscopic voxels

Coefficient of variation for glx, glutamate, and GABA levels

Test-retest reliability (coefficient of variation (CV)) for glutamate and GABA was calculated from the HCs that were re-scanned 6 weeks after baseline examinations as part of the larger PECANSII project. CV was

5.2% for glutamate and 6.9% for glx in dACC (N=47), 8.9% for glutamate and 10.2% for glx in left thalamus (N=33), and 8.3% for GABA levels in dACC (N=34).

Supplemental Results

1H-MRS quality and visual inspection

For the PRESS acquisitions in thalamus and dACC, the signal-to-noise ratio (SNR) and FWHM are provided in Table S1 together with Cramér-Rao lower bound (CRLB) values for all metabolites. There was a small but significant difference between full-width half-maximum (FWHM) values for dACC in patients compared with HCs (Patients: 0.030±0.008; HCs: 0.026±0.006, p=0.002), but all FWHM values were below the 0.086ppm used as cut-off value in the LCModel manual. For the MEGAPRESS acquisition in dACC, the SNR, FWHM, and fit error for GABA and glx are provided in Table S2.

Thalamus

Four spectra (3 HCs and 1 ANP) were excluded after visual inspection, and data from one HC were not transferred due to a technical error, leaving a total of 55 ANPs and 47 HCs PRESS spectra from thalamus of good quality. Exclusion of individual metabolites due to CRLB >20% was done for one glutamate (HC) and 94 glutamine data, why glutamine data are not reported for left thalamus. The segmentation of the T1w structural image was excluded for one ANP due to poor quality and it was therefore not possible to calculate concentrations in IU for this subject. Six spectra were excluded from the main analyses because participants used benzodiazepines but are included in supplementary analyses in the supplementary results. In total, 48 ANPs and 47 HCs spectra of good quality were analyzed for glx IU, and 48 ANPs and 46 HCs for glutamate IU. For creatine scaled values 49 ANPs and 47 HCs spectra were included in analyses with glx/Cr and 49 ANPs and 46 HCs spectra included in glutamate/Cr analyses.

Dorsal ACC

For the dACC PRESS sequence, one spectrum was excluded after visual inspection (1 ANP) leaving 55 ANPs and 51 HCs spectra of good quality. Mean CRLBs were <6% for all metabolites except glutamine and exclusion due to CRLBs >20% was only done for glutamine (8 ANPs and 11 HCs). Six spectra were excluded from the main analyses because participants used benzodiazepines but are included in supplementary analyses in the supplementary results. The segmentation of the T1w structural image was excluded for one ANP due to poor quality, and it was therefore not possible to calculate concentrations in IU for this subject. In total, 48 ANPs and 51 HCs spectra of good quality were analyzed for glx and glutamate IU values in dACC, and 49 ANPs and 51 HCs spectra included in analyses with glx/Cr and glutamate/Cr.

For the dACC MEGAPRESS sequence, 6 spectra were excluded after visual inspection (4 ANPs and 2 HCs), the MEGAPRESS sequence was not acquired in 11 participants (9 ANPs and 2 HCs), for 1 ANP the water was not quantifiable (why only the GABA/Cr value could be used), and the segmentation of the T1w structural image was excluded for one ANP. Five ANPs were excluded from the analyses because they received benzodiazepines but are included in supplementary analyses in the supplementary results. No data were excluded due to a fit error > 15% leaving 37 ANPs and 47 HCs spectra of good quality for analyses of IU values, and 39 ANPs and 47 HCs spectra for analyses using GABA/Cr.

Glx, glutamate and GABA levels adjusted for gray matter fraction in antipsychotic-naïve patients and healthy controls

Dorsal ACC

Glx: The levels of glx did not differ between antipsychotic-naïve patients and HCs (main effect of diagnosis insignificant: $F(1,98)=0.84$, $p=0.36$), and adjusting for covariates did not change the results ($F(1, 98)=0.65$, $p=0.42$). Also, there was no difference when only analyzing patients with a schizophrenia diagnosis ($p=0.78$; after including covariates: $p=0.89$).

Glutamate: The levels of glutamate did not differ between antipsychotic-naïve patients and HCs (main effect of diagnosis insignificant: $F(1,98)=2.02$, $p=0.16$), and adjusting for covariates did not change the

results (F(1, 98)=1.84, p=0.18). Also, there was no difference when only analyzing patients with a schizophrenia diagnosis (p=0.44; after including covariates: p=0.64).

