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Imaging Neuroinflammation in Gray and White Matter in Schizophrenia: An In-Vivo PET Study With [^{18}F]-FEPPA

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Neuroinflammation and abnormal immune responses have been implicated in schizophrenia (SCZ). Past studies using positron emission tomography (PET) that examined neuroinflammation in patients with SCZ in vivo using the translocator protein 18 kDa (TSPO) target were limited by the insensitivity of the first-generation imaging agent [^{11}C]-PK11195, scanners used, and the small sample sizes studied. Present study uses a novel second-generation TSPO PET radioligand *N*-acetyl-*N*-(2-[^{18}F]fluoroethoxybenzyl)-2-phenoxy-5-pyridinamine ([^{18}F]-FEPPA) to evaluate whether there is increased neuroinflammation in patients with SCZ. A cross-sectional study was performed using [^{18}F]-FEPPA and a high-resolution research tomograph (HRRT). Eighteen patients with SCZ with ongoing psychotic symptoms and 27 healthy volunteers (HV) were recruited from a tertiary psychiatric clinical setting and the community, respectively. All participants underwent [^{18}F]-FEPPA PET and magnetic resonance imaging, and PET data were analyzed to obtain [^{18}F]-FEPPA total volume of distribution (V_T) using a 2-tissue compartment model with an arterial plasma input function, as previously validated. All subjects were classified as high-, medium- or low-affinity [^{18}F]-FEPPA binders on the basis of rs6971 polymorphism, and genotype information was incorporated into the analyses of imaging outcomes. No significant differences in neuroinflammation indexed as [^{18}F]-FEPPA V_T were observed between groups in either gray ($F_{(1,39)} = 0.179$, $P = .674$) or white matter regions ($F_{(1,38)} = 0.597$, $P = .445$). The lack of significant difference in neuroinflammation in treated patients with SCZ in the midst of a psychotic episode and HV suggests that neuroinflammatory processes may take place early in disease progression or are affected by antipsychotic treatment.

Key words: translocator protein 18 kDa (TSPO)/ inflammation/positron emission tomography/psychosis/rs6971 polymorphism/microglia

Introduction

Several lines of evidence point toward a role of immune response and neuroinflammation in the pathogenesis of schizophrenia (SCZ),[1–5] including reports of elevations in peripheral levels of proinflammatory cytokines,[1–4] prostaglandin 2,[5] and C-reactive protein[6,7] in patients with SCZ. Additionally, several studies have shown that anti-inflammatory agents, administered as adjuvants to antipsychotic therapy, can improve positive, cognitive, and global symptoms.[8–10] Interactions between the immune system and risk of SCZ starts before birth, with epidemiological studies consistently showing a parallel relationship between exposure to prenatal inflammation and increased risk of developing SCZ in offspring.[11]

Microglia act as resident macrophages of the central nervous system and first responders mobilizing the inflammatory response to brain insults[12] by undergoing activation from the quiescent ("sentry") state, migrating to the site of neuronal injury and releasing inflammatory cytokines.[12–15] Importantly, activated microglia express a mitochondrial translocator protein 18 kDa (TSPO),[16] previously called peripheral-type benzodiazepine receptor. Microglial activation and the associated elevations in TSPO levels have been recognized as quantifiable indices of neuroinflammation.[12] Microglial activation, and neuroinflammation, may also be involved in the structural alterations in brain structure, possibly underlying the gray matter loss observed in a number of disorders,[17–20] including SCZ.[21–23] While cognitive and depressive symptoms of SCZ in particular have been linked with markers of inflammation,[6,10,24,25] to our knowledge, no study has addressed this question in brain in living humans. Furthermore, alterations in white matter tracts and changes in regional connectivity have also been implicated in the pathogenesis of SCZ. Diffusion tensor

imaging (DTI) studies have identified differences in fractional anisotropy between patients with SCZ and healthy volunteers (HV) in corpus callosum,[26] cingulum bundle,[27] superior longitudinal fasciculus (SLF),[28] and the posterior limb of the internal capsule (PLIC).[29]

