



In-vivo imaging of brain microglial activity in antipsychotic-free and medicated schizophrenia: a [¹¹C](R)-PK11195 positron emission tomography study

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Title Page

Title

In-vivo imaging of brain microglial activity in antipsychotic-free and medicated schizophrenia: a [¹¹C](R)-PK11195 positron emission tomography study

Running title

PET in unmedicated and medicated schizophrenia

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Abstract

Positron emission tomography (PET) imaging of the 18kDa translocator protein (TSPO) has been used to investigate whether microglial activation, an indication of neuroinflammation, is evident in the brain of adults with schizophrenia. Interpretation of these studies is confounded by potential modulatory effects of antipsychotic medication on microglial activity. In the first such study in antipsychotic-free schizophrenia, we have used [^{11}C](*R*)-PK11195 PET to compare TSPO availability in a predominantly antipsychotic-naïve group of moderate-to-severely symptomatic unmedicated patients ($n=8$), similarly symptomatic medicated patients with schizophrenia taking risperidone or paliperidone by regular intramuscular injection ($n=8$), and healthy comparison subjects ($n=16$). We found no evidence for increased TSPO availability in antipsychotic-free patients compared to healthy controls (mean difference 4%, $p=0.981$). However, TSPO availability was significantly elevated in medicated patients (mean increase 88%, $p=0.032$) across prefrontal (dorsolateral, ventrolateral, orbital), anterior cingulate and parietal cortical regions. In the patients, TSPO availability was also strongly correlated with negative symptoms measured using the Positive and Negative Syndrome Scale across all the brain regions investigated ($r=0.651-0.741$). We conclude that the pathophysiology of schizophrenia is not associated with microglial activation in the 2-6 year period following diagnosis. The elevation in the medicated patients may be a direct effect of the antipsychotic, although this study cannot exclude treatment resistance and/or longer illness duration as potential explanations. It also remains to be determined whether it is present only in a subset of patients, represents a pro- or anti-inflammatory state, its association with primary negative symptoms, and whether there are significant differences between antipsychotics.

Introduction

Schizophrenia affects approximately 0.5-1% of the population worldwide ¹. It can be characterised by three symptom domains: positive symptoms (delusions and hallucinations), negative symptoms (lack of motivation, anhedonia, flat affect, social withdrawal) and cognitive impairments, all of which can have profound effects on functioning and quality of life. The efficacy of antipsychotics is predominantly against positive symptoms, while the negative and cognitive symptoms remain relatively poorly modified and contribute significantly to the impaired functioning and wider burden of the disorder. Therefore novel avenues of research need to be explored to elucidate the pathophysiological mechanisms underlying all symptom domains and to inform the development of more effective and tolerable medications.

Convergent evidence suggests that inflammation may play a key role in the pathophysiology of schizophrenia and as a potential target for drug development ²⁻¹⁰. In particular, peripheral markers of inflammation are raised in patients with schizophrenia ¹¹ including first-episode antipsychotic-naïve patients ^{12,13}, and there is some evidence for increased inflammatory markers post-mortem ¹⁴⁻¹⁶. A crucial question though, is whether there is evidence of neuroinflammation in schizophrenia in-vivo.

Evidence for neuroinflammation can be measured in-vivo using positron emission tomography (PET) and radioligands that bind to the 18kDa translocator protein (TSPO), which is increasingly expressed when microglia become activated. To date, PET studies in schizophrenia have shown mixed results. Increased TSPO expression has been reported using the first-generation radiotracer [¹¹C](R)-PK11195 ^{17,18} and the second-generation tracer [¹¹C]PBR28 ¹⁹, but not replicated in other second-generation tracer studies using [¹¹C]DAA1106 ²⁰, [¹⁸F]FEPPA ²¹, and [¹¹C]DPA-713 ²². The discrepant findings may reflect differences in methodology, radiotracers, brain regions, and aspects of the patient populations including severity and duration of illness. However, importantly, all of the schizophrenia patients in these PET studies were taking antipsychotic medication at the time of scanning, introducing a potential confound due to their effects on the immune system. The majority of in vitro studies of microglia suggest that antipsychotics are anti-inflammatory ²³ although there are several contradictory reports ^{23,24} and the relevance of the in vitro data to the context of the in vivo brain in

schizophrenia is not certain. In normal rats, the effect of chronic treatment with antipsychotics on post mortem TSPO binding density varies between brain regions and individual drugs²⁵, while morphological evidence for microglial activation has been found following treatment with either haloperidol or olanzapine at clinically relevant doses²⁶. However, this study was unable to differentiate whether the activation represented a pro- or anti-inflammatory biochemical phenotype²⁷, and we are not aware of comparable published reports in non-human primates or humans.