GABA: The levels of GABA were lower in the antipsychotic-naïve patients compared with HCs (main effect of diagnosis: F(1,83)= 7.10, p=0.01) also when adjusting for sex, age, and smoking status (F(1,83)= 6.19, p=0.015). Similarly, there were borderline significantly lower levels when restricting the analysis to only include patients with a schizophrenia diagnosis (F(1,72)=3.70, p=0.06) but not after including covariates (p=0.13).

Left thalamus

Glx: Levels of glx in left thalamus of all antipsychotic-naïve patients compared with HCs were not significantly higher before adjustment for covariates (F(1,94)=0.25, p=0.62) or after including age, sex, and smoking status (p=0.32). Similarly, there was no significant difference between thalamic levels of glx in the subgroup with a schizophrenia diagnosis and HCs before (p=0.58) or after adjusting for covariates (p=0.23).

Glutamate: Levels of glutamate in left thalamus of all antipsychotic-naïve patients compared with HCs were not significantly higher before adjustment for covariates (F(1,93)=1.12, p=0.29) or after including age, sex, and smoking status (p=0.12). Similarly, there was no significant difference between thalamic levels of glutamate in the subgroup with a schizophrenia diagnosis and HCs before adjusting for covariates (p=0.30), but a trend for higher glutamate levels in patients after adjustment for age, sex, and smoking status (p=0.09).

Glx, glutamate and GABA levels in antipsychotic-naïve patients and healthy controls scanned after a scanner upgrade

A scanner upgrade was performed after inclusion of the first 14 participants (10 patients and 4 HCs).

Glx and glutamate levels in dACC in 8 healthy volunteers scanned before and after the upgrade did not differ significantly (Glx: T(7)=0.93, p=0.38); glutamate: (T(7)=1.26, p=0.25). Analyses with exclusion of the 10 patients and 4 HCs scanned before the upgrade are reported below.

Dorsal ACC

Glx: The levels of glx did not differ between antipsychotic-naïve patients and HCs scanned after the upgrade (main effect of diagnosis insignificant: F(1,86)=0.23, p=0.63), and adjusting for covariates did not change the results (p=0.68). Also, there was no difference when only analyzing patients with a schizophrenia diagnosis (p=0.55; after including covariates: p=0.38).

Glutamate: The levels of glutamate did not differ between antipsychotic-naïve patients and HCs scanned after the upgrade (main effect of diagnosis insignificant: F(1,86)=0.40, p=0.53), and adjusting for covariates did not change the results (p=0.58). Also, there was no difference when only analyzing patients with a schizophrenia diagnosis (p=0.59; after including covariates: p=0.42).

GABA: The levels of GABA were lower at trend-level in the antipsychotic-naïve patients compared with HCs scanned after the upgrade (main effect of diagnosis: F(1,81)= 3.17, p=0.08) also when adjusting for sex, age, and smoking status (p=0.08), but not when restricting the analysis to only include patients with a schizophrenia diagnosis (p=0.50; after including covariates: p=0.60).

Thalamus

Glx: Levels of glx in left thalamus were not significantly different in all antipsychotic-naïve patients compared with HCs scanned after the upgrade (F(1,83)=0.75, p=0.75) and adjustment for age, sex, and smoking status did not alter the results (p=0.59). Also, there was no difference when only analyzing patients with a schizophrenia diagnosis (p=0.27; after including covariates: p=0.16).

Glutamate: Levels of glutamate in left thalamus were not significantly different in all antipsychotic-naïve patients compared with HCs scanned after the upgrade (F(1,83)=1.23, p=0.27) and adjustment for age, sex, and smoking status did not alter the results (p=0.18). There was a trend for higher levels of glutamate in the thalamus of antipsychotic-naïve patients with a schizophrenia diagnosis compared with healthy controls (p=0.07), which became significant when adjusting for age, sex, and smoking status (F(1,72)=4.79, p=0.03).