While 3 early postmortem studies have detected increased presence of microglia in the hippocampal and cortical tissues of patients with SCZ,[30–32] other studies have failed to detect any difference[33–36] or even reported a decrease in microglial activation.[37] Positron emission tomography (PET), though, avoids the limitations of postmortem studies and provides an in vivo index of neuroinflammation in SCZ. Early studies using TSPO radiotracer [^{11}C]-PK11195 have reported increased neuroinflammation in total gray matter[38] and the hippocampus[39] of antipsychotic-treated patients. The only in vivo study in SCZ using a new generation TSPO ligand, [^{11}C]-DAA1106, reported no difference in tracer binding between patients with SCZ and matched controls, but did find tracer binding to correlate significantly with positive psychotic symptoms and duration of illness. However, this initial study used an older HR+ PET scanner with lower resolution and sensitivity,[40] examined a smaller sample size, did not investigate white matter regions or correlation with cognitive deficits, and did not correct for genotype, which is known to significantly affect TSPO-binding outcomes of second-generation radioligands [^{11}C]-DPA-713, [^{11}C]DAA1106, [^{11}C]PBR28, and *N*-acetyl-*N*-(2-[^{18}F]fluoroethoxybenzyl)-2-phenoxy-5-pyridinamine ([^{18}F]-FEPPA).[41–44]

[^{18}F]-FEPPA is a novel radioligand exhibiting optimal chemical, pharmacokinetic, and pharmacodynamic properties for applications in imaging TSPO. Preclinical tracer characterization studies have shown [^{18}F]-FEPPA to exhibit moderate brain uptake, slow washout, and regional distribution that matches previously demonstrated levels of TSPO.[45] Recent evaluations in HV have shown that [^{18}F]-FEPPA total distribution volumes (V_T) can be quantified with excellent identifiability using a 2-tissue compartment model.[46] Thus, in the present study, we used [^{18}F]-FEPPA to examine neuroinflammation in both gray and white matter regions in patients with SCZ with ongoing psychotic symptoms. Moreover, we explored the association between neuroinflammation and gray matter volumes to evaluate the involvement of inflammatory processes in brain regional atrophy. In addition, we explored the associations between neuroinflammation and severity of psychopathology as well as neuropsychological deficits in patients with SCZ, given reported associations between peripheral markers of inflammation and poorer cognitive performance.[6,10,24]

Methods

Subjects

After 1 subject was excluded for excessive head motion and another participant was determined to be a low-affinity binder (LAB), 16 patients with SCZ currently undergoing antipsychotic therapy (11 male participants; 42.6 ± 14.1 years old) and 27 HV (10 male participants; 43.5 ± 16 years old) between the ages of 18 and 65 were included in this study. Patients met the following inclusion criteria: diagnosis of SCZ, as evaluated with the Structured Clinical Interview for DSM-IV[47] with no other Axis I disorders such as depression which may also be associated with neuroinflammation[48]; with ongoing psychotic symptoms, as confirmed with a minimum score of 5 on 1 item or 4 on 2 items of the Positive and Negative Syndrome Scale (PANSS)[49]; on stable antipsychotic therapy, and with no use of clozapine or benzodiazepines which have been shown to affect TSPO,[50,51] except clonazepam, which exhibits low affinity for TSPO.[52,53] HV had no history of psychiatric illness and had no first-degree relatives with a major mental disorder. Exclusion criteria for all subjects included: current diagnosis of substance abuse or a positive urine drug screen; pregnancy or current breastfeeding; clinically significant medical illness; and the presence of metal implants precluding an magnetic resonance imaging (MRI) scan. All subjects provided written consent after being informed of the study procedures. Neurocognitive performance was assessed in patients with SCZ using the Repeatable Battery for the Assessment of Neuropsychological Status.[54,55] Clinical status was also assessed with the PANSS, Calgary Depression Scale, Snaith-Hamilton Pleasure Scale, Scale for the Assessment of Negative Symptoms, and Apathy Evaluation Scale, while movement disorders were evaluated with the Barnes Akathisia Rating Scale, and Abnormal Involuntary Movement Scale. Assessments are described in more detail in supplementary methods.

This study was approved by the Research Ethics Board at the Centre for Addiction and Mental Health and the University of Toronto.

