Increased ventral striatal CB₁ receptor binding is related to negative symptoms in drug-free patients with schizophrenia

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ABSTRACT

Increasing animal genetic, post-mortem and pharmacological evidence supports a role for the cerebral type 1 cannabinoid (CB₁) receptor in the pathogenesis of schizophrenia (SCZ) and/or neural circuit dysfunctions responsible for its symptomatology. Moreover, since important interspecies differences are present in CB₁ receptor expression, in vivo human data are of direct interest. We investigated an in vivo CB₁ receptor expression in SCZ patients compared to healthy controls (CON), and in relation with psychopathological symptom severity using positron emission tomography (PET) and the selective high-affinity radioligand [18F]MK-9470. A total of sixty-seven patients with SCZ, with (SCZ-T, n = 51) and without (SCZ-F, n = 16) antipsychotic treatment, and 12 age and gender-matched CON were investigated with [18F]MK-9470 PET. Parametric modified standardized uptake value (mSUV) images, reflecting CB₁ receptor binding, were compared and related to psychopathological symptoms. Compared to CON, there was a significant increase of CB₁ receptor binding in SCZ patients in the nucleus accumbens, insula, cingulate cortex, inferior frontal cortex, parietal and mediotemporal lobe. Furthermore, in the SCZ-F group only, CB₁ receptor binding was negatively correlated to negative symptoms and depression scores, especially in the nucleus accumbens. Present findings strongly support that CB₁ receptor binding is altered in the mesocorticolimbic circuitry of both SCZ-T and SCZ-F patients, especially in the nucleus accumbens. In SCZ-F patients, it is associated with negative symptoms and depression scores.

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Introduction

Schizophrenia (SCZ) is one of the most common severe psychiatric disorders with a lifetime prevalence of about 1% (Van Os and Kapur, 2005). Existing antipsychotics reduce psychotic symptoms but are generally not very effective in treating negative symptoms, and a significant portion of patients are refractory to all current treatments. Despite the availability of treatments that reduce psychotic symptoms, a clear biochemical basis of SCZ has not been identified yet.

Several neurobiological, pharmacological and epidemiological studies support an association between SCZ and the endogenous cannabinoid system (ECS) (Leweke and Koethe, 2008). The ECS mediates the psychotomimetic effects of Δ⁹-tetrahydrocannabinol (Δ⁹-THC), the major psychoactive ingredient of Cannabis sativa, by activation of the type 1 cannabinoid (CB₁) receptor. CB₁ receptor activation modulates synaptic release of other transmitters such as glutamate, dopamine, and γ-aminobutyric acid (GABA) (Wilson and Nicoll, 2002).

High rates of cannabis consumption in normal volunteers induce cognitive impairment resembling of SCZ patients (Henquet et al., 2005). In SCZ patients, cannabis use is associated with a reduction in age at onset (De Hert et al., 2011). Moreover, several studies have suggested that frequent cannabis use is associated with increased risk for psychotic disorder and symptoms (Moore et al., 2007; Van Winkel and GROUP investigators, 2011). Postmortem binding studies in SCZ patients showed an increase of CB₁ receptor binding in the posterior and anterior cingulate cortex (ACC) (Newell et al., 2006; Zavitsanou et al., 2004). [³H]CP-55940 binding was also increased in the dorsolateral prefrontal cortex (DLPFC) in SCZ patients, whereas no changes in CB₁ receptor binding were found in the striatum or temporal lobe (Dean et al., 2001). This was confirmed in two recent postmortem human tissue studies that reported a 22% increase in [³H]CP-55940 binding and a 20% increase in [³H]MePPEP binding in the DLPFC in schizophrenic patients (Dalton et al., 2011; Jenko et al., 2013).
From postmortem immunohistochemistry and in situ hybridization experiments, conflicting results have been found. Reduced CB1 receptor messenger RNA and protein expression were detected in the postmortem DLPFC of SCZ patients (Eggan et al., 2008), and reduced immunodensity of CB1 receptor was in the PFC of antipsychotic-treated schizophrenics but not in drug-free patients (Uriguen et al., 2009). Additional arguments for involvement of the ECS in SCZ include endogenous ligand studies where elevated anandamide levels were demonstrated in the cerebrospinal fluid of SCZ patients (Giuffrida et al., 2004; Koethe et al., 2009; Leweke et al., 1999). These were inversely correlated with psychotic symptoms and negative symptoms in particular and suggest that the ECS system, acting through one of its major endogenous ligands, represents a protective and counterbalancing system toward psychosis (Leweke, 2012).

