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Imaging Microglial Activation in Individuals at Clinical High Risk for Psychosis: an *In Vivo* PET Study with [^{18}F]FEPPA

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Several lines of evidence implicate microglial activation and abnormal immune response in the etiology of psychosis. Previous positron emission tomography (PET) neuroimaging studies of the translocator protein 18 kDa, TSPO, were limited by low affinity of the first-generation radioligand, low-resolution scanners, and small sample sizes. Moreover, there is a dearth of literature on microglial activation in individuals at clinical high risk (CHR) for psychosis. We used a novel second-generation TSPO radioligand, [^{18}F]FEPPA, to examine whether microglial activation is elevated in the dorsolateral prefrontal cortex (DLPFC) and hippocampus of antipsychotic-naive CHR. Twenty-four CHR (antipsychotic-naive $n=22$) and 23 healthy volunteers (HV) completed a high resolution [^{18}F]FEPPA PET scan and MRI. The PET data were analyzed using the validated two-tissue compartment model with arterial plasma input function with total volume of distribution (V_T) as outcome measure. All analyses were controlled for the TSPO rs6971 polymorphism. We did not observe any significant differences in microglial activation, as indexed by [^{18}F]FEPPA V_T, between CHR and HV in either the DLPFC ($F_{(1,\ 44)}=0.41$, $p=0.52$) or the hippocampus ($F_{(1,\ 44)}=2.78$, $p=0.10$). Exploratory associations show that in CHR, [^{18}F]FEPPA V_T was positively correlated with apathy (DLPFC: $r=0.55$, $p=0.008$; hippocampus: $r=0.52$, $p=0.013$) and state anxiety (DLPFC: $r=0.60$, $p=0.003$; hippocampus: $r=0.48$, $p=0.024$). The lack of significant group differences in [^{18}F]FEPPA V_T suggests that microglial activation is not significantly elevated in the clinical high risk state that precedes psychosis.
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INTRODUCTION

A role of the immune system in the etiology of schizophrenia is supported by convergent evidence from genetic, epidemiology, and preclinical studies (Kirkpatrick and Miller, 2013). Genome-wide association studies have found associations between several inflammation-related genes and risk of schizophrenia (Sekar *et al*, 2016; Shi *et al*, 2009; Stefansson *et al*, 2009), suggesting a link between the immune system and schizophrenia. Supporting these findings, several studies have reported increased level of inflammatory markers (eg, pro-inflammatory cytokines and C-reactive protein) in individuals at elevated clinical risk for schizophrenia (Perkins *et al*, 2015; Stojanovic *et al*, 2014) and in patients with psychosis (Fernandes *et al*, 2016). Moreover, epidemiologic and preclinical studies have suggested an association between early-life infection and risk of schizophrenia (Brown, 2011). Clinical relevance of such links is supported by clinical trials showing a potential role for anti-inflammatory agents in alleviating psychotic symptoms (Khandaker *et al*, 2015; Sommer *et al*, 2014).

Microglia, the resident macrophages of the central nervous system, are the key components of the brain's immune defense system. Following a brain injury they become activated and transform morphologically from ramified to amoeboid form (Perry *et al*, 2007). An important characteristic of activated microglia is a significant increase in expression of the mitochondrial 18 kDa translocator protein, also known as TSPO, thus making TSPO a suitable target for imaging microglial activation(Venneti *et al*, 2009).

Several postmortem studies have examined markers of microglial activation including TSPO in patients with schizophrenia, however, there is a wide variability across different studies, as about half of studies showed an increase in microglial activation markers and 40% showed no difference (Trepanier *et al*, 2016). Given this inconsistency and to avoid limitations of postmortem studies, microglial activation can be quantified in-vivo with positron emission tomography (PET) by using radioligands that target TSPO.

Studies on TSPO have shown a single gene polymorphism in the gene of this protein (rs6971) that affects the binding affinity of second-generation TSPO radioligands such as [^{18}F]FEPPA, [^{11}C]DPA-713 and [^{11}C]PBR28 (Kreisl *et al*, 2013a; Mizrahi *et al*, 2012; Owen *et al*, 2012). On the basis of

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this polymorphism individuals can be classified as high-affinity binder (HAB), mixed-affinity binder (MAB), or low-affinity binder (LAB).

