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# Neuroinflammation in bipolar disorder – A [$^{11}$C]-(*R*)-PK11195 positron emission tomography study

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ABSTRACT

*Background:* The "monocyte-T-cell theory of mood disorders" regards neuroinflammation, i.e. marked activation of microglia, as a driving force in bipolar disorder. Microglia activation can be visualized *in vivo* using [$^{11}$C]-(*R*)-PK11195 PET. Indirect evidence suggests the hippocampus as a potential focus of neuroinflammation in bipolar disorder. We aim to determine if there is increased [$^{11}$C]-(*R*)-PK11195 binding to activated microglia in the hippocampus of patients with bipolar I disorder when compared to healthy controls.

*Material and methods:* Fourteen patients with bipolar I disorder and eleven healthy controls were included in the analyses. Dynamic 60-min PET scans were acquired after the injection of [$^{11}$C]-(*R*)-PK11195. All subjects underwent psychiatric interviews as well as an MRI scan, which was used for anatomic co-registration in the data analysis. The data from the PET scans was analyzed with a two-tissue-compartment model to calculate the binding potential, using the metabolite-corrected plasma and blood curve as input.

*Results:* A significantly increased [$^{11}$C]-(*R*)-PK11195 binding potential, which is indicative of neuroinflammation, was found in the right hippocampus of the patients when compared to the healthy controls (1.66 (CI 1.45–1.91) versus 1.33 (CI 1.16–1.53); $p = 0.033$, respectively). Although the same trend was observed in the left hippocampus, this difference was not statistically significant.

*Conclusion:* This study is the first to demonstrate the presence of focal neuroinflammation in the right hippocampus in bipolar I disorder.



## 1. Introduction

The pathophysiology of bipolar disorder (BD) is complex and its neurobiology remains largely unknown (Schulze, 2010). Both the stress system and the immune system interact with the brain and are influenced by the environment. Their interactions can be regarded as possible linking pins. The "monocyte-T-cell theory of mood disorders" (Maes et al., 1995) considers an activated inflammatory response system (IRS) in mood disorders to be the driving force behind these illnesses. IRS activation can be regarded as a disbalance in immune regulatory processes. In BD this theory is supported by altered concentrations of immune related peripheral bio-assays, e.g. elevated serum or plasma levels of pro-inflammatory cytokines, aberrant expression of pro-inflammatory genes in circulating monocytes (Padmos et al., 2008), alterations in the kynurenine pathway (Myint et al., 2007) and a modulating effect of several psychopharmaceuticals on the immune system (Drzyzga et al., 2006; Haarman et al., 2013; Padmos et al., 2008; Pollmächer et al., 2000; Rybakowski, 2000).

Activation of the IRS is thought to correspond to neuroinflammation, which is reflected by an increase in activated microglia, the resident macrophages of the brain (Beumer et al., 2012). Indirect evidence gathered from post-mortem studies, corticosteroid

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treatment related hippocampal changes and animal models suggest the hippocampus as a potential focus of neuroinflammation in BD. Post-mortem studies in humans demonstrated an increased expression of inflammation related pro-apoptosis genes (Benes et al., 2006) and oxidative damage (Che et al., 2010) to the RNA in the hippocampus of BD patients as well as a decrease in growth-associated protein (Tian et al., 2007) that has been proposed to be related to neuroinflammation (Kato et al., 2003). In addition medical treatment with corticosteroids, known for their anti-inflammatory effects and associated with not only depressive symptoms but also manic symptoms, is related to both functional and morphological changes in the hippocampus (Brown, 2009). Furthermore, multiple rodent studies demonstrate a relationship between a disturbed microglia function in the hippocampus and other pathophysiological changes which are thought to relate to neuropsychiatric disorders(Beumer et al., 2012; Costello et al., 2011; Kreisel et al., 2013; Macchi et al., 2013; Roumier et al., 2008, 2004). In a recent rodent study stress-induction led to dynamic microglia changes in the hippocampus alone, which were associated with depressive-like behavior (Kreisel et al., 2013). Another study demonstrated an increased cytokine response to lipopolysaccharide challenge in the hippocampus of *SERT* mutant rats (Macchi et al., 2013).