MRI Data Acquisition

T1- and proton density (PD)-weighted brain MRIs (T1: echo time [TE] = 5.3–15, repetition time [TR] = 8.9–12, field of view [FOV] = 20 cm, matrix = 256 × 256, slice thickness = 1.5 mm, number of excitations (NEx) = 2; proton density: TE = 17, TR = 6000, FOV = 22 cm, matrix = 256 × 256, slice thickness = 2 mm, number of excitations = 2) were obtained for each subject using a 1.5 T Signa scanner (General Electric Medical Systems) for 16 of the HV subjects and 12 of the SCZ subjects. MRI images for the remaining 4 SCZ and 11 HV were acquired using the 3 T MR-750 scanner (General Electric Medical Systems), with T1 parameters: slice thickness = 0.9 mm, TR = 8.2 ms, TE = min full, flip angle = 8°, NEx = 1, acquisition matrix = 256 × 228, and FOV = 28 cm and PD parameters: slice thickness = 2 mm, TR = 6000, TE = min full, number of acquisitions = 1, and FOV = 22. MRI images were used for the anatomical delineation of regions and quantification of PET images, as well

as determination of regional volumes.[46,56] Differences in MRI acquisition parameters did not have a significant effect on [^{18}F]-FEPPA outcome measures (data not shown).

PET Data Acquisition

All [^{18}F]-FEPPA PET scans were performed using a high-resolution CPS-HRRT PET scanner (Siemens Molecular Imaging), which measures radioactivity in 207 1.2-mm thick slices. Following a transmission scan, each subject was administered 177.77 ± 21.43 MBq of [^{18}F]-FEPPA by intravenous bolus injection and scanned for 125 min. The images were reconstructed into 34 time frames: 1 frame of variable length, 5 × 30, 1 × 45, 2 × 60, 1 × 90, 1 × 120, 1 × 210, and 22 × 300 s.

Input Function Measurement

Arterial blood was collected for the first 22.5 min after radiotracer injection at a rate of 2.5 ml/min and blood radioactivity levels were measured using an automatic blood sampling system (Model PBS-101; Veenstra Instruments). Additionally, 7 ml samples were drawn at −5, 2.5, 7, 12, 15, 20, 30, 45, 60, 90, and 120 min, relative to time of injection. The relative proportion of radiolabeled metabolites was measured using high-performance liquid chromatography and dispersion- and metabolite-corrected plasma input function were generated as previously described.[44,46]

Image Analysis

MRI volumes and time-activity curves (TACs) were extracted for hippocampus (HC), medial prefrontal cortex (mPFC), temporal cortex, dorsolateral PFC (DLPFC), and the striatum regions of interest (ROI) using in-house imaging pipeline ROMI.[56] All gray matter ROI were delineated using T1 MRI images, except for the striatum which was delineated using PD images.[57] Johns Hopkins University DTI atlas in ICBM-152 space was used to define white matter ROIs: corpus callosum, cingulum bundle, SLF, and PLIC, as previously described.[58]

In addition to the ROI-based analysis, PET images were also analyzed using a voxel-based method by generating parametric images using the Logan graphical analysis. Voxel-wise differences between images of HV and SCZ participants were evaluated using a 2-sample t test in SPM8, including rs6971 polymorphism as a covariate.

Kinetic parameters of [^{18}F]-FEPPA were derived from the TACs using 2-tissue compartment model and plasma input function to obtain the binding outcome measure V_T for each ROI, which has been validated for [^{18}F]-FEPPA quantification.[46] Kinetic analysis of gray and white matter incorporated a 5% and 2.7% contribution from the blood in the vascular lumen, respectively,[59] in the fitting of the 2-compartment kinetic model using PMOD software (PMOD Technologies). PET images were corrected for partial volume effect using the Muller-Gartner approach,[60] and the results are presented with and without the partial volume correction.

rs6971 Polymorphism Genotyping

Genomic DNA was obtained from peripheral leukocytes, using high salt extraction method.[61] The rs6971 polymorphism, known to affect binding of second-generation TSPO PET radioligands,[41–44] was genotyped using a TaqMan assay on demand C_2512465_20 (Applied Biosystems), as previously described. Based on their genotypes, study participants were classified as high-affinity binder, mixed-affinity binder, and LAB, respectively, as previously described.[43,44]