Several radioligands for in vivo imaging of the CB1 receptor have been developed recently that can be used for quantitative positron emission tomography (PET). Berding et al. (2006) showed the feasibility of CB1 receptor imaging with [11C]AM281 PET in a SCZ patient; however, the poor signal-to-noise ratio and the high radiation burden of the ligand constituted a major difficulty to extend the investigation to a larger number of subjects. Using [11C]OMAR in 9 treated SCZ patients, Wong et al. (2010) demonstrated elevated CB1 receptor binding across all the brain regions studied. Furthermore significant correlations between the ratio of positive versus negative symptoms and CB1 receptor binding were found.

Since SCZ is a heterogeneous disorder and antipsychotic treatment can lead to bias in observed findings, we have investigated cerebral CB1 receptor binding of 67 SCZ patients using the selective CB1 receptor radioligand [11C]MK-9470 (Burns et al., 2007) and PET. As most of the postmortem and in vivo studies showed an increase in CB1 receptor binding as outlined above, the primary outcome was to test the hypothesis that in vivo CB1 receptor binding is increased in antipsychotic-free and/or SCZ patients on antipsychotic monotherapy in comparison to healthy controls. As a second objective, we tested the hypothesis that regional CB1 receptor binding was related to the severity of SCZ symptoms.

### Material and methods

**Participants**

A total of 67 patients with schizophrenia (SCZ) (age: 36.7 ± 9.2 years) were recruited in a prospective study: an antipsychotic-free group (SCZ-F, n = 16), consisting of first-episode antipsychotic-naive patients (n = 10) and patients after treatment washout (n = 6; duration of the washout period = 9.4 ± 4.0 months), as well as five groups of patients treated (SCZ-T, n = 51) under stable treatment with one of the following drugs: amisulpride (n = 11), risperidone (n = 10), clozapine (n = 10), olanzapine (n = 10), and aripiprazole (n = 10) (Table 1). These drugs were selected because they not only represent commonly prescribed SGAs but also cover the range of pharmacological profiles for such compounds.

Patients with SCZ were recruited from the clinic of the Department of Psychiatry at U.C. St. Jozef of Kortenberg (Belgium), the Department of Psychiatry and Psychotherapy of the University of Cologne, and the Department of Psychiatry and Psychotherapy of the Central Institute of Mental Health of Mannheim (both Germany). Patient scans were all performed at the Department of Nuclear Medicine of Mannheim University Hospital (both Germany).

**Table 1**

<table>
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<tr>
<th>Cognitive tests</th>
<th>CON</th>
<th>SCZ-T</th>
<th>SCZ-F</th>
<th>Amisulpride (n = 11)</th>
<th>Aripiprazole (n = 10)</th>
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Data are given as means ± standard deviation. CON = controls, SCZ-T = schizophrenic patients treated with antipsychotics, SCZ-F = schizophrenic patients antipsychotic-free, M = male, F = female, y = years, Y = yes, PANSS = positive and negative symptom scales, PANSS-P = positive PANSS, PANSS-N = negative PANSS, PANSS-G = general PANSS, PANSS-T = total PANSS, CGI = Clinical Global Impression, GAF = Global Assessment of Functioning, RAVLT = Rey Auditory Verbal Learning Test, TMT = Trial Making Test, VMS-WMS-R = Visual Memory Span Wechsler Memory Scale Revised, COVAT = Controlled Oral Word Association Test, SS-WAIS = Symbol Substitution Wechsler Adult Intelligence Scale, LNS-WAIS = Letter-number sequencing-Wechsler Adult Intelligence Scale, CPT-IP = Continuous Performance Test-Identical Pairs version, NA = not applicable.