Glx, glutamate, and GABA levels in antipsychotic-naïve patients compared with healthy controls after exclusion of participants with excessive motion

Motion in antipsychotic-naïve patients and healthy controls was indirectly assessed by the frequency correction output from the Gannet analyses of the MEGAPRESS data in that greater correction implies greater motion. There was significantly higher frequency correction in the patient group (N=37) compared with healthy controls (N=47) (mean ANPs: 0.55±0.9 Hz; mean HCs: 0.43±0.15 Hz; T(82)=2.39, p=0.02). A Cooks distance outlier analysis identified two ANPs with extreme values. The group difference in frequency correction was no longer significant after exclusion of these two subjects (T(80)=1.71, p=0.09), although the mean frequency correction remained larger in the patient group (mean ANPs: 0.50±0.20 Hz; mean HCs: 0.43±0.15 Hz) .

Excluding the two ANPs with excess motion from the glx, glutamate, and GABA group analyses did not alter the results as described below.

dACC

Glx: Glx levels in IU in dACC did not differ between antipsychotic-naïve patients and HCs (p=0.84) and adjusting for covariates did not change the results (p=0.85). Also, there was no difference when only analyzing patients with a schizophrenia diagnosis (p=0.40; after including covariates: p=0.30).

Glutamate: Glutamate levels in IU in dACC did not differ between antipsychotic-naïve patients and HCs (p=0.46) and adjusting for covariates did not change the results (p=0.44). Also, there was no difference when only analyzing patients with a schizophrenia diagnosis (p=0.76; after including covariates: p=0.73).

GABA: GABA levels in IU in dACC were lower in the ANPs compared with HCs (F(1,81)= 4.96, p=0.03) also after adjustment for age, sex, and smoking status (F(1,81)= 4.97, p=0.03). However, when excluding the analyses to ANPs with a schizophrenia diagnosis only, the GABA levels were not significantly lower before (p=0.30) or after adjustment for co-variates (p=0.33).

Thalamus

Glx: Glx levels in IU in thalamus did not differ significantly between antipsychotic-naïve patients and HCs (p=0.75; after adjustment for co-variates: p=0.62). Also, no group difference was found when only analyzing ANPs with a schizophrenia diagnosis (p=0.28; after adjustment for co-variates: p=0.20).

Glutamate: Glutamate levels in IU in thalamus did not differ significantly between antipsychotic-naïve patients and HCs (p=0.26; after adjustment for co-variates: p=0.13). However, when excluding the analyses to ANPs with a schizophrenia diagnosis only, the levels of thalamic glutamate were higher at trend-level before adjusting for co-variates (p=0.07) and significantly higher in the ANPs after adjustment for age, sex, and smoking status (F(1,80)= 4.48, p=0.02).

Glx, glutamate and GABA levels in dorsal ACC in all antipsychotic-naïve patients including participants receiving benzodiazepines compared with healthy controls

dACC

Glx: The levels of glx did not differ between antipsychotic-naïve patients including those receiving benzodiazepines and HCs (main effect of diagnosis insignificant: F(1,103)=0.03, p=0.87), and adjusting for covariates did not change the results (p=0.92). Including the use of benzodiazepines as an additional co-variate did not alter the results (p=0.72). There was no significant main effect of benzodiazepine use (p=0.20). Also, there was no difference when only analyzing patients with a schizophrenia diagnosis (p=0.74; after including covariates: p=0.66).

Glutamate: The levels of glutamate did not differ between antipsychotic-naïve patients including those receiving benzodiazepines and HCs (main effect of diagnosis insignificant: F(1,103)=0.38, p=0.54), and adjusting for covariates did not change the results (p=0.54). Including the use of benzodiazepines as an additional co-variate did not alter the results (p=0.35) but revealed a non-significant trend for higher levels of glutamate in benzodiazepine users (main effect of benzodiazepine use: p=0.10). Also, there was no difference when only analyzing patients with a schizophrenia diagnosis (p=0.74; after including covariates: p=0.66).

GABA: The levels of GABA in IU in the entire sample of antipsychotic-naïve patients including those receiving benzodiazepines (N=42) were lower compared with HCs (mean patients: 2.275±0.365, mean HCs: 2.403±0.332) but not to a significant extend (main effect of diagnosis: $F(1,88)= 2.98$, $p=0.09$). A similar result was found when sex, age, and smoking status were adjusted for ($p=0.08$). When including the use of benzodiazepines as an additional co-variate, the levels of GABA were significantly lower in the patient group ($F(1,88)= 4.94$, $p=0.03$), and a borderline significant main effect of benzodiazepines on GABA levels was found due to higher levels in benzodiazepine users ($p=0.07$).