Statistical Analysis

All statistical analyses were performed using SPSS (version 19.0; IBM). Independent sample *t* tests were performed to test for differences between the demographic measures of the patient and control groups. A 2-factor ANOVA with ROI as within-subject factor, group as between-subject factor, and controlling for the TSPO genotype was carried out to test for differences in [^{18}F]-FEPPA V_T between groups. This analysis was also carried out using age as a covariate. Furthermore, a factorial ANOVA with TSPO genotype as a fixed variable and age as a covariate was used to compare regional differences in [^{18}F]-FEPPA V_T between HV and patients with SCZ in individual ROIs. *P* values below .05 were considered significant and Bonferroni correction was used for multiple comparisons. Partial correlations were used to examine the association between [^{18}F]-FEPPA V_Ts and MRI volumes for each ROI after normalization to the total intracranial volume (ICV) and controlling for the effects of TSPO (rs6971) polymorphism. A similar approach was used to evaluate correlations between [^{18}F]-FEPPA V_Ts and duration of untreated psychosis, length of illness, age of disease onset, number of acute crises, chlorpromazine equivalents, clinical presentation, and neuropsychological measures.

Results

Demographic characteristics of the study population and the clinical characteristics of the SCZ group are presented in table 1. All participants in the SCZ group were on treatment with a single antipsychotic agent, with 14 subjects receiving atypical antipsychotics and 2 receiving typical antipsychotic treatment. PET radiotracer injection parameters did not differ between the 2 groups (all $P > .149$). Considering the [^{18}F]-FEPPA PET imaging data, no significant effect of clinical group (HV vs SCZ) was detected on [^{18}F]-FEPPA V_Ts (figure 1) when controlling for genetics ($F_{(1,39)} = 0.179$, $P = .674$), as well as with incorporation

Table 1. Mean Demographic Characteristics for Healthy Volunteers (HV) and Patients (SCZ)

	Demographics	HV (n = 27)	SCZ (n = 16)	
	Age (years)	43.5 ± 15.5	42.5 ± 14.0	F = 0.263, P = .611
Gender	Male/female	10/17	10/6	X^2 = 2.618, P = .106
Genotype	HAB/MAB/LAB	19/8/0	10/6/1	X^2 = 1.904, P = .386
PET measures	Amount injected (mCi)	4.81 ± 0.36	4.79 ± 0.84	F = 0.027, P = .869
	Specific activity (mCi/μmol)	4071.7 ± 3622.0	3589.9 ± 1492.7	F = 0.426, P = .518
	Mass injected (μg)	0.91 ± 0.76	0.80 ± 0.63	F = 2.19, P = 0.149
	Age at SCZ onset		29.1 ± 8.3	
	Number of episodes		7.3 ± 11.7	
	Length of untreated illness (months)		43.4 ± 63.0	
	Length of illness (months)		177.3 ± 105.7	
	CDS		3.8 ± 2.9	
	AES		33.8 ± 8.0	
PANSS	Total		70.2 ± 9.7	
	Positive		19.3 ± 2.2	
	Negative		18.6 ± 5.0	
	General		31.6 ± 6.3	
RBANS	Total		76.3 ± 18.2	
	Immediate memory		81.9 ± 22.9	
	Visuospatial ability		79.8 ± 21.5	
	Language		83.9 ± 20.1	
	Attention		75.4 ± 14.5	
	Delayed memory		84.8 ± 19.1	
Treatment	Typical antipsychotics		2	
	Atypical antipsychotics		14	
	Antidepressants		5	
	Clonazepam		7	
	Anti-Parkinsonian agents		2	
	CPZ equivalents		300.0 ± 237.0	

Note: AES, Apathy Evaluation Scale; CDS, Calgary Depression Scale; CPZ, chlorpromazine; HAB, high-affinity binder; LAB, low-affinity binder; MAB, mixed-affinity binder; PANSS, Positive and Negative Syndrome Scale; PET, positron emission tomography; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; SCZ, schizophrenia.

