Citation for published version (APA): Egerton, A., Modinos, G., Ferrera, D., & McGuire, P. (2017). Neuroimaging studies of GABA in schizophrenia: A systematic review with meta-analysis. *Translational psychiatry, 7*(6), [e1147]. https://doi.org/10.1038/tp.2017.124
REVIEW

Neuroimaging studies of GABA in schizophrenia: a systematic review with meta-analysis

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Data from animal models and from postmortem studies suggest that schizophrenia is associated with brain GABAergic dysfunction. The extent to which this is reflected in data from in vivo studies of GABA function in schizophrenia is unclear. The Medline database was searched to identify articles published until 21 October 2016. The search terms included GABA, proton magnetic resonance spectroscopy (1H-MRS), positron emission tomography (PET), single photon emission computed tomography (SPECT), schizophrenia and psychosis. Sixteen GABA 1H-MRS studies (538 controls, 526 patients) and seven PET/SPECT studies of GABA/βenzodiazepine receptor (GABA/βZR) availability (118 controls, 113 patients) were identified. Meta-analyses of 1H-MRS GABA in the medial prefrontal cortex (mPFC), parietal/occipital cortex (POC) and striatum did not show significant group differences (mFC: g = −0.3, 409 patients, 495 controls, 95% confidence interval (CI): −0.6 to 0.1; POC: g = −0.3, 139 patients, 111 controls, 95% CI: −0.9 to 0.3; striatum: g = −0.004, 123 patients, 95 controls, 95% CI: −0.7 to 0.7). Heterogeneity across studies was high (I2 > 50%), and this was not explained by subsequent moderator or meta-regression analyses. There were insufficient PET/SPECT receptor availability studies for meta-analyses, but a systematic review did not suggest replicable group differences in regional GABA/βZR availability. The current literature does not reveal consistent alterations in in vivo GABA neuroimaging measures in schizophrenia, as might be hypothesized from animal models and postmortem data. The analysis highlights the need for further GABA neuroimaging studies with improved methodology and addressing potential sources of heterogeneity.

Translational Psychiatry (2017) 7, e1147; doi:10.1038/tp.2017.124; published online 6 June 2017

INTRODUCTION

One of the most consistent findings from postmortem studies in schizophrenia is a reduction in the GABA-synthesizing enzyme, GAD67 mRNA and protein.1–3 Expression of GAD67 is activity regulated4,5 and GAD67 is responsible for over 90% of all (cytosolic) GABA production.6 In contrast to GAD67, inconsistent findings in schizophrenia are reported for the GAD65 isoform,7–10 which is involved in vesicular, synaptic GABA production during intense periods of neural activity.11,12 The potential effects of a reduction in GAD67 on cortical excitatory/inhibitory networks is a key component in some neurobiological models of schizophrenia.13 In particular, GABA dysfunction is thought to lead to the disinhibition of glutamatergic pyramidal neurons and a loss of synchronous cortical activity.14,15 Postmortem studies also suggest that schizophrenia is associated with dysfunctional GABA signalling at the postsynaptic receptor level. Receptor autoradiography using 3H-muscimol, an agonist at the GABA binding site on the GABA/β benzodiazepine receptor (GABA/βZR) complex, has consistently shown an increase in binding density in the prefrontal, cingulate and temporal cortices and caudate nucleus.16–22 In contrast, density of binding to the BZ binding site of the GABA/βZR complex has been found unaltered, increased or decreased postmortem.22–27 Postmortem investigations of GABA, α subunit expression have found reductions in α1 (refs 28,29) and increases in α2 (refs 29,30) expression in schizophrenia, but inconsistent results for the α5 subunit.29,31,32

GABA function in schizophrenia can be assessed in vivo using neuroimaging techniques. Proton magnetic resonance spectroscopy (1H-MRS) optimized for GABA detection can measure GABA concentrations within a voxel of interest. This approach measures total (intracellular and extracellular) GABA and macromolecules (denoted GABA+) across all tissue content in a relatively large voxel. An alternative neuroimaging approach is to use positron emission tomography (PET) or single photon emission computed tomography (SPECT) in conjunction with specific radiotracers that bind to GABA or BZ receptors.33 However, all the PET/SPECT radiotracers currently available for human use bind to the BZ rather than to the GABA site of GABA/βZR receptors. The PET/SPECT radiotracers iomazenil and flumazenil have limited subunit selectivity, binding GABA/β/BZ receptors containing α1, α2, α3 and α6 subunits, whereas Ro15-4513 has more selectivity for α1 and α5.34