A Indicates the total number of word correctly recalled on RAVLT trials 1-5.
B Indicates the seconds required to complete the TMT.
C Indicates the percentile equivalents for the VMS-WMS-R.
D Indicates the total number of words created after three COVAT trials.
E Indicates the number of correct symbols drawn within 120 s (scaled score) S-WAIS.
F Indicates the total correct answers (scaled score) LNS-WAIS.
G Indicates d-prime and omission error totals from the CPT-IP.
H Results surviving Bonferroni correction for multiple comparisons (p < 0.05) for the comparison SCZ-F Drug Naïve > any other other SCZ-T group.
I Results surviving independent-sample t tests (p < 0.05) for the comparison Amisulpride-treated SCZ-T > any other SCZ-T group.
performed at the UZ Leuven (Belgium). All SCZ patients fulfilled the diagnostic criteria from the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (DSM-IV) (*American Psychiatry Association, 2000*), by consensus of at least two board-certified psychiatrists. All SCZ patients, except the SCZ-F antipsychotic-free patients, had psychotic symptoms for at least a year. SCZ-T patients were clinically stable and were maintained on a stable dose of antipsychotic monotherapy for at least 3 months prior to screening.

Exclusion criteria for SCZ patients included: history of neurological dysfunction, other major psychiatric or internal disorders; history of serious suicide attempt(s) or clinically significant suicidal ideation were excluded; or current or recent (3 months) drug abuse, including cannabis. Because of the low likelihood that tobacco smoking interferes with CB₁ receptor binding as demonstrated in animal studies (*Gérard et al., 2010*), and no changes in anandamide were found in CSF (*Leweke et al., 2007*), current tobacco smoking was not considered an exclusion criterion.

A population of 12 cannabis-naïve healthy subjects matched by age, gender and educational level, was used as control group (CON) (Table 1). These subjects were recruited in response to advertisements in the departmental homepage and local community newspapers, and they were selected randomly from different previous PET studies (*Sanabria-Bohorquez et al., 2010; Van Laere et al., 2008*). All volunteers were free of diagnosable psychopathology according to DSM-IV criteria and inclusion and exclusion criteria were as described previously (*Van Laere et al., 2008*).

All patients and healthy subjects gave a written informed consent according to the World Medical Association Declaration of Helsinki. The study was approved by the local ethics committees. The absence of any drug use at the time of PET scanning was confirmed by blood and urine testing that included general screening and toxicology for benzodiazepines, neuroleptics, opiates, cocaine and metabolites, amphetamines and cannabinoids on the same day as the PET scan in all SCZ patients and controls. Additionally, in order to exclude any structural brain abnormalities and to anatomically coregister with the PET data, each subject received a standard magnetic resonance imaging (MRI) scan, both T1-weighted Magnetization Prepared Rapid Acquisition Gradient Echo (3D-MPRAGE) and T2-weighted. MRI was acquired on a 1.5 Tesla Vision Scanner (Siemens, Germany).

**Psychiatric and neurocognitive assessment**

The severity of psychotic symptoms in SCZ patients was evaluated using the positive and negative syndrome scale (PANSS) (*Kay et al., 1987*). To calculate PANSS scores, the 30 PANSS items were grouped into three categories: positive (PANSS-P), negative (PANSS-N) and general (PANSS-G) symptoms. Additionally, as several studies have suggested that a five-factor model (positive, negative, excited, depressed and disorganized) may better reflect the PANSS structure in SCZ patients, data were also characterized using these five PANSS factors (*Llorca et al., 2012; Wallwork et al., 2012*). Moreover, the Clinical Global Impressions — severity of illness (CGI) (*Guy, 1976*) and Global Assessment of Functioning (GAF) (*Jones et al., 1995*) were also administered to provide additional information on level of functioning.

A neurocognitive test battery was also used with national validated versions of the tests to assess memory functioning, psychomotor and processing speed, attention and vigilance (*Comblatt et al., 1988; Guy, 1976; Jones et al., 1995; Reitan and Wolfson, 1993; Rey, 1964; Spreen and Strauss, 1998; Wechsler, 1974, 1981*). Further details are provided in the Supplementary data. The test battery was not used for SCZ-F antipsychotic-naïve given the potential confounding effects of acute symptomatology and conceptual disorganization on test performance, and for CON because they were screened for previous PET studies, which did not require the same neurocognitive evaluation. The remaining 57 SCZ patients completed mainly all the tests (n = 54), or at least 5 of the 7 neurocognitive tests (n = 3).