Microglia activation can be visualized *in vivo* with the radiopharmaceutical [$^{11}$C]-(*R*)-PK11195 by means of positron emission tomography (PET). This radiopharmaceutical binds to the translocator protein (TSPO), a receptor that is upregulated in the mitochondria of activated microglia cells (Doorduin et al., 2008). [$^{11}$C]-(*R*)-PK11195 has been utilized successfully in models of central inflammation, such as following an injection of the endotoxin lipopolysaccharide in animal models (Choi et al., 2011; Dickens et al., 2014; Dobos et al., 2012) and following infections of the central nervous system in humans (Garvey et al., 2014; Grover et al., 2012; Wiley et al., 2006). In various psychiatric and neurodegenerative disorders [$^{11}$C]-(*R*)-PK11195 PET has proven to be a useful tool for imaging neuroinflammation (Banati, 2002; Doorduin et al., 2009; Folkersma et al., 2011; van Berckel et al., 2008). Using this radiopharmaceutical Doorduin et al. demonstrated the hippocampus to be the primary focus of neuroinflammation in schizophrenia-related psychosis (Doorduin et al., 2009).

New radiopharmaceuticals such as [$^{11}$C]-PBR28 have been developed that are potentially more sensitive for imaging of neuroinflammation. However, these revealed substantial heterogeneity in binding affinity due to polymorphisms in the TSPO, resulting in so-called high-, median- and low-affinity binders, complicating data interpretation (Kreisl et al., 2013). Since [$^{11}$C]-(*R*)-PK11195 binding is not affected by polymorphisms we have selected this radiopharmaceutical for our study.

In the current study we aim to determine if there is an increased [$^{11}$C]-(*R*)-PK11195 binding to activated microglia in BD patients in comparison to a healthy control group. We *a priori* hypothesized the hippocampus to be the main focus of neuroinflammation in BD. In a second model we explored the presence of neuroinflammation in other brain regions. In addition, we examined whether clinical characteristics would be associated with neuroinflammation.

## 2. Materials and methods

### 2.1. Participants

For the present cross-sectional case-control study we included 15 preferably euthymic patients with bipolar I disorder (BD-I) and a group of 12 controls demographically similar in age and sex that participated in the MOODINFLAME study. MOODINFLAME is a large-scale European medical scientific project aiming to advance early diagnosis, treatment and prevention of mood disorders targeting the activated inflammatory response system. The patients for the present study were recruited from an outpatient clinic for bipolar disorder. The healthy controls were recruited via advertisements, recruitment posters and by contacting healthy controls from previous studies that gave their consent to be asked for future studies. For the MOODINFLAME study adult male and female subjects were included who were free of inflammation related symptoms, including fever and infectious or inflammatory disease. Furthermore, they were free of uncontrolled systemic disease, uncontrolled metabolic disease or other significant uncontrolled somatic disorder known to affect mood. They did not use somatic medication known to affect mood or the immune system, such as corticosteroids, non-steroid anti-inflammatory drugs and statins. Female candidates who were pregnant or recently gave birth were excluded. Patients and controls were free of benzodiazepines at least in the last week prior to the PET-scan. They were also free of anticoagulant use or presence of coagulation disease, did not suffer from palmar arc artery insufficiency, did not participate in a prior research study involving radiation less than a year ago, and did not have any contraindication for MRI scanning.

Patients were allowed to continue their regular psychopharmaceutical treatment. They were neither in a depressed nor (hypo-)manic episode at the time of scanning as indicated by an Inventory of Depressive Symptoms – Clinician Version (IDS-$C_{30}$) score <22 and Young Mania Rating Scale (YMRS) score <12, respectively. Patients with any other current primary major psychiatric diagnosis were excluded including: schizophrenia, schizoaffective disorder, anxiety disorder and substance use disorders. Healthy controls did not have any current or lifetime psychiatric diagnosis.

Nine patients were excluded due to protocol violations (claustrophobia (3), presence of ferromagnetic objects (2), palmar arc artery insufficiency (1), coagulation disorder (1), use of benzodiazepines (1) and pregnancy (1)). Four healthy controls were excluded due to protocol violations (claustrophobia (2) and presence of ferromagnetic objects (2)). After completing inclusion one participant admitted to having used a benzodiazepine on the evening prior to the PET-scan. In another participant experienced technical difficulties with the automatic blood sampling system prohibited valid determination of the input function. These two subjects were removed from the subsequent analyses.