Neuroimaging of GABA function is potentially important because several hypotheses around the role of GABA dysfunction in schizophrenia can only be tested in vivo. Evidence that GABA dysfunction has a role in the pathophysiology of schizophrenia has also led to interest in the therapeutic potential of pharmacological compounds that act on GABA function, and data from animal studies suggest that administration of benzodiazepines can prevent the development of neuroanatomical and neurophysiological abnormalities associated with schizophrenia.35,36
Although there have been several neuroimaging studies of GABA in schizophrenia, the nature of GABAergic abnormalities in schizophrenia in vivo remains unclear. The present study aims to address this issue by conducting a systematic review and a meta-analysis of \(^1\)-H-MRS and PET/SPECT studies of GABA in schizophrenia. In our primary analyses as well as studies of patients with schizophrenia, we included studies of subjects at high clinical or genetic risk for the disorder, as GABAergic dysfunction may be a ‘trait’ characteristic, arising through the influences of genetic variation during development.\(^37\) The potential influence of clinical subgroups,\(^38,39\) medication status,\(^38,40\) symptom severity,\(^38,40,41\) age\(^42,43\) and gender\(^44\) were investigated using subsequent moderator analyses and meta-regression.

MATERIALS AND METHODS

Study selection

The meta-analysis and systematic review was performed in accordance with the guidelines of the PRISMA group.\(^45\) The Medline electronic database was searched to identify journal articles published from 1 January 1950 until 21 October 2016, using the following MeSH and freeform search terms: (‘GABA’) AND (‘MRS’ OR ‘spectroscopy’ OR ‘positron emission tomography’ OR ‘single photon emission tomography’ OR ‘single photon emission computed tomography’ OR ‘PET’ OR ‘SPECT’ OR ‘SPECT’) AND (‘schizophrenia’ OR ‘psychosis’ OR ‘schizophreniform disorder’ OR ‘psychosis risk’). Reference lists of the returned articles were hand searched for further relevant publications. Two authors independently performed the searches and identified articles for inclusion (AE and DF).

Inclusion required that articles were published in peer-reviewed journals in English or English translation. Inclusion also required that articles reported GABA measures in vivo, in a group with clinical diagnosis of schizophrenia, schizoaffective disorder or first episode psychosis, or a group at clinical or genetic risk for schizophrenia, compared with a healthy volunteer (control) group. \(^1\)-H-MRS studies were excluded if they reported the GABA signal only as the combined signal with glutamate (Glx). PET/SPECT studies were excluded if they investigated translocator protein, which mediates various mitochondrial functions and was previously described as the peripheral benzodiazepine receptor.\(^46\) Where articles reported overlapping samples, only data from the article reporting the largest sample was included.

Outcome measures

The primary outcome measure was the control and patient mean and standard deviation (s.d.) \(^1\)-H-MRS GABA\(^+\) concentration in each voxel, or GABA\(_2\)/BZR availability in each region of interest. Where these values were not reported in the published article, the authors were contacted or values were estimated from figures using a freely available ruler for Mac OS X (http://www.pascal.com/software/freeruler/). Where values were reported in each hemisphere separately, the mean of these values was calculated. For \(^1\)-H-MRS studies, due to partially overlapping voxel locations and to provide sufficient data for meta-analysis, data were combined into the medial frontal cortex (mFC), parietal and occipital cortices and striatum. All the variables were extracted independently by two authors (AE and DF) and cross-checked for accuracy.

Meta-analysis

Inclusion in the meta-analyses required availability of data in a given brain region from five or more studies.\(^47\) Where there were insufficient data for meta-analysis, the findings were summarized. Where articles included more than one patient or control group, these groups were entered separately in the analyses. For each variable, the effect size statistic Hedges’ \(g\) was calculated. Hedges’ \(g\) is the Cohen’s effect size incorporating a correction for bias from small sample sizes.\(^48\)

The meta-analysis for each variable was performed using STATA/IC, version 14, using the METAN command (StataCorp LP, College Station, TX, USA). A random-effects inverse-variance model\(^49\) was used to calculate the pooled effect size to adjust for study heterogeneity. Significance was assessed using two-sided 95% confidence intervals.

Heterogeneity was measured using the \(I^2\) value, which indicates the percentage variance due to heterogeneity between studies compared with chance.\(^50\) Where \(I^2\) values indicated substantial heterogeneity (\(I^2 > 50\%\)), potential sources of heterogeneity were investigated by using sensitivity analysis to assess potential influences of single studies, and Egger’s test\(^51\) to investigate potential publication bias.

Moderator analyses

Potential influences of study characteristics were investigated using moderator analyses. Subgroup analyses investigated the following dichotomous characteristics of data sets: (1) clinical category of subjects (first episode psychosis or schizophrenia patients versus clinical risk or genetic risk groups); (2) explicitly stated absence of GABAergic (benzodiazepine or anticonvulsant) medication at the time of scanning; (3) presence (in > 90% of the sample) or absence (in 100% of the sample) of antipsychotic medication at the time of imaging. For mFC \(^1\)-H-MRS studies, subgroup analyses additionally investigated potential influences of mFC voxel location (Figure 1).