**PET imaging**

The radiotracer [¹⁸F]MK-9470 is an inverse agonist with a high-affinity and specificity for the human CB₁ receptor. Its synthesis is described in the Supplemental material. All subjects were fasted for at least 4 h prior to the PET acquisition. PET scans were acquired on an ECAT EXACT HR + (Siemens, Erlangen, Germany) in a three-dimensional mode. Subjects received on average 301.8 ± 54.0 MBq of [¹⁸F]MK-9470 in slow bolus intravenous injection. Before the dynamic PET emission scan, the subject was positioned in the scanner with the head fixed in a vacuum cushion to minimize head motions during the imaging acquisition. Dynamic PET scans were started 120 min after radioligand injection with 60-min scanning session (twelve 5-min frames). PET images were reconstructed using the three-dimensional filtered backprojection algorithm correcting for attenuation (attenuation scan performed using a ⁶⁸Ge source) and scatter. The spatial resolution of the reconstructed images was 4 mm full-width at half-maximum.

**Image processing**

The index of CB₁ receptor binding was quantified using the modified standard uptake value (mSUV), a non-invasive simplified quantification method also used in previous human [¹⁸F]MK-9470 studies (*Burns et al., 2007; Gérard et al., 2011; Van Laere et al., 2008, 2009, 2010, 2011*). mSUV images were obtained by summation of the activity concentration between 120 and 180 min postinjection, corrected for tracer injected and subject's body weight (*Thie et al., 2007*). It has been recently demonstrated that this approach allows a more practical acquisition protocol in patients and is a valid simplified quantification providing an index of tracer binding, provided no group differences in peripheral metabolism is present (*Gérard et al., 2011; Van Laere et al., 2010, 2011*). In order to assess whether the mSUV method was also appropriate in the SCZ group, the fractional uptake ratio (FUR), which is an index strongly proportional to total distribution volume Vₚ of [¹⁸F]MK-9470 (*Sanabria-Bohorquez et al., 2010*), was calculated and compared to mSUV values. Validation and further quantification details are given as Supplementary data (Fig. S1 and Fig. S2).

Parametric mSUV images were generated using PMOD v. 2.9 (PMOD Technologies, Zurich, Switzerland). For each subject, PET frames were realigned and coregistered to the individual MRI using SPM2 (Statistical Parametric Mapping, Wellcome Department of Imaging Neuroscience, London, UK). The coregistered [¹⁸F]MK-9470 mSUV images were then spatially normalized to a specific in-house created CB₁ receptor template (*Van Laere et al., 2006*) constructed in Montreal Neurological Institute (MRI) (space 2 × 2 × 2 mm) in SPM2 using nonlinear warping. A predefined volume-of-interest (VOI) analysis was performed using an in-house previously created set of VOIs defined on the CB₁ receptor template (*Van Laere et al., 2008*). Additionally, subcortical brain areas (caudate nucleus, putamen, nucleus accumbens, pallidum, thalamus, and hypothalamus) were individually adjusted by delineating these regions manually on the transverse slices of T1 images. The personalized VOI map was then loaded on the corresponding normalized mSUV PET image, and the average mSUV values within the VOIs were then determined using PMOD. CB₁ receptor binding values of cortical Brodmann areas (BA) were also grouped into larger anatomical regions on the base of the number of voxels.