The aims of the current study were therefore to further investigate the evidence for neuroinflammation in schizophrenia by comparing antipsychotic-free patients, medicated patients, and matched healthy volunteers, using increased TSPO availability measured by [¹¹C](R)-PK11195 PET as evidence for microglial activation. To the best of our knowledge this is the first report of TSPO availability in vivo in drug-free schizophrenia using PET. Due to potential variability of effects across different antipsychotics, and to minimise additional variability from poor oral drug compliance, we studied patients taking risperidone by long acting intramuscular injection (LAI). Patients taking paliperidone LAI were also included on the basis that paliperidone is the main metabolite (9-OH-risperidone) of risperidone and is pharmacologically active in all patients taking risperidone²⁸. Longitudinal MRI studies of cerebral grey matter suggest that the pathological process in schizophrenia is widespread in frontal, temporal and parietal lobes²⁹ and it is of great topical importance to understand whether neuroinflammation is associated with this process. Our hypotheses were therefore based on these brain regions. On the basis of the literature available at the time of developing the protocol, we hypothesised that TSPO availability would be elevated in antipsychotic-free patients compared to healthy volunteers. For medicated patients, in the absence of a clear directional hypothesis from the literature we further proposed to test the null hypothesis that TSPO availability does not significantly differ between medicated and drug-free patients. A secondary aim was to examine correlations between brain TSPO availability, symptom domains of schizophrenia, and peripheral (blood) markers of inflammation.

Subjects and Methods

Participants

Nineteen patients with a diagnosis of schizophrenia were recruited from mental health services in the Manchester region of the UK. PET data could not be acquired for 3 (1 failed intravenous cannulation, 2 anxiety before or during scan) and 16 patients (11 males; mean \pm SD age 33 \pm 9 yr; range 18-46 yr) completed the study. Of these, 8 were not taking antipsychotics (6 antipsychotic-naïve and 2 antipsychotic-free for 12 and 60 months respectively) and 8 were taking either risperidone (n=7) or paliperidone (n=1) by LAI. The majority of patients (11/16) were taking other medications, mainly antidepressants (for details of current and past medications, see Supplementary Material Section B3).

Diagnosis was confirmed using the Structured Clinical Interview for the DSM-IV (SCID-I)³⁰.

Symptom severity was assessed using the Positive and Negative Syndrome Scale (PANSS)³¹. All patients had moderate to severe symptom severity (mean \pm SD PANSS total score 85 \pm 9) and were experiencing psychosis (PANSS positive score 21 \pm 4). For demographic and clinical characteristics see Table 1. Patients were sex- and age-matched (\pm 6 yr) with 16 healthy volunteers (11 males; age 33 \pm 10 yr; range 20-50 yr) scanned with the same protocol. All participants were medically healthy on the basis of clinical history, physical examination, routine blood tests and negative urine toxicology. Additional measures included body mass index (BMI) and markers of inflammation in plasma (TNF- α , IFN- γ , IL-6, IL-8, IL-1 β and CRP).

Exclusion criteria for all participants included substance abuse in the previous year, lifetime history of substance dependence, anti-inflammatory medications in the previous month, another Axis I disorder, pregnancy, and history of neurological or autoimmune disorder. The study was approved by the Greater Manchester East Research Ethics Committee and the United Kingdom Administration of Radioactive Substances Advisory Committee. All subjects provided written informed consent.

MRI and PET acquisition

A 1.5 Tesla T1-weighted MRI brain scan, to exclude significant abnormality and for identification of regions of interest (ROI), was acquired as described previously³². [¹¹C](R)-PK11195 (1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinoline carboxamide) was synthesised onsite as described previously³³. Following intravenous injection of the radiotracer (dose range 400-740 MBq) PET emission data were acquired for 60 minutes on a high-resolution research tomograph (HRRT; Siemens/CTI, Knoxville, Tennessee) with intrinsic spatial resolution of ~2.5mm full width at half the maximum (FWHM), as described previously³⁴. PET data were reconstructed with 3D iterative ordered subset expectation maximisation³⁵ (OSEM; 12 iterations and 16 subsets) without resolution modelling using the HRRT user community software which employs an ultrafast ordinary Poisson OSEM algorithm (OP-OSEM)³⁶.

