INTRODUCTION

Schizophrenia is characterized by positive and negative symptoms, as well as by cognitive deficits, in particular in domains related to attention and verbal working memory.\(^1,2\) For half a century, the dopamine hypothesis has dominated theories regarding the pathophysiology of schizophrenia. However, although many symptoms can be linked to dopaminergic dysregulation, it has been suggested that causative abnormalities may lie elsewhere.\(^3\) In this regard, focus has been directed to glutamatergic dysregulation and, in particular, to an N-methyl-D-aspartate receptor hypofunction.\(^4,5\) Thus, patients with schizophrenia show elevated levels of post-mortem brain and cerebrospinal fluid (CSF) kynurenic acid, an endogenous N-methyl-D-aspartate receptor antagonist.\(^6,7\) Several lines of research have also implicated the inhibitory neurotransmitter γ-aminobutyric acid (GABA) in the pathophysiology of schizophrenia and a number of studies have identified deficits in parvalbumin containing GABA neurons in schizophrenia.\(^11\) One of the most consistent post-mortem findings in schizophrenia is a decreased expression of the 67 kDa isoform of glutamic acid decarboxylase, a key enzyme in the biosynthesis of GABA.\(^1,2,12\) In line with this, several studies have shown an association between GAD1, the gene for the enzyme 67 kDa isoform of glutamic acid decarboxylase, and schizophrenia.\(^16,17\) Furthermore, congruent with a reduced expression of 67 kDa isoform of glutamic acid decarboxylase, post-mortem studies reveal lower GABA levels in multiple brain regions including the nucleus accumbens, thalamus, amygdala and hippocampus in patients with schizophrenia.\(^19,23\)

In contrast to genetic and post-mortem studies, in vivo studies of GABA in schizophrenia have been inconclusive. With proton magnetic resonance spectroscopy (\(^1\)H), some studies found a decrease,\(^24–26\) some an increase,\(^27,28\) and yet others found no changes in GABA levels\(^29–31\) in patients with schizophrenia. Differences in GABA levels between patients and unaffected controls appear dependent on the brain area investigated, the duration of illness, as well as on the medication.\(^25–28,30,31\) Recently, a study using positron emission tomography utilizing \([\text{\textsuperscript{11}C}]\) flumazenil suggested an impaired GABA neurotransmission in patients with schizophrenia, a finding that was also associated with positive symptoms.\(^32\) Further, several studies analyzing CSF GABA in patients with schizophrenia, most of them performed during the 1980s, have yielded mostly negative and partly inconsistent results.\(^33–41\)

Taken together, there is an increasing body of evidence from genetic and post-mortem studies implicating an altered GABA transmission as a significant component of schizophrenia pathophysiology. However, robust evidence from CSF studies of an involvement of GABA is still lacking. We here analyze CSF GABA and four other amino acids, that is, glutamate, glycine, taurine and tyrosine, with a sensitive analytical assay, in well-characterized groups of healthy controls and patients with first-episode psychosis (FEP), most of them drug naive to antipsychotic medication. We hypothesize that CSF GABA is reduced in FEP patients, and that low levels of GABA associate to worse symptoms and cognitive deficits.

MATERIALS AND METHODS

Subject population

The study was approved by the Regional Ethics Committee in Stockholm and conformed to the tenets of the Declaration of Helsinki. All subjects were included from March 2011 through January 2014, after providing written informed consent. This study formed part of the Karolinska Schizophrenia Project (KaSP) and was funded by the Swedish Research Council, the Swedish Medical Products Agency and the European Commission (7th Framework Programme). The Karolinska Schizophrenia Project (KaSP) Consortium includes the following institutions: Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden; Department of Clinical Neuroscience, Centre for Psychiatry Research, Karolinska Institutet, Stockholm, Sweden; Neuroimmunology Unit, Department of Clinical Neuroscience, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden and Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, MA, USA. Correspondence: Professor G Engberg, Department of Physiology and Pharmacology, Karolinska Institutet, Nanna Svartz väg 2, Stockholm 171 77, Sweden. E-mail: goran.engberg@ki.se

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Schizophrenia Project, a multidisciplinary research consortium that investigates the pathophysiology of schizophrenia.