Thus far, several PET studies have examined microglial activation in schizophrenia whereas a few have examined the disease at very early stages such as first-episode psychosis. Early PET studies of schizophrenia using the prototypical radioligand for TSPO, [^{11}C]PK11195, showed increased binding of this radioligand in treated schizophrenia patients compared to healthy volunteers, respectively, in total gray matter and hippocampus (Doorduin *et al*, 2009; van Berckel *et al*, 2008). The interpretation of these studies are, however, limited due to the known technical limitations of [^{11}C]PK11195 (Vivash and O'Brien, 2016). These limitations promoted the development of second-generation TSPO radioligands with greater advantages for quantifying TSPO expression *in vivo* (Doorduin *et al*, 2009; van Berckel *et al*, 2008). The first study using a second-generation TSPO radioligand, [^{11}C]DAA1106, found no significant difference in binding between chronic medicated schizophrenia and healthy volunteers; however, [^{11}C]DAA1106 binding in schizophrenia patients was significantly correlated with duration of illness and severity of positive psychotic symptoms (Takano *et al*, 2010). Recently, Coughlin and colleagues (Coughlin *et al*, 2016) using PET and another second-generation TSPO radioligand, [^{11}C]DPA-713, also found no difference in microglial activation between recent-onset schizophrenia ($n=12$) and healthy volunteers ($n=14$). Our study in treated chronic schizophrenia ($n=16$), and more recently in untreated first-episode psychosis (total $n=19$, $n=14$ antipsychotic naive) using [^{18}F]FEPPA did not observe significant group differences between patients and matched healthy volunteers or significant associations between [^{18}F]FEPPA binding and length of illness, severity of symptoms, or neuropsychological measures when controlling for multiple testing (Hafizi *et al*, 2016; Kenk *et al*, 2015). Most recently, Collste *et al* (2017) using another second-generation TSPO radioligand, [^{11}C]PBR28, reported significantly lower binding of [^{11}C]PBR28 in antipsychotic-naive first-episode psychotic patients ($n=16$) as compared to matched healthy volunteers.

To date there is only one other study that examined microglial activation in CHR ($n=14$) which reported elevated [^{11}C]PBR28 distribution volume ratio (DVR) in the gray matter compared to healthy volunteers ($n=14$; Bloomfield *et al*, 2015). In the same study and in a separate cohort they reported an elevated DVR in the gray matter of chronic treated schizophrenia ($n=14$) as compared to matched healthy volunteers. However, using DVR as outcome measure poses several important limitations (Narendran and Frankle, 2016). In fact, when the [^{11}C]PBR28 data of Bloomfield *et al* are analyzed using the outcome measure, total distribution volume (V_T) with the validated two tissue compartment model (2TCM) (Fujita *et al*, 2008; Owen *et al*, 2012), there were no significant group differences between CHR and healthy volunteers or schizophrenia patients *versus* matched healthy volunteers.

In the current study, we investigated microglial activation in the largest CHR sample so far ($n=25$, $n=22$ antipsychotic naive), using the gold standard [^{18}F]FEPPA V_T and a high-resolution research tomograph (HRRT). We also examined associations between microglial activation and severity of symptoms in addition to neuropsychological measures.

MATERIALS AND METHODS

Subjects

Twenty-five CHR and 24 matched healthy volunteers were initially enrolled and scanned in this study. One healthy volunteer and one CHR were excluded from all the analyses due to having the low-affinity binder genotype that did not allow [^{18}F]FEPPA PET quantification. Most of the participants in the CHR group were antipsychotic-naive ($n=22$). Fifteen healthy volunteers of the total of 24 have been included in our previous cohorts (Hafizi *et al*, 2016), whereas none of the CHR individuals have been previously reported.