PET image analysis

Individual MRI scans were co-registered with the summed PET images using a mutual information algorithm³⁷ then segmented into grey matter (GM) and white matter (WM) probability maps³⁸. The inverse transformation parameters from segmentation were then used to spatially normalise a maximum probability brain atlas^{39, 40} into individual space. Binding potential (BP_{ND}), representing the ratio at equilibrium of specifically bound radioligand to that of non-displaceable radioligand in tissue⁴¹, was calculated using a GM cerebellum reference input function and the simplified reference tissue model (SRTM)⁴² (see Supplementary Material Sections A1-3 for further methodological considerations, including support for the use of cerebellum as reference region). Parametric maps of BP_{ND} were generated with a basis-function implementation of the SRTM using in-house kinetic modelling software⁴³. The individualised GM brain atlases were then projected onto these parametric maps to obtain mean BP_{ND} values for cortical regions of interest (ROIs). TSPO availability was too low in temporal cortex to accurately quantify^{44, 45} (see Supplementary Material Section A4) so we report 5 cortical ROIs: dorsolateral prefrontal [DLPFC], ventrolateral [VLPFC], orbitofrontal [OFC], anterior cingulate [ACC], and parietal.

Statistical analysis

Statistical analysis was performed in SPSS Statistics Version 22 (Armonk, NY: IBM Corp).

Independent-samples *t*-tests and one-way analysis of variance (ANOVA) were used to assess differences between demographic, clinical and radiotracer characteristics across groups. Categorical data (sex, smoking status) were compared between groups using Fisher's exact test (2-tailed).

Differences in [¹¹C](R)-PK11195 BP_{ND} (the dependent variable) between healthy controls and patients were assessed using a factorial repeated measures ('split-plot' or mixed between-within subjects) ANOVA with group as between-subjects factor (3 levels: healthy controls, antipsychotic-free patients and medicated patients) and brain region as within-subjects factor (5 levels, one for each ROI, treated as repeated measures). The normal distribution of BP_{ND} for each combination of the levels of the between- and within-subjects factors was confirmed by Shapiro-Wilk's test ($p > 0.05$) and Normal Q-Q Plot. Equality of covariance was confirmed by Box's test. Sphericity of the data was checked by Mauchly's test and corrected by the Greenhouse-Geisser procedure if violated. Homogeneity of variances was checked by Levine's test. Post-hoc correction for multiple comparisons in the between-subjects factor (3 groups) was by Tukey's HSD test. Correlations were measured by Pearson's *r* (2-tailed). Findings were considered significant at the $p < 0.05$ level, with correction for multiple comparisons where warranted.

Results

Patients and healthy controls were well matched for age and sex (Table 1). Patients had a higher BMI than controls, and the majority of patients (11 of 16) were smokers while none of the controls smoked. However, antipsychotic-free and medicated patients were well matched for BMI and smoking status in addition to sex, age at onset of psychosis, PANSS positive and PANSS general score. Medicated patients were older and had a longer duration of illness than antipsychotic-free patients, and there was a trend for them to have lower PANSS positive and higher PANSS negative scores. The injected mass of PK11195 was very low (in the order of 2 μg per subject) and did not significantly differ between the antipsychotic-free, medicated and healthy control groups ($F_{2,29}=0.510$, $p=0.60$). Combining all patients and controls there was no main effect of age ($F_{1,28}=1.376$, $p=0.251$), BMI ($F_{1,28}=2.518$, $p=0.124$) or sex ($F_{1,28}=0.379$, $p=0.543$) so these were not included as factors or covariates in subsequent analyses.

BP_{ND} was significantly different between the three groups (main effect of group: $F_{1,29}=3.843$, $p=0.033$). Visual inspection of the data (Figure 1) suggested that BP_{ND} was higher in the medicated group compared to the other two groups and very similar between the unmedicated and control groups, across all ROIs. Pairwise comparisons showed that BP_{ND} in the medicated patients was significantly higher than in the controls ($p=0.032$) by a mean of 88% (Table 2, Figure 2A), not different between antipsychotic-free patients and controls (mean 4% higher; $p=0.981$), and although BP_{ND} was higher in medicated than antipsychotic-free patients by a mean of 83% this reached only trend statistical significance ($p=0.097$). Region*group interaction was not statistically significant ($F_{5,7,82.3}=1.399$, $p=0.216$), confirming the visual impression from Figure 1 that the pattern of BP_{ND} differences between the groups did not differ across the five ROIs. Given the group differences, the significance of the elevations in individual ROIs in the medicated group was tested post hoc by t-test (1-tailed) for independent samples with unequal variance. BP_{ND} was significantly higher in the medicated than the control group in the ACC ($p=0.035$), DLPFC ($p=0.035$) and VLPFC ($p=0.036$), and reached trend significance in OFC ($p=0.056$) (Table 2, Figure 2B). BP_{ND} was significantly higher

in the medicated than the antipsychotic-free patients in the OFC ($p=0.043$) and reached trend significance in the VLPFC ($p=0.051$) and DLPFC ($p=0.068$). For further data excluding age and BMI as significant factors in the finding, see Supplementary Material Section B1.