### 2.2. Ethical considerations

The Medical Ethical Review Committee of the University Medical Center Groningen approved the protocol, which was performed in accordance with the Helsinki Declaration of 1975. Written informed consent was obtained from all participants.

### 2.3. Assessment

All subjects underwent a Mini-International Neuropsychiatric Interview 5.0.0 (MINI) to confirm the bipolar I disorder diagnosis in the patient group and the absence of psychiatric disorders in the healthy control group (Sheehan et al., 1998). Clinical features were extracted from the interviews held according to the MOODINFLAME protocol. This included the Patient Questionnaire of the former Stanley Foundation Bipolar Network, the YMRS, the IDS-$C_{30}$ and a somatic illness questionnaire.

The Patient Questionnaire includes separate clinician and patient chapters covering a spectrum of clinical features including vocational, educational and economic status, onset and course of illness, family history, past treatment, cycling and seasonal patterns, medical problems, medications, ability to function and symptomatic status, precipitants of illness (e.g. substance use), treatment adherence and insight into the illness (Leverich et al.,

2001). In the event of a mismatch of results from the MINI in relation to the Patient Questionnaire, diagnoses were checked with the treating physician. The YMRS is an eleven-item, multiple-choice questionnaire to assess manic symptoms (Young et al., 1978). The IDS-$C_{30}$ is a thirty-item, multiple-choice questionnaire to assess depressive symptoms of all symptom domains of depression (Rush et al., 1986). The YMRS and IDS-$C_{30}$ were assessed shortly before the scans and used in the relevant analyses. The somatic illness questionnaire is a MOODINFLAME specific checklist exploring all the organ systems for current and lifetime medical symptoms.

### 2.4. Radiochemistry

[$^{11}$C]-(*R*)-PK11195 was labeled as described previously (Doorduin et al., 2009). [$^{11}$C]-(*R*)-PK11195 was obtained in 32 ± 18% radiochemical yield ($n$ = 27). The quality control was performed by HPLC, using a Novapak C18 column (150 × 3.9 mm) with acetonitrile/25 mM $NaH_2PO_4$ (pH 3.5) (60/40) as the eluent at a flow of 1 ml/min. The radiochemical purity was always >95% and the specific activity was 111 ± 130 GBq/μmol. No differences were found between healthy volunteers and patients for the injected dose (390 ± 18 vs. 355 ± 63 MBq, $p$ = 0.088) and injected mass (0.75 ± 0.58 vs. 0.67 ± 0.40 mg/L, $p$ = 0.696).

### 2.5. Positron emission tomography

For arterial blood sampling a catheter was inserted in the radial artery after testing for collateral circulation with the Allen test and injection of 1% lidocaine (Fresenius Kabi Nederland BV, 's Hertogenbosch, The Netherlands) for local anesthesia. In the other arm, a venous catheter was inserted in the antebrachial vein for injection of [$^{11}$C]-(*R*)-PK11195. Positron emission tomography imaging was performed with the ECAT EXACT HR+ camera (Siemens, Knoxville, Tenessee). Head movement was minimized with a head-restraining adhesive band and a neuroshield was used to minimize the interference of radiation from the subject's body. A 60-min emission scan in 3D-mode was performed, starting simultaneously with the intravenous injection of [$^{11}$C]-(*R*)-PK11195. The tracer was injected at a speed of 0.5 ml/s (total volume of 8.3 ml).

After radiotracer injection, arterial blood radioactivity was continuously monitored with an automated blood sampling system (Veenstra Instruments, Joure, The Netherlands). Five extra blood samples were collected at 10, 20, 30, 45 and 60 min after [$^{11}$C]-(*R*)-PK11195 injection to determine the amount of radioactivity in blood and plasma to calibrate the sampling system. The arterial blood samples that were collected at 20, 45 and 60 min after [$^{11}$C]-(*R*)-PK11195 injection were also used for metabolite analysis. The metabolite analysis was performed as described previously (Doorduin et al., 2009).