Meta-regressions were conducted to explore potential influences of continuous variables relating to patient characteristics (age, percentage male in sample, illness duration, Positive and Negative Syndrome Scale total score), voxel grey matter content and publication year on GABA measures. Symptoms rated using the BPRS were converted to Positive and Negative Syndrome Scale scores using the established conversion scale of Leucht et al.\(^52\) Meta-regression analyses were performed in STATA/IC version 14 using the METAREG command, with Hedges’ \(g\) as the outcome variable. To reduce the likelihood of chance findings, both subgroup analyses and meta-regressions required a minimum of five data sets. In all cases, the threshold for statistical significance was \(P < 0.05\).

Study quality and methodological characteristics

The methodological characteristics of \(^1\)-H-MRS and PET/SPECT studies are summarized in Supplementary Tables 1 and 2, respectively. Although there are no established criteria for formal quality assessment of \(^1\)-H-MRS and PET/SPECT studies, key factors that may impact on data quality are discussed in the Supplementary Information.

RESULTS

\(^1\)-H-MRS studies

Nineteen articles describing \(^1\)-H-MRS studies of GABA\(^+\) in schizophrenia were identified (Supplementary Figure 1). Of these, the article by Chen et al.\(^53\) was excluded due to partial overlap with the larger sample reported in Kegeles et al.\(^40\) Similarly, the article by Rowland et al.\(^54\) was excluded due to overlap with the larger sample reported in Rowland et al.\(^43\) Data from a single study were reported across two articles\(^55,56\) from which data extraction was combined. The clinical characteristics of the \(^1\)-H-MRS samples are provided in Table 1. Data used to calculate effect sizes are available in Supplementary Table 1. The methodological characteristics are provided in Supplementary Table 2.

Medial frontal cortex

Twelve articles\(^38,40,42,43,55,57–60\) involved 17 data sets for GABA\(^+\) in the mFC, providing data from a total of 409 patients and 495 controls. Meta-analysis returned a summary effect size of \(g = -0.3\), which was nonsignificant (95% confidence interval: −0.6 to 0.1,
$P = 0.1$, Figure 2a). The $I^2$ value was 84%, indicating a significant ($P < 0.001$) and considerable heterogeneity across data sets. Visual inspection of the Forrest plot (Figure 2a) shows that one study was clearly an outlier, and that of the remaining studies, approximately half reported higher GABA+ levels in patients than controls, while the other half reported the opposite. The recalculated summary effect size after removal of the outlying controls, while the other half reported the opposite. The recalculated summary effect size after removal of the outlying data set was $g = -0.1$, which was also nonsignificant (95% confidence interval: $-0.4$ to $0.2$, $P = 0.5$, $I^2 = 69$%). Sensitivity analyses did not return significant results on any iteration, and the Eggers test did not suggest publication bias.

Available data sets permitted a series of subgroup analyses, which involved studies that (i) only included patients with a first episode of psychosis or schizophrenia (14 data sets$^{38,40,42,43,55,57,59,60}$); (ii) explicitly excluded patients taking benzodiazepine or anticonvulsant medication (eight data sets$^{38,41,43}$); (iii) included patients of whom greater than 90% were being treated with antipsychotic medication (12 data sets$^{38,40,42,43,55,57,59,60,62}$); (iv) excluded subjects who had taken antipsychotic medication (five data sets$^{38,40,41,62}$); or (v) included only 1H-MRS voxels in the medial prefrontal area of the medial frontal cortex (Figure 1; 10 data sets$^{38,40,42,43,55,57,59,60}$). All of these subgroup analyses returned nonsignificant summary effect sizes and $I^2$ values $>50$. Meta-regression did not reveal any significant relationships between mFC GABA+ and age, illness duration, symptom severity, percentage grey matter in the voxel or publication date. There was a significant association with percentage of males in the sample (17 observations, $\beta = -0.04$; $t = -2.5$; $P = 0.03$), but this was driven by outlying values from one study that included only male subjects. The effect was no longer significant when this study had been removed ($\beta = -0.004$; $t = -0.4$; $P = 0.7$).

Parietal/occipital cortex

Meta-analysis of GABA+ in the parietal/occipital cortex included seven observations across six articles$^{39,55,57,59,63,64}$ providing data from a total of 139 patients and 111 controls. The summary effect size was nonsignificant ($g = -0.3$; 95% confidence interval: $-0.9$ to $0.3$, $P = 0.3$, $I^2 = 80$%; Figure 2b) with no indication of publication bias. Limiting the analysis to observations in first episode psychosis or schizophrenia (six observations$^{39,55,57,59,63,64}$) also returned nonsignificant summary effect sizes. There were insufficient data to investigate further subgroups. All meta-regression analyses were nonsignificant.