An assumption of ANOVA is that the variance of the dependent variable should be equal between the groups of the between-subjects factor. Observation of the data (Figure 1) suggested that the spread of the regional BP_{ND} values was moderately higher in the patient groups than the controls and this was confirmed by a significant Levene's test (p -values: DLPFC 0.043; VLPFC <0.001 ; OFC 0.033; ACC 0.008; parietal cortex <0.001).

We found no significant correlations between [^{11}C](R)-PK11195 BP_{ND} and scores on PANSS total, positive or general subscales. However, there were highly significant positive correlations with PANSS negative scores in DLPFC ($r=0.651$, $p=0.006$), VLPFC ($r=0.741$, $p=0.001$), OFC ($r=0.706$, $p=0.002$) and ACC ($r=0.694$, $p=0.003$). Figure 3 illustrates the correlation for VLPFC BP_{ND} .

Separating the patients into medicated and unmedicated subgroups, correlations between regional BP_{ND} and PANSS negative score were significant in the medicated patient group (e.g. for mean BP_{ND} across the 5 ROIs: $r=0.819$, $p=0.013$), but not the unmedicated patient group (mean BP_{ND} : $r=0.290$, $p=0.486$), suggesting that the correlation in the schizophrenia group as a whole (Figure 3) is predominantly due to the medicated patients. There were no significant correlations between BP_{ND} in any of the ROIs and duration of illness (whether in the medicated group, the antipsychotic-free group, or all patients together) (Supplementary Material Section B2) or any of the peripheral inflammatory markers. Nor did we find any differences in concentration of peripheral inflammatory markers between the two patient subgroups.

Discussion

This is the first PET study, to our knowledge, to investigate microglial activation in antipsychotic-free (predominantly antipsychotic-naïve) patients with schizophrenia and to compare the findings between antipsychotic-free and medicated patients. We found no evidence for increased TSPO availability in antipsychotic-free patients in the 2-6 year period following diagnosis. However, TSPO availability was significantly elevated in frontal and parietal cortical regions in patients taking risperidone or paliperidone LAI, providing evidence for neuroinflammation and associated microglial activation in this group. In the patients there was also a strong correlation between TSPO availability and PANSS negative score across these brain regions.

A strength of our study is that the majority of the unmedicated patients were antipsychotic-naïve and the two previously medicated patients had been antipsychotic-free for one and five years respectively. Previous PET studies of microglial activation in schizophrenia¹⁷⁻²² have all been in medicated patients which may potentially confound their interpretation. Our finding suggests that schizophrenia is not associated with microglial activation, at least not in the relatively early stages. Nevertheless, PET evidence has recently been reported for microglial activation in antipsychotic-naïve people at ultra-high risk for psychosis¹⁹, a proportion of whom can be expected to develop schizophrenia over the following few years. Indeed, the one patient who had transitioned to schizophrenia by the time of report had the highest TSPO availability in the cohort. The method for determining TSPO availability in this study has been challenged⁴⁶, but if the interpretation is valid the apparent discrepancy with our findings prompts the conjecture that microglial activation may be a dynamic process along the timeline of the prodrome, early and established phases of schizophrenia, and that microglial activation in the prodrome may no longer be apparent in the early stages of schizophrenia. However, it emphasises the need for both studies to be independently replicated before drawing firm conclusions.

A further strength is that medication was standardised in the medicated patient group and variability in compliance was minimised by intramuscular LAI. Our finding of significantly increased TSPO availability in this group is consistent with several¹⁷⁻¹⁹, but not all²⁰⁻²², of the previous PET studies,