FEP patients

Forty-one FEP patients (25 male and 16 female) who met the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for schizophrenia (n = 12), schizophrreniform disorder (n = 14), severe depression with psychotic features (n = 1), delusional disorder (n = 3), brief psychotic disorder (n = 1), psychotic disorder not otherwise specified (n = 9) or schizoaffective syndrome (n = 1) were recruited from psychiatric emergency wards and 3 psychiatric outpatient clinics in Stockholm. Diagnosis was established based on a structured clinical interview of the DSM-IV or a consensus diagnostic procedure. All patients were re-assessed after approximately 1.5 years and were then found to meet the criteria for the following DSM-IV diagnoses: schizophrenia (n = 25), psychotic disorder not otherwise specified (n = 5), delusional disorder (n = 4), brief psychotic disorder (n = 1), schizoaffective syndrome (n = 3) and no diagnosis (n = 3). Exclusion criteria were neurologic or severe somatic illness, substance abuse and autism spectrum disorder. Absence of major brain abnormalities was confirmed using magnetic resonance imaging. All patients underwent an extensive clinical characterization, including the Global Assessment of Functioning (GAF; where symptom and functioning dimensions were assessed separately), the Positive and Negative Syndrome Scale (PANSS), Clinical Global Impression (CGI), Alcohol Use Disorders Identification Tests and Drug Use Disorders Identification Tests. All patients included in this study were somatically healthy and free from any substance abuse disorder. Tobacco use was permitted and 11 of the 41 patients (27%) used tobacco (smoking or snuff). Occasional medication with sedatives and anxiolytics were allowed during the course of the study. At the time of CSF sampling, 12 out of 41 patients (29%) were treated with benzodiazepines (BZDs). Eighteen out of 41 patients (44%) were under antipsychotic treatment at the time of CSF sampling (mean time (± s.e.m.) 7.2 ± 1.82 days). Patients with more than 1 month of treatment with antipsychotics were not included in the study, with the exception of the inclusion of one patient that had been treated for 57 days. Twelve out of 41 patients were naive to all medications. Antipsychotics used were olanzapine, aripiprazole, risperidone, quetiapine or haloperidol (see Supplementary Table S1). Individual medication was maintained in all patients throughout the test period, although the dosages of anxiolytics/hypnotics may have been slightly adjusted. Duration of untreated psychosis was based on information from the patients or his/her relatives. For most patients (n = 34), GAF, PANSS, cognitive testing and lumbar puncture were all performed within a 10-day period (mean time (± s.e.m.): 5.5 ± 0.4 days), whereas seven of the patients underwent these investigations during a period from 14 to 40 days (mean time (± s.e.m.): 19.4 ± 3.6 days).

Healthy control subjects

Twenty-one healthy control subjects (11 males and 10 females) were recruited by advertisement. Medical examination was made by routine laboratory blood and urine tests, physical examination, as well as a brain magnetic resonance imaging examination. The Mini International Neuropsychiatric Interview was used to exclude previous or current psychiatric illness. Further, exclusion criteria were previous or current use of illegal drugs and first-degree relatives with psychiatric illness. All participants were free from medication and any form of substance abuse evaluated with Alcohol Use Disorders Identification Tests/Drug Use Disorders Identification Tests at the time of the study. None of the subjects had any first-degree relative with a psychiatric diagnosis. In all but one case, no structural brain abnormalities was detected using magnetic resonance imaging, as evaluated by an experienced neuroradiologist at the MR Centre, Karolinska University Hospital, Solna. This individual exhibited signs of demyelinating disease on magnetic resonance imaging, but did not fulfill criteria for a clinically isolated syndrome or multiple sclerosis, as the clinical neurological exam was normal and there was no history of relevant neurological symptoms. CSF examination revealed oligoclonal bands, but no other abnormalities. Test results were similar to other controls and therefore this subject was not excluded from the analysis. For all healthy controls, cognitive test session and lumbar puncture were all performed within mean time (± s.e.m.): 14.5 ± 3.01 days.

CSF collection

Efforts were made to reduce confounding factors of the lumbar puncture procedure that could influence analysis of CSF amino acids.32 These efforts include the use of a disposable atraumatic needle (22G Sprotte, Geisingen, Germany) that was inserted at the L 4-5 level with all individuals in the right decubitus position. Further, the same volume of CSF (18 ml) was allowed to drip into a plastic test tube, protected from light. CSF supernatant from all subjects was divided into 10 aliquots that were frozen at −80 °C within 1 h of sampling following centrifugation (Sigma 5810R, Eppendorf, Hamburg, Germany) at 3500 r.p.m. (1438 g) for 10 min) to separate cells and supernatant, respectively. The majority of subjects (n = 27; 23 patients and 14 controls) underwent the lumbar puncture between 0745 and 2200 h after a night’s sleep. Owing to clinical routines, morning sampling was not possible in the remaining FEP patients (n = 18). To control for this confounding factor, seven controls also underwent lumbar puncture during the same time interval (that is, 1030 and 1315 h).

All subjects were instructed to avoid physical activity during the preceding 8 h; however, it was not feasible to monitor rest or posture in this regard. Importantly, no correlation between CSF GABA levels and the point of time for lumbar puncture was observed (Pearson: all: r = −0.14, P = 0.28; controls: r = −0.06, P = 0.81; patients: r = −0.17, P = 0.29). This is in analogy with BenMenachem et al.,43 showing no differences in CSF GABA in healthy controls between lumbar puncture in the afternoon and next morning sampling.

A fresh sample was analyzed for cell numbers, albumin, immunoglobulin G and the presence of immunoglobulin M antibodies to Borrelia, as well as with immune electrophoresis.

Analysis of CSF GABA

Samples were subsequently analyzed for GABA (and additional amino acids, that is, glutamate, taurine, glycine and tyrosine) with a gradient elution reversed-phase high pressure liquid chromatography system, including a gradient pump (Spectra System P4000, Waltham, MA, USA), a degasser (Spectra System SCM 400), a Luna 100 C18(2) column (50 × 2 mm i.d., 5 μm particle size, Phenomenex, Torrance, CA, USA) and a fluorescence detector (Jasco FP-920, Tokyo, Japan) operating at excitation and emission wavelengths of 344 and 495 nm, respectively. The chromatographic separation was performed at room temperature (22 °C). CSF from FEP patients and healthy controls were derivatized for 60 s at room temperature with O-phthalaldehyde/2-mercaptoethanol reagent. The reagent was prepared by dissolving 27 mg O-phthalaldehyde in 0.5 ml ethanol (99.5%), 4.5 ml borate buffer (0.4 μM boric acid adjusted to pH 10.4 with sodium hydroxide) and 20 μl 2-mercaptoethanol was added.