Left thalamus

Glx: The levels of glx in left thalamus did not differ between all antipsychotic-naïve patients compared with HCs ($F(1,100) = 0.08$, $p=0.78$) and adjustments for co-variates did not alter the result ($p=0.66$), neither did inclusion of benzodiazepine use as an additional co-variate ($p=0.63$) and the main effect of benzodiazepine use was insignificant ($p=0.94$). Also, there was no difference when only analyzing patients with a schizophrenia diagnosis ($p=0.31$; after including covariates: $p=0.22$).

Glutamate: There was a trend for higher levels of glutamate in left thalamus of all antipsychotic-naïve patients compared with HCs after adjustment for the covariates ($F(1,99)= 3.22$, $p=0.08$) also after including benzodiazepine use as an additional co-variate ($p=0.09$). There was no main effect of benzodiazepine use ($p=0.64$). The increase of glutamate levels was borderline significant when restricting the analysis to only include patients with a schizophrenia diagnosis ($p=0.05$) and significant after adjusting for covariates ($F(1,87)= 6.98$, $p=0.0099$).

Glx, glutamate and GABA scaled to total creatine in antipsychotic-naïve patients and healthy controls

Mean values for glx, glutamate, GABA, and other neurometabolites scaled to total creatine (/Cr) in dACC and left thalamus are summarized in supplementary Table S5 together with statistics comparing levels in antipsychotic-naïve patients with psychosis or schizophrenia with healthy controls. Briefly, in dACC GABA/Cr in antipsychotic-naïve patients was lower ($p=0.005$) and glx/Cr from the larger MEGAPRESS voxel was lower as well ($p=0.0003$). However, there was no group difference in glx/Cr in the PRESS voxel

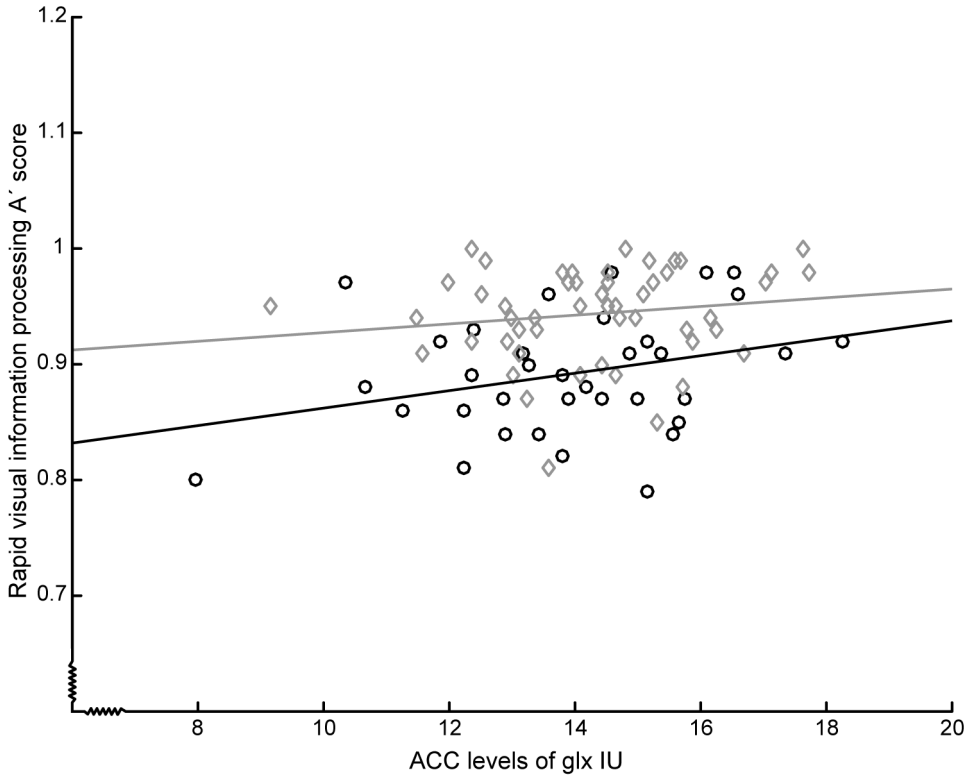
(p=0.38), but there was a trend for lower glutamate/Cr (0.06). In left thalamus, glutamate/Cr was higher in patients, although not to a significant extend (p=0.19), and there was no group difference in thalamic glx/Cr (p=0.78).