and the homogeneous nature of the increase seen across cortical regions is also consistent with the global increase in microglial activation seen in rats treated chronically (8 weeks) with clinically-relevant doses of antipsychotics²⁶. Although an earlier study in rats did not find increased cortical TSPO binding following chronic risperidone treatment²⁵, clinically comparable antipsychotic doses were not used⁴⁷ and our study supports such an increase at clinical doses. We should be cautious about extrapolating uncritically to other drugs; nor can we exclude the possibility that the intramuscular route of administration contributed to our findings until a comparison is made with oral administration. Patients in the other PET studies were taking a diverse range of first and second generation antipsychotics, and it may be hypothesised that the variability in the PET studies is at least in part due to variability in the effects on microglia of different antipsychotics or doses. Further variability may come from differences in radiotracer characteristics and clinical severity, and it will be important for future studies to investigate other antipsychotics while controlling for all these variables. Additionally, Figure 1 shows extensive overlap between the medicated patients and the other groups but also several medicated patients with BP_{ND} values well above the normal range, suggesting that inflammation may only be evident in a subgroup of patients with schizophrenia, an idea supported by post-mortem data¹⁵. The only conspicuous clinical characteristic of these patients was that they had the highest scores on the PANSS negative scale (see Figure 3 and further discussion below) and there were no other obvious clinical associations with high BP_{ND} that we could identify (see Supplementary Materials Section B3).

TSPO are expressed predominantly⁴⁸, but not exclusively^{48,49}, on microglia and increased TSPO availability is interpreted to indicate the presence of activated microglia. However, activated microglia have a range of pro- and anti-inflammatory chemical phenotypes including classical M1 (cytotoxic), M2a (repair and regeneration) and M2b/c (immunomodulatory)²⁷. The majority of studies specifically investigating risperidone suggest it is anti-inflammatory⁵⁰⁻⁵³, though neutral²⁵ and pro-inflammatory⁵⁴ effects have also been reported. Current PET TSPO radioligands are unable to differentiate between these activation states and we are unable to determine whether the increased TSPO availability seen in the medicated patients in our study represents a pro- or anti-inflammatory

state. It is now appreciated that at least a proportion of the longitudinal GM volume decreases in schizophrenia may be associated with higher cumulative exposure to antipsychotics over time⁵⁵. This is consistent with post-mortem data in non-human primates⁵⁶ and in vivo MRI studies in rats⁵⁷⁻⁵⁹ reporting brain volume reductions following chronic exposure to clinically relevant doses of antipsychotics, most prominently in frontal^{56,57} and parietal⁵⁶ cortices as well as ACC⁵⁹ where evidence for microglial activation was also found. This cautions against an assumption that our finding of increased TSPO in these areas in the medicated patients is functionally benign. In this regard, the strong correlation between severity of negative symptoms and TSPO availability in the patients is predominantly due to an association in the medicated subgroup which is not seen in the unmedicated patients. Identification of the chemical phenotype of antipsychotic-associated microglial activation should be a priority for future studies. In a preliminary post hoc analysis of BP_{ND} in the striatum, a critical site of therapeutic action of antipsychotics^{60,61}, TSPO availability was increased in the medicated patient group to only a small and non-significant extent. Although limited to a single subcortical site, these data tentatively suggest that effects of antipsychotic medication on TSPO may be more prominent in cortical than subcortical regions (see Supplementary Materials Section B4 for further details).

While the increased BP_{ND} in the medicated group may be a direct effect of medication, alternative explanations for this finding in our study are possible. In order to remove symptom severity as a confounding factor, both patient groups were similarly symptomatic. The medicated patients were therefore psychotic despite treatment and may be treatment-resistant. We therefore cannot exclude the possibility that the increased TSPO availability in the medicated group is related to a difference in schizophrenia pathophysiology between the groups rather than medication. Of potential relevance, a higher baseline inflammatory state has recently been shown to be predictive of worse clinical outcomes and poorer response to antipsychotics⁶², although in our schizophrenia cohort concentrations of peripheral inflammatory markers were not higher in the medicated group. Also, the medicated patients had a longer duration of illness than the antipsychotic-free patients, and we are unable to exclude this as a possible cause of their increased TSPO availability. However, BP_{ND} was

not correlated with duration of illness, potentially making medication or treatment-resistance more likely explanations.

Our study has several other limitations. In the absence of prior data on medication-free patients in the literature, no formal a priori power calculation was performed and group sizes (n=16) were chosen in line with similar PET studies. However, the small size of our schizophrenia subgroups limits the power to identify small between-group differences in TSPO availability, and it is likely that our finding of only trend significant difference between them is a Type II error. Nevertheless, Type II error is unlikely to explain the lack of difference between the antipsychotic-free patients and healthy controls given the extremely small difference in BP_{ND} .