To be eligible, CHR individuals had to meet the following inclusion criteria: fulfillment of diagnostic criteria for prodromal syndrome as per the Criteria of Prodromal Syndromes (Miller *et al*, 2002) with no current Axis I disorders, as determined with the Structured Clinical Interview for DSM-IV (SCID; First *et al*, 1995), such as depression which was shown to be associated with microglial activation (Setiawan *et al*, 2015). Healthy volunteers did not have any history of psychiatric illness, psychoactive drug use, and/or first-degree relatives with a major mental disorder. Participants were excluded for any of the following: current or past history of 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 MRI scan. In CHR, clinical status and severity of symptoms (eg, psychosis-risk symptoms) were assessed with the structured interview for psychosis-risk syndromes (SIPS), scale of psychosis-risk symptoms (SOPS; Miller *et al*, 2002), Calgary Depression Scale (depression scale), Snaith-Hamilton Pleasure Scale (pleasure scale), Global Assessment of Functioning scale (global functioning), state-trait anxiety inventory (anxiety scale) and Apathy Evaluation Scale (apathy scale). Neurocognitive performance was assessed using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS; Randolph, 1998; Wilk *et al*, 2004). Assessments are described in detail in the method section of the Supplementary Material.

This study was approved by the Research Ethics Board at the Centre for Addiction and Mental Health. All subjects provided written informed consent after being informed of all study procedures.

PET and MRI Data Acquisition and Analysis

Details of PET and MRI data acquisition have been described elsewhere (Kenk *et al*, 2015) and are summarized below and in the method section of the Supplementary Material. Proton density-weighted (PD) brain MRI scan was obtained for each subject using a 1.5T General Electric Signa scanner (General Electric Medical Systems, Milwaukee, WI, USA) for four healthy volunteers and two CHR. For the remaining 23 CHR and 20 healthy volunteers, PD MRI images were acquired using a 3T MR-750 scanner (General Electric Medical Systems). All [^{18}F]FEPPA PET scans were performed using a high-resolution neuro-PET camera system (HRRT,

Siemens Molecular Imaging, Knoxville, TN, USA) for 125 min following an intravenous bolus injection of 183.74 ± 12.14 (mean ± SD) MBq of [^{18}F]FEPPA. Arterial blood samples were collected automatically using an automatic blood sampling system (Model PBS-101, Veenstra Instruments, Joure, Netherlands) for the first 22.5 min after radiotracer injection at a rate of 2.5 ml/min and manually at − 5, 2.5, 7, 12, 15, 20, 30, 45, 60, 90, and 120 min to measure radioactivity in blood and determine the relative proportion of radiolabeled metabolites. Dispersion- and metabolite-corrected plasma input function was generated as previously described (Rusjan *et al*, 2011).

Image processing and calculation of total distribution volumes (V_T). Time-activity curves were extracted for dorsolateral prefrontal cortex (DLPFC), hippocampus, medial prefrontal cortex, temporal cortex, total gray matter, and whole brain using validated in-house imaging pipeline ROMI (Rusjan *et al*, 2006). All regions of interest were delineated using individual proton density (PD) MRI (Rusjan *et al*, 2006). Kinetic parameters of [^{18}F]FEPPA were derived from the time-activity curves using the two-tissue compartment model (2TCM) and plasma input function to obtain the total distribution volume (V_T) for each region of interest, which has been validated for [^{18}F]FEPPA quantification and described elsewhere (Kenk *et al*, 2015; Rusjan *et al*, 2011). PET images were also corrected for partial volume effect using the Muller-Gartner approach (Muller-Gartner *et al*, 1992), and the results are presented in the Supplementary Materials (Supplementary Figure 1 in the Supplementary Material).

For exploratory purposes, we investigated the difference between clinical groups using DVR as an outcome measure. DVR is defined as regional V_T normalized by V_T in the cerebellum, gray matter, or whole brain (DVR = V_T _Region/ V_T _k, where k represents cerebellum, gray matter, or whole brain). The results of DVR analyses are presented in details in the Supplementary Materials.

Voxel-based PET image analysis. Parametric images of [^{18}F]FEPPA V_T were generated using the Logan graphical analysis method, to examine voxel-wise group comparisons of V_T between groups. More details are provided in the method section of the Supplementary Material.