### 2.6. Magnetic resonance imaging

Axial T1 (gradient echo T1 3D, slice thickness 1.2 mm isotropic) and T2 Flair (3 mm) weighed images were acquired using a 3T MRI scanner and an eight-channel head coil (3T Intera, Philips, Best, The Netherlands). The T1 images provided the input for the normalization of the PET scans to standard brain morphology of all subjects. Both the MRI scan and the PET scan were preferably made on the same day, and no more than 1 week apart.

### 2.7. Image analysis

Attenuation correction was performed with the separate ellipse algorithm. Images were reconstructed by filtered back projection in 21 successive frames of increasing duration (6 × 10 s, 2 × 30 s, 3 × 1 min, 2 × 2 min, 2 × 3 min, 3 × 5 min, 3 × 10 min). MRI images were co-registered to the sum of all frames of the PET scan, resulting in the most optimal co-registration, using statistical parametric mapping (SPM8; Welcome Trust Center Neuroimaging, University College London, UK). Grey matter regions of interest (ROIs) were defined by the co-registered MRI images using a probability map that was based on automatic delineation of ROIs with the PVElab software (Svarer et al., 2005). The ROIs were transferred to the dynamic PET images and time-activity curves were calculated. In total 15 ROIs were included: left and right hippocampus, left and right frontal cortex, left and right dorsolateral prefrontal cortex (PFC), left and right temporal cortex, left and right parietal cortex, bilateral occipital cortex, bilateral anterior cingulate, bilateral posterior cingulate, bilateral cerebellum and basal ganglia.

The time–activity curves of all ROIs were used for kinetic modeling with software developed in Matlab 7.1 (Mathworks, Natick, Massachusets). The individual delay was corrected for the delay in radioactivity measurements in blood, as a result of the distance between the subject and the automated blood sampling system. A two-tissue compartment model was used to calculate the $k_1$–$k_4$ with the metabolite corrected plasma and blood curve as an input function, correcting for the individual delay and a free blood volume. The binding potential was defined as $k_3/k_4$ and was calculated for each ROI individually.

### 2.8. Statistical analysis

Statistical analyses were performed using Stata Statistical Software, release 11 (StataCorp. 2009, College Station, TX).

The differences in demographic data between the groups were investigated with Student's t-test (age), Pearson's chi-squared test (gender) and Kruskal–Wallis equality-of-populations rank test (IDS-$C_{30}$ score).

Student's *t*-test was used to determine differences in whole-brain grey matter binding potential between the patient and healthy control group.

Statistical analyses of the binding potentials in the examined brain regions were performed using two general linear models. The first model investigated the hypothesis that the hippocampi are the focus of neuroinflammation, incorporating the binding potentials of the left and right hippocampus as dependent variables. In the second model, exploring the other brain regions, binding potentials of all investigated brain regions were added as dependent variables. In both models the whole-brain grey matter binding potential was used as a covariate to normalize for individual global cerebral [$^{11}$C]-(*R*)-PK11195 uptake variations. Beforehand inverse square root transformation was applied to the binding potentials to meet the normality assumption in the general linear models.

Results of the general linear models are presented as back-transformed means of the individual ROIs with 95% confidence intervals (CI). The level of significance was defined as 0.05, two-sided, in the first (hypotheses driven) model. To correct for multiple comparisons in the second (explorative) model false discovery rate (FDR < 0.1) correction was applied to the results, as described by Hochberg and Benjamini (1990).

In order to rule out possible epilepsy associated inflammation effects (Aronica and Crino, 2011) *post hoc* analyses were performed excluding the patient with this comorbidity. Furthermore, to increase the homogeneity of the sample additional *post hoc* analyses were performed excluding the patient with mild depressive symptoms and the medication free patient.

Correlations between the binding potentials and the IDS-$C_{30}$ score, YMRS score, age at onset, number of episodes and duration of illness were assessed with Spearman's rho.

