Research Paper

GABA levels and TSPO expression in people at clinical high risk for psychosis and healthy volunteers: a PET-MRS study

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Published online first on Sept. 26, 2018; subject to revision

Background: γ-Aminobutyric acidergic (GABAergic) dysfunction and immune activation have been implicated in the pathophysiology of schizophrenia. Preclinical evidence suggests that inflammation-related abnormalities may contribute to GABAergic alterations in the brain, but this has never been investigated in vivo in humans. In this multimodal imaging study, we quantified cerebral GABA plus macromolecule (GABA+) levels in antipsychotic-naive people at clinical high risk for psychosis and in healthy volunteers. We investigated for the first time the association between GABA+ levels and expression of translocator protein 18 kDa (TSPO; a marker of microglial activation) using positron emission tomography (PET). **Methods:** Thirty-five people at clinical high risk for psychosis and 18 healthy volunteers underwent 3 T proton magnetic resonance spectroscopy to obtain GABA+ levels in the medial prefrontal cortex (mPFC). A subset (29 people at clinical high risk for psychosis and 15 healthy volunteers) also underwent a high-resolution [^{18}F]FEPPA PET scan to quantify TSPO expression. Each participant was genotyped for the TSPO *rs6971* polymorphism. **Results:** We found that GABA+ levels were significantly associated with TSPO expression in the mPFC ($F_{1,40}$ = 10.45, p = 0.002). We found no significant differences in GABA+ levels in the mPFC ($F_{1,51}$ = 0.00, p > 0.99) between people at clinical high risk for psychosis and healthy volunteers. We found no significant correlations between GABA+ levels or residuals of the association with TSPO expression and the severity of prodromal symptoms or cognition. **Limitations:** Given the cross-sectional nature of this study, we could determine no cause-and-effect relationships for GABA alterations and TSPO expression. **Conclusion:** Our findings suggest that TSPO expression is negatively associated with GABA+ levels in the prefrontal cortex, independent of disease status.

Introduction

γ-Aminobutyric acidergic (GABAergic) dysregulation and immune activation have been separately implicated in the pathophysiology of schizophrenia, but the in vivo relationship between these 2 systems has never been investigated in parallel in the human brain.

Postmortem and neuroimaging studies support a role for dysregulation of the primary inhibitory neurotransmitter system, γ-aminobutyric acid (GABA), in the pathophysiology of schizophrenia.[1,2] Postmortem studies have consistently reported deficits in the expression of fast-spiking, parvalbumin-positive GABAergic interneurons in the prefrontal cortex of patients with schizophrenia and reductions in mRNA and protein levels of GAD67, a major GABA-synthesizing enzyme.[3–6] In addition, a recent study reported reduced cerebrospinal fluid concentrations of GABA in patients following a first episode of psychosis compared with healthy controls.[7] Recent advances in magnetic resonance spectroscopy (^{1}H-MRS) allow for the in vivo quantification of cerebral GABA levels. Several GABA ^{1}H-MRS studies have been published in schizophrenia, particularly in the medial prefrontal cortex (mPFC).[8] While some studies report reductions in GABA levels in patients with schizophrenia,[9–12] others have reported increased[13–15] or unaltered levels[9,11,13,16–18] in the mPFC. However, these discrepancies appear to reflect differences in voxel placement and/or whether the patients had previous exposure to antipsychotics. This idea is supported by a study that reported elevated GABA levels in the mPFC of unmedicated patients with schizophrenia, but in not in medicated patients.[13] Further, a recent study reported elevated GABA levels in antipsychotic-naive patients following a first episode of psychosis that normalized after 4 weeks of antipsychotic treatment.[19] Differences in methodology, duration, phase of illness or demographics (e.g., age, sex or smoking status) may also explain these inconsistencies. In this context, studying people at clinical high risk (CHR) for psychosis provides an unparalleled opportunity to investigate the neurobiological

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Submitted Oct. 11, 2017; Revised Apr. 15, 2018; Accepted May 13, 2018

DOI: 10.1503/jpn.170201

changes underlying schizophrenia, without the confounding influence of antipsychotic medication. Only 3 studies have investigated GABA levels in people at CHR for psychosis. Wang and colleagues[20] reported unaltered GABA levels in the mPFC of antipsychotic-naive people at CHR (21 people at CHR and 23 healthy volunteers). Similarly, a recent ^{1}H-MRS study also failed to detect differences in GABA levels in the mPFC of antipsychotic-naive people at CHR (21 people at CHR and 20 healthy volunteers),[21] whereas another study reported increased GABA levels in the mPFC (23 people at CHR and 24 healthy volunteers).[22]