Detection of amino acid gradients was performed with two degassed mixture mobile phases. Mobile phase A consists of 0.04 M sodium acetate buffer (pH 6.95) containing 2.5% (v/v) of methanol, 2.5% (v/v) of tetrahydrofuran and mobile phase B consists of methanol. The flow rate of the mobile phase was 0.7 ml min−1 throughout the analysis and all gradient changes were linear. The gradient conditions were as follows: initial conditions are 100% mobile phase A; from time 0 to 11 min the gradient changes to 70% mobile phase A and 30% mobile phase B; from 11 min to 16 min the gradient changes to 10% mobile phase A and 90% mobile phase B; from 16 min the gradient changes to 100% mobile phase A and remains in this condition until the next injection. Samples of 20 μl were manually injected into the system. The signals from the fluorescence detector were transferred to a computer and analyzed by DataLys Azur Software (Grenoble, France). Approximate retention time of GABA was 9.8 min (glutamate 1.2 min; taurine 5.5 min; tyrosine 11.5 min).

Cognitive testing

The Measurement and Treatment Research to Improve Cognition in Schizophrenia Consensus Cognitive Battery was used to evaluate cognitive function. This battery captures key cognitive domains relevant to schizophrenia. The Measurement and Treatment Research to Improve Cognition in Schizophrenia Consensus Cognitive Battery includes 10 tests that measure 7 cognitive domains: Speed of processing (Brief Assessment of Cognition in Schizophrenia: Symbol Coding, Category Fluency: Animal Naming, Trail Making Test: Part A); Attention/vigilance (Continuous Performance Test-Identical Pairs); Working memory (Wechsler Memory Scale-3rd Edition: Spatial Span, Letter-Number Span); Verbal learning (Hopkins Verbal Learning Test-Revised); Visual learning (Brief Visuospatial Memory Test-Revised); Reasoning and problem solving (Neuropsychological Assessment Battery: Mazes) and Social cognition (Mayer–Salovey–Caruso Emotional Intelligence Test: Managing Emotions). One psychologist (HFB) administered all the tests.
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Statistical analysis
The normality of data was determined using D’Agostino and Pearson’s omnibus normality test. One-tailed tests of significance (Mann–Whitney U-test) were performed in the comparison between CSF GABA levels in FEP patients and healthy controls, and in the correlation analyses as a directional change, that is, decreased GABA levels in FEP patients, could be hypothesized at this stage. Two-tailed tests were performed to determine the possible effects of various medications on CSF GABA (Table 2), as the direction of any change in CSF GABA levels could not be anticipated. To assess the relative importance of potential confounders, we used binary logistic regression or χ²-test, as well as the R package ‘relaimpo’.45 Here individual regressor’s contribution to a multiple linear regression model is quantified using six different methods. Although the different methods produced similar results, we here report an approach based on sequential unpaired t-test with equal s.d. Reported correlation coefficients are Pearson’s r. Bonferroni correction was used in the comparison of different cognitive tests between healthy controls and FEP patients, giving an α-threshold of 0.005 (0.05/10). Only those cognitive tests that remained significant after the Bonferroni correction was used for the comparison with CSF GABA in FEP patients giving an α-threshold of 0.003 (0.05/6). With regard to the correlation studies between CSF GABA and cognitive tests in healthy controls, the α-threshold was set to 0.005 (0.05/10). Symptom ratings were highly correlated (see Supplementary Table S2) and therefore not corrected for repeated measure. To confirm the association between CSF GABA and clinical symptoms, we applied a principal component analysis (Supplementary Information). All analyses were performed using Prism version 6.0 (GraphPad Software, La Jolla, CA, USA), SPSS Statistics version 20.0 (IBM, Armonk, NY, USA), or R statistics (R Development Core Team, Vienna, Austria). Statistical significance was considered when P < 0.05.

RESULTS
Participants
Clinical and demographic characteristics of participants are presented in Table 1. There was no significant difference in age, gender or body mass index between patients and healthy controls. Duration of untreated psychosis was 10.5 ± 1.88 (mean ± s.e.m.) months and total PANSS score was 73.9 ± 3.42 (mean ± s.e.m.). Eighteen patients (44%) received antipsychotic medication, 12 patients (29%) BDZs, 10 patients (24%) zopiclone, 5 patients (12%) antidepressants and 11 patients (27%) received phenothiazine derivatives. Twenty-nine patients (71%) received a combination of some of these drugs at the time of CSF sampling. Twelve out of 41 patients (29%) were naive to all medications (see Supplementary Table S1).

CSF GABA in FEP patients versus healthy controls
The CSF levels of GABA in FEP patients and healthy controls are displayed in Figure 1.