of age as a covariate ($F_{(1,38)}$ = 0.159, P = .692). Percent differences between the diagnostic groups (HV vs SCZ) were estimated at −15.6%, 3.9%, 0.2%, −1.4%, and −0.54% in the HC, mPFC, DLPFC, temporal cortex, and striatum, respectively. The lack of significant differences between groups was unchanged following correction for partial volume effects ($F_{(1,39)}$ = 0.401, P = .530 and $F_{(1,38)}$ = 0.381, P = .541). Using the same statistical approach, no difference was observed between the clinical groups when considering V_T values measured in the white matter tracts (figure 2; $F_{(1,38)}$ = 0.597, P = .445; $F_{(1,37)}$ = 0.574, P = .453 with age added as a covariate). Performing an ANOVA to explore differences between clinical groups in individual white and gray matter yielded similar results (table 2). Furthermore, lack of significant difference between the 2 groups was confirmed by voxel-wise analysis, supporting our observations and suggesting that ROI delineation did not affect the findings.

When considering gray matter regions of all 38 subjects, no correlation was observed between the ROI volumes normalized to ICV and the [^{18}F]-FEPPA V_T in respective regions when controlling for the TSPO genotype (all P > .436). Similarly, no correlations were observed when patients with SCZ were considered separately, with or without controlling for age (P > .05). This lack of correlation was confirmed using data corrected for partial volume effect.

None of the disease parameters correlated with regional [^{18}F]-FEPPA V_T, except for a negative correlation between striatal V_T and number of crises, which appeared to be driven by a single outlier, and neither surpassed Bonferroni correction for multiple comparisons (P < .01). Exploration of correlations between [^{18}F]-FEPPA V_Ts and measures of psychopathology and cognition did not detect any significant associations (supplementary tables 1–6).

Discussion

Early brain PET imaging of patients with SCZ using prototypical first-generation TSPO radioligand [^{11}C]PK11195 was hindered by the low signal-to-noise ratio, low brain uptake, and high levels of nonspecific binding of the radiotracer.[62] These limitations were overcome by the use of the newer TSPO ligand [^{18}F]-FEPPA, which presents optimal chemical, pharmacokinetic, and pharmacodynamic

