Data Supplement for Marenco et al., Prefrontal GABA Levels Measured With Magnetic Resonance Spectroscopy in Patients With Psychosis and Unaffected Siblings. Am J Psychiatry (doi: 10.1176/appi.ajp.2015.15020190)

Supplementary Methods

Details of patients scanned off medication

The patients and nursing staff administering clinical ratings were blind to medication or placebo status. Time off psychotropics varied between 14 and 29 days for the patients in the "coded medications protocol", while the remaining eight patients had been medication free for 90 days (N=1), in excess of ten months (N=6) and for an unknown amount of time (N=1). The average time off medication was 3.7±5 months.

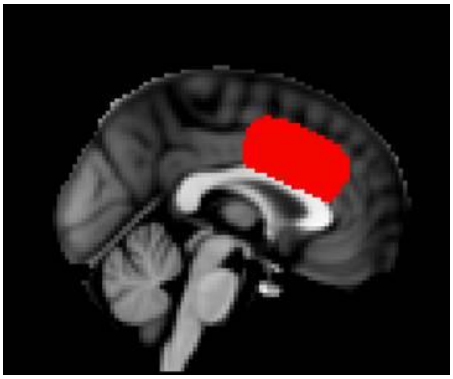
Further exclusions

Probands with a prior history of substance abuse or dependence lasting more than 5 years or who had relapsed in the last six months were excluded. When data were available for more than one sibling in the same family, only a randomly chosen one was included (six siblings were dropped because of this). Three patients with schizophrenia were dropped from the sample because they were taking prescribed amphetamine derivatives at the time of the study. Three patients diagnosed with delusional disorder were dropped because this diagnostic group was too small to justify a separate analysis. Healthy volunteers were excluded if they had a first degree relative with psychosis.

Details of voxel placement

The structural image that serves to localize the voxel was first tilted in order to be parallel to the dorsal anterior cingulate. The voxel was then placed straddling symmetrically the midline, with its anterior edge aligned to the genu of the corpus callosum, and its lower bound just above the corpus callosum.

In order to check for consistency in positioning across groups, we registered the structural scans used for voxel placement to the MNI template (affine registration with SPM5) and applied the transformation to the binarized image of the voxel. We then calculated the x, y, z axis coordinates of the centroids of the spatially "normalized" voxel for each individual. We also derived a mean voxel image of all voxels in standard space and thresholded this image at 0.4 (40% overlap of all voxels). This generated a mask, represented in red in the figure below that was used to calculate %overlap for each voxel. This measure was used to exclude some cases where voxel placement was erroneous (not included in the reported sample), with a minimum overlap of 46% (average 85% ± 9 SD).



Details of the acquisition technique

The J based editing method exploits the J coupling between the GABA-4 protons at 3.0ppm and the GABA-3 protons at 1.9ppm. Two types of spectra were acquired: editing-pulse-on, and editing-pulse-off (editing pulses centered at a frequency 2000 Hz higher than the water resonance).

The GABA-4 resonance is a triplet. In the editing-pulse-off spectra, J-coupling inverts the outer lines of the triplet. In the editing-pulse-on spectra, the editing pulses, which are separated by half the echo time, invert the GABA-3 protons, resulting in a spectrum in which the outer lines of the GABA-4 resonance are in phase. The editing-pulse-off spectrum is then subtracted from the editing-pulse-on spectrum to yield an edited or difference spectrum in which the creatine and other metabolite signals at 3.0 ppm cancel but the outer two lines of the GABA-4 signal add.

Data processing

A fully automated nonlinear fitting program written in IDL (ITT Visual Information Solutions, White Plains, NY) was used to quantify the spectral peaks. Pairs of acquisitions with variations larger than 10% in the amplitude of the residual water peak, an indicator of subject movement, were eliminated. Phase errors were automatically corrected for the zero and first order in the time domain with the phase and offset of the residual water signal. The "edited" signal was zero-order phased by hand for maximal flatness of the baseline around the GABA resonance. The residual water signal that remained in the signal was removed. The fitting algorithm was a Marquardt-Levenberg routine. By fixing the frequency position and the lineshape, the GABA+ and Glx amplitudes remained the only free parameters to be determined from the fit. Metabolite peaks were fitted using a Voigt lineshape, fixed relative frequency separation values derived from literature, and the assumption of a common shared lineshape.