The CSF GABA concentration was significantly lower in FEP patients compared with healthy controls (median 2.88 μM, interquartile range 2.02–6.57 μM, n = 41 vs median 4.11 μM, interquartile range 2.68–5.13 μM, n = 21, P = 0.042). No significant associations were found between CSF GABA levels and age, gender, body mass index or tobacco use (see Supplementary Table S3). CSF GABA levels did not differ between FEP patients that were naive to antipsychotic treatment and those on antipsychotic treatment (P = 0.85). Neither did CSF GABA levels differ between treated and untreated FEP patients with regard to BDZs (P = 0.30), zopiclone (P = 0.47), antidepressants (P = 0.37), phenothiazine derivatives (P = 0.62) or all drugs combined (P = 0.15) (see Table 2). To further investigate the impact of potential confounders, an R package relaimpo was also used to assess the relative importance of the regressors diagnosis (yes/no), medication (yes/no), age, gender, body mass index and tobacco use (yes/no) in a linear model predicting CSF GABA levels.

### Table 1. Demographic and clinical characteristics of the study population

| Characteristic            | Healthy controls (n = 21) | FEP patients (n = 41) | P-value |
|---------------------------|---------------------------|-----------------------|---------|
| Age (years)               | 25.9 ± 1.11 (21)          | 29.2 ± 1.06 (41)      | 0.06*   |
| Gender (male/female)      | 11/10                     | 25/16                 | 0.52†   |
| BMI (kg/m²)               | 22.2 ± 0.55 (20)          | 23.1 ± 0.62 (39)      | 0.33§   |
| Tobacco users             | 0%                        | 27%                   |         |
| DUP (months)              | —                         | 10.5 ± 1.88 (37)      |         |
| Days of antipsychotic treatment | —                       | 7.2 ± 1.82 (18)      |         |

| Medication                |                           |                       |
|---------------------------|---------------------------|-----------------------|
| Antipsychotics            | 0%                        | 44%                   |         |
| Benzodiazepines           | 0%                        | 29%                   |         |
| Zopiclone                 | 0%                        | 24%                   |         |
| Antidepressants           | 0%                        | 12%                   |         |
| Phenothiazine derivatives | 0%                        | 27%                   |         |

| PANSS                      |                           |                       |
|---------------------------|---------------------------|-----------------------|
| Positive                  | —                         | 19.5 ± 0.86 (41)      |         |
| Negative                  | —                         | 15.9 ± 1.21 (41)      |         |
| General                   | —                         | 38.5 ± 1.87 (41)      |         |
| Total                     | —                         | 73.9 ± 3.42 (41)      |         |

| Level of functioning      |                           |                       |
|---------------------------|---------------------------|-----------------------|
| GAF symptoms              | 35.6 ± 1.99 (41)          |         |
| GAF functioning           | 46.2 ± 2.17 (41)          |         |
| CGI score                 | 4.4 ± 0.17 (41)           |         |

Abbreviations: BMI, body mass index; CGI, Clinical Global Impression; DUP, duration of untreated psychosis; FEP, first-episode psychosis; GAF, Global Assessment of Functioning; PANSS, Positive and Negative Syndrome Scale. *Unless otherwise indicated. †Binary Logistic regression. ‡χ²-test.

Figure 1. GABA in the CSF of healthy controls (HC, n = 21) and (FEP, n = 41) patients. Each point represents the concentration of a single CSF sample and the horizontal lines represent the median for each group. Statistical differences between controls and FEP patients were determined using Mann–Whitney U-test. *P < 0.05. CSF, cerebrospinal fluid; FEP, first-episode psychosis; GABA, γ-aminobutyric acid.
GABA was found to correlate positively to scores of the symptom dimension of GAF (r = 0.31, P = 0.02). With regard to scores of the functioning dimension of GAF a trend towards statistical significance was observed (r = 0.24, P = 0.06). A negative correlation between CSF GABA and CGI scores (r = −0.38, P = 0.007) was detected.

Notably, ratings for PANSS, GAF and CGI scales were highly correlated (see Supplementary Table S2), making a Bonferroni correction for repeated measures inappropriate. To overcome the problem with adequate control of type I and II errors, a principal component analysis was performed (for details, see Supplementary Information) followed by linear regression modeling of extracted individual principal component scores. This confirmed an association between symptoms and low CSF GABA levels (β = −0.27, P = 0.020; Table 4).

**DISCUSSION**

The present study shows a reduction in CSF GABA levels in patients with FEP compared with healthy controls. Furthermore, CSF GABA concentration, which was unrelated to antipsychotic and/or anxiolytic medication, was found to correlate with general and total score of PANSS, as well as to illness severity, such that lower CSF GABA levels predicted higher symptom levels.

Mounting clinical and experimental data suggest a role of GABA in the pathophysiology of schizophrenia (cf. Introduction). However, evidence for this is mainly indirect and analyses of GABA in CSF of patients with schizophrenia have failed to give a conclusive result in this regard. Although Van Kammen et al. found significantly lower CSF GABA levels in young women with schizophrenia, most previous studies analyzing GABA in the CSF from patients with schizophrenia have failed to observe any difference compared with controls.33–40 These studies, all performed in the 70s or 80s, have been limited by the lack of a control group of age-matched healthy volunteers or by the sensitivity of the GABA assay (enzymatic fluorometric method, ion-exchange column chromatography or radio-receptor assay). Thus, discrepancies between present data, utilizing a well-characterized cohort, healthy volunteers as controls and top-of-the-art high-performance liquid chromatography analysis of GABA and previous literature may be explained by differences in study design and methods of GABA analysis.