Striatum

Five data sets reported GABA+ in the striatum across four articles$^{39,41,55,58}$. The summary effect size was not significant (123 patients, 95 controls, $g = -0.004$; 95% confidence interval: $-0.7$ to $0.7$, $P < 0.1$, $I^2 = 82$% Figure 2c), with no indication of publication bias. There were insufficient data for subgroup analyses and meta-regression returned nonsignificant findings.

Other brain regions

One study$^{40}$ examined GABA+ in the dorsolateral prefrontal cortex, one$^{42}$ examined GABA in the centrum semiovale and one$^{65}$ examined GABA+ in the left hippocampus (Table 1, Figure 2d). There were insufficient data for meta-analysis and no significant group differences in GABA+ were reported for these brain regions.

GABA$_A$/BZR availability

Ten articles were initially identified, which reported GABA$_A$/BZR availability in schizophrenia$^{66-73}$. Of these, three were excluded: one because it was a conference abstract rather than a paper, one because it was a conference abstract rather than a paper, one because it was a conference abstract rather than a paper, and one because it was a conference abstract rather than a paper.
Table 1. 1H-MRS GABA data sets: clinical characteristics of the samples

| Region   | First author | Year | Patient group | g | Sample size | SCZ age mean | %M | %AP | %BZ | PANSS | DOI |
|----------|--------------|------|---------------|---|-------------|--------------|-----|-----|-----|-------|-----|
| mFC      | Goto         | 2009 | FEP           | -0.2 | 18 18 50 29 | 100 NR        | 68 0.5 |
|          | Ongür        | 2010 | SCZ           | 0.5  | 19 21 67 39 | 95 76 51 21  |
|          | Tasaki       | 2010 | SCZ           | -0.3 | 29 38 53 34 | 100 42 51 21 |
|          | Kegeles      | 2012 | SCZ unmed     | 0.7  | 11 16 69 32 | 0 19 71 7    |
|          | Kegeles      | 2012 | SCZ med       | 0.2  | 11 16 69 32 | 100 19 57 9  |
|          | Rowland      | 2013 | SCZ young     | 0.0  | 10 11 82 30 | 100 0 63 2.7 |
|          | Rowland      | 2013 | SCZ old       | -1.0 | 10 10 70 51 | 100 0 57 25.5|
|          | Marsman      | 2014 | SCZ           | -0.8 | 19 13 76 28 | 100 35 53 6.5|
|          | De la Fuente | 2015 | CHR           | 0.7  | 24 23 65 21 | 0 0 NR       |
|          | Brandt       | 2016 | SCZ           | 0.3  | 24 24 79 38 | 100 17 NR    |
|          | Marenco      | 2016 | Siblings      | -0.7 | 61.3 31 55 30 | 0 0 NR       |
|          | Marenco      | 2016 | SCZ unmed     | -0.4 | 61.3 25 72 28 | 0 0 NR       |
|          | Marenco      | 2016 | SCZ med       | -0.3 | 61.3 70 71 31 | 100 36 NR    |
|          | Menschikov   | 2016 | CHR           | -4.1 | 26 21 100 NR | NR NR NR     |
|          | Rowland      | 2016 | SCZ young     | 0.0  | 40 29 69 26 | 93 0 5.6     |
|          | Rowland      | 2016 | SCZ old       | -0.7 | 37 31 61 48 | 90 0 24     |
|          | Yang         | 2016 | FEP           | 0.8  | 23 22 41 26 | 0 NR 1.6     |
| POC      | Goto         | 2009 | FEP           | 0.2  | 18 18 50 29 | 100 NR        | 68 0.5 |
|          | Ongür        | 2010 | SCZ           | 0.4  | 19 21 67 39 | 95 76 51 21  |
|          | Yoon         | 2010 | FEP/SCZ       | -2.6 | 13 13 85 28 | 62 NR 73     |
|          | Kelemen      | 2013 | FEP           | -0.7 | 20 28 64 25 | 0 0 88 0.8   |
|          | Marsman       | 2014 | SCZ           | 0.3  | 19 15 76 28 | 100 35 53 6.5|
|          | Thakkar      | 2016 | Sibling       | 0.0  | 12 23 53 31 | 0 0 NR       |
|          | Thakkar      | 2016 | SCZ           | -0.5 | 12 21 71 36 | 100 24 49 14 |
| Striatum | Goto         | 2009 | FEP           | -0.8 | 18 18 50 29 | 100 NR        | 68 0.5 |
|          | Tasaki       | 2010 | SCZ           | -0.2 | 29 38 53 34 | 100 42 51 21 |
|          | De la Fuente | 2015 | CHR           | 1.3  | 24 23 65 21 | 0 0 NR       |
|          | Thakkar      | 2016 | Sibling       | -0.3 | 12 23 53 31 | 0 0 NR       |
|          | Thakkar      | 2016 | SCZ           | -0.1 | 12 21 71 36 | 100 24 49 14 |
| dIPFC    | Kegeles      | 2012 | SCZ unmed     | -0.1 | 11 16 69 32 | 0 19 71 7    |
|          | Kegeles      | 2012 | SCZ med       | -0.4 | 11 16 69 32 | 100 19 57 9  |
| Hippocampus | Stan       | 2015 | SCZ           | -0.2 | 16 18 78 42 | 61 28 NR     |
| CSO      | Rowland      | 2013 | SCZ younger   | 0.2  | 10 11 82 30 | 100 0 63 7.7 |
| CSO      | Rowland      | 2013 | SCZ older     | -0.7 | 10 10 70 51 | 1000 0 57 25.5|