Converging evidence from preclinical, genetic and peripheral studies have implicated immune-related abnormalities in the pathophysiology of schizophrenia.[23] Microglia play a critical role in both healthy and diseased states of the central nervous system.[24] In the developing and mature central nervous system, microglia are involved in synaptic pruning and maturation, and in maintaining synaptic plasticity.[24] On the other hand, microglia also function as inflammatory cellular mediators in response to tissue damage or brain insult.[25] Neuroinflammation is characterized (at least in part) by microglial activation.[26] Microglial activation is associated with elevated expression of a mitochondrial protein, translocator protein 18 kDa (TSPO), making TSPO a suitable marker for quantifying microglial activation in vivo.[27] Currently, we can quantify TSPO expression in vivo using positron emission tomography (PET). The most replicated finding across TSPO PET studies in schizophrenia suggests no differences in TSPO expression using second-generation radioligands,[28–31] although a recent study reported a significant reduction in patients with schizophrenia compared with healthy volunteers,[32] also supported by a recent meta-analysis.[33] Three other studies reported elevated TSPO expression in people with schizophrenia and first-episode psychosis,[34–36] but these studies used the first-generation radioligand [^{11}C]PK11195, which has several known limitations.[37] Only 2 studies have investigated TSPO expression in people at CHR for psychosis, and both reported a lack of group differences between people at CHR and healthy volunteers using total distribution volume (V_T), a validated outcome measure for second-generation TSPO radioligands.[38,39]

Accumulating evidence suggests that GABAergic signalling can modulate inflammatory processes, and that immune processes can induce reciprocal changes in the GABAergic system, providing a potential link between immune activation and GABAergic alterations in schizophrenia.[40] Preclinical studies have consistently shown alterations in gene expression across GABAergic pathways and reductions in GABAergic parvalbumin-positive interneurons following prenatal exposure to inflammatory stimuli.[41–43] Activation of GABA receptor activity attenuates immune activation, while inhibition of receptor activity increases inflammatory responses.[44–46] In fact, studies have shown that microglial activation is negatively regulated by GABAergic neurotransmission.[45,47] As well, GABA receptors are present on immune cells, including astrocytes and microglia, suggesting a potential role for GABA in neuroimmune responses.[48,49] Moreover, GABAergic deficits can lead to glutamatergic hyperactivity, resulting in toxicity-mediated neuronal death, excessive glial activation and neuroinflammation.[50,51] Although there is a close relationship between GABAergic and immune dysfunction in schizophrenia, the in vivo relationship between these 2 systems has yet to be investigated in humans. In the present study, we investigated GABA plus macromolecule (GABA+) in the mPFC of antipsychotic-naive people at CHR for psychosis, making this the largest GABA ^{1}H-MRS study in this population (35 people at CHR and 18 healthy volunteers). We also investigated, to our knowledge for the first time, the in vivo association between GABA+ levels and TSPO expression using 3 T ^{1}H-MRS and high-resolution PET, respectively. Based on previous evidence, we hypothesized that there would be a negative association between GABA+ levels and TSPO expression in the brain.

Methods

Participants

We initially enrolled 36 people at CHR for psychosis and 18 healthy volunteers. Most of the people in the CHR group were antipsychotic-naive (n = 30).

People in the CHR group were included if they met diagnostic criteria for prodromal risk syndrome as assessed by the Criteria of Prodromal Syndromes.[52] They were excluded if they had a current axis I disorder as determined by the Structured Clinical Interview[53] for DSM-IV Axis I disorders (SCID-I). Healthy volunteers were included if they did not have a history of past psychoactive drug use and/or first-degree relatives with a major mental disorder. All participants were excluded if they had a clinically significant medical illness, had a current diagnosis of alcohol or substance use/dependence, were pregnant or breastfeeding, or had metal implants that precluded an MRI scan. In the CHR group, we assessed clinical status and the severity of prodromal symptoms using the Structured Interview for Psychosis-Risk Syndromes, the Scale of Psychosis-Risk Symptoms, the Calgary Depression Scale for Schizophrenia for depression symptoms and the Apathy Evaluation Scale for apathy. We assessed neurocognitive performance using the Repeatable Battery for the Assessment of Neuropsychological Status.[54]

This study was approved by the research ethics board at the Centre for Addiction and Mental Health in Toronto, Canada. All participants provided written informed consent after procedures had been explained thoroughly.