The present finding of lower CSF GABA levels in FEP patients likely reflects a reduced overall GABAergic neurotransmission in the brain. The observed direction of changes is in line with previous post-mortem studies showing a reduced GABA synthesis in schizophrenia (cf. Introduction). Further, in excellent agreement with present findings, a recent positron emission tomography study, utilizing the GABA-A receptor ligand [11C]Flumazenil, indicate an impaired GABA transmission in the orbital frontal cortex in patients with schizophrenia.52 Our findings also reveal negative correlations between CSF GABA and total and general PANSS, such that low CSF GABA levels predicted high general severity of illness. In addition, low CSF GABA concentrations were associated with reduced scores of the symptom and functioning dimensions of GAF, as well as with high CGI scores, indicating that symptoms and illness severity associate with lower levels of CSF GABA. In line with a large body of studies, investigating cognitive functions in schizophrenia, FEP patients showed a significant reduction in performance compared with healthy controls in all parts of our cognitive test battery (Table 3). Moreover, CSF GABA levels were found to be positively correlated with Continuous Performance Test-Identical Pairs (although it did not fully meet the Bonferroni-corrected significance threshold of P < 0.008), a cognitive test that measures attention, a cognitive domain impaired in patients with schizophrenia.245,47 A relationship between CSF GABA and cognitive performance is in line with a

| Table 2. CSF GABA levels (µM) with regard to medication |
|---------------|---------------|-------------|
| Medication     | Patient on drug | Patient off drug | P-value* |
| Antipsychotics  | 3.1 ± 0.29 (18) | 3.2 ± 0.37 (23) | 0.85     |
| Benzodiazepines | 2.8 ± 0.25 (12) | 3.4 ± 0.32 (29) | 0.30     |
| Zopiclone      | 3.5 ± 0.40 (14) | 3.1 ± 0.29 (31) | 0.47     |
| Antidepressants | 2.6 ± 0.48 (5)  | 3.3 ± 0.26 (36) | 0.37     |
| Phenothiazine derivatives | 3.0 ± 0.31 (11) | 3.3 ± 0.31 (30) | 0.62     |
| Any treatment  | 3.0 ± 0.20 (29) | 3.7 ± 0.60 (12) | 0.15     |

Abbreviations: CSF, cerebrospinal fluid; GABA, γ-aminobutyric acid. *Unpaired t-test with equal s.d. All antipsychotics combined. All benzodiazepines combined. All antidepressants combined. *Either antipsychotics, benzodiazepines, zopiclone, antidepressants and phenothiazine derivatives or a combination of these. Patients off drug were all drug naive.
Recent magnetic resonance spectroscopy study showing that GABA predicts working memory. Notably, in contrast to patients, healthy controls showed a negative correlation between CSF GABA and performance in the social cognition test. This finding is in line with several clinical reports showing that increased GABA transmission, induced by BZDs or vigabatrin (an inhibitor of GABA transaminase, thereby increasing GABA levels throughout the brain) is associated with cognitive impairments.

A strength of the present study is that the majority of the patients were drug naïve with respect to antipsychotics at the time of the lumbar puncture. No differences in CSF GABA levels were observed between patients on treatment and those without antipsychotic treatment (Table 2), indicating that low CSF GABA levels are a consequence of antipsychotic treatment. It is generally accepted that these drugs are primarily used for anxiolysis and do not affect positive or negative symptoms of the disease. Although a few studies report some favorable effects of BZDs on psychotic symptoms, recent meta-analysis give no evidence for antipsychotic efficacy of additional benzodiazepine medication. Further, administration of GABA-A receptor antagonists is not typically associated with psychotomimetic symptoms. Similarly, clinical trials with GABA-B receptor agonists, such as baclofen or γ-hydroxybutyric acid, have given no evidence for antipsychotic efficacy.

**Table 3. Comparison of different cognitive tests between healthy controls and FEP patients**

| Test       | Cognitive domain               | Mean ± s.e.m. | Healthy Controls (n = 21) | FEP patients (n = 40) | P-value* |
|------------|--------------------------------|---------------|---------------------------|-----------------------|----------|
| CPT-IP     | Attention/vigilance            | 3.0 ± 0.08    | 2.19 ± 0.11               | < 0.0001              |
| TMT        | Speed of processing            | 23.2 ± 1.13   | 32.8 ± 2.13               | 0.003                 |
| BACS-SC    | Speed of processing            | 61.2 ± 1.18   | 46.0 ± 1.87               | < 0.0001              |
| Fluency    | Speed of processing            | 25.2 ± 1.28   | 21.6 ± 0.90               | 0.02                  |
| WMS-III    | Working memory (non-verbal)    | 18.5 ± 0.60   | 15.9 ± 0.50               | 0.003                 |
| LNS        | Working memory (verbal)        | 15.5 ± 0.55   | 13.3 ± 0.50               | 0.008                 |
| NAB: MAZES | Reasoning and problem solving  | 22.9 ± 0.89   | 19.1 ± 0.88               | 0.007                 |
| BVMT-R     | Visual learning                | 29.5 ± 1.10   | 22.4 ± 1.10               | < 0.0001              |
| MSCEIT     | Social cognition               | 98.0 ± 1.23   | 89.9 ± 2.0                | 0.007                 |
| HVLT-R     | Verbal learning                | 28.7 ± 0.65   | 24.0 ± 0.73               | < 0.0001              |