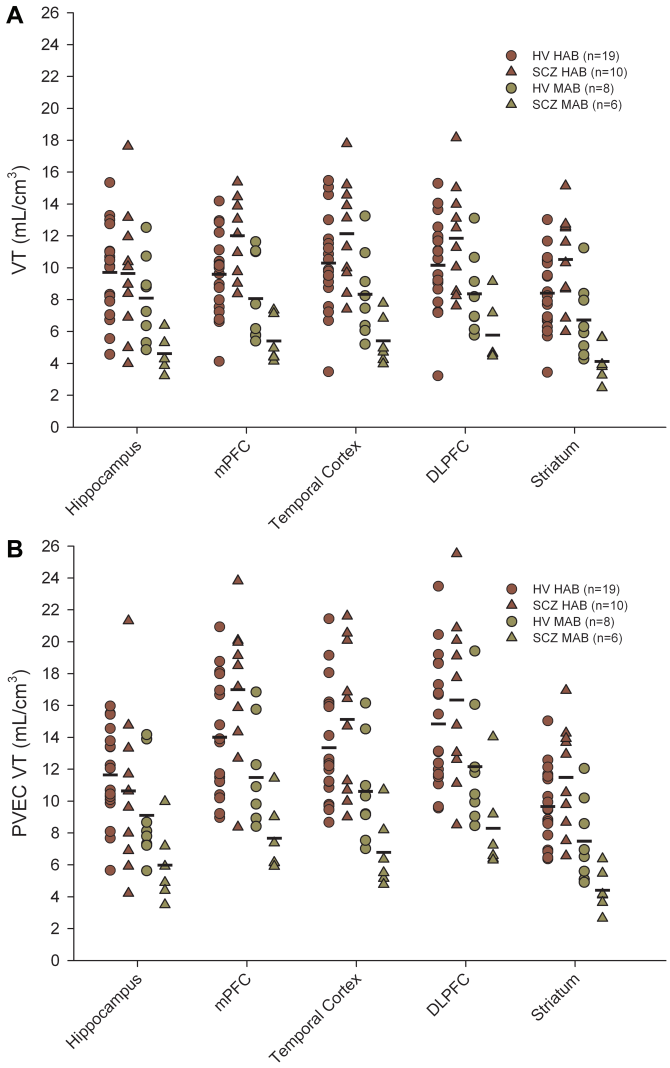

Fig. 1. Total volumes of distribution (V_T) of [^{18}F]-FEPPA in each of the studied ROIs (hippocampus; mPFC, medial prefrontal cortex; temporal cortex; DLPFC, dorsolateral PFC; and striatum). Data are presented as partial volumes uncorrected V_Ts (A) and partial volume effect-corrected V_Ts (B). No significant effect of clinical group (HV vs SCZ) was detected on [^{18}F]-FEPPA V_Ts when controlling for genetics ($F_{(1,39)} = 0.179$, $P = .674$). These findings were unchanged following correction for partial volume effects ($F_{(1,39)} = 0.401$, $P = .530$). [^{18}F]-FEPPA, *N*-acetyl-N-(2-[^{18}F] fluoroethoxybenzyl)-2-phenoxy-5-pyridinamine; HV, healthy volunteer; ROI, regions of interest; SCZ, schizophrenia.

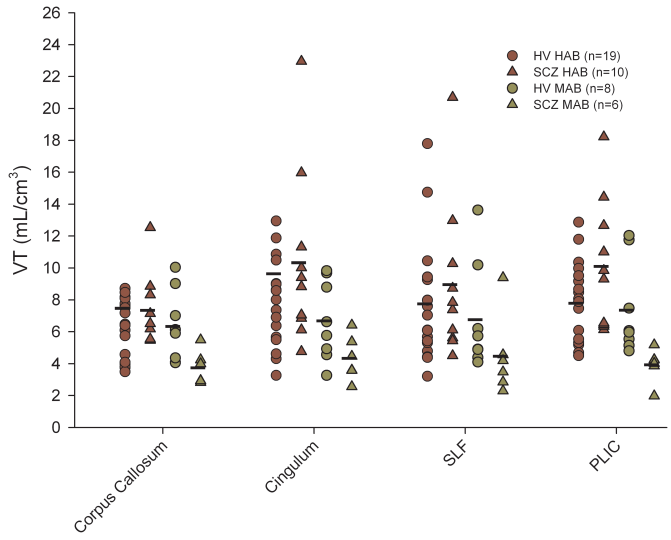

Fig. 2. Total volumes of distribution (V_T) of [^{18}F]-FEPPA measured in white matter tracts, with TAC derived from the ROI analysis on corpus callosum, cingulum bundle, superior longitudinal fasciculus (SLF), and the posterior limb of the internal capsule (PLIC). No difference was observed between the clinical groups when considering V_T values measured in the white matter tracts ($F_{(1,38)} = 0.597$, $P = .445$). [^{18}F]-FEPPA, *N*-acetyl-*N*-(2-[^{18}F]fluoroethoxybenzyl)-2-phenoxy-5-pyridinamine; ROI, regions of interest; TAC, time-activity curve.

properties for TSPO imaging,[45] including 3-fold higher potency compared with PBR28, and is an order of magnitude more potent than DPA713 or PK11195.[45]

Despite the challenges of using [^{11}C]PK1195, PET imaging studies have reported increased tracer binding potential in the hippocampal tissue of patients with SCZ during psychosis[39] and in the whole-brain gray matter of patients with recent-onset SCZ.[38] In the current study, despite use of a second-generation TSPO radioligand and scanning patients with ongoing psychotic symptoms in an high-resolution research tomograph (HRRT) scanner, we observed no significant differences in [^{18}F]-FEPPA binding between HV participants and patients with SCZ. While differences were initially expected in the HC and the DLPFC on the basis of past findings,[39,63] we also evaluated the [^{18}F]-FEPPA binding in the mPFC, temporal cortex, and the striatum and found no significant effects. Comparing the present findings with other investigations using second-generation TSPO ligands, our results are in line with those of a recent study that demonstrated no differences in [^{11}C]DAA1106 binding in chronic SCZ patients,[40] confirming these findings with a larger sample, correcting for genotype, using the HRRT scanner, and including patients with ongoing psychotic symptoms of moderate severity. From a methodological perspective, the present study utilized an F-18 radioligand and the HRRT scanner that has been demonstrated to improve PET quantification[64,65]