Quality control

Visual quality control was performed on all GABA scans by JWvdV (a physicist with 25 years of experience) and SM. This consisted of inspection of plots represented in Figures S1 and S2. All spectral reviews were performed blind to the diagnosis of the subject. When there was disagreement between the two raters, a discussion resulted in a consensus rating. Spectra were assigned three levels of quality: excellent, good or reject. The first two categories are used in this report. In addition, signal to noise ratio

(SNR) was calculated in the frequency domain for NAA and GABA and Cramer Rao lower bounds (CRLB) were calculated in the time domain for all metabolites. These measures were used to document the quality of the fitting procedure. CRLBs were expressed as a percentage of the metabolite amplitudes.

Correction of the water signal

The relaxometry porperties of brain tissues of interest at 3T are:

n_gray= 0.82, T2_gray= 91 ms and T1_gray= 1425 ms; n_white= 0.7, T2_white= 70 ms and T1_white= 881 ms; n_csf= 1, T2_csf= 503 ms and T1_csf= 4163 ms, where n stands for proton density. These values were obtained by averaging values found in the following references (1-15).

The following calculations were performed, based on (16):

a_gray= n_gray*EXP(-68/T2_gray)*(1-2*EXP(-(1500-1.5*68)/T1_gray)+2*EXP(-(1500-68/2)/T1_gray)-EXP(-1500/T1_gray))

a_white= n_white*EXP(-68/T2_white)*(1-2*EXP(-(1500-1.5*68)/T1_white)+2*EXP(-(1500-68/2)/T1_white)-EXP(-1500/T1_white))

a_csf= n_csf*EXP(-68/T2_csf)*(1-2*EXP(-(1500-1.5*68)/T1_csf)+2*EXP(-(1500-68/2)/T1_csf)-EXP(-1500/T1_csf))

a_g=a_gray/a_csf

a_w=a_white/a_csf

h=1-((1-a_w)*wm_fraction+(1-a_g)*gm_fraction)

corrected water signal=water signal/h

This calculation generates a value for the water signal as if all the voxel were occupied by CSF.

Rationale for choice of dependent variables

In MRS, a normalization of the raw signal derived from the metabolite of interest is necessary for technical reasons, but also introduces complexities in interpreting the results since the effect of the independent variables on the denominator of the ratios is ultimately unknown. Therefore, the interpretation of MRS findings relies on the relative directionality of the results for all three variables mentioned above. Ideally, if GABA/Creatine and GABA/water have similar directionality, the results can be attributed to the effects of GABA; if GABA/Creatine and Creatine/water have opposite directionality, the results can be attributed to the effects of Creatine; and if GABA/water and Creatine/water have similar directionality, the water signal may be responsible for the finding.

Multiple comparison correction for the analysis of effects of symptoms on GABA levels

To reduce the number of multiple comparisons involved (n=18), we ran the analyses first with all six symptom dimensions as dependent variables and followed up interesting leads with specific analyses of a single symptom profile and metabolite.

Supplementary Discussion

Factors contributing to difference in directionality of findings in treated patients

The data by Ongur et al. were acquired in a location consistent with ours, but the data acquisition sequence differed from ours in some respects, patients were older, had a longer illness duration (20 years, compared with <10 years in patients reported here) and most of them were treated with anticonvulsants. When the 6 patients not treated with anticonvulsants in (17) were compared with controls, no significant difference emerged, however this comparison was clearly underpowered. The report by Kegeles et al. (18) found no change in GABA levels in the dorso-lateral prefrontal cortex in patients with schizophrenia, but elevated levels of GABA in the medial prefrontal cortex in untreated patients. Their measurements were located more anteriorly than ours, and their untreated patients had been off medications for 20 months on average, compared with 3.7 in our study.