Data acquisition and analysis: ^{1}H-MRS

All participants underwent a ^{1}H-MRS scan on a 3 T MR-750 scanner (General Electric Medical Systems) equipped with an 8-channel head coil. Head position was fixed at the centre of the head coil with tape strapped across the forehead and soft padding to minimize head motion. A 24 mL (20 × 40 × 30 mm^3) MRS voxel was positioned as shown in Figure 1A. The voxel called mPFC was composed of 65% cingulate gyrus and 28% superior frontal gyrus (Appendix 1, Fig. 2S, available at jpn.ca/170201-a1). We performed shimming using the

manufacturer-supplied automated shimming routine (AUTOSHIM) to adjust magnet homogeneity. We measured GABA levels using a modification of standard single-voxel, double-spin echo data acquisition by inserting a pair of frequency-selective radiofrequency (RF) pulses before and after the second volume-selective, 180° RF pulse (MEGA-PRESS).[13,55–59] This J-editing approach used frequency-selective RF pulses to alter the temporal evolution of the strongly coupled spins in the GABA C2, C3 and C4 multiplets. The frequencies of these editing RF pulses were centred to suppress the C3 resonance of GABA at 1.9 ppm in the "on" condition and at 7.5 ppm in the "off" condition (Fig. 1B). This editing RF pulse pair inhibited and allowed J-modulation of the coupled GABA spin system, such that subtraction of the 2 subspectra yielded the J-edited GABA resonance free from the overlapping creatine and aspartate resonances. However, our data acquisition did not exclude the underlying macromolecule, so the GABA observed in this study was GABA+. We averaged 8 excitations for each "on" and "off" frequency cycle, resulting in 66 data frames. We combined these multichannel raw MRS data sets in the time domain based on coil sensitivity derived from unsuppressed water signal, weighted by the sum of the squares of the signal intensities from each channel (Fig. 1B).[60] Subtraction of the "on" from the "off" condition yielded only the J-edited GABA C4 resonance (Fig. 1C). Acquisition parameters for the measurements were as follows: echo time 68 ms, repetition time 1500 ms, bandwidth = 5 kHz, number of excitations = 528 (512 water-suppressed, 16 water-unsuppressed), data points = 4096. The data acquisition implementation used in this study has been validated in a large-scale, multivendor, multisite study, with the aim of understanding the factors that impact GABA measurement outcome with a coefficient of variation of the entire healthy volunteer cohort of 12% for GABA.[61]

All postprocessing and analysis were performed with Gannet[62,63] using Gaussian line shape fitting, with modifications to allow the output of GABA+ area (Fig. 1D). The data were zero-padded to 32 000 points, and we used line-broadening of 3 Hz. The ratio of GABA+ to unsuppressed water peak areas has been reported.[64] Spectra that exceeded a full width at half maximum of unsuppressed water resonance greater than 10 Hz were excluded from further analysis.

To estimate the fractions of grey matter, white matter and cerebrospinal fluid in the voxel, we created volume images and a segmentation mask for the mPFC from the MRS raw data file and 3D T_1-weighted images using an in-house MATLAB-based code. We then performed segmentation using statistical parametric mapping software (SPM8; Wellcome Centre for Human Neuroimaging) to determine fractional tissue composition in the voxel.

Data acquisition and analysis: PET and structural MRI

Data from PET and MRI were available for a subset of our sample (29 people at CHR and 15 healthy volunteers). As well, 26 people at CHR and 13 healthy volunteers were