**Table 1**
Subject characteristics.

| No | Sex | Age | Age at onset | No episodes (depressed/(hypo)manic) | IDS-$C_{30}$ score | YMRS score | Medical comorbidities[a] | Medication (time since last medication switch (months)) |
|---|---|---|---|---|---|---|---|---|
| *Patients* | | | | | | | | |
| 1 | F | 52 | 14 | (>5/>11) | 14 | 0 | – | Valproate, Thyroxine, Trazodone, Lamotrigine, Quetiapine (11) |
| 2 | F | 53 | 18 | (>11/>5) | 2 | 0 | DH; Hypothyroidism | Lithium, Valproate, Thyroxine, Omeprazol (32) |
| 3 | M | 61 | 25 | (>5/3) | 0 | 0 | – | Lithium, Lamotrigine (63) |
| 4 | F | 36 | 17 | (>11/>11) | 1 | 0 | Hypothyroidism | Lithium, Thyroxine, COCP (5) |
| 5 | F | 41 | 16 | (>5/>5) | 2 | 0 | – | Trazodone (43) |
| 6 | F | 49 | 17 | (>20/>20) | 3 | 0 | – | Carbamazepine, Citalopram (14) |
| 7 | M | 37 | 12 | (>20/>20) | 3 | 0 | – | Lithium, Lamotrigine (25) |
| 8 | M | 58 | 21 | (>5/3) | 5 | 0 | – | Valproate, Quetiapine (11) |
| 9 | M | 55 | 43 | (>11/2) | 4 | 0 | – | Lithium, Trazodone (13) |
| 10 | F | 50 | 15 | (4/4) | 11 | 0 | – | Valproate (30) |
| 11 | M | 55 | 18 | (>20/>20) | 2 | 0 | – | Lithium, Quetiapine (4) |
| 12 | M | 24 | 22 | (0/1) | 0 | 0 | – | – (21) |
| 13 | M | 40 | 30 | (1/2) | 5 | 0 | Epilepsy | Valproate, Levetiracetam (10) |
| 14 | F | 36 | 21 | (1/>11) | 6 | 0 | – | Lithium (10) |
| *Healthy controls* | | | | | | | | |
| 1 | F | 45 | | | 0 | 0 | | – |
| 2 | F | 56 | | | 2 | 0 | – | – |
| 3 | F | 64 | | | 0 | 0 | – | – |
| 4 | M | 66 | | | 0 | 0 | – | – |
| 5 | F | 56 | | | 0 | 0 | – | – |
| 6 | F | 21 | | | 0 | 0 | – | – |
| 7 | F | 50 | | | 0 | 0 | – | – |
| 8 | F | 25 | | | 3 | 0 | – | – |
| 9 | M | 21 | | | 2 | 0 | – | – |
| 10 | M | 26 | | | 12 | 0 | – | – |
| 11 | M | 22 | | | 4 | 0 | – | – |

Overview of the characteristics of the BD-I patients and healthy controls.
[a] Current medical comorbidities requiring medical care. IDS-$C_{30}$ – Inventory of Depressive Symptoms Clinician version; YMRS – Young Mania Rating Scale; DH – diaphragmatic hernia; COCP – combined oral contraceptive pill.

## 3. Results

### 3.1. Demographics

Subject characteristics are displayed in Table 1. While all patients but one were euthymic (IDS-$C_{30}$ score <12), a statistical difference in the IDS-$C_{30}$ score between the patient and healthy control groups was observed ($H = 4.676$; $p = 0.031$). Differences between the groups in gender or age were not statistically significant.

### 3.2. [$^{11}$C]-(R)-pk11195 PET

There was no statistical difference in the whole-brain grey matter binding potential of [$^{11}$C]-(*R*)-PK11195 in patients versus controls (1.28 (CI 1.07–1.50) versus 1.35 (CI 1.10–1.60); $p = 0.70$).

The hypothesis driven general linear model with the left and right hippocampi binding potentials as the dependent variables demonstrated a significant increased [$^{11}$C]-(*R*)-PK11195 binding potential in the right hippocampus of the patients when compared to the healthy controls (1.66 (CI 1.45–1.91) versus 1.33 (CI 1.16–1.53); $p = 0.033$; Fig. 1). The difference between the [$^{11}$C]-(*R*)-PK11195 binding potential of the left hippocampus of BD-I patients compared to the healthy controls was not statistically significant (1.55 (CI 1.30–1.90) versus 1.20 (CI 1.00–1.46); $p = 0.071$).