The impact of glutamate and glx levels in dorsal ACC on cognitive performance

Graphical presentations of the significant associations between glx levels in dACC and cognitive performance in tests of attention and spatial working memory are presented in supplementary figure S4 and S5, respectively.

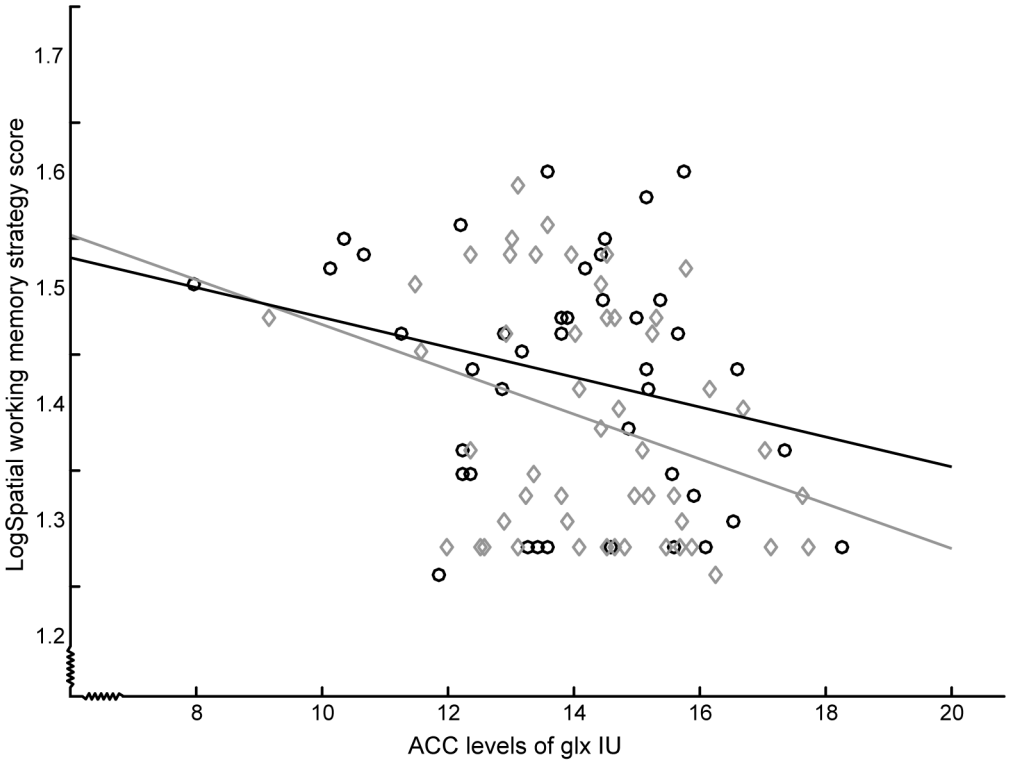
Higher levels of glutamate in dorsal ACC were also significantly associated with better performance in tests of spatial working memory and attention as shown in supplementary table S4.

Supplementary figure S4. The association between glx levels in dorsal ACC and performance in a test of sustained visual attention



The figure shows a significant positive association between glx levels in dorsal ACC and performance in a test of attention in both antipsychotic-naïve patients with schizophrenia or psychosis (black circles) and healthy controls (grey diamonds). $N_{Patients}$=36, N_{HC}=51, b1=0.056, R^2=28.7%, T(86)=2.14, p=0.035. Glx: glutamate + glutamine. ACC: Anterior cingulate cortex. IU: Institutional units.

Supplementary figure S5. The association between glx levels in dorsal ACC and performance in a test of spatial working memory



The figure shows a significant positive association between glx levels in dorsal ACC and performance in a test of spatial working memory (logarithmically transformed, higher score indicates worse performance) in both antipsychotic-naïve patients with schizophrenia or psychosis (black circles) and healthy controls (grey diamonds). $N_{Patients}$=43, N_{HC}=51, b1=-0.016, R^2=11.4%, T(93)=-2.86, p=0.005. Glx: glutamate + glutamine. ACC: Anterior cingulate cortex. IU: Institutional units.