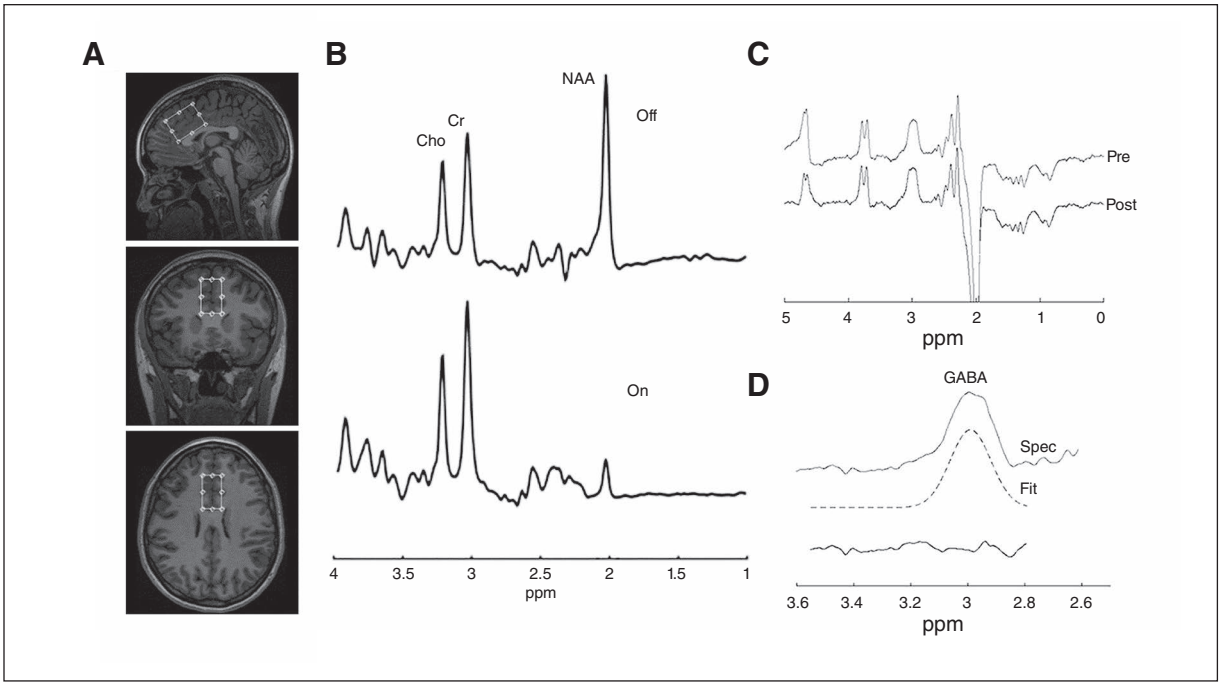

Fig. 1: (**A**) Axial, sagittal and coronal views of the voxel placement in the medial prefrontal cortex. (**B**) Typical fitting results from a patient: magnetic resonance spectroscopy acquired for the "off" and "on" conditions (see text). (**C**) Difference between the "on" and "off" spectra before (pre) and after (post) signal alignment using Gannet software. (**D**) Gaussian lineshape fitting of edited GABA resonance showing GABA resonance at 3.0 ppm, fitted signal and residual. Cho = choline; Cr = creatine; fit = model of best fit; GABA = γ-aminobutyric acid; NAA = *N*-acetylaspartate; ppm = parts per million; pre = before spectral alignment; post = after spectral alignment; spec = GABA-edited spectrum.

included in our previous cohorts,[39] including 1 manuscript in submission. The PET and MRI scans were obtained on average 16.09 days apart. The PET data acquisitions have been described in detail elsewhere.[29,30] Briefly, we obtained proton-density-weighted and T_1-weighted brain magnetic resonance images for each participant using a 3 T MR-750 scanner. We performed all [^{18}F]FEPPA scans using a high-resolution research tomograph (CPS/Siemens). Each participant was given an intravenous bolus injection of 186.53 ± 11.01 MBq of [^{18}F]FEPPA for 125 min. Arterial and manual blood samples were taken to measure radioactivity in blood and the relative proportion of radiolabelled metabolites. We collected arterial blood for the first 22.5 min at a rate of 2.5 mL/min after radioligand injection, using an automatic blood sampling system (Model PBS-101, Veenstra Instruments). We took manual blood samples at –5, 2.5, 7, 12, 15, 20, 30, 45, 60, 90 and 120 min relative to the time of injection. Dispersion and metabolite-corrected plasma input function were generated as previously described.[30] We extracted time–activity curves for the mPFC using a validated in-house imaging pipeline regions of mental interest.[65] The region of interest was delineated using proton-density MRIs. We derived V_T in the mPFC from the time–activity curve and plasma input function using a 2-tissue compartment model, which has been validated for [^{18}F]FEPPA quantification.[66]

Genotyping: rs6971 *polymorphism*

A polymorphism in the TSPO gene (*rs6971*) affects the binding affinity of second-generation radioligands, including [^{18}F]FEPPA.[67,68] We genotyped all participants on the basis of this polymorphism as high-affinity (C/C), mixed-affinity (C/T) or low-affinity (T/T) binders, as described elsewhere.[68,69]