**Table 4. Correlations between CSF GABA, clinical symptoms and cognitive performance in FEP patients**

|                      | r     | P-value* |
|----------------------|-------|----------|
| PANSS                 |       |          |
| Positive             | −0.26 | 0.05     |
| Negative             | −0.19 | 0.12     |
| General              | −0.31 | 0.02     |
| Total                | −0.30 | 0.03     |
| Severity of illness  |       |          |
| GAF symptom dimension| 0.31  | 0.02     |
| GAF functioning dimension| 0.24 | 0.06     |
| CGI score            | −0.38 | 0.007    |
| Cognitive tests      |       |          |
| CPT-IP               | 0.37  | 0.01     |
| TMT                  | −0.038| 0.41     |
| BACS-SC              | −0.028| 0.43     |
| WMS-III              | 0.016 | 0.46     |
| BVMT-R               | −0.095| 0.28     |
| HVLT-R               | −0.12 | 0.24     |

Abbreviations: BACS-SC, Brief Assessment of Cognition in Schizophrenia Symbol Coding; BVMT-R, Brief Visuospatial Memory Test-Revised; CPT-IP, Continuous Performance Test-Identical Pairs; FEP, first-episode psychosis; HVLT-R, Hopkins Verbal Learning Test-Revised; LNS, Letter-Number Span; MSCEIT, Mayer–Salovey–Caruso Emotional Intelligence Test; NAB: MAZES, Neuropsychological Assessment Battery: Mazes; TMT, Trail Making Test; WMS-III, Wechsler Memory Scale-3rd Edition. *Unpaired t-test with equal s.d. **Significant after Bonferroni-correction, α-value = 0.005.
unanimous picture for antipsychotic effects of these drugs.\textsuperscript{57,58} Indeed, the most common adverse effects observed of vigabatrin is behavioral disturbances, ranging from irritability and confusion to psychotic reactions.\textsuperscript{59} Thus, the many experimental and post-mortem studies, suggesting a role of GABA in the pathophysiology of schizophrenia is not supported by the clinical experience of medication with GABA receptor agonists. Although CSF GABA levels are elevated in the cerebrospinal fluid of male patients with schizophrenia. \textit{Schizophr Res} 2005; \textbf{80}: 315–322.

8. Nilsson LX, Linderholm KR, Engberg G, Paulsson L, Blennow K, Lindström LH et al. Elevated levels of kynurenine in the cerebrospinal fluid of male patients with schizophrenia. \textit{Schizophr Res} 2005; \textbf{80}: 315–322.

9. Linderholm KR, Skogh E, Olsson SK, Dahl ML, Holtze M, Engberg G et al. Increased levels of kynurenine and kynuric acid in the CSF of patients with schizophrenia. \textit{Schizophr Bull} 2012; \textbf{38}: 426–432.

10. Schwarcz R, Russoupopou A, Wu HQ, Medoff D, Tamminga CA, Roberts RC. Increased cortical kynurenate content in schizophrenia. \textit{Biol Psychiatry} 2001; \textbf{50}: 521–530.

11. Lewis DA, Curley AA, Glaser JR, Volk DW. Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia. \textit{Trends Neurosci} 2012; \textbf{35}: 57–67.

12. Akbarian S, Huang HS. Molecular and cellular mechanisms of altered GAD1/GAD67 expression in schizophrenia and related disorders. \textit{Brain Res Rev} 2006; \textbf{52}: 29–54.

13. Curley AA, Arion D, Volk DW, Asafu-Adjei JK, Sampson AR, Fish KN et al. Cortical deficits of glutamic acid decarboxylase 67 expression in schizophrenia: clinical, protein, and cell type-specific features. \textit{Am J Psychiatry} 2011; \textbf{168}: 921–929.

14. Thompson Ray M, Weikert CS, Wyatt E, Webster MJ. Decreased BDNF, trkB-TK+ and GAD67 mRNA expression in the hippocampus of individuals with schizophrenia and mood disorders. \textit{J Psychiatry Neurosci} 2011; \textbf{36}: 195–203.

15. Volk DW, Matsubara T, Li S, Sengupta EJ, Georgiev D, Minabe Y et al. Deficits in transcriptional regulators of cortical parvalbumin neurons in schizophrenia. \textit{Am J Psychiatry} 2012; \textbf{169}: 1082–1091.

16. Addington AM, Gornick M, Duckworth J, Sporn A, Gogtay N, Bobb A et al. GAD1 (2q31.1), which encodes glutamic acid decarboxylase (GAD67), is associated with childhood-onset schizophrenia and cortic gray matter volume loss. \textit{Mol Psychiatry} 2005; \textbf{10}: 581–588.