Abbreviations: %AP, percentage of SCZ group currently taking antipsychotic medication; %BZ, percentage of SCZ group currently taking benzodiazepine or anticonvulsant medication; C, control; CHR, clinical high risk; CSO, centrum semiovale; dIPFC, dorsolateral prefrontal cortex; DOI, mean duration of illness in years; FEP, first episode psychosis; g, Hedge’s effect size; %M, percentage of male in the SCZ sample; mFC, medial frontal cortex area; NR, not reported; PANSS, Positive and Negative Syndrome Scale mean total symptom score; POC, parietal/occipital cortex; SCZ, schizophrenia or schizophreniform disorder; sibling, healthy siblings of patients with SCZ. Age is expressed in years (mean).
Figure 2. (a–d) Forest plots showing effect sizes (Hedge’s g) for ¹H-MRS GABA studies in schizophrenia versus control. Error bars represent 95% confidence intervals. Black squares indicate data from clinical patient samples (FEP or SCZ) while white squares indicate data from CHR or sibling samples. CHR, clinical high risk; FEP, first episode psychosis; ¹H-MRS, proton magnetic resonance spectroscopy; SCZ, schizophrenia or schizoaffective disorder; sibling, unaffected siblings of patients with schizophrenia; med, currently receiving antipsychotic medication; unmed, currently unmedicated with antipsychotics.


**DISCUSSION**

The main finding of this article is that meta-analyses of $^{1}$H-MRS studies found no evidence for significantly altered GABA$^{+}$ concentrations in patients with schizophrenia compared with healthy volunteers in the medial frontal cortex, parieto-occipital cortex or striatum. Analyses revealed a substantial level of heterogeneity across studies, which may relate to differences in patient samples and $^{1}$H-MRS methodological characteristics. Although there were insufficient studies of GABA$\alpha_1$/BDZ availability to perform meta-analysis, a systematic review of these studies

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**Table 2.** PET/SPECT GABA$\alpha_1$/BDZ receptor availability data sets: clinical characteristics of the samples

| First author | Year | Patient group | Sample size | SCZ%M | SCZ% age | %AP | %BZ | PANSS | DOI |
|--------------|------|---------------|-------------|-------|-----------|-----|-----|-------|-----|
| Busatto$^{67}$ | 1997 | SCZ           | 12          | 15    | 93        | 29  | 60  | NR    | 6.5 |
| Verhoeff$^{70}$ | 1999 | SCZ           | 24          | 25    | 100       | 41  | 80  | 0     | NR  |
| Asai$^{71}$    | 1999 | SCZ           | 16          | 16    | 100       | 44  | 69  | 0     | NR  |
| Lee$^{72}$     | 2008 | SCZ           | 11          | 12    | 55        | 33  | 0   | 0     | 90.4|
| Kang$^{73}$    | 2014 | CHR           | 15          | 11    | 66        | 19  | 18  | 0     | NR  |
| Frankie$^{75}$ | 2015 | SCZ           | 22          | 17    | 65        | 28  | 0   | NR    | 83  |

Abbreviations: %AP, percentage of SCZ group currently taking antipsychotic medication; %BZ, percentage of SCZ group currently taking benzodiazepine or anticonvulsant medication; C, control; CHR, clinical high risk; DOI, mean duration of illness in years; %M, percentage of male in SCZ sample; NR, not reported; PANSS, Positive and Negative Syndrome Scale mean total symptom score; SCZ, schizophrenia. Age is expressed in years, mean; regional effect sizes are provided in Figure 2.

**Figure 3.** Illustration of effect sizes (Hedge’s $g$) for PET/SPECT studies of regional GABA$\alpha_1$/BDZ receptor availability in schizophrenia versus control. Error bars represent 95% confidence intervals. ACC, anterior cingulate cortex; Cing. Ctx, cingulate cortex; dlPFC, dorsolateral prefrontal cortex; EC, entorhinal cortex; FC, frontal cortex; Hip, hippocampus; $^{1}$H-MRS, proton magnetic resonance spectroscopy; mFC, medial frontal cortex; mTC, medial temporal cortex; NAc, nucleus accumbens; OFC, orbitofrontal cortex; ParaHip, parahippocampus; PFC, prefrontal cortex; TC, temporal cortex. Studies reporting only voxel-wise analyses$^{70,74}$ are excluded from the figure.
found no consistent evidence for altered GABA/BZR availability in schizophrenia.