Table 1 Demographic Characteristics of the Participants and Radioligand Injection Parameters

Demographics		**HV (n = 23)**	**CHR (n = 24)**	
Age (years)		23.04 ± 3.20	21.21 ± 3.35	F = 3.69, P = 0.061
Gender	Male/Female	9/14	11/13	χ^2 = 0.22, P = 0.64
Genotype[a]	HAB/MAB/LAB	19/4/1	18/6/1	χ^2 = 0.41, P = 0.52
Drug[b] (current use)				
	Nicotine	0	5	
	Cannabis	0	0	
Lifetime recreational drug use history (> 10 times)				
	Cannabis	0	10	
	MDMA	0	1	
Anti-psychotic use[c]		0	3	
PET measures	Amount injected (mCi)	4.87 ± 0.37	5.06 ± 0.26	F = 3.94, P = 0.053
	Specific activity (mCi/μmol)	3536.16 ± 3896.46	1675.07 ± 1508.41	F = 4.74, P = 0.035
	Mass injected (μg)	1.25 ± 0.94	1.86 ± 1.40	F = 3.003, P = 0.090
	Depression scale		6.50 ± 4.20	
	Apathy scale		38.88 ± 11.55	
	Pleasure scale		3.75 ± 3.23	
	Global functioning		53.83 ± 7.38	
SOPS	Total		33.58 ± 10.80	
RBANS	Total		88.79 ± 16.06	
Anxiety scale[d]				
	State		46.04 ± 14.20	
	Trait		55.65 ± 10.81	

Abbreviations: Anxiety scale, state-trait anxiety inventory; Apathy scale, Apathy Evaluation Scale; Depression scale, Calgary Depression Scale; HAB, high-affinity binder; LAB, low-affinity binder; Global functioning, Global assessment of functioning; MAB, mixed-affinity binder; PET, positron emission tomography; Pleasure scale, Snaith-Hamilton Pleasure Scale; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; SOPS, Scale of Psychosis-risk Symptoms.
[a]Two low-affinity binders (one in each diagnostic group) were excluded from all the analyses listed in the table.
[b]All the participants in both HV and CHR groups had negative urine drug screening test for ethanol, methadone, benzodiazepines, opiate, cannabis, and cocaine.
[c]Three CHR individuals were taking antipsychotic medications at a very low dose: risperidone 0.5 mg, 1 mg and aripiprazole 5 mg, respectively.
[d]Anxiety scores were not available for one clinical high risk individual.

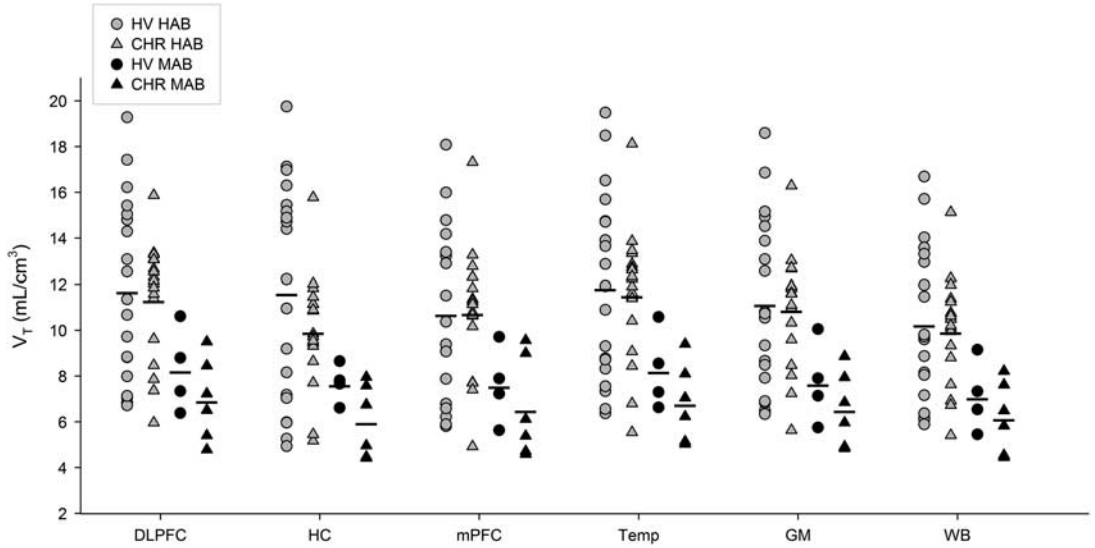

Figure 1 Total distribution volume of [^{18}F]FEPPA (V_T) in CHR and healthy volunteers across different ROIs. DLPFC, hippocampus, mPFC, Temporal cortex, total gray matter, and whole brain.

rs6971 Polymorphism Genotyping

The participants were categorized based on the TSPO rs6971 as high-(C/C), mixed-(C/T), and low-affinity (T/T) binders, as described elsewhere (Mizrahi *et al*, 2012; Owen *et al*, 2012). Details of genotyping procedures are provided in the Supplementary Material.