Our findings, however, are in contrast with a recent report of a 16% increase in TSPO binding observed in postmortem tissues using tritium-labeled second-generation TSPO ligand PBR28.[63] These differences may result from the unique properties of radioligands used in the studies, or from differences in patient populations, treatment status, and methodology (in vivo vs postmortem). While our current kinetic modeling approach has been previously validated,[46] further refinements that would incorporate a possible slow irreversible binding compartment[66] are undergoing evaluation for future TSPO-imaging studies with [^{18}F]-FEPPA. It should be noted that

Table 2. Regional [^{18}F]-FEPPA V_Ts in Gray and White Matter ROIs of Patients With Schizophrenia (SCZ) and Healthy Volunteers (HV)

		HV (n = 27)		SCZ (n = 16)		Model		Diagnostic Effect		Genetic Effect		Age Effect	
	Gray Matter ROI	Adjusted Mean	SE	Adjusted Mean	SE	$F_{(3,39)}$	P	$F_{(1,39)}$	P	$F_{(1,39)}$	P	$F_{(1,39)}$	P
V_T	Hippocampus	11.45	2.03	8.03	2.65	1.501	.230	1.045	.313	1.801	.187	0.997	.324
	Medial prefrontal cortex	9.03	0.52	9.69	0.67	**5.968**	**.002**	0.602	.442	**15.606**	**<.001**	1.012	.321
	Temporal cortex	9.59	0.60	9.78	0.78	**5.101**	**.004**	0.037	.847	**14.173**	**.001**	0.394	.534
	Dorsolateral prefrontal cortex	9.51	0.57	9.83	0.76	**4.521**	**.008**	0.110	.742	**11.472**	**.002**	1.120	.296
	Striatum	8.17	0.47	8.38	0.61	**6.151**	**.002**	0.075	.786	**18.443**	**<.001**	0.307	.583
V_T, corrected for partial volume effect	Hippocampus	13.08	2.02	9.19	2.63	1.570	.212	1.374	.249	2.227	.144	0.473	.496
	Medial prefrontal cortex	13.10	0.77	13.76	1.00	**5.460**	**.003**	0.274	.604	**15.869**	**<.001**	0.057	.813
	Temporal cortex	12.39	0.75	12.24	0.98	**5.152**	**.004**	0.014	.907	**14.878**	**<.001**	0.034	.854
	Dorsolateral prefrontal cortex	13.90	0.84	13.56	1.10	**3.905**	**.016**	0.062	.804	**11.397**	**.002**	0.028	.869
	Striatum	8.903	0.54	9.03	0.70	**6.900**	**.001**	0.020	.888	**20.652**	**<.001**	0.230	.634
	White Matter ROI	**HV (n = 27)**		**SCZ (n = 15)**		$F_{(3,38)}$	P	$F_{(3,38)}$	P	$F_{(3,38)}$	P	$F_{(3,38)}$	P
V_T	Corpus callosum	7.05	0.82	6.05	1.10	1.127	.350	0.521	.475	2.071	.158	0.299	.588
	Cingulum	8.60	1.06	7.37	1.43	2.066	.121	0.482	.492	3.414	.072	1.371	.249
	SLF	7.37	0.63	6.52	0.85	1.624	.200	0.641	.428	2.559	.118	0.911	.346
	PLIC	7.57	0.56	7.22	0.74	2.314	.092	1.208	.149	**6.406**	**.016**	0.400	.531

Note: Factorial ANOVA comparisons were performed for each ROI to compare differences between diagnostic groups, with genotype (HAB vs MAB) and age added as covariates. White matter data obtained from one subject from the SCZ group was excluded, since no reliable kinetic model fit could be achieved in the white matter ROIs (coefficient of variation [COV] > 50). [^{18}F]-FEPPA, *N*-acetyl-*N*-(2-[^{18}F]fluoroethoxybenzyl)-2-phenoxy-5-pyridinamine; HAB, high-affinity binder; MAB, mixed-affinity binder; PLIC, posterior limb of the internal capsule; ROI, regions of interest; SLF, superior longitudinal fasciculus. Significant values are provided in bold.

the variability in our current study, as with other second-generation TSPO ligands, is relatively high even with the incorporation of the genetic factor. Using the mean and variability of V_T from our data, ~21 subjects per group would be needed to detect a 20% difference between the groups using F test ANOVA (alpha = .05 and power = 0.8); however, exposing an additional 5 SCZ patients to radioactivity and the inconvenience of arterial PET studies with no observed trend for an effect with 16 subjects would be unethical and uneconomical.