Table S1. Spectral quality for PRESS acquisitions in dorsal ACC and left thalamus

	Dorsal ACC			Left thalamus		
	ANPs Mean ± SD, n[a]	HCs Mean ± SD, n[a]	Statistics	ANPs Mean ± SD, n[a]	HCs Mean ± SD, n[a]	Statistics
FWHM (ppm)	0.030±0.008, n=53	0.026±0.006, n=51	T(102)= 3.17, p=0.002	0.047±0.006, n=55	0.047±0.007, n=47	T(100)=-0.05, p=0.96
Signal to noise ratio	31.6±3.0, n=53	31.9±3.0, n=51	T(102)= -0.50, p=0.62	15.9±3.9, n=55	16.4±3.4, n=47	T(100)= -0.65, p=0.52
CRLB (%) Glutamate	5.2±0.6, n=53	5.1±0.5, n=51	T(102)=0.48, p=0.63	10.0±2.3, n=55	10.0±2.1, n=46	T(99)=-0.03, p=0.29
CRLB (%) Glx	5.0±0.8, n=53	4.9±0.6, n=51	T(102)=0.29, p=0.77	8.6±1.5, n=55	8.7±1.7, n=47	T(100)=-0.46, p=0.65
CRLB (%) Glutamine	16.4±2.4, n=45	16.3±2.2, n=40	T(83)=0.29, p=0.77	-	-	-
CRLB (%) NAA	3.0±0.4, n=53	3.1±0.3, n=51	T(102)=-0.30, p=0.76	5.3±1.5, n=55	5.1±0.9, n=47	T(100)=0.90, p=0.37
CRLB (%) PCr+Cr	2.9±0.2, n=53	3.0±0.0, n=51	T(102)=-1.73, p=0.09	4.1±1.0, n=55	3.9±0.8, n=47	T(100)=1.23, p=0.22
CRLB (%) Myo-inositol	4.1±0.4, n=53	4.1±0.5, n=51	T(102)= 0.16, p=0.87	8.9±2.7, n=55	8.3±1.8, n=47	T(100)=1.36, p=0.18
CRLB (%) Choline	3.1±0.3, n=53	3.1±0.2, n=51	T(102)= 1.26, p=0.21	4.7±1.3, n=55	4.4±0.8, n=47	T(100)=1.23, p=0.22

Abbreviations: ANPs: Antipsychotic-naïve patients; HCs: Healthy controls; SD: Standard deviation; FWHM: Full-width half-maximum; CRLB: Cramér-Rao lower bound; Glx: Glutamate+glutamine; NAA: N-acetyl aspartate; PCr+Cr: Phosphocreatine+creatine. Ppm: Parts per million. [a]: N states the number of spectra analysed.

Table S2. Spectral quality for MEGAPRESS acquisitions in dorsal ACC

	ANPs Mean ± SD, n[a]	HCs Mean ± SD, n[a]	Statistics
FWHM (Hz)	19.8±2.4, n=38	20.0±1.7, n=47	T(83)=-0.40, p=0.69
Signal to noise ratio	19.9±4.0, n=38	21.0±4.7, n=47	T(83)=-1.13, p=0.26
Fit error (%) GABA	6.8±1.6, n=38	6.6±1.6, n=47	T(83)=0.62, p=0.54
Fit error (%) Glx	3.7±1.4, n=38	3.2±1.0, n=47	T(83)=1.59, p=0.12

Abbreviations: ANPs: Antipsychotic-naïve patients; HCs: Healthy controls; ACC: Anterior cingulate cortex; SD: Standard deviation; FWHM: Full-width half-maximum; GABA: Gamma-aminobutyric acid; Glx: Glutamate+glutamine. Hz: Hertz. [a]: N states the number of spectra analysed.