Statistical Analysis

Demographic measures were examined for any group differences using analysis of variance (ANOVA, continuous variables) or chi-square tests (categorical variables). Multivariate analysis of variance (MANOVA), with regional V_Ts as the dependent variables, group (CHR individuals *vs* healthy volunteers) as the independent variable, and the TSPO genotype (rs6971) as a covariate were carried out to test for differences in [^{18}F]FEPPA V_Ts between clinical groups. Partial correlations controlling for the effects of TSPO rs6971 polymorphism were used to explore the association between [^{18}F]FEPPA V_Ts and clinical and neuropsychological measures. All statistical analyses were performed using SPSS (version 22.0; IBM, Armonk, NY, USA), with $p<0.05$ two-tailed considered significant. Bonferroni correction was used to correct for multiple comparisons in regions we set out to test (ie dorsolateral prefrontal cortex and hippocampus). For descriptive purposes, we also report differences in medial prefrontal cortex, temporal cortex, total gray matter, and whole brain, with V_T data.

RESULTS

Demographics and Injection Parameters

Demographic and clinical characteristics of groups are presented in Table 1. Most of the participants in the CHR group were antipsychotic naive ($n=22$). Three out of 24 CHR individuals were taking antipsychotic medications at a very low dose: risperidone 0.5 mg, 1 mg and aripiprazole 5 mg, respectively. Six CHR individuals were taking antidepressants (SNRI or SSRI). Compared to the healthy volunteers, CHR received significantly lower specific activity ($F=4.74$, $p=0.035$). All other PET radiotracer injection parameters and demographics did not differ between the two groups (all $p>0.05$).

Differences in [^{18}F]FEPPA V_T between CHR and Healthy Volunteers

After controlling for the rs6971 polymorphism, no significant effect of clinical group (healthy volunteer *vs* CHR) was detected on [^{18}F]FEPPA V_Ts (Figure 1; $F_{(2,\ 43)}=2.70$, $p=0.08$; Hippocampus: $F_{(1,\ 44)}=2.78$, $p=0.10$, 15.61% higher in healthy volunteers than CHR; DLPFC: $F_{(1,\ 44)}=0.41$, $p=0.52$, 5.27% higher in healthy volunteers than CHR). The lack of group effect was not altered after controlling for age, tobacco, and/or anti-depressant use, excluding the CHR individuals that were on antidepressants and also with the correction for partial volume effects (Supplementary Figure 1). These results were consistent with other exploratory regions of interest (Supplementary Table 1). However, after removing an outlier (a CHR participant with hippocampus V_T value 2 standard deviation above the mean), we found a trend toward significance ($F_{(2,\ 42)}=3.12$, $p=0.054$; Hippocampus: $F_{(1,\ 43)}=3.83$, $p=0.057$, 18.13% higher in healthy volunteers than CHR; DLPFC: $F_{(1,\ 43)}=0.78$, $p=0.38$, 7.19% higher in healthy volunteers than CHR; Supplementary Table 9, and Figure 3). The CHR outlier was drug-naive, medically healthy and did not have any comorbidity.

In addition, we found no significant effect of clinical group with any of the DVR methods used. Results of the DVR method obtained before and after correction for partial volume effects and also other brain regions are reported in Supplementary Tables 2–4.

Voxel-Based Analyses

In line with results of the region of interest analyses, we did not find any group differences using the ROI-independent voxel-based analyses, confirming the lack of difference in [^{18}F]FEPPA V_T between CHR and healthy volunteers (Supplementary Figure 2).