**PET data analysis and statistics**

For VOI-based analysis, the average mSUV values were compared using analyses of variance (ANOVARAs and MANOVARAs) and LSD post-hoc tests. A SPM analysis was additionally performed comparing the SCZ-T and SCZ-F groups to CON. Before performing a statistical SPM analysis, spatially normalized mSUV PET images were masked within the brain 80% isoscontour of the CB₁ receptor template and smoothed with a full-width-at-half maximum of 10 mm. A proportional scaling was
used and a relative gray matter analysis threshold of 80% of the mean was adopted to exclude extracerebral activity. Data were explored at a cluster-level \( p_{\text{cluster}} < 0.05 \) (corrected for multiple comparisons) and voxel-level \( p_{\text{height}} < 0.001 \) (uncorrected for multiple comparisons). The extent threshold \( k_{\text{ext}} \) was set at 50 voxels (approximately 0.4 cm\(^3\)) to reduce the chance of false-positive clusters. To exclude the influence of confounders such as age, gender, smoking and cannabis use, analyses were done with and without these parameters as nuisance variables.

Group comparisons on clinical symptoms were assessed with ANOVA and Bonferroni post-hoc tests. Correlations between clinical measures and cognitive scores were calculated using Pearson correlation coefficients and Bonferroni correction. The significance threshold was set at \( p < 0.05 \). Statistical analyses were conducted using Statistica version 9.0 (Statsoft Inc., Tulsa Oklahoma, USA).

**Results**

**Clinical characteristics and treatment effects**

Table 1 presents clinical characteristics and neurocognitive performance of SCZ patients and controls. Significant differences were present between SCZ-T and SCZ-F antipsychotic-naïve on all psychopathological symptoms (\( p \) from .01 to \( 10^{-4} \)). Globally, SCZ-F antipsychotic-naïve had the highest PANSS total score (PANSS-T) among all SCZ subgroups. Within the SCZ-T group, ANOVA revealed no differential effect of antipsychotic treatment on symptom domains. Moreover, no difference in clinical symptoms was found among the five SCZ-T groups and the SCZ-F drug-washout group. The mean scores for neurocognitive performances for each individual SCZ group are reported in Table 1. Additional results on behavioral and neurocognitive performance are given in the Supplemental material (see Inline Supplementary Table S1).

Inline Supplementary Table S1 can be found online at http://dx.doi.org/10.1016/j.neuroimage.2013.04.052.

**CB\(_1\) receptor group comparison**

Compared to CON, there was a global gray-matter increase of CB\(_1\) receptor binding in the SCZ-F group (+10.1%), which was also present but less pronounced in the SCZ-T group (+5.5%) (mSUVT \( = 1.14 \pm 0.16 \); mSUVC \( = 1.19 \pm 0.20 \); mSUVC\(_{\text{CON}} \) = 1.08 \pm 0.17). A VOl-based analysis demonstrated that regional [\(^{18}\)F]MK-9470 binding values in SCZ (both SCZ-F and SCZ-T) were higher in all brain regions, in comparison to CON (Fig. 1 and Fig. 2). The increased CB\(_1\) receptor binding found across cortical and subcortical gray matter regions was higher for SCZ-F, especially in the NuAc (+15.5%, \( p = 0.02 \)) and parietal cortex (+10.4%, \( p < 0.001 \)). In addition, CB\(_1\) receptor change was also significant in the insula (+6.6%, \( p = 0.04 \)) and inferior frontal gyrus (+12.0%, \( p = 0.01 \)). In SCZ-T, this increase was significant in the NuAc (+12.9%, \( p = 0.02 \)), insula (+7.7%, \( p = 0.01 \)), and in larger anatomical regions such as cingulate cortex (+6.1%, \( p = 0.002 \)), mesiotemporal lobe (+6.7%, \( p = 0.03 \)), and parietal cortex (+4.3%, \( p < 0.001 \)) (Fig. 2).

As a secondary analysis, we explored also the possible effect of type of antipsychotic drug on the CB\(_1\) receptor binding (see Supplemental data). No treatment-specific changes in CB\(_1\) receptor binding were observed between SCZ-T and SCZ-F groups and compared to CON. However, comparing relative [\(^{18}\)F]MK-9470 binding normalized to the average brain binding, antipsychotic monotherapy significantly altered CB\(_1\) receptor binding in the mesocorticolimbic circuitry (\( p_{\text{cluster}} < 0.05 \) corrected for multiple comparisons; Fig 3; Inline Supplementary Table S2). These results remained when analysis with age, gender, past cannabis use and current tobacco smoking as nuisance variables was done.