17. Straub RE, Lipska BK, Egan MF, Goldberg TE, Callcott JH, Mayhew MB et al. Allentric variation in GAD1 (GAD67) is associated with schizophrenia and influences cortical function and gene expression. \textit{Mol Psychiatry} 2007; \textbf{12}: 854–869.

18. Du J, Duan S, Wang H, Chen W, Zhao X, Zhang A et al. Comprehensive analysis of polymorphisms throughout GAD1 gene: a family-based association study in schizophrenia. \textit{J Neurotransm (Vienna) 2008; }\textbf{115}: 513–519.

19. Perry TL, Kish SJ, Buchanan J, Hansen S. Gamma-aminobutyric-acid deficiency in brain of schizophrenic patients. \textit{Lancet} 1979; \textbf{1}: 237–239.

20. Spokes EG, Garrett NJ, Rossor MN, Iversen LL. Distribution of GABA in post-mortem brain tissue from control, psychotic and Huntington’s chorea subjects. \textit{J Neurol Sci} 1980; \textbf{48}: 303–313.

21. Toru M, Watanabe S, Shibuya H, Nishikawa T, Noda K, Mitsuhashi H et al. Neurotransmitters, receptors and neuropeptides in post-mortem brains of chronic schizophrenic patients. \textit{Acta Psychiatr Scand} 1978; \textbf{88}: 121–137.

22. Kutay FZ, Pogun S, Hariri NI, Peker G, Ercanin S. Free amino acid level determinations in normal and schizophrenic brain. \textit{Prog Neuropsychopharmacol Biol Psychiatry} 1988; \textbf{13}: 119–126.

23. Ohnума T, Augood SJ, Arai H, McKenna PJ, Emson PC. Measurement of GABAergic parameters in the prefrontal cortex in schizophrenia: focus on GABA content, GABA(A) receptor alpha-1 subunit messenger RNA and human GABA transporter-1 (HGAT-1) messenger RNA expression. \textit{Neuroscience 1999; }\textbf{93}: 441–448.

24. Rowland LM, Krause BW, Witjensburg SA, McMahon RP, Chiappelli J, Nugent KL et al. Medial frontal GABA is lower in older schizophrenia: a MEGA-PRESS with macromolecule suppression study. \textit{Mov Psychiatry} 2016; \textbf{21}: 198–204.

25. Goto N, Yoshimura R, Moriya J, Kakeda S, Ueda N, Ikenouchi-Sugita A et al. Reduction of brain gamma-aminobutyric acid (GABA) concentrations in early-stage schizophrenia patients: 3T Proton MRS study. \textit{Schizophr Res} 2009; \textbf{112}: 192–193.

26. Yoon JH, Maddock RJ, Rokem A, Silver MA, Minzenberg MJ, Ragland JD et al. GABA concentration is reduced in visual cortex in schizophrenia and correlates with -orientation-specific surround suppression. \textit{J Neurosci} 2010; \textbf{30}: 3777–3781.

27. Ongur D, Prescot AP, McCarthy J, Cohen BM, Renshaw PF. Elevated gamma-aminobutyric acid levels in chronic schizophrenia. \textit{Biol Psychiatry} 2010; \textbf{68}: 667–670.

28. Kegeles LS, Mao X, Stanford AD, Girgis R, Oeij N, Xu X et al. Elevated prefrontal cortex gamma-aminobutyric acid and glutamate-glutamine levels in schizophrenia measured in vivo with proton magnetic resonance spectroscopy. \textit{Arch Gen Psychiatry} 2012; \textbf{69}: 449–459.

29. Marenco S, Meyer C, Kuo S, van der Veen JW, Shen J, De Jong K et al. Prefrontal GABA levels measured with magnetic resonance spectroscopy in patients with psychosis and unaffected siblings. \textit{Am J Psychiatry} 2016; \textbf{173}: 527–534.

30. Tayoshi S, Nakatani M, Sumitani S, Taniguchi K, Shibuya-Tayoshi S, Numata S et al. GABA concentration in schizophrenia patients and the effects of antipsychotic medication: a proton magnetic resonance spectroscopy study. \textit{Schizophr Res} 2010; \textbf{117}: 83–91.

31. Goto N, Yoshimura R, Kakeda S, Moriya J, Hori H, Hayashi K et al. No alterations of brain GABA after 6 months of treatment with atypical antipsychotic drugs in
early-stage first-episode schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry, 2010; 34: 1480–1483.

32 Frankie WG, Cho RY, Prasad KM, Mason NS, Paris J, Himes ML et al. In vivo measurement of GABA transmission in healthy subjects and schizophrenia patients. Am J Psychiatry, 2015; 172: 1148–1159.

33 Lichtshtein D, Dobkin J, Ebstein RP, Biederman J, Rimon R, Belmaker RH. Gamma-aminobutyric acid (GABA) in the CSF of schizophrenic patients before and after neuroleptic treatment. Br J Psychiatry, 1978; 132: 145–146.

34 Gold BI, Bowers MB Jr., Roth RH, Sweeney DW. GABA levels in CSF of patients with psychiatric disorders. Am J Psychiatry, 1980; 137: 362–364.