Alterations in white matter tracts and changes in regional connectivity are increasingly recognized as key factors in the pathogenesis of SCZ. White matter regions assessed in the current study are based on published reports of abnormalities in white matter tracts of patients with SCZ, as detected by measuring fractional anisotropy.[29,67] In addition to the diffuse nature of white matter abnormalities in SCZ, the lack of any significant difference in V_T values of the white matter tracts may reflect lack of effects of neuroinflammation in these tracts, antipsychotics use or challenges inherent in measuring TSPO binding in these tissues.[58]

In SCZ, meta-analyses have shown a progressive reduction in cerebral volume and an increase in ventricular volume over the course of the illness, from first-episode[21,68] to chronic disease.[22] Neuroinflammation, indexed using [^{18}F]-FEPPA V_T values in the current study, did not correlate with regional volumes, possibly due to the complicating factor of antipsychotic treatment and the time of assessment (eg, in the chronic stage of disease). Timing of the scan in the course of illness may be relevant, and in our sample only 5 of the 18 participants with SCZ were scanned within the first 5 years of disease onset; of note, excluding these patients did not alter the results (data not shown). The effect of antipsychotic drugs on regional volume is unclear at this time, with some studies showing reductions in gray matter and increased ventricular fluid volume,[69] while other groups report increases in gray matter volumes.[70,71] Since there is evidence that changes in regional volumes occur at different time points following the initiation of antipsychotic treatment,[70] further exploration on how neuroinflammation and regional volume change over the progression of the disease, before and during the course of antipsychotic treatment are warranted. While benzodiazepines may affect [^{18}F]-FEPPA binding to TSPO,[50,51] clonazepam does not seem to exhibit affinity.[52,53] Importantly, removing the 7 participants who were treated with clonazepam did not alter our findings, but the remaining group size is likely too small to draw conclusions.

In neurochemical brain imaging studies, sample size routinely represents a potential limitation; however, to our knowledge this study is the largest PET study to date investigating neuroinflammation in SCZ, particularly in patients exhibiting psychotic symptoms and controlling for rs6971. Furthermore, although we did not observe an effect of antipsychotic dose in the present study, it is still

possible that antipsychotic medications influenced the results. In vitro studies have demonstrated an inhibitory effect for a number of antipsychotic medications on the release of proinflammatory cytokines and nitric oxide by microglia,[72,73] thus, antipsychotics may exert inhibitory effects on microglia that could mask any increases in neuroinflammation, accounting for the lack of differences in [^{18}F]-FEPPA binding between groups. Ideally, future studies with antipsychotic-naive patients can address this issue.

In our current study, V_T was used as an outcome measure, representing a ratio between the concentration of the radioligand in the tissue and its concentration in the plasma. We did not delineate the individual contributions of the specific binding (V_S) and the combination of nonspecific binding and free tracer (V_{ND}) to the total V_T,[74] since V_T has been previously shown to be highly identifiable (lower coefficient of variance compared with V_S and BP_{ND}) and a reliable measure of [^{18}F]-FEPPA retention.[44] The use of this outcome measure is not likely to affect the findings, since the nonspecific-binding component would not be expected to be altered in patients with SCZ. Finally, the development and characterization of an ^{18}F-labeled tracer provides a TSPO radioligand with a long half-life, providing flexibility to the data acquisition, as well as allowing the tracer to be distributed to centers without a cyclotron.

In conclusion, we found no evidence of increased neuroinflammation as indexed with [^{18}F]-FEPPA binding in the gray and white matter brain regions of patients with SCZ who were experiencing psychotic symptoms despite treatment with antipsychotic medications. No correlations were observed between [^{18}F]-FEPPA binding and psychopathological indices, length of illness, neuropsychological measures, or regional MRI volumes.

Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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