TABLE S1. Paired on-off medication studies participants: demographic and clinical characteristics

	demog	on	off	p
Sex (M/F)	11/6			
Age	29.5±9.5			
Ethnicity (Cauc./other)	12/5			
Smoking (Y/N)	11/6			
Diagnosis (N SCZ/ SCZAFF/NOS)	11/3/3			
Prior SA	35%			
Illness duration (yrs.)	8±8			
CPZE (mg)		368±155		
phase		10 on-first	7 off-first	
Panss Negative		2.4+0.96	2.4+1.1	0.84
Panss Positive		2.1+0.57	2.4+1	0.03
Panss Disorganized		2.6+0.87	3.1+0.94	0.01
Panss distress		2.1+0.74	2.1+0.54	0.74
Panss excitement-hostility		1.3+0.56	1.6+0.84	0.07
Panss suspiciousness		2.6+0.8	2.9+1.3	0.21

The antipsychotics taken during the "active" phase of the protocol varied across patients: Risperidone (N=10), Aripiprazole (3), Olanzapine (2), Ziprasidone (1) Quetiapine (1). Values are means ± SD or frequency counts. Abbreviations: M=male, F=female, Cauc.=Caucasian, Y=yes, N=no, SCZ=schizophrenia, SCZAFF= schizo-affective disorder, NOS=psychosis not otherwise secified, SA=substance abuse, Panss=positive and negative symptom scale.

TABLE S2. Demographics and clinical characteristics of the whole treated patients group

All treated SCZ	
N	83
sex	69% M
age	30.7±9
Ethnicity: %Cauc	75%
family SES	49.7±13
employed	27.7%
Education: HS-college-graduate	27-58-15%
smoking	33%
% GM /tissue	69.1%±6
% CSF	14.4%±6
Diagnosis (N SCZ/ SCZAFF/NOS)	66/10/7
Prior SA	31%
Illness duration	9.2+7.3
Age of onset	22.7+5.6
CPZE (mg)	585+325
Clozapine +	10
N Atyp/Typ/both	68/7/7
N Antidep/ BDZ/ anticonv/ Li++/ antichol/ other/ none	32/ 14/ 16/ 9/ 16/ 11/ 23
Panss Negative	2.4+1.5
Panss Positive	2.1+1.1
Panss Disorganized	2.3+1.3
Panss distress	1.8+0.7
Panss excitement-hostility	1.2+0.33
Panss suspiciousness	2.6+1.6
GAF	43.5+11.9

Chlorpromazine equivalents are based on 78 patients, due to missing information on dose in two and concomitant medications such as carbamazepine and primidone in three patients. Abbreviations as in previous Table, SES=socio-economic status, GM=gray matter, CSF=cerebro-spinal fluid, Atyp=atypical antipsychotics, Typ=Typical antipsychotics, Antidep=antidepressants, BDZ=benzodiazepines, anticonv=anticonvulsants, Li++=Lithium, antichol=anticholinergics, GAF=global assessment of function.

TABLE S3. Results of main analysis after removing statistical outliers, three participants with poor CRLB/SNR for GABA, patients with psychosis not otherwise specified and their siblings

	Overall adj. R^2	Overall F(df), p	dx	age	sex	%GM/ tissue	MNI y pos
Gaba/Cre	R^2=0.09	$F_{(7,286)}$=5.2, p<0.00002	p<0.0007	p<0.00014, ß= - 0.22	p<0.41	p<0.73	p<0.1
Gaba/water	R^2=0.06	$F_{(7,286)}$=3.6, p<0.00095	p<0.002	p<0.74	p<0.02 F>M	p<0.90	p<0.083
Cre/water	R^2=0.18	$F_{(7,286)}$=10.3, p<0.000001	p<0.48	p<0.000002, ß=0.26	p<0.000008, F>M	p<0.49	p<0.000005 ß=0.26