35 Gerner RH, Hare TA. CSF GABA in normal subjects and patients with depression, schizophrenia, mania, and anorexia nervosa. Am J Psychiatry, 1981; 138: 1098–1101.

36 Zimmer R, Teelken AW, Meier KD, Ackenheil M, Zander KJ. Preliminary studies on CSF gamma-aminobutyric acid levels in psychiatric patients before and during treatment with different psychotropic drugs. Prog Neuropsychopharmacol Biol Psychiatry, 1980; 4: 613–620.

37 McCarthy BW, Gomes UR, Neethling AC, Shanley BC, Taljaard JJ, Potgieter L et al. Gamma-aminobutyric acid concentration in cerebrospinal fluid in schizophrenia. J Neurochem, 1981; 36: 1406–1408.

38 van Kammaen DP, Sternberg DE, Hare TA, Waters BN, Bunney WE Jr. CSF levels of gamma-aminobutyric acid in schizophrenia. Low values in recently ill patients. Arch Gen Psychiatry, 1982; 39: 91–97.

39 Gerner RH, Fairbanks L, Anderson GM, Young JG, Scheinin M, Linnoila M et al. CSF neurochemistry in depressed, manic, and schizophrenic patients compared with that of normal controls. Am J Psychiatry, 1984; 141: 1533–1540.

40 Perry TL, Hansen S, Jones K. Schizophrenia, tardive dyskinesia, and brain GABA. Biol Psychiatry, 1989; 25: 200–206.

41 van Kammaen DP, Petty F, Kelley MB, Kramer GL, Barry EJ, Yao JK et al. GABA and brain abnormalities in schizophrenia. Psychiatry Res, 1998; 82: 25–35.

42 Teunissen CE, Petzold A, Bennett JL, Berven FS, Brundin LR, Comabella M et al. A consensus protocol for the standardization of cerebrospinal fluid collection and biobanking. Neurology, 2009; 73: 1914–1922.

43 Ben Menachem E, Persson L, Schechter PJ, Haegele KD, Huebert N, Hardenberg J. Cerebrospinal fluid parameters in healthy volunteers during serial lumbar punctures. J Neurochem, 1989; 52: 632–635.

44 Nuechterlein KH, Green MF, Kern RS, Baade LE, Barch DM, Cohen JD et al. The MATRICS Consensus Cognitive Battery, part 1: test selection, reliability, and validity. Am J Psychiatry, 2008; 165: 203–213.

45 Gropming U. Relative importance for linear regression in R: the package relaimpo. J Stat Softw 2006; 17: 1–27.

46 Heinrichs NW, Zakanis KS. Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. Neuropsychology, 1998; 12: 426–445.

47 Mesholam-Gately RI, Giuliano AJ, Goff KP, Faraone SV, Seidman LJ. Neurocognition in first-episode schizophrenia: a meta-analytic review. Neuropsychology, 2009; 23: 315–336.

48 Stewart SA. The effects of benzodiazepines on cognition. J Clin Psychiatry, 2005; 66: 9–13.

49 Barker MJ, Greenwood KM, Jackson M, Crowe SF. Cognitive effects of long-term benzodiazepine use: a meta-analysis. CNS Drugs, 2004; 18: 37–48.

50 Cavanna AE, Ali F, Rickards HE, McCorry D. Behavioral and cognitive effects of anti-epileptic drugs. Discov Med, 2010; 9: 138–144.

51 Gattaz WF, Roberts E, Beckmann H. Cerebrospinal fluid concentrations of free GABA in schizophrenia: no changes after haloperidol treatment. J Neural Transm, 1986; 66: 69–73.

52 Loscher W, Schmidt D. Diazepam increases gamma-aminobutyric acid in human cerebrospinal fluid. J Neurochem, 1987; 49: 152–157.

53 Waddington JL, Longden A. Rotational behaviour and cGMP responses following manipulation of nigral mechanisms with chlordiazepoxide. Evidence for enhancement of GABA transmission by benzodiazepines. Naunyn Schmiedebergs Arch Pharmacol, 1977; 300: 233–237.

54 Haefely W, Kulcsar A, Mohler H, Pieri L, Polc P, Schaffner R. Possible involvement of GABA in the central actions of benzodiazepines. Adv Biochem Psychopharmacol, 1975; 14: 131–151.

55 Wolkowitz OM, Pickar D. Benzodiazepines in the treatment of schizophrenia: a review and reappraisal. Am J Psychiatry, 1991; 148: 714–726.

56 Dold M, Li C, Gillies D, Leucht S. Benzodiazepine augmentation of antipsychotic drugs in schizophrenia: a meta-analysis and Cochrane review of randomized controlled trials. Eur Neuropsychopharmacol, 2013; 23: 1023–1033.

57 Daskalakis ZJ, George TP, Clozapine, GABA(B), and the treatment of resistant schizophrenia. Clin Pharmacol Ther, 2009; 86: 442–446.

58 Kantrowitz J, Citrome L, Javitt D. GABA(B) receptors, schizophrenia and sleep dysfunction: a review of the relationship and its potential clinical and therapeutic implications. CNS Drugs, 2009; 23: 681–691.

59 Sander JW, Hart YM. Vigabatrin and behaviour disturbances. Lancet, 1990; 335: 57.