Inline Supplementary Table S2 can be found online at http://dx.doi.org/10.1016/j.neuroimage.2013.04.052.

**Correlation between clinical measures and CB\(_1\) receptor binding**

In the whole SCZ group, we found no correlations between CB\(_1\) receptor and PANSS, CGI and GAF scores. However, modest inverse correlations were observed between CB\(_1\) receptor binding and PANSS subscales for negative and general symptoms in SCZ-F, but no significant correlations were present for SCZ-T.

We found that, in SCZ-F patients, CB\(_1\) receptor binding was negatively correlated with the negative PANSS items “social withdrawal” (PANSS-N4) and “stereotyped thinking” (PANSS-N7), and with the two general PANSS items “depression” (PANSS-G6) and “active social avoidance” (PANSS-G16). Inline Supplementary Table S3 gives the correlation coefficients for a whole-brain VOI, lobar areas, and NuAc, using both PANSS dimensions and PANSS factors. These indicate that, although the strength of the correlation is strongest in the NuAc, the observed correlations are mainly global. Individual data for the NuAc are shown in Fig. 4 (PANSS-N4: \( r = -0.63, p = 0.009 \); PANSS-N7: \( r = -0.56, p = 0.02 \); PANSS-G6: \( r = -0.58, p = 0.02 \); PANSS-G16: \( r = -0.60, p = 0.01 \)). There were no significant relationships between CB\(_1\) receptor availability and PANSS-N and PANSS-G total score, although total PANSS-N showed a trend toward significance in the NuAc (\( r = -0.48, p = 0.06 \); Inline Supplementary Table S3). Additionally, PANSS factor analyses confirmed that PANSS negative factor was correlated with CB\(_1\) receptor binding in the NuAc (\( r = -0.51, p = 0.04 \); Inline Supplementary Table S3). The correlation with CB\(_1\) receptor binding in the NuAc and PANSS depressive factor still showed a trend toward significance (\( r = -0.45, p = 0.08 \); Inline Supplementary Table S3).

Inline Supplementary Table S3 can be found online at http://dx.doi.org/10.1016/j.neuroimage.2013.04.052.

**Effects of past cannabis use on CB\(_1\) receptor binding**

Overall, 52.0% of the SCZ patients consumed cannabis at least once in their life (Table 1). The lifetime history of cannabis use was comparable between SCZ-T (51.0%) and SCZ-F (56.3%). The last cannabis consumption dated back to at least six months before the PET investigations (SCZ-T: 7.0 \( \pm 6.1 \) years; SCZ-F: 6.0 \( \pm 4.8 \) years). SCZ patients were classified as “heavy cannabis users” when the frequency of past cannabis use during the period of heaviest cannabis consumption was several times a day, according to CIDI definitions. Cannabis dose, duration of the period of most intensive use, and age of the first cannabis exposure were not correlated to any psychotic symptoms. SCZ patients with a history of heavy cannabis exposure did not show different CB\(_1\) receptors binding from SCZ patients with moderate (\( p = 0.16 \)), low (\( p = 0.38 \)) or an absence of lifetime exposure to cannabis (\( p = 0.80 \)) (Fig. S3), suggesting that the lifetime cannabis use did not have a significant effect on CB\(_1\) receptor binding.