We are unable in our medicated patients to distinguish between primary negative symptoms and so-called 'pseudo-negative' symptoms secondary to antipsychotic side effects and untreated positive symptoms. Our data therefore cannot be used to support a link between activated microglia and whatever pathophysiology underlies primary negative symptoms. In separate clinical disorders minocycline has been shown to attenuate brain microglial activation⁶³ and to reduce negative symptoms of schizophrenia in particular³⁻⁵, so the association is plausible. A future TSPO PET study could investigate this in a large group of similarly medicated patients stratified by high and low PANSS negative score.

We were unable to accurately quantify TSPO availability in temporal cortex with our methodology. This is an important site of longitudinal grey matter loss in schizophrenia and changes in superior temporal gyrus volume have been related to positive symptom severity²⁹.

Although we attempted to control medication as much as possible, some patients were taking other medications including antidepressants (see Supplementary Materials Section B3). Antidepressants have anti-inflammatory effects on microglia in vitro⁶⁴, although this is unlikely to have confounded our conclusions as a similar proportion of patients in both groups were taking an antidepressant (antipsychotic-free n=3; medicated n=4). Where it could be determined, the reasons included relatively mild subjective low mood and as an augmentation strategy for the treatment of negative

symptoms⁶⁵. Major depressive disorder, in which microglial activation has been reported⁶⁶, was an exclusion factor in our study. One patient in the medicated group was taking diazepam, which is a ligand at the TSPO. Extrapolation from in vitro data suggests that high doses of diazepam (>30mg daily) may reduce TSPO availability by ~9% in human PET studies⁶⁷. This patient's dose was 5mg daily, wasn't taken every day, and was omitted for 24 hours before PET scanning, making an appreciable effect unlikely.

With regard to the lack of homogeneity of variances between the groups, ANOVA assumes that the means of the groups are the same (null hypothesis) and the variances within the groups are equal. Using these assumptions a p value of <0.05 was obtained meaning that one of these assumptions is invalid. Failure of either of these assumptions still demonstrates differences between the groups. Looking at the data in Figure 1, it may well be the case that there is an increase in both the mean value and variance of the medicated group, for instance through inter-individual variation in the presence or extent of an inflammatory state. Nevertheless, the statistical test is valid in demonstrating that this distribution is not by chance, and is supported by the significant elevations found by t-test in the majority of the individual ROIs in the medicated group compared to controls.

As TSPO expression is ubiquitous throughout the brain there is no ideal reference region for PET studies with TSPO radioligands. However, a standard alternative is to use a pseudo-reference region and our methodology used the cerebellum, supported by studies showing its superiority over alternative data-driven approaches^{34, 68} and consistent with our previous [¹¹C](R)-PK11195 studies on the HRRT^{34, 69-71}. We found no evidence in our data for a significant difference in cerebellar TSPO availability between the schizophrenic patients and controls, which is consistent with a similar finding in the PET study by Bloomfield et al¹⁹, and further validates the use of cerebellar GM in schizophrenia (for further details, see Supplementary Material Section A3).

In conclusion, our data in antipsychotic-free patients with schizophrenia suggest for the first time that significant microglial activation is not present in the early years of the disorder. In contrast, evidence for microglial activation is present in patients medicated with risperidone or paliperidone LAI and correlates with the severity of PANSS negative score. While this may be a direct effect of the

medication, our study cannot exclude treatment-resistance and/or longer duration of illness as contributory or alternative explanations, and it will be important for future studies to clarify these issues. It will also be important to investigate whether microglial activation changes longitudinally in schizophrenia unrelated to medication; the pro- or anti-inflammatory phenotype of the microglial activation seen in our medicated patients and how that may differ across antipsychotic drugs; and any association between microglial activation and primary negative symptoms, offering a target for a symptom domain that is largely untreated by current antipsychotics.

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Conflict of Interest

The authors declare no conflict of interest.

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Figure legends

Figure 1: Regional [^{11}C](*R*)-PK11195 BP_{ND} in antipsychotic-free patients with schizophrenia, patients with schizophrenia treated by risperidone or paliperidone long acting injection (LAI), and healthy controls. Horizontal bars indicate means. Open circles represent controls (n=16), closed circles represent antipsychotic-free patients (n=8) and triangles represent medicated patients (n=8).

Figure 2: [^{11}C](*R*)-PK11195 binding in antipsychotic-free and medicated patients with schizophrenia compared to healthy controls. (A) Mean cortical BP_{ND} compared between groups, showing statistically significant elevation in medicated patients compared to controls, no difference between antipsychotic-free patients and controls, and trend difference between the two patient groups; (B) BP_{ND} in individual ROIs compared between groups. * indicates a significant elevation in medicated patients compared to controls at $p < 0.05$; # indicates significant elevation in medicated patients compared to antipsychotic-free patients at $p < 0.05$.