To our knowledge, this is the first meta-analysis of 1H-MRS GABA studies in schizophrenia. Postmortem studies in schizophrenia find reductions in GAD67,1–3 which is responsible for the majority of basal GABA synthesis in the cortex. The 1H-MRS GABA signal may reflect the entire GABA content of the voxel (that is, intracellular and extracellular, and involved in metabolism or neurotransmission). Recent work argues that the 1H-MRS GABA signal predominantly relates to extracellular, extra-synaptic GABA providing tonic inhibitory tone, rather than GABA involved in phasic synaptic neurotransmission.76,77 Theoretically, the 1H-MRS GABA signal should therefore be sensitive to GAD67 reduction. However, our meta-analysis of in vivo 1H-MRS GABA studies in schizophrenia found that, although in cortical regions the summary effect sizes were consistent with lower GABA levels, these effect sizes were small and nonsignificant.

An absence of large, detectable differences in GABA concentrations in schizophrenia in vivo could reflect normalization by compensatory mechanisms at the cellular or network level,15 and it is unknown whether GAD67 reduction in schizophrenia is primary, or secondary to other pathological mechanisms such as glutamatergic dysfunction.78,79 Furthermore, one limitation of 1H-MRS is that it measures total GABA concentrations within a relatively large voxel (mean 30 ml in the studies included in this article), which is determined a priori, and cannot discriminate between GABA levels in different cell types. This limits the application of 1H-MRS in addressing the cell- and network-specific GABA abnormalities hypothesized to occur in schizophrenia.15

The 1H-MRS meta-analysis also reflects several limitations in the currently available literature. Sixteen studies contributed to the meta-analysis, but there were relatively few investigations in each brain region, with non-overlapping voxel placements (for example in the mFC), variability between clinical samples and 1H-MRS methodological approaches and high heterogeneity. Meta-analysis revealed substantial variability in the findings across studies. For example, there were approximately equal numbers of studies reporting increases of GABA+ in the medial frontal cortex in schizophrenia as there were studies reporting reductions (Figure 2a), and all meta-analyses were associated with significant and high levels of heterogeneity. This may reflect between-study differences in patient samples, methodological approaches or relate to inconsistency in 1H-MRS GABA measurement. Regional brain GABA levels in schizophrenia may vary with the stage of the disorder, as has been reported in some individual studies,8,40,42,43 and appears to be evident for brain glutamate levels.80 Our analysis was limited in that there were too few studies to perform meta-analyses of all patient subgroups in all regions. However, exclusion of data sets from ‘at risk’ participants (and thus restricting the analysis to patients with schizophrenia) did not change our findings. Similarly, the findings in the mFC remained nonsignificant and heterogeneous when the analysis was limited to either antipsychotic unmedicated or treated patients, or restricted to the prefrontal part of the medial frontal region. Moreover, meta-regression found no effect of duration of illness, participant age or symptom severity on GABA effect sizes. Nevertheless, there are several other clinical and methodological variables that might contribute to heterogeneity, such as the duration of treatment, time off medication or substance use, which we were not able to investigate in this meta-analysis. It is also possible that more complex relationships exist between two or more study variables on the GABA effect size, for example the location of GABA dysfunction within the mFC may vary with age or illness stage.

Owing to limited data availability, our meta-analysis did not account for the several methodological differences between studies that may have impacted on data quality. Differences in field strength, voxel size and acquisition times will translate to large between-study differences in the signal to noise ratio, and it was not possible to evaluate spectral quality in 7 of the 16 included articles (see Supplementary Information). Only two recent studies included methodology to isolate the GABA signal from macromolecule contamination,59,43 which is a key area for future methodological development. Therefore, while the meta-analyses did not indicate differences in regional GABA levels between patients and controls, this interpretation is limited pending publication of further individual studies. Future studies should directly compare different patient samples, maximize signal to noise ratio, address macromolecule contamination and include detailed and transparent reporting of spectral quality. On the basis of postmortem evidence, we suggest that key regions for investigation include the dorsolateral prefrontal cortex, anterior cingulate cortex and hippocampus.3,10,17,81–83

Our systematic review of PET/SPECT studies examining GABA/BZR availability also suggested an overall lack of evidence for differences in patients compared with controls, with no significant regional group differences in four out of seven studies.77,71,72,75 However, the three voxel-wise studies all reported lower GABA/BZR availability in patients compared with controls, but there was no consistency across studies in the regions where these differences were detected.70,73,74 All identified studies applied PET/SPECT radiotracers with affinity at the BZ site of the GABA/BZR complex, and postmortem autoradiography studies of availability of BZ binding sites have also produced inconsistent results.22–27 This contrasts with reports of increases in availability of the GABA binding site on the GABA/BZR in schizophrenia postmortem,16–22 for which in vivo radiotracers are currently unavailable. A further consideration is that several PET/SPECT studies estimated regional GABA/BZR availability relative to white matter,57,72–74 which may be confounded by the presence of white matter abnormalities in schizophrenia (see ref. 84).