The subsequent explorative general linear model adding all the investigated brain regions revealed a lower [$^{11}$C]-(*R*)-PK11195 binding potential in the left dorsolateral PFC of the BD-I patients when compared to healthy controls (1.18 (CI 1.09–1.27) versus 1.40 (CI 1.28–1.53); $p = 0.009$). However this difference did not survive correction for the false discovery rate (Table 2).

*Post hoc analyses* that were performed excluding the patient with epilepsy demonstrated an increased [$^{11}$C]-(*R*)-PK11195 binding potential in both the right hippocampus (1.69 (CI 1.47–1.96) versus 1.33 (CI 1.17–1.54); $p = 0.029$) as well as the left hippocampus (1.63 (CI 1.35–2.00) versus 1.21 (CI 1.02–1.47); $p = 0.042$) of the BD-I patients when compared to healthy controls. In the explorative model the results were comparable to the original analyses in effect size and statistical significance.

*In post hoc* analyses excluding the patient with mild depressive symptoms and the medication free patient the results were comparable to the original analyses in effect size and statistical significance in both models.

### 3.3. Association with clinical features

Correlations between the hippocampi binding potentials and the total IDS-$C_{30}$ score or individual items were not statistically significant in both the patient and healthy control group. All patients and healthy controls scored 0 on the YMRS. Therefore, correlations could not be calculated with manic symptoms.

The correlations between the hippocampal binding potentials and the illness progression characteristics (number of depressive or manic episodes, total number of mood episodes) were also not statistically significant.

## 4. Discussion

To our knowledge this is the first study to reveal actual neuroinflammation *in vivo* in BD. We partly confirmed our a-priori hypothesis, demonstrating a statistically significant increased binding potential of [$^{11}$C]-(*R*)-PK11195 in the right hippocampus of BD-I patients as compared to healthy controls. The left hippocampus [$^{11}$C]-(*R*)-PK11195 binding potential showed the same trend as the right hippocampus, with a comparable increase in

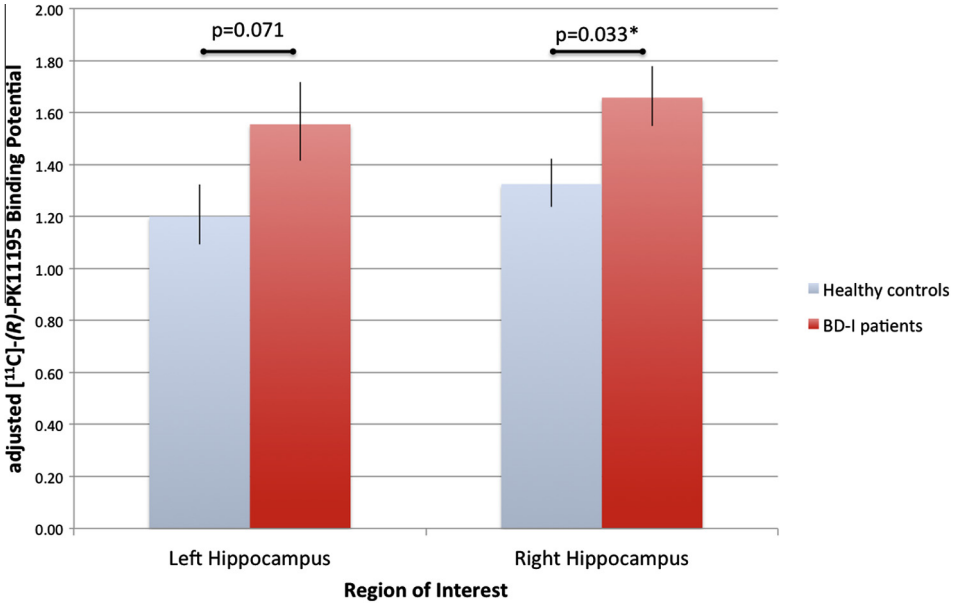


**Fig. 1.** Results of the hypothesis driven analysis. Mean adjusted [$^{11}$C]-(R)-PK11195 binding potentials in the left and right hippocampus of healthy controls and BD-I patients. Statistical analysis on the binding potentials was performed using a general linear model, with the whole-brain grey matter binding potential as a covariate to correct for global [$^{11}$C]-(R)-PK11195 uptake. Beforehand inverse square root transformation was applied to the binding potentials to meet the normality assumption in the general linear model. Results are presented as bars (back-transformed means) with error stripes (standard error). A significantly increased [$^{11}$C]-(R)-PK11195 binding potential was observed in the right hippocampus of the patients when compared to the healthy controls ($p = 0.033$). * statistically significant $p < 0.05$.