Table S3. Neurometabolite levels in dorsal ACC and thalamus of antipsychotic-naïve patients with schizophrenia and healthy controls

	Dorsal ACC			Left thalamus		
	ANSPs Mean±SD, n[a]	HCs Mean±SD, n[a]	Statistics[b]	ANSPs Mean±SD, n[a]	HCs Mean±SD, n[a]	Statistics[b]
PRESS sequences						
Glutamate IU	10.88±1.48, n=37	10.91±1.17, n=51	F(1,87)=0.00, p=0.96	7.12±0.89, n=37	6.75±0.82, n=46	F(1,82)=6.21, p=0.01
Glx IU	14.47±2.10, n=37	14.30±1.69, n=51	F(1,87)=0.50, p=0.48	10.14±1.75, n=37	9.78±1.35, n=47	F(1,82)=1.67, p=0.20
Glutamine IU	3.79±0.72, n=32	3.65±0.60, n=40	F(1,75)=2.66, p=0.11	-	-	-
NAA IU	8.87±1.01, n=37	8.84±0.82, n=51	F(1,87)=0.08, p=0.77	7.17±0.71, n=37	7.30±0.51, n=47	F(1,82)=0.11, p=0.74
Myo-inositol IU	5.98±0.80, n=37	5.96±0.67, n=51	F(1,87)=0.01, p=0.92	3.55±0.50, n=37	3.63±0.48, n=47	F(1,82)=0.28, p=0.60
Choline IU	2.00±0.32, n=37	2.03±0.28, n=51	F(1,87)=0.08, p=0.78	1.67±0.21, n=37	1.71±0.15, n=47	F(1,82)=0.14, p=0.71
PCr+Cr IU	7.27±0.74, n=37	7.07±0.64, n=51	F(1,87)=1.56, p=0.22	5.64±0.72, n=37	5.55±0.32, n=47	F(1,82)=0.63, p=0.43
Gray matter (%)	67.1±7.4, n=37	69.1±6.0, n=51	F(1,87)=1.06, p=0.31	19.0±7.5, n=37	18.5±5.6, n=47	F(1,82)=1.18, p=0.28
White matter (%)	14.7±4.8, n=37	13.4±3.7, n=51	F(1,87)=0.98, p=0.33	80.0±8.0, n=37	81.3±5.3, n=47	F(1,82)=1.73, p=0.19
CSF (%)	18.2±7.3, n=37	17.5±5.9, n=51	F(1,87)=0.19, p=0.66	1.1±3.3, n=37	0.2±0.7, n=47	F(1,82)=0.62, p=0.43

	Dorsal ACC			Left thalamus		
	ANSPs Mean±SD, n[a]	HCs Mean±SD, n[a]	Statistics[b]	ANSPs Mean±SD, n[a]	HCs Mean±SD, n[a]	Statistics[b]
MEGAPRESS sequence						
GABA IU	2.32±0.31, n=26	2.40±0.33, n=47	F(1,72)=1.04, p=0.32	-	-	-
Glx IU	10.71±1.67, n=26	10.97±1.21, n=47	F(1,72)=0.23, p=0.63	-	-	-
PCr+Cr IU	14.57±1.24, n=26	13.92±1.32, n=47	F(1,72)=3.03, p=0.09	-	-	-
Gray matter (%)	50.9±5.0, n=26	53.8±5.3, n=47	F(1,72)=2.86, p=0.10	-	-	-
White matter (%)	32.3±4.3, n=26	30.8±4.7, n=47	F(1,72)=0.42, p=0.52	-	-	-
CSF (%)	16.8±6.3, n=26	15.3±5.1, n=47	F(1,72)=1.06, p=0.31	-	-	-

Abbreviations: ANSPs: Antipsychotic-naïve schizophrenia patients; HCs: Healthy controls; ACC: Anterior cingulate cortex; SD; Standard deviation; IU: institutional units; Glx: Glutamate+glutamine; NAA: N-acetyl aspartate; PCr+Cr: Phosphocreatine+creatine; GABA: Gamma-aminobutyric acid; PRESS: Point-resolved spectroscopy (2*2*2cm^3 voxel in dorsal ACC and 2*1.5*2cm^3 voxel in left thalamus); MEGAPRESS: Mescher–Garwood Point-resolved spectroscopy (3*3*3cm^3 voxel in dorsal ACC). [a]: N denotes the number of analyzed spectra (detailed information is provided in the supplemental results). [b]: Results are corrected for age, sex, and smoking status.