Figure 3: Correlation between [^{11}C](*R*)-PK11195 BP_{ND} and scores on the negative subscale of the PANSS in the VLPFC across antipsychotic-free (circles) and medicated (triangles) patients with schizophrenia.

Tables

Table 1: participant characteristics

Characteristic	All subjects		<i>p</i> -value	Schizophrenia patients		<i>p</i> -value
	Patients (n=16)	Healthy controls (n=16)		Antipsychotic -free (n=8)	Medicated (n=8)	
Gender (M:F)	11:5	11:5	1.00	4:4	7:1	0.28
Age (yr)	33 ± 9	33 ± 10	0.67	27 ± 7	38 ± 8	0.01
BMI (kg/m ²)	28 ± 6	24 ± 4	0.02	27 ± 6	30 ± 4	0.20
Smoking status (Yes:No)	11:5	0:16	<0.0001	5:3	6:2	1.00
Age at onset of psychosis (yr)	23 ± 5	-	-	24 ± 6	23 ± 5	0.90
Duration of illness (yr)	9 ± 7	-	-	4 ± 2 (range 2-6)	15 ± 7 (range 2-22)	<0.001
PANSS total	85 ± 9	-	-	86 ± 9	85 ± 10	0.94
PANSS positive	21 ± 4	-	-	23 ± 3	20 ± 4	0.08
PANSS negative	20 ± 5	-	-	18 ± 4	22 ± 5	0.08
PANSS general	44 ± 5	-	-	45 ± 5	44 ± 4	0.51
Injected mass of PK11195 (µg)	1.9 ± 0.8	2.1 ± 1.8	0.88	2.3 ± 0.7	1.6 ± 0.8	0.09

Values presented as mean ± SD

Table 2: ANOVA of regional BP_{ND} for antipsychotic-free and antipsychotic-treated patients with schizophrenia compared with healthy controls

Region	Healthy controls (n=16)	Antipsychotic-free patients (n=8)	Medicated patients (n=8)	Antipsychotic-free patients vs controls		Medicated patients vs controls		Medicated vs antipsychotic-free patients	
				Difference (%)	Signif (p) ^a	Difference (%)	Signif (p) ^a	Difference (%)	Signif (p) ^a
DLPFC	0.065 (0.051)	0.069 (0.076)	0.133 (0.087)	6%	0.453	105%	0.035*	95%	0.068
VLPCF	0.128 (0.038)	0.131 (0.063)	0.211 (0.111)	3%	0.443	66%	0.036*	61%	0.051
OFC	0.078 (0.057)	0.069 (0.052)	0.155 (0.117)	-12%	0.350	103%	0.056	125%	0.043*
ACC	0.098 (0.061)	0.133 (0.079)	0.194 (0.126)	37%	0.142	99%	0.035*	46%	0.133
Parietal	0.066 (0.025)	0.059 (0.065)	0.109 (0.085)	-11%	0.380	65%	0.100	86%	0.102
Mean	0.087 (0.053)	0.092 (0.072)	0.161 (0.108)	4%	0.981^b	88%	0.032^b	83%	0.097^b

Values presented as mean (SD). ^afor individual ROIs the significance is derived by t-test (1-tailed) for independent samples with unequal variance;

^bsignificance derived by pairwise comparison of significant main effect of group from factorial repeated measures ANOVA, with correction for multiple comparisons by Tukey's HSD. * significant at p≤0.05. DLPFC: dorsolateral prefrontal cortex; VLPCF: ventrolateral prefrontal cortex; OFC: orbitofrontal cortex; ACC: anterior cingulate cortex.