Statistical analysis

We tested differences in participant characteristics between the CHR group and healthy volunteers using χ^2 tests for categorical variables (e.g., sex) and analysis of variance for continuous variables (e.g., age). To test for differences in GABA+ levels in mPFC between groups, we used univariate analysis of variance, with GABA+ levels as the dependent variable and group (CHR v. healthy volunteers) as a fixed factor. To test our hypothesis regarding the association between [^{18}F]FEPPA V_T and GABA+ levels in mPFC, we used a general linear model with [^{18}F]FEPPA V_T in mPFC as the dependent variable; group and TSPO genotype (categorical variable, high-affinity binders or mixed-affinity binders) as fixed factors; and GABA+ levels as a covariate. We tested the main effects of group, TSPO genotype and GABA+ levels, and the interaction between GABA+ levels and group. We removed the GABA+ × group interaction from the final model because it was not significant. Owing to the large-scale difference between GABA+ levels (10^{-5} range) and [^{18}F]FEPPA V_T (10^1 range), we standardized GABA+ levels before the analysis by subtracting the mean and dividing the difference by the standard deviation. To test for potential confounding effects of sex and age on GABA+ levels and association with TSPO expression, we added both factors as covariates to the model. We also explored the associations between the residuals of the general linear model and clinical and neuropsychological measures in people at CHR for psychosis using bivariate correlations. We performed statistical analyses using SPSS version 23.0 (IBM Inc.). We set the significance level at $p < 0.05$.

Results

The demographic and clinical characteristics of the participants are shown in Table 1. One member of the CHR group was excluded because of motion during the ^{1}H-MRS scan that could not be corrected. We found no significant differences in sex and age between the CHR group and the healthy volunteers (Table 1), or in TSPO genotype or PET parameters (Appendix 1, Table S1). Five members of the CHR group were on low-dose antipsychotic treatment with risperidone (1 taking 0.5 mg and 2 taking 1 mg), quetiapine (75 mg) or aripiprazole (5 mg) at the time of the PET scan. All participants had had a negative urine drug screen, except for 1 member of the CHR group, who had a positive urine drug screen for benzodiazepines (1-time use) and 4 members of the CHR group who had a positive urine drug screen for cannabis, but no other drugs. The averaged unsuppressed water line width for GABA+ data acquisition, full width at half maximum, was 7.39 ± 0.85 Hz for healthy volunteers and 7.57 ± 1.14 Hz for the CHR group. We observed no significant differences in full width at half maximum between groups (Appendix 1, Table S2).

GABA+ levels in the mPFC

We found no significant differences in GABA+ levels in mPFC between the CHR group and healthy volunteers (35 CHR and 18 healthy volunteers; $F_{1,51} = 0.00$, p . 0.99; 2.14% higher in the CHR group v. the healthy volunteers; Fig. 2). We obtained similar results after controlling for sex and age ($F_{1,49} = 0.00$, $p > 0.99$). Results remained the same when excluding members of the CHR group who were tobacco users, or were positive for cannabis, benzodiazepine or antipsychotic use. We found no significant differences in grey matter, white matter or cerebrospinal fluid fractions in the mPFC voxel between the CHR group and the healthy volunteers (Appendix 1, Table S2). We also found no significant correlations between GABA+ levels and symptom severity or clinical and neuropsychological measures (Appendix 1, Table S3).

GABA+ and [^{18}F]FEPPA V_T in the mPFC

We observed a significant negative association between GABA+ levels and [^{18}F]FEPPA V_T in the mPFC ($\beta = -1.47$, SE = 0.45; $F_{1,40} = 10.45$, $p = 0.002$; Fig. 3). We obtained similar results after controlling for sex and age ($\beta = -1.43$, SE = 0.46; $F_{1,38} = 9.42$, $p = 0.004$). Results remained the same when excluding members of the CHR group who were tobacco users, or were positive for cannabis, benzodiazepine or antipsychotic use. We found no significant correlations between residuals of the model and symptom severity or clinical and

neuropsychological measures. The lack of group differences in [^{18}F]FEPPA V_T between CHR and healthy volunteers has been reported elsewhere (Appendix 1, Table S1).[39]

Discussion

This is, to our knowledge, the first in vivo study to investigate GABA+ levels in the mPFC of people at CHR for psychosis and their association with TSPO expression. As previously reported, we observed no significant differences in GABA+ levels or [^{18}F]FEPPA V_T in the mPFC between members of the CHR group and healthy volunteers. We reported a significant negative association between GABA+ levels and TSPO expression in the mPFC. We found no correlations between GABA+ levels in the mPFC or residuals of the model and severity of prodromal symptoms or neuropsychological scores.