**Discussion**

Several lines of experimental evidence point to a dysregulation of the functions of the CB\(_1\) receptor in SCZ. The majority of the studies investigating CB\(_1\) receptor in SCZ examined CB\(_1\) receptor changes using radioligand binding and quantitative autoradiography in postmortem tissue (Dean et al., 2001; Eggan et al., 2008; Newell et al., 2006; Urquen et al., 2009; Zavitsanou et al., 2004). Concerning in vivo investigations, few quantitative CB\(_1\) receptor PET studies in the brain of SCZ patients have been carried out either using first-generation CB\(_1\) receptor radiotracers in only one patient (Berding et al., 2006) or involving a low number of patients (Wong et al., 2010). We have investigated the CB\(_1\) receptor binding in SCZ patients both with and without different antipsychotic treatments, which has not been reported before. Moreover, we have correlated the CB\(_1\) receptor changes with the severity of symptoms.
Compared to controls, we found a widespread moderate increase in CB₁ receptor binding in the whole group of SCZ patients. This may suggest that the CB₁ receptor is involved in the pathophysiology of SCZ and/or in the neural mechanisms underlying its symptoms (Dean et al., 2001; Newell et al., 2006; Zavitsanou et al., 2004). The upregulation of the CB₁ receptor binding was statistically significant in the parietal and mesotemporal lobe, inferior frontal gyrus, and especially in selected mesocorticolimbic brain areas such as the NuAc, insula and ACC. The NuAc is an important key region of the mesolimbic system involved in both psychosis and substance use disorders (Chambers et al., 2001). It has been suggested that dopamine release in the NuAc caused by cannabinoids and other drugs used by patients, is a final common mechanism for the reinforcing effects of psychostimulants and other drugs (Di Chiara and Imperato, 1988). Nevertheless, it is of interest that the antipsychotic effects of the CB₁ receptor inverse agonist rimonabant in animal models are due to changes in glutamatergic transmission in the NuAc, without dopaminergic participation (Soria et al., 2005). Additionally, we found a relevant CB₁ receptor alteration in the ACC of SCZ patients. Based on its rich interconnections with prefrontal, limbic and dopaminergic brain areas, the ACC region is involved in normal cognition, particularly in relation to attention and motivation. Impairments of cognitive functioning in these areas have been seen in long-term cannabis users (Solowij et al., 2002) and similar cognitive impairments also resemble core negative/cognitive symptoms of SCZ. Therefore, changes in CB₁ receptor in the ACC may be of relevance in SCZ, principally in relation to negative/cognitive symptoms.

Our findings are in line with previous reports that in SCZ an increased CB₁ receptor binding is observed, either by autoradiography, especially in the cingulate cortex and DLPFC (Dalton et al., 2011; Dean et al., 2001; Jenko et al., 2012; Newell et al., 2006; Zavitsanou et al., 2004), or in vivo where significant elevated CB₁ receptor binding of [¹¹C]OMAR was found in the pons of SCZ patients (Wong et al., 2004).
At odds with these results, Eggan et al. (2008) found a reduced CB1 receptor mRNA and immunocytochemical receptor protein expression in the DLPFC of SCZ patients, and another immunohistochemical study showed no alteration of density of CB1 receptor immunopositive cells in the ACC of SCZ patients (Koethe et al., 2007). Also, Uriguen et al. (2009) and Dalton et al. (2011) reported unchanged CB1 receptor mRNA expression in the DLPFC of drug-free SCZ patients post-mortem. The discrepancy between ligand binding and immunocytochemistry approaches to CB1 receptor protein measurement might be due to post-mortem tissue changes, to differences between cell surface receptor availability and total CB1 receptor protein because there is a large intracellular reserve in CB1 receptor protein (Coutts et al., 2001), or methodological differences, such as reported by Dalton et al. (2011), as CB1 receptor antibodies may not qualitatively or quantitatively stain receptors in all cell types or subcellular compartments. Indeed, CB1 receptor antibodies used in earlier studies could exclusively label inhibitory synapses by electron microscopy (Eggan and Lewis, 2007; Eggan et al., 2008). Therefore the antibodies recognize CB1 receptors on GABAergic axon terminals only and may therefore fail to detect level of CB1 receptors in...
excitatory glutamatergic terminal receptors which require different, more sensitive antibodies to be visualized and which are detectable using a radioligand binding approach.