Figures

Figure 1

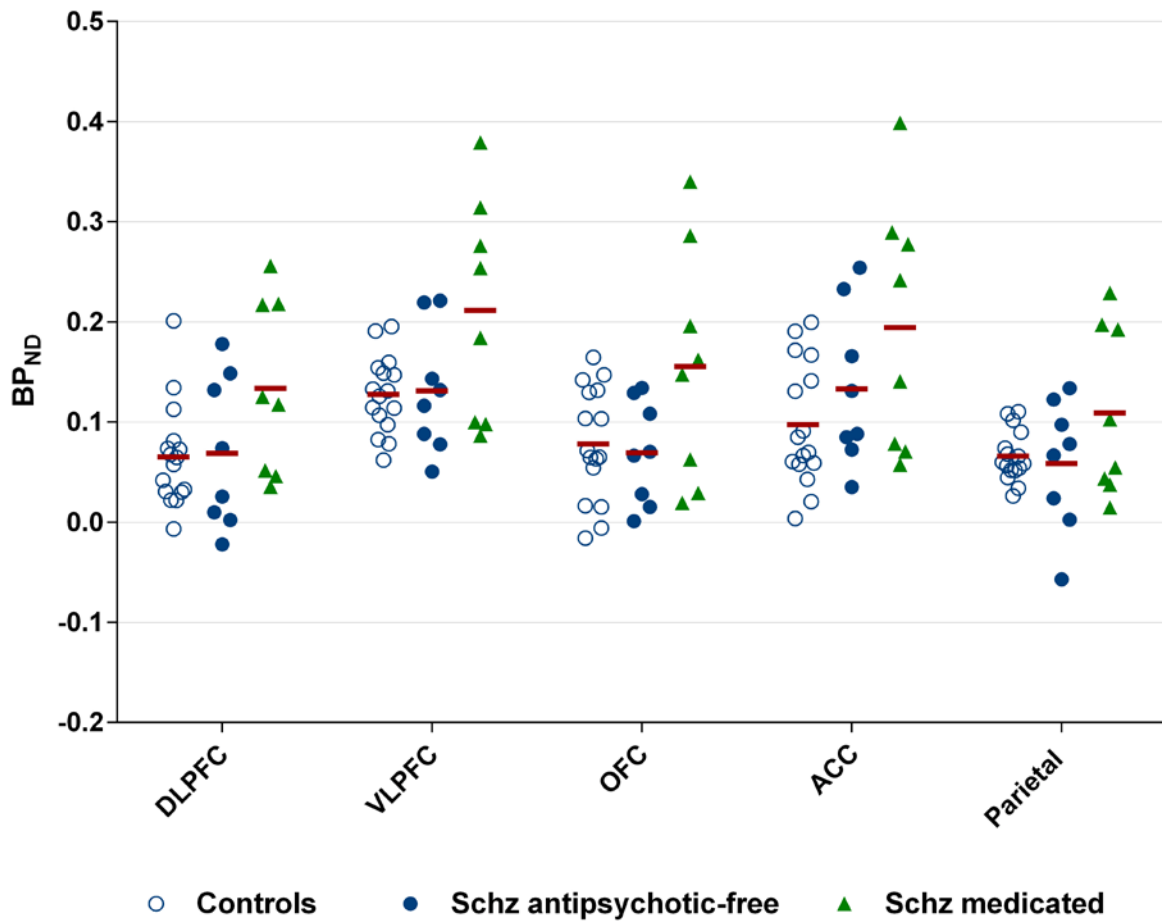


Figure 2

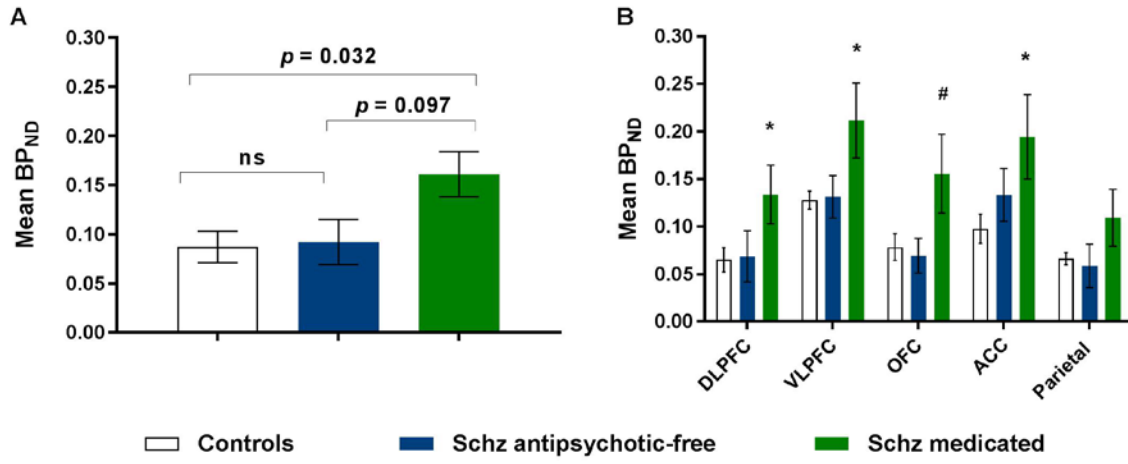


Figure 3