GABA+ levels in the mPFC

We found no significant differences in GABA+ levels in the mPFC between the CHR group and the healthy volunteers (35 people at CHR and 18 healthy volunteers). We also found no associations between GABA+ levels and the severity of prodromal symptoms or clinical and neuropsychological measures. Of the 3 ^{1}H-MRS GABA studies in people at CHR for psychosis, 2 studies reported unaltered GABA levels in the mPFC, and the other reported elevated GABA levels in people at CHR compared with healthy volunteers. Such discrepancies in the findings are most likely due to differences in the positioning of the ^{1}H-MRS voxel in the mPFC. A recent study using a smaller sample and a voxel that overlapped with ours (i.e., it was more dorsally placed in the mPFC) reported unaltered GABA levels in antipsychotic-naive people at CHR compared with healthy controls.[21] Of the 2 studies with the ^{1}H-MRS voxel more ventrally placed in the mPFC, 1 reported unaltered GABA levels,[20] but the other reported increased GABA levels[22] in people at CHR compared with healthy volunteers. This discrepancy could be attributable to methodological rigour, given that the MEGA-PRESS spectrum shown in the former study was of poorer quality (a very broad GABA resonance)[20] than the spectrum shown in the study that reported mPFC GABA elevations.[15] Future studies should consider standardizing voxel placement and size, naming voxels consistently and (if possible) quantifying GABA levels in both dorsal and ventral brain regions to clarify whether regional differences exist in the mPFC in people at CHR for psychosis. However, consistent with our findings, a recent

Table 1: Demographic and clinical characteristics of study participants

Measure	Healthy volunteers (n = 18)	People at clinical high risk for psychosis (n = 35)	Statistical test	p value
Age, yr, mean ± SD	21.28 ± 1.99	20.57 ± 1.63	F = 1.91	p = 0.17
Sex, male: female	6:12	19:16	χ^2 = 2.09	p = 0.15
Current drug use, n*				
Nicotine	0	8	—	—
Cannabis	0	4	—	—
Lifetime history of recreational drug use (use > 10 times), n				
Cannabis	0	17	—	—
MDMA	0	1	—	—
Cocaine	0	1	—	—
LSD	0	1	—	—
Barbiturate	0	1	—	—
Antipsychotic use, n†	0	5	—	—
SOPS, mean ± SD				
Total	—	36.03 ± 11.81	—	—
Positive	—	11.20 ± 3.76	—	—
Negative	—	11.37 ± 5.94	—	—
Disorganization	—	3.74 ± 2.65	—	—
General	—	8.86 ± 4.31	—	—
AES, mean ± SD‡	—	39.06 ± 9.19	—	—
CDSS, mean ± SD	—	5.51 ± 4.03	—	—
RBANS, total, mean ± SD	—	90.94 ± 13.95	—	—

AES = Apathy Evaluation Scale; CDSS = Calgary Depression Scale for Schizophrenia; CHR = clinical high risk for psychosis; LSD = lysergic acid diethylamide; MDMA =3,4-methylenedioxymethamphetamine (ecstasy); SOPS = Scale of Psychosis-Risk Symptoms; RBANS = Repeatable Battery for the Assessment of Neuropsychological Status; SD = standard deviation.
*All participants underwent a urine drug screen at baseline for cannabis, ethanol, methadone and cocaine. Baseline results were negative, except for 1 member of the CHR group with a positive result for benzodiazepine, and 4 members of the CHR group with a positive result for cannabis.
†Participants at clinical high risk for psychosis who were currently on antipsychotic treatment: 0.5 mg risperidone (n = 1), 1.0 mg risperidone (n = 2), 75 mg quetiapine (n = 1), and 5 mg aripiprazole (n = 1).
‡Score missing for 2 participants.

meta-analysis of ^{1}H-MRS GABA in schizophrenia failed to reveal group differences in the mPFC.[8] Longitudinal clinical follow-up revealed that 7 of the 35 people at CHR in this study (20%) converted to psychosis. Although this was a cross-sectional study and conclusions were limited by small sample size, we found no significant differences in GABA+ levels in the mPFC between converters at CHR and nonconverters ($F_{1,35}$ = 1.53, p = 0.23; Appendix 1, Figure S1).