Abbreviations: Cre=Creatine, F=female, M=male, MNI= Montreal Neurological Institute.
Post-hoc tests for the effect of diagnosis were as follows:
GABA/Creatine: controls>siblings p<0.003; controls>treated patients p<0.047.
GABA/water: controls>siblings p<0.00034; treated patients>siblings p<0.047.
Statistical outliers were defined as exceeding mean ±3SD for the whole group. Criterion for exclusion based on Cramer Rao Lower Bound for GABA was > mean+3SD for the whole group.
Patients with psychosis not otherwise specified were all Caucasian and differed from the racial mix of the rest of the patients (X^2=4.2, p=0.04). They also differed clinically from other patients in that they had more prevalent histories of substance abuse in remission (X^2=5.4, p=0.02), and shorter duration of illness (2.1 vs. 4.6 years t-test, p=0.03 uncorrected for multiple comparisons). No other clinical or demographic variables differed between patients with psychosis not otherwise specified and the other patients.

TABLE S4. Intra-class correlation (ICC) results after adding eight sibling-proband pairs

	GABA/Cre	GABA/water	Cre/water
ICC (N=24)	0.51, p<0.0022	0.4, p<0.014	0.18, p<0.15

The 8 siblings added for this analysis had prior psychiatric disorders in remission (three with depressive disorders, two with substance abuse, one with social phobia, one with attention deficit disorder) or were taking antidepressants at the time of the study (two cases, in one of which the indication was irritable bowel syndrome).

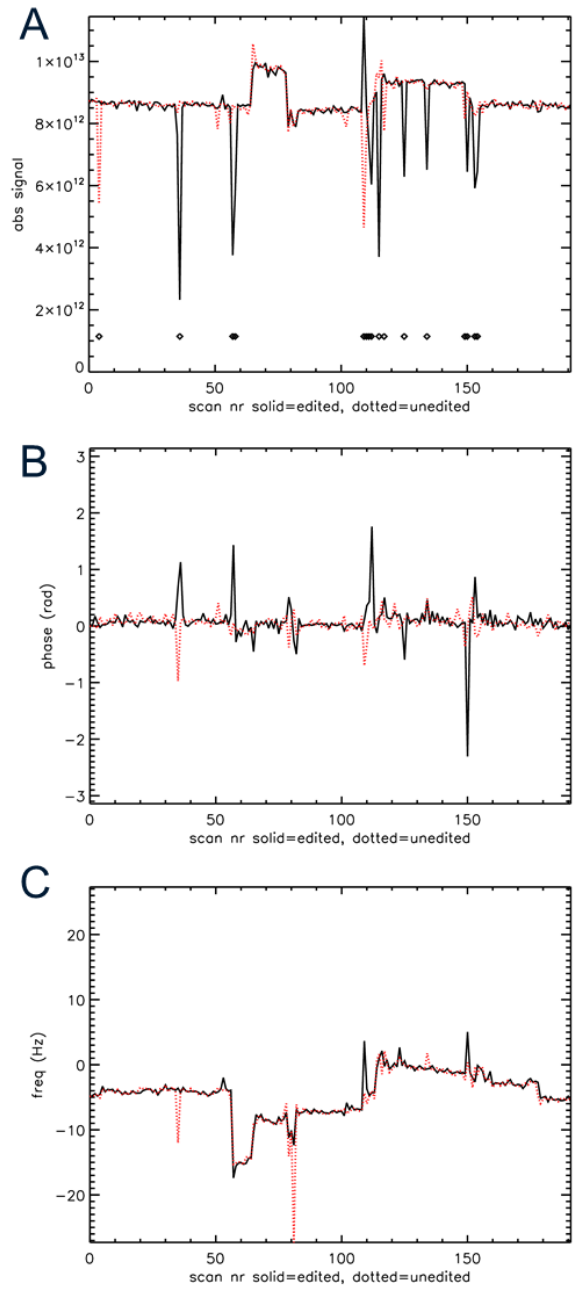
TABLE S5. Results of paired t-test OFF-ON medications

	Mean +SD ON antipsychotics	Mean +SD OFF antipsychotics	t	p
Gaba/Cre	0.098+0.011	0.093+0.009	-1.9	0.07
Gaba/water	16.9+1.8	16.4+1.7	-1.02	0.32
Cre/water	174.2+15.8	177+15.8	0.72	0.48