In contrast to 1H-MRS, GABA/BZR PET imaging may be able to measure changes in synaptic GABA concentrations. Frankle et al.75 used this approach to examine the increase in GABA following administration of the presynaptic GABA reuptake inhibitor tiagabine. Although they found no difference between patients with schizophrenia and controls, when the analysis was restricted to the subgroup of patients that were antipsychotic-naïve, the increase in GABA following tiagabine was significantly diminished. Tiagabine-induced increases in cortical GABA are not detectable using 1H-MRS,85,86 which is consistent with the view that the GABA 1H-MRS signal principally reflects nonsynaptic GABA. Pharmacologically induced alterations in synaptic GABA may be more sensitively imaged with 11C-Riluzole PET, because it is a GABA/BZR inverse agonist with greater selectivity for intra-synaptic receptors.87 In the future, combination of this approach with 1H-MRS in the same subjects, and potentially during the same scanning session on combined PET-MR platforms, might investigate dysfunction of synaptic versus nonsynaptic GABA in schizophrenia.

Cluster analyses of postmortem data find that GABAergic deficits are not present in all schizophrenia patients, but characterize a patient subgroup of approximately 50% of the postmortem sample.88,89 This postmortem ‘Low GABA Marker’ (LGM) phenotype89 does not readily relate to illness severity, psychoactive medication or substance use at the time of death.89,89 If a LGM subgroup could similarly be identified using in vivo biomarkers, this might lead to a stratified approach to treatments that address GABAergic dysfunction. It is possible that the heterogeneity in 1H-MRS studies may also reflect GABAergic subgroups of patients, either within- or between-study samples, which are again not readily identifiable by clinical variables. However, unlike postmortem studies, GABA imaging studies did not show consistently higher variability in GABA measurements in the patient compared with the control group (Supplementary Table 2). Combination of GABA 1H-MRS or GABA PET/SPECT with electroencephalogram gamma-band oscillations in schizophrenia,23,75,90 which reflect on parvalbumin neuron
activity, may help determine whether such GABAergic subgroups of patients are identifiable in vivo.

In conclusion, at present, the neuroimaging literature suggests that brain GABA function, as indexed by \(^{1}H\)-MRS GABA concentrations and GABA\(_{BZ}/BZR\) BZ site availability, does not provide a consistent pattern of alteration in schizophrenia. However, the total number of studies completed in this field is still relatively small, and most studies to date have involved small patient samples (typically 15–30 patients), and varying data quality (see Supplementary Information for discussion). It remains unclear if the absence of overall differences reflects confounding effects of age, stage of illness, medications or other unknown factors. Further studies using larger and more homogeneous samples may therefore be useful, as would studies directly comparing specific patient subgroups. Advances in both \(^{1}H\)-MRS and PET methodologies may reveal specific aspects of GABA dysfunction in vivo in schizophrenia within the next few years.

**CONFLICT OF INTEREST**
The authors declare no conflict of interest.
37 Marin O. Interneuron dysfunction in psychiatric disorders. Nat Rev Neurosci 2012; 13: 107–120.

38 Marenco S, Meyer C, Kuo S, van der Veen JW, Shen J, DeJong K et al. Frontal glutamate and gamma-aminobutyric acid levels and their associations with mismatch negativity and digit sequencing task performance in schizophrenia. JAMA Psychiatry 2016; 73: 166–174.

39 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. Schizophr Res 2009; 112: 192–193.

40 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.

41 Ono D, Steiner EC, Ulrich CF, van der Veen JW, Shen J, DeJong K et al. Correlation between reduced in vivo benzodiazepine receptor binding and severity of psychotic symptoms in schizophrenia. Am J Psychiatry 1997; 154: 56–63.

42 Schroder J, Bubec B, Demisch S, Sauer H. Benzodiazepine receptor distribution and diazepam binding in schizophrenia: an exploratory study. Psychiatry Res 1997; 68: 125–131.

43 Bull S, Busatto GF, David AS, Jones SH, Hemsley DR, Pilowsky LS et al. Cognitive functioning and GABA/benzodiazepine receptor binding in schizophrenia: a 123I-ioflupane SPECT study. Biol Psychiatry 1998; 43: 107–117.