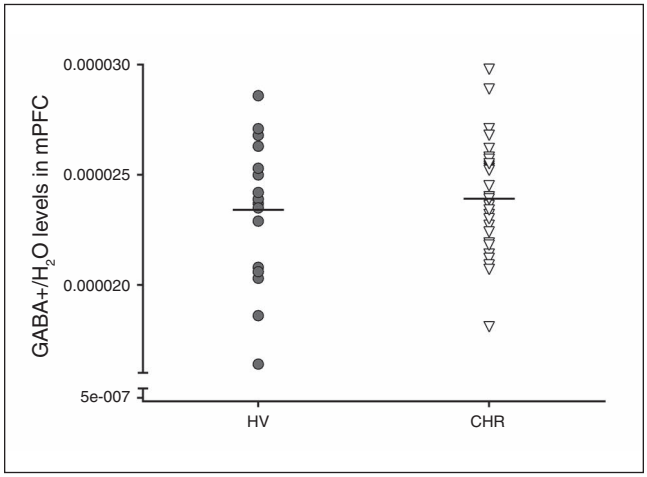

Fig. 2: GABA+/H_2O levels in the medial prefrontal cortex (mPFC) of healthy volunteers (HV; n = 18) and people at clinical high risk (CHR) for psychosis (n = 35). GABA+ = γ-aminobutyric acid plus macromolecule.

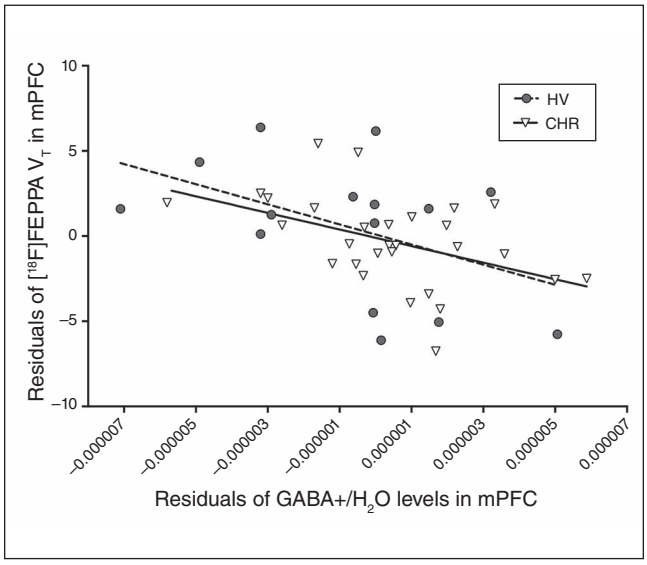

Fig. 3: Associations between residuals of regressing [^{18}F]FEPPA V_T on TSPO genotype and residuals of regressing GABA+/H_2O levels in medial prefrontal cortex (mPFC) on TSPO genotype in people at clinical high risk (CHR) for psychosis (r = –0.49, p = 0.008) and healthy volunteers (HV; r = –0.53, p = 0.053). GABA+ = γ-aminobutyric acid plus macromolecule; TSPO = translocator protein 18 kDa; V_T = total distribution volume.

GABA+ and [^{18}F]FEPPA V_T in the mPFC

We found a significant negative association between GABA+ levels and [^{18}F]FEPPA V_T, suggesting that regardless of clinical diagnosis, higher TSPO expression is associated with lower GABA levels. Although this is the first in vivo study to investigate the link between immune activation and the GABAergic system in the brain, accumulating evidence from preclinical studies suggests a potential link between these 2 systems. Preclinical studies have consistently shown that immune activation alters components of the GABAergic system in the brain, including reductions in GABA receptor expression and function,[70,71] neuronal density[72,73] and the expression of GAD65 and GAD67, 2 major GABA-synthesizing enzymes.[71,74] Similarly, reduced GABA levels in the prefrontal cortex of mice have been reported following prenatal exposure to inflammatory stimuli.[75] Further, supporting our results, overexpression of interleukin-6, specifically in glial cells, decreased GABA-positive and parvalbumin-positive neurons in adult mouse hippocampus.[76] In addition, $GABA_A$ and $GABA_B$ receptor agonists can reduce microglial activation[45] and attenuate the release of proinflammatory cytokines,[44] while inhibition of GABA receptor activity increases inflammatory responses.[46] Our results provide, to our knowledge, the first in vivo evidence linking GABA levels and TSPO expression, but future studies should investigate the mechanisms underlying this association.