TABLE S6. Effect size of contrasts between diagnostic groups (adjusted LS means)

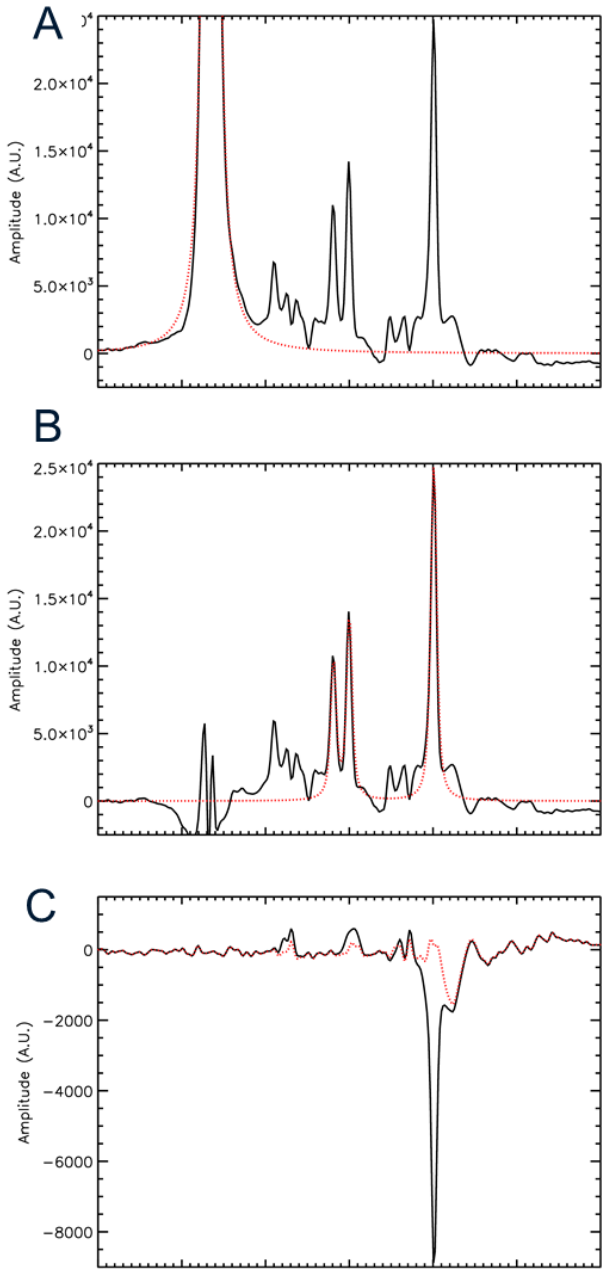
	HV-SIB	HV-SCZ	HV-SCZ_UTR	SCZ_ON-OFF
GABA/Cre	0.74	0.35	0.43	0.46
GABA/water	0.79	0.24	0.26	0.25
Cre/water	0.17	-0.14	-0.23	-0.17

FIGURE S1. Visual quality control procedure (part I)