**Table 2**
Results of the explorative analysis.

| Region of interest | Adjusted [$^{11}$C]-(*R*)-PK11195 binding potential | | *p* |
|---|---|---|---|
| | Healthy controls (mean (CI)) | BD-I patients (mean (CI)) | |
| Left hippocampus | 1.20 (1.00–1.46) | 1.55 (1.30–1.90) | 0.071 |
| Right hippocampus | 1.33 (1.16–1.53) | 1.66 (1.45–1.91) | 0.033 |
| Left frontal cortex | 1.24 (1.10–1.41) | 1.24 (1.12–1.39) | 0.184 |
| Left dorsolateral PFC | 1.40 (1.28–1.53) | 1.18 (1.09–1.27) | 0.009 |
| Left temporal cortex | 1.19 (1.09–1.29) | 1.26 (1.17–1.36) | 0.315 |
| Left parietal cortex | 1.37 (1.26–1.50) | 1.33 (1.23–1.44) | 0.638 |
| Right frontal cortex | 1.20 (1.12–1.28) | 1.27 (1.20–1.35) | 0.184 |
| Right dorsolateral PFC | 1.25 (1.12–1.42) | 1.25 (1.13–1.39) | 0.970 |
| Right temporal cortex | 1.21 (1.13–1.31) | 1.32 (1.24–1.42) | 0.111 |
| Righ parietal cortex | 1.40 (1.27–1.57) | 1.40 (1.28–1.54) | 0.956 |
| Occipital cortex | 1.28 (1.19–1.37) | 1.40 (1.31–1.49) | 0.081 |
| Anterior cingulate | 1.39 (1.24–1.56) | 1.29 (1.17–1.43) | 0.397 |
| Posterior cingulate | 1.26 (1.10–1.46) | 1.36 (1.20–1.55) | 0.456 |
| Cerebellum | 1.01 (0.94–1.10) | 1.13 (1.05–1.22) | 0.055 |
| Basal ganglia | 1.24 (1.16–1.31) | 1.24 (1.18–1.31) | 0.891 |

Mean adjusted [$^{11}$C]-(*R*)-PK11195 binding potentials in the left hippocampus, right hippocampus, left frontal cortex, left dorsolateral prefrontal cortex (PFC), left parietal cortex, left temporal cortex, right frontal cortex, right dorsolateral PFC, right parietal cortex, right, temporal cortex, occipital cortex, anterior cingulate, posterior cingulate, basal ganglia and cerebellum of healthy controls and BD-I patients. Statistical analysis on the binding potentials was performed using a general linear model, with the whole-brain grey matter binding potential as a covariate to correct for global [$^{11}$C]-(*R*)-PK11195 uptake. Beforehand inverse square root transformation was applied to the binding potentials to meet the normality assumption in the general linear model. Results are presented as back-transformed means with confidence interval (CI). A lower [$^{11}$C]-(R)-PK11195 binding potential in the left dorsolateral PFC of the patients when compared to healthy controls (1.18 (CI 1.09–1.27) versus 1.40 (CI 1.28–1.53); $p = 0.009$) was revealed. However this difference did not survive correction for the false discovery rate (FDR < 0.1).

binding potential, but it was not statistically significant. Although the effect size had the same magnitude as the right hippocampus, a slightly larger standard error was observed in the calculations and the study may have been underpowered to demonstrate a difference in the left hippocampus between patients and controls. This supposition is supported by a *post hoc* analysis excluding the patient with epilepsy that demonstrated a significant difference in the left hippocampus as well.