Association between [^{18}F]FEPPA V_T and Severity of Symptoms, Clinical and Neuropsychological Measures in CHR

There were no significant correlations between [^{18}F]FEPPA V_T (before and after partial volume correction) and severity of psychosis-risk symptoms as measured by the SOPS, cognitive function as measured by RBANS, depression as measured by Calgary depression scale (depression scale), anhedonia as measured by Snaith-Hamilton pleasure scale (pleasure scale), and global functioning as measured by Global Assessment of Functioning (global functioning) (Supplementary Tables 5). Interestingly, apathy scores as measured by Apathy Evaluation Scale were significantly correlated with [^{18}F]FEPPA V_T in our primary regions of interest, hippocampus ($r=0.52$, $p=0.013$) and DLPFC ($r=0.55$, $p=0.008$) (Figure 2), and also in most exploratory brain regions, such that higher [^{18}F]FEPPA V_T was associated with higher apathy (Supplementary Table 7). Moreover, we observed a positive correlation between state anxiety score as measured by the state sub-scale of state-trait anxiety inventory and [^{18}F]FEPPA V_T in our primary regions of interest, hippocampus ($r=0.48$, $p=0.024$) and DLPFC ($r=0.60$, $p=0.003$; Figure 3), and also in all the exploratory brain regions, such that higher [^{18}F]FEPPA V_T was associated with greater state anxiety (Supplementary Table 8). All the correlations were exploratory and remained after removing the outlier.

DISCUSSION

In this study we observed no significant differences in microglial activation in the DLPFC and hippocampus, as indexed by the gold standard [^{18}F]FEPPA V_T, between CHR and healthy volunteers. However, after removing an outlier, we observed a trend toward significant lower [^{18}F]FEPPA binding in CHR as compared to healthy volunteers ($p=0.054$). There was no significant effect of clinical group on [^{18}F]FEPPA V_T in other brain regions (ie, the medial prefrontal cortex, the temporal cortex, total gray matter, and the whole brain). The results of voxel wise analyses were consistent with our ROI-based results, suggesting that our findings were not affected by ROI delineation. In addition, we observed positive associations between microglial activation in DLPFC and hippocampus and apathy and anxiety scores in CHR.

Our results in CHR are in line with four recent PET studies that examined microglial activation in psychosis using second-generation TSPO radioligands including a PET study using [^{11}C]DAA1106 (Takano *et al*, 2010) and our previous study using [^{18}F]FEPPA that found no significant differences between chronic treated schizophrenia and matched healthy volunteers (Kenk *et al*, 2015). A study using another second-generation TSPO radioligand, reported no significant differences in [^{11}C]DPA-713 binding between recent-onset treated schizophrenia and healthy volunteers (Coughlin *et al*, 2016). More recently we found similar results in a larger group of untreated ($n=14$ antipsychotic naive) patients with first-episode psychosis using [^{18}F]FEPPA (Hafizi *et al*, 2016). These findings along with the results of our current study, suggest that microglial activation is not significantly elevated in CHR or first-episode psychosis relative to healthy volunteers. The trend toward significance that we observed after removing the outlier, is consistent with a recent TSPO PET study using [^{11}C]PBR28 which reported significantly lower TSPO radioligand binding in antipsychotic-naive first-episode psychotic patients as compared to matched healthy volunteers (Collste *et al*, 2017).

The findings of the present study, however, are in contrast with two early PET studies using [^{11}C]PK11195 that reported higher microglial binding in the total gray matter of recent-onset schizophrenia (van Berckel *et al*, 2008) and in the hippocampus of treated schizophrenia (Doorduin *et al*, 2009). Nevertheless, [^{11}C]PK11195 is known to have several limitations such as high non-specific binding, low brain penetration, and low signal-to-noise ratio. Our results are also in apparent contrast with the only other study in CHR