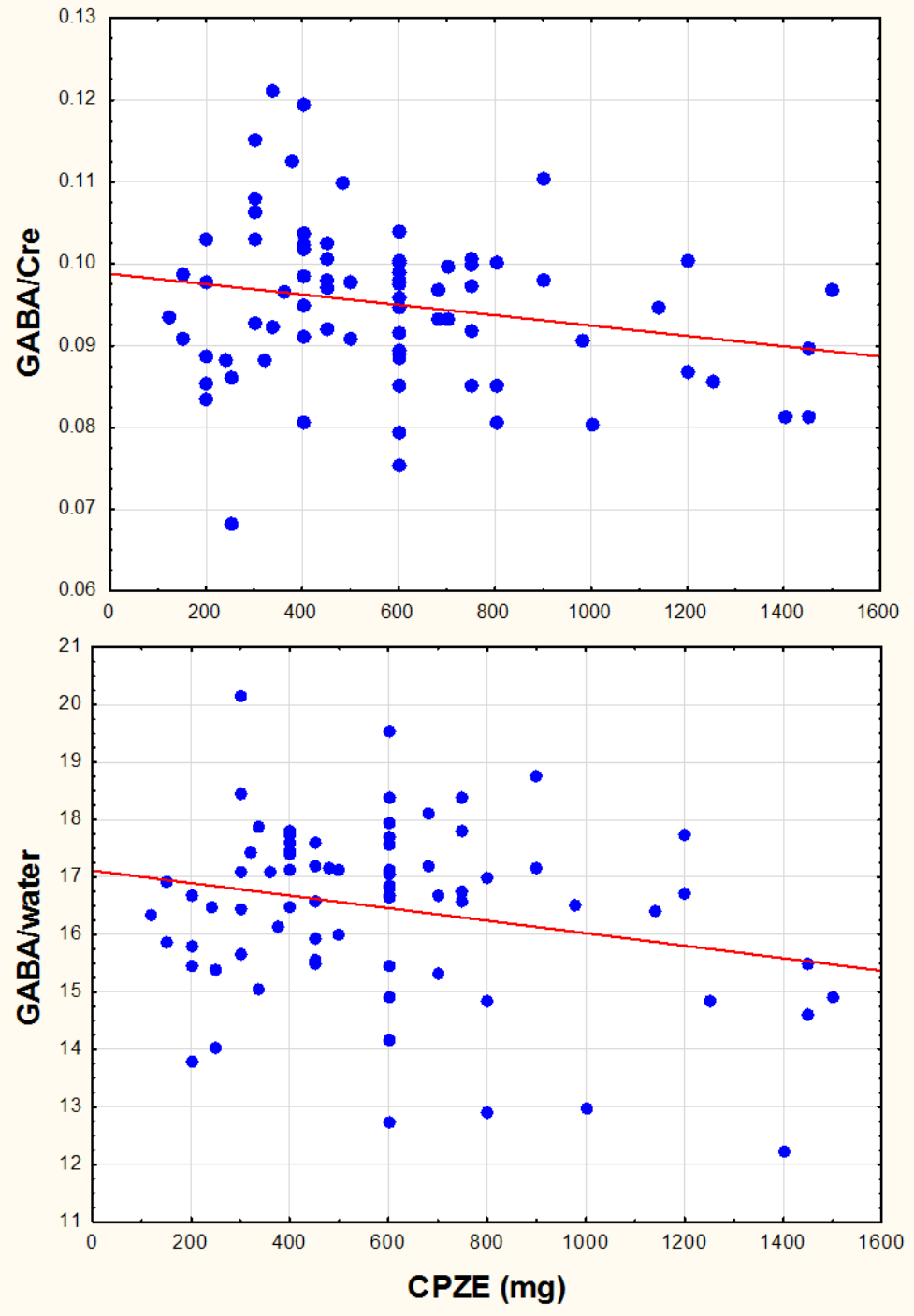
Panel A shows the difference in water amplitude between alternating pulse editing on and off scans. When this difference exceeded 10%, this was considered an indication of movement and the pair of scans was removed from the calculation of average spectra. When the movement was particularly severe, it was also reflected in panels B and C, where phase and frequency, respectively, of the water signal over the course of the scan are represented. Scans with excessive movement, particularly at the beginning of the scan were not analyzed. This is a case that was rejected due to excessive movement.

FIGURE S2. Visual quality control procedure (part II)



Plots in Figure S2 allow to assess the quality of the fitting procedure: panel A shows the fitting of the residual water peak, which is subtracted from the spectrum, panel B shows the fitting of NAA, Creatine and Cho, and panel C shows the fitting of the editing pulse off-on difference spectrum. Red dotted lines represent the fitted data while the raw data are in black. Note how in panel C the fitting procedure fails due to the movement present during the acquisition (visible in Fig. S1).

FIGURE S3. Relationship between GABA and chlorpromazine equivalents



Raw values were plotter. GABA/water is in arbitrary units *10000. Detailed results for GABA/Creatine: overall model: adjusted R^2=0.17, $F_{(5,73)}$=4.2, p<0.003; effect of chlorpromazine equivalents p<0.04, ß=-0.22, partial eta^2=0.056.

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