Table S4. The effect of levels of glutamate in dorsal ACC on cognition

Cognitive domain (outcome measure)	**N** Cognitive test		**Mean test score** ± SD or 25th-75th percentile		**Main effect** All participants	
	ANPs	**HCs**	**ANPs**	**HCs**	**Glutamate**	
					F-value	P-value
Sustained visual attention (RVP A')	38	51	0.89±0.05	0.94±0.04	4.59	0.035
Spatial working memory[a,b] (SWM strategy)	45	51	27.6 (22.0-32.0)	23.0 (19.0-30.0)	6.73	0.010
Premorbid IQ (DART)	44	51	17.1±6.4	21.5±5.3	0.29	0.59

Abbreviations: ANPs: Antipsychotic-naïve patients; HCs: Healthy controls; SD: Standard deviation, RVP A: Rapid visual information processing A; SWM: Spatial working memory, IQ: Intelligence quotient. [a]: Data were logarithmically transformed prior to statistical analyses due to non-normality. [b]: High score indicates worse performance.

Table S5. Neurometabolites scaled to total creatine in antipsychotic-naïve patients and healthy controls

	Dorsal ACC			Left thalamus		
	ANPs Mean±SD, n[a]	HCs Mean±SD, n[a]	Statistics[b]	ANPs Mean±SD, n[a]	HCs Mean±SD, n[a]	Statistics[b]
PRESS sequences						
Glutamate/Cr	1.50±0.13, n=49	1.54±0.11, n=51	F(1,99)=3.60, p=0.06	1.26±0.15, n=49	1.22±0.16, n=46	F(1,94)=1.74, p=0.19
Glx/Cr	1.99±0.20, n=49	2.02±0.18, n=51	F(1,99)=0.78, p=0.38	1.79±0.27, n=49	1.77±0.25, n=47	F(1,95)=0.08, p=0.78
Glutamine/Cr	0.51±0.10, n=41	0.52±0.08, n=40	F(1,80)=0.11, p=0.74	-	-	-
NAA/Cr	1.23±0.06, n=49	1.25±0.06, n=51	F(1,99)=1.98, p=0.16	1.31±0.13, n=49	1.32±0.10, n=47	F(1,95)=0.12, p=0.73
Myo-inositol/Cr	0.83±0.07, n=49	0.85±0.07, n=51	F(1,99)=0.78, p=0.38	0.64±0.09, n=49	0.65±0.08, n=47	F(1,95)=0.85, p=0.36
Choline/Cr	0.28±0.03, n=49	0.29±0.03, n=51	F(1,99)=1.25, p=0.27	0.30±0.03, n=49	0.31±0.03, n=47	F(1,95)=0.40, p=0.53
PCr+Cr[c]	5.34±0.24, n=49	5.29±0.25, n=51	F(1,99)=0.88, p=0.35	5.52±0.66, n=49	5.53±0.32, n=47	F(1,95)=0.04, p=0.85
MEGAPRESS sequence						
GABA/Cr	0.100±0.015, n=39[d]	0.109±0.014, n=39, n=47	F(1,85)=8.51, p=0.005	-	-	-
Glx/Cr	0.108±0.014, n=39[d]	0.117±0.011, n=47	F(1,85)=14.64, p=0.0003	-	-	-
PCr+Cr[c]	10.89±0.62, n=38[d]	10.83±0.74, n=47	F(1,84)=0.00, p=0.99	-	-	-

Abbreviations: ANPs: Antipsychotic-naïve patients; HCs: Healthy controls; ACC: Anterior cingulate cortex; SD; Standard deviation; IU: institutional units; Glx: Glutamate+glutamine; NAA: N-acetyl aspartate; PCr+Cr: Phosphocreatine+creatine; GABA: Gamma-aminobutyric acid. PRESS: Point-resolved spectroscopy (2*2*2cm^3 voxel in dorsal ACC and 2*1.5*2cm^3 voxel in left thalamus); MEGAPRESS: Mescher–Garwood Point-resolved spectroscopy (3*3*3cm^3 voxel in dorsal ACC). [a]: N denotes the

number of analyzed spectra. [b]: Results are corrected for age, sex, and smoking status. [c]: Total creatine+phosphocreatine scaled to water and used as reference by LCModel or Gannet. [d]: For one Gannet-output only the creatine scaled value, but not the water scaled value, could be used.

Supplemental References

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