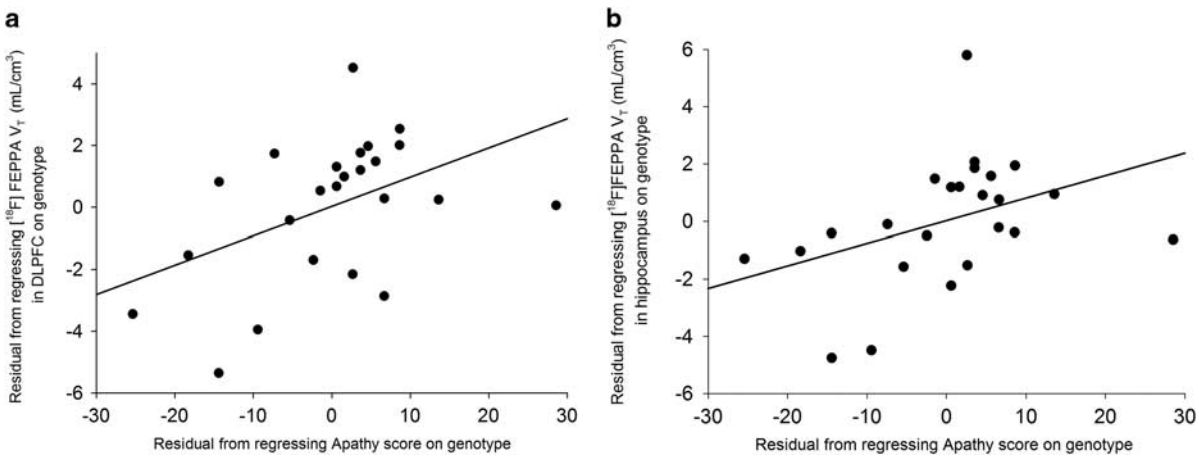

Figure 2 Relationship between apathy score and [^{18}F]FEPPA (V_T) in DLPFC ($r=0.55$, $p=0.008$) (a) and hippocampus ($r=0.52$, $p=0.013$) (b) in CHR.

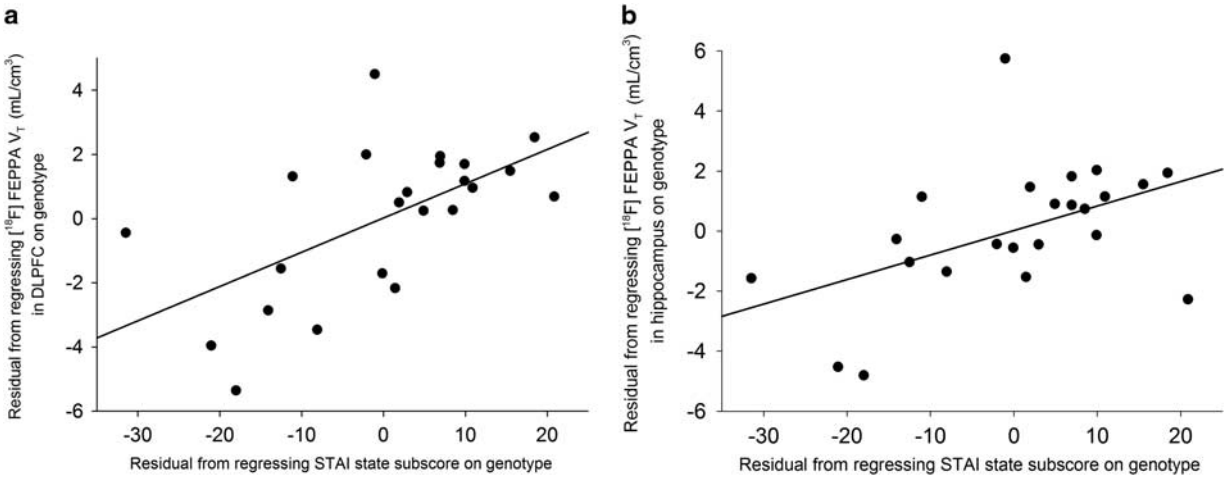

Figure 3 Relationship between the state subscore of State-Trait Anxiety Inventory (STAI) and [^{18}F]-FEPPA (V_T) in DLPFC ($r = 0.60$, $p = 0.003$) (a) and hippocampus ($r = 0.48$, $p = 0.024$) (b) in CHR.

that showed higher DVR in CHR and patients with chronic schizophrenia, as compared to healthy volunteers (Bloomfield *et al*, 2015). However, there are several differences between our study and Bloomfield and colleagues study, particularly the use of an alternative outcome measure, which for radioligands without a reference region is always controversial (Cannon, 2015; Narendran and Frankle, 2016). Notably in the Bloomfield *et al* [^{11}C]PBR28 study, using the validated V_T with 2TCM (Fujita *et al*, 2008), the authors did not find any significant difference in microglial activation between CHR/ chronic schizophrenia and healthy volunteers. Further, our sample size was substantially larger and we used a high resolution PET scanner (HRRT) with superior sensitivity and resolution.