44 Verheijen NP, Soares JC, D’Souza CD, Gil R, Degen K, Abi-Dargham A et al. [123I]Ioflupane SPECT benzodiazepine receptor imaging in schizophrenia. Psychopharmacology 1999; 165: 165–173.

45 Abi-Dargham A, Laruelle M, Krystal J, D’Souza C, Zoghbi S, Baldwin RM et al. No evidence of altered in vivo benzodiazepine receptor binding in schizophrenia. Neuropsychopharmacology 1999; 20: 650–661.

46 Assaf A, Takano I, Hsu H, Matsuura M, Otsuka A et al. GABA/Benzodiazepine receptor binding in patients with schizophrenia using [11C]Ro15-4513, a radioligand with relatively high affinity for alpha5 subunit. Schizophr Res 2008; 99: 1–3.

47 Kang J, Park PJ, Kim SJ, Kim KR, Lee SY, Lee E et al. Reduced binding potential of GABA-A/benzodiazepine receptors in individuals at ultra-high risk for psychosis: an [18F]fluoroflumazenil positron emission tomography study. Schizophr Bull 2014; 40: 548–557.

48 Lee JS, Lee JK, Park PJ, Oh MK, Chun JW, Kim SJ et al. Is the GABA system related to the social competence improvement effect of aripiprazole? An [18F]Fluoro-flumazenil PET study. Psychiatry Investig 2013; 10: 75–80.

49 Franklin 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.

50 Stagg CJ. Magnetic resonance spectroscopy as a tool to study the role of GABA in motor-cortical plasticity. Neuroimage 2014; 86: 19–27.

51 Dyke K, Pepes SE, Chen C, Kim S, Sigurdsson HP, Draper A et al. Comparing GABA-dependent physiological measures of inhibition with proton magnetic resonance spectroscopy measurement of GABA using ultra-high-field MRI. Neuroimage 2017; 152: 360–370.

52 Belforte JE, Zsiovos V, Sklar ER, Jiang Z, Yu G, Li Y et al. Postnatal NMDA receptor ablation in corticobasal interneurons confers schizophrenia-like phenotypes. Nat Neurosci 2010; 13: 76–83.

53 Turner CP, DeBenedetto D, Ware E, Stowe R, Lee A, Swanson J et al. Postnatal exposure to MRB1 induces selective changes in GAD67 or parvalbumin. Exp Brain Res 2010; 201: 472–488.

54 Maldonado-Avilés JG, Curley AA, Hashimoto T, Morrow AL, Ramsey AJ, O’Donnell P et al. Altered markers of tonic inhibition in the dorsolateral prefrontal cortex of subacute schizophrenia: a fMRI study. Schizophrenia Research 2009; 106: 450–453.

55 Benes FM, Lim B, Matzevich D, Walsh JP, Subaraju S, Minns M et al. Regulation of the GABA cell phenotype in hippocampus of schizophrenia and bipolar. Proc Natl Acad Sci USA 2007; 104: 10164–10169.

56 Knable MB, Barci BM, Webster MJ, Meador-Woodruff J, Torrey EF, Stanley Neurosci 2007; 10: 969–972.

57 Welfafang M, Velakoulis D, Whitef JD, Pantelis C. Understanding aberrant white matter development in schizophrenia: an avenue for therapy? Expert Rev Neurother 2011; 11: 971–987.
85 Myers JF, Evans CJ, Kalk NJ, Edden RA, Lingford-Hughes AR. Measurement of GABA using J-difference edited 1H-MRS following modulation of synaptic GABA concentration with tiagabine. Synapse 2014; 68: 355–362.

86 Waschkies CF, Bruns A, Muller S, Kapps M, Borroni E, von Kienlin M et al. Neuropharmacological and neurobiological relevance of in vivo (1)H-MRS of GABA and glutamate for preclinical drug discovery in mental disorders. Neuropsychopharmacology 2014; 39: 2331–2339.

87 Stokes PR, Myers JF, Kalk NJ, Watson BJ, Erritzoe D, Wilson SJ et al. Acute increases in synaptic GABA detectable in the living human brain: a [(1)(1)C]Ro15-4513 PET study. Neuroimage 2014; 99: 158–165.

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

89 Volk DW, Sampson AR, Zhang Y, Edelson JR, Lewis DA. Cortical GABA markers identify a molecular subtype of psychotic and bipolar disorders. Psychol Med 2016; 46: 2501–2512.

90 Rowland LM, Edden RA, Kontson K, Zhu H, Barker PB, Hong LE. GABA predicts inhibition of frequency-specific oscillations in schizophrenia. J Neuropsychiatry Clin Neurosci 2013; 25: 83–87.

91 Sohal VS, Zhang F, Yizhar O, Deisseroth K. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. Nature 2009; 459: 698–702.