The finding of neuroinflammation in BD corroborates previous studies which used less direct indicators of immune activation: an increase in peripheral TSPO receptors in platelets of BD patients, described by Marazziti (Marazziti et al., 2005); peripheral blood monocyte gene expression found to be related to hemodynamic changes measured by functional MRI in the hippocampus of a combined sample of unipolar and bipolar depressed patients described by Savitz et al. (2013) and multiple studies investigating immune system related peripheral blood derived bio-assays, described above (Beumer et al., 2012; Haarman et al., 2013; Myint et al., 2007; Padmos et al., 2008). To increase the understanding of the role of immune activation in the pathophysiology of BD further research on the relationship between the peripheral blood derived bioassays and central nervous system neuroinflammation is necessary.

The same holds true on the relationship between the various functional neuroimaging observations and neuroinflammation. Animal model studies can be directive in this regard. These demonstrated that microglia have an active role in the development of

mature synapses during embryogenesis (Paolicelli et al., 2011), pruning synapses postnatally (Schafer et al., 2012), regulating neurogenesis (Sierra et al., 2010) and inducing apoptosis (Beumer et al., 2012) in the hippocampus as well as other regions. It is tempting to speculate that these cellular processes (partially) explain the metabolic disturbances (Deckersbach et al., 2006; Gonul et al., 2009) and the decreased neuronal viability observed in neuroimaging studies (Deicken et al., 2003).

The decreased [$^{11}$C]-(*R*)-PK11195 binding potential in the left dorsolateral PFC of BD-I patients compared to controls in the explorative analysis is possibly a false-positive finding as it was no longer statistically significant after FDR correction for multiple testing. However, a possible differential [$^{11}$C]-(*R*)-PK11195 binding potential between the right hippocampus and the left dorsolateral PFC could also be regarded in the view of recent resting state fMRI connectivity studies that demonstrated aberrant connectivity between the PFC regions and limbic system regions in BD (Vargas et al., 2013).

It must be noted that our patients were almost all in the euthymic state, so they were not markedly depressed. It remains uncertain whether the inflammatory response would be greater during a depressive or manic episode.

Previously neuroinflammation PET studies have been performed in schizophrenia and unipolar major depressive disorder (MDD). Our finding corresponds with the result in the study by Doorduin et al. (2009), albeit that the effect size in the right hippocampus in BD is smaller than that was found in schizophrenia-related psychotic patients, possibly related to the more extensive symptomatology of these patients. Another study on TSPO binding in patients with mild or moderate MDD using the radiopharmaceutical [$^{11}$C]-PBR28 did not demonstrate a difference between patients and controls (Hannestad et al., 2013). Although the hippocampus was not a specific region of interest in their study and subjects with a high sensitive C-reactive protein level of more than 5 mg/l were excluded, it could be argued that perhaps neuroinflammation plays a more important role in BD than in MDD.

The present study has several inevitable limitations. Increased [$^{11}$C]-(*R*)-PK11195 binding to the TSPO receptor in the brain is traditionally related to microglia activation (Beumer et al., 2012). It is important to note that the TSPO receptor can also be expressed in astrocytes, potentially influencing the [$^{11}$C]-(*R*)-PK11195 binding potential signal (Lavisse et al., 2012). However, because both cells are known to contribute to neuroinflammation (Hostenbach et al., 2013), it can be argued that regardless of activated microglia cells or astrocytes being responsible for the increased TSPO expression, the increased [$^{11}$C]-(*R*)-PK11195 binding most likely represents a neuroinflammatory process either way.

The naturalistic design of the study does not take the possible confounding effect of concomitant medication use into account. It is known that most mood stabilizing medications, including lithium, anticonvulsants and antipsychotics, have an effect on the immune system (Drzyzga et al., 2006; Haarman et al., 2013; Padmos et al., 2008; Pollmächer et al., 2000; Rybakowski, 2000). However, their effects are generally immunosuppressive in nature. It can be argued that in the present study most medications would actually have diminished the effect of the observed neuroinflammation, so the amount of microglia activation in medication-free euthymic BD-patients could be even larger, compared to controls.

## 5. Conclusion

In conclusion, this study demonstrates the presence of focal neuroinflammation in the right hippocampus of BD-I patients, being a point of departure for unraveling the role of *in vivo* immune activation in the pathophysiology of BD.

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