In CHR, we observed a significant positive association between [^{18}F]FEPPA V_T in DLPFC and state anxiety, suggesting that higher microglial activation in the DLPFC is associated with higher anxiety. Preclinical studies support the role of activated microglia and immune mediators in anxiety-like behaviors (Sawada *et al*, 2014; Wohleb *et al*, 2013). Clinical studies show a reciprocal relationship between anxiety and immune response, such that inducing an immune response in healthy humans can increase anxiety (Reichenberg *et al*, 2001) and people with anxiety have impaired immune response (Salim *et al*, 2012). We also observed a positive association between [^{18}F]FEPPA V_T in DLPFC and hippocampus and apathy in CHR. This is in line with a growing body of literature supporting a link between inflammatory markers (eg proinflammatory cytokines) and negative symptoms of schizophrenia (Khandaker *et al*, 2015; Meyer *et al*, 2011). The positive association between microglial activation and apathy is also consistent with the current evidence on the role of microglial activation in Alzheimer's disease (Kreisl *et al*, 2013b; Suridjan *et al*, 2015) and the fact that apathy is considered as an early index of inflammatory state in the central nervous system in chronic neurodegenerative diseases (Perry *et al*, 2007). However, this is speculation at this point and needs to be further studied.

The results of this study should be interpreted considering the limitations that are inherent to neurochemical PET studies. First, in this study CHR received significantly lower specific activity compared to the healthy volunteers. However, there were neither a significant difference in any other PET parameters (Table 1) nor any significant associations between specific activity and V_T in any of our regions of interest ($p > 0.3$). Second, while small sample size is a potential limitation in molecular imaging studies, our study is thus far the largest PET study examining microglial activation in clinical populations. Third, although an increase in [^{18}F]FEPPA binding is mostly attributed to microglial activation, studies show that astrocytes and vascular endothelial cells also express TSPO (Notter *et al*, 2017). Further, TSPO expression may not directly relate with other signs of low-grade inflammation, such as inflammatory cytokines (Notter *et al*, 2017). However, this would not affect the overall conclusion of this study. Fourth, all the correlations were exploratory in nature, and controlling for potential confounders (ie, age, drug use, and medications) did not alter the outcome. Finally, the variability of V_T in our current study, as with other second-generation TSPO radioligands, is relatively high even after controlling for the effect of rs6971 polymorphism, suggesting that larger samples would be needed to find a significant effect between groups. A sample size calculation using the data from the current study showed that to detect group effects between CHR and matched controls in the DLPFC (effect size was 0.19) or the hippocampus (effect size was 0.49), 444 and 67 participants per group, respectively, would be needed (two tailed test at $\alpha = 0.05$ and 80% power). After removing the outlier, a similar sample size calculation revealed that to detect a significant effect of diagnostic group in the DLPFC (effect size = 0.26) or hippocampus (effect size = 0.58), we would need, respectively, 231 or 49 participants per group. Despite this variability, the binding of [^{18}F]FEPPA was increased during induced inflammation in animals (Zhang *et al*, 2012), Major Depressive Episode (MDE) patients (Setiawan *et al*, 2015), meningioma (Ko *et al*, 2013), and also Alzheimer's disease in humans (Kreisl *et al*, 2013b; Suridjan *et al*, 2015; Yasuno *et al*, 2008). Also, in this study we did not correct V_T for the plasma free fraction of the radioligand (f_p), as it was previously shown to substantially increase the variability (Hines *et al*, 2013). More studies are needed to

determine whether correcting V_T values for f_p can improve the ability of [^{18}F]FEPPA to distinguish alterations in microglial activation in CHR. Further, due to this variability in [^{18}F]FEPPA binding, it is also possible that only a subgroup of CHR would present a neuroinflammatory phenotype, or that TSPO imaging cannot accurately capture low-grade inflammatory processes such as those present in psychosis-related disorders (as compared to AD or MDE).

In conclusion, our results showed no evidence of increased microglial activation as quantified with [^{18}F]FEPPA binding, in the DLPFC and the hippocampus of CHR as compared with healthy volunteers.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)