Limitations

Interpretation of the results of this study should consider the following limitations. First, the proton MRS voxels are relatively large because of the low concentration of GABA in the human brain. Our mPFC voxel contained approximately 65% cingulate gyrus and 28% superior frontal gyrus (Appendix 1, Figure S2). In addition, the GABA quantification reported in this study was a combination of GABA molecule and mobile macromolecule (up to 50% of total GABA signal),[77] termed GABA+. The contribution of this macromolecule to the total GABA signal in healthy participants has been documented using the same J-editing approach.[78] However, it is not known whether this mobile macromolecule might differ in people at CHR for psychosis.

Second, there have been inconsistencies in the placement of ^{1}H-MRS voxels across GABA studies in schizophrenia, particularly in the mPFC.[8] We acknowledge that the failure of this study to detect group differences in GABA+ levels between people at CHR for psychosis and healthy volunteers may be due to the more dorsal placement of our ^{1}H-MRS voxel in the mPFC — consistent with Modinos and colleagues[21] — compared with a more ventral placement.[22]

Third, in neurochemical brain-imaging studies, relatively small sample sizes represent a potential limitation; however, to our knowledge, this is the largest GABA ^{1}H-MRS study in people at CHR for psychosis. In addition, given the novelty of our findings in linking TSPO expression and GABA levels, this study can serve as a framework for future studies.

Fourth, although we acquired scans on 2 separate days, both [^{18}F]FEPPA PET and GABA ^{1}H-MRS have satisfactory

test–retest reliability, and no changes in PET and MRI machine stability and CHR pathophysiology are expected in our time interval (about 16 days).

Fifth, the voxel used for ^{1}H-MRS in mPFC was larger than the region of interest used with PET and also included the dorsal part of the anterior cingulate cortex, but this was unlikely to affect the results presented.

Sixth, although an increase in [^{18}F]FEPPA binding was mostly attributed to microglial activation, studies show that astrocytes and vascular endothelial cells also express TSPO.[79] However, this does not affect the overall conclusion of our study, given that astrocytes are also involved in the immune response. Further studies are needed to evaluate the contribution of these components to the TSPO signal. In addition, the link between TSPO expression and microglial activation is not yet well understood. Several studies have shown marked elevations in TSPO expression in activated microglia,[80–82] including a study by Sandiego and colleagues[83] that reported robust increases in [^{11}C]PBR28 binding (a second-generation TSPO radioligand) following a lipopolysaccharide challenge, confirming that TSPO is upregulated during immune activation; however, other studies have shown a decrease in TSPO in proinflammatory states.[84]

Seventh, the [^{18}F]FEPPA outcome measure, V_T, is a composite measure that includes both specific and nonspecific-plus-free binding, and to date, it is unknown whether the nonspecific-plus-free binding is different between groups. Further, in this study we did not correct [^{18}F]FEPPA V_T for the plasma-free fraction of the radioligand (f_p), because it has been shown to substantially increase the variability.[85]

Finally, we acknowledge that GABA does not provide a full picture of excitatory and inhibitory balance in the brain. However, exploratory analysis in our data set revealed no significant group differences between mPFC glutamine + glutamate or inhibitory index,[86] or associations with TSPO expression in the mPFC (Appendix 1, Table S4, Table S5, Figure S3, Figure S4). Similarly, we found no group differences in mPFC *N*-acetylaspartate/creatine or choline/creatine ratios between people at CHR for psychosis and healthy volunteers, or associations with TSPO expression in the mPFC (Appendix 1, Table S4, Table S5).

Conclusion

The results of this study suggest a link between GABA+ levels and TSPO expression in the mPFC, independent of disease status.

Acknowledgments: The authors thank the excellent staff of the Research Imaging Centre at the Centre for Addiction and Mental Health — in particular Peter Truong for his Gannet GABA processing and for generating the brain region composition of the magnetic resonance spectroscopy voxel, and the Focus on Youth Psychosis Prevention clinic. The authors are grateful to Felix Raschke of National Centre for Radiation Research in Oncology, Dresden, Germany, for the voxel masking code.

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Competing interests: R. Mizrahi has received speaker fees from Otsuka Lundbeck Canada. No other competing interests declared.

Funding: This work was supported by the National Institutes of Health (NIH) R01 grant MH100043 to R. Mizrahi.

Contributors: R. Mizrahi designed the study. All authors acquired and analyzed the data. N. Sailasuta and R. Mizrahi wrote the article, which all authors reviewed. All authors approved the final version to be published and can certify that no other individuals not listed as authors have made substantial contributions to the paper.

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