We also investigated whether different antipsychotic treatments could alter CB₁ receptor density. Although D₂/₃ receptors are the main target of antipsychotics, the increased CB₁ receptor binding found in SCZ was modulated by antipsychotics. These findings represent a further indirect evidence regarding the functional complexes that dopamine receptors form with CB₁ receptors (Kearn et al., 2005), and may point to interactional linkage between the cannabinoid receptor systems in relation to psychotic symptoms (Leweke and Koethe, 2008). Indeed, the dopamine hypothesis of SCZ, postulating that increased brain dopaminergic activity causes psychotic symptoms of SCZ, may lead to or be a consequence of the overactivity of the ECS. The exaggerated dopamine release onto postsynaptic D₂-like receptors in SCZ might trigger release of anandamide, which then acts as a retrograde messenger to and may indirectly induce a CB₁ receptor-mediated attenuation of dopamine release. This hypothesis is supported by Giuffrida et al. (2004) who proposed a model on a dopaminergic–endocannabinoid interaction in SCZ, where (over)activation of D₂ receptors was associated with (increased) release of anandamide, counterbalancing dopamine-mediated psychotic symptoms by strengthening the endogenous adaptive feedback loop, through CB₁ receptor activation. This model is further supported by the fact that higher values of cerebrospinal anandamide levels are associated with lower negative symptoms and the fact that SCZ patients treated primarily with a selective D₂ receptor antagonist show markedly lower anandamide levels in CSF than SCZ patients treated with antipsychotics targeting multiple receptor systems including D₂/₃ and serotonin receptors. However, the differences found in the CB₁ receptor binding between patients treated with antipsychotics and antipsychotic-free patients might be an indirect treatment effect due to the antipsychotic medication, but might also be an effect of the ongoing disease itself, or a combination of both.

Our second aim was to investigate the relationship between CB₁ receptor binding and severity of clinical symptoms. In antipsychotic-free schizophrenic patients, we found that elevated [¹⁸F]MK-9470 binding was inversely correlated with negative and general symptoms, but we did not find such correlations in patients under monotherapy. Overall, looking at PANSS scores over the groups, only the SCZ-F antipsychotic-naïve patients were more severely ill than the other groups. In the SCZ-T groups, which were stable, there does not seem to be a great variance in symptomatology, and this lack of variance might have obscured some of the possible correlations. The inverse correlation with negative symptoms is consistent with the trend level found in Wong et al. (2010) for some cortical areas and the deduction made from the relationship found between CB₁ receptor binding and the ratio of brief psychiatric rating score (BPRS) positive symptoms versus negative symptoms, suggesting that it might be possible to characterize the CB₁ receptor binding as it relates to severity of schizophrenic symptoms. The more pronounced psychopathology was clearly dominated by positive and negative symptoms. The majority of the SCZ patients (87%) had a depression severity ranging from absent to mild, although 50% of the SCZ-F antipsychotic-naïve patients scored in the moderate/severe range. However, the negative correlations observed between increased CB₁ receptor binding and psychotic symptoms are clearly in line with previous findings by Giuffrida et al. (2004) and Leweke et al. (2007) indicating that an increased activation of the ECS is associated with less pronounced symptoms. Given our findings, it may be possible that the entire ECS is region specifically upregulated in acute schizophrenia serving an adaptive if not protective role toward psychotic symptoms.

Further studies may clarify the mechanism by which the results discussed here are linked to limbic hyperdopaminergic or prefrontal hypodopaminergic activity and involvement of the glutamatergic system. In particular, they might investigate whether the reported upregulation of CB₁ receptors in the mesocorticolimbic regions in SCZ reflects a primary pathology or a compensatory homeostatic adaptation of the ECS to other neurotransmitter imbalances.

Cannabis consumption is an important confounding variable in this population, therefore we investigated the lifetime cannabis consumption on CB₁ receptor binding in SCZ patients, since it was reported that in patients with high-frequency cannabis use, cerebrospinal anandamide levels were more than tenfold lower than in patients with low-frequency use (Leweke et al., 2007), even though more than 20 times of lifetime cannabis use were already suggested “high frequency”. Moreover, Hirvonen et al. (2012) found reversible and regionally selective downregulation CB₁ receptor binding in heavy daily cannabis smokers. However, our data showed that the lifetime cannabis use did not affect CB₁ receptor density since no difference in CB₁ receptor binding was observed between the SCZ patients with frequent and no exposure to cannabis (Fig. S3). Furthermore, to exclude such potential confounding influence, we have included past cannabis use as nuisance variable in the analysis, but found no differences with and without nuisance variable inclusion.

Given the demonstrated role for the CB₁ receptor in the pathology of SCZ (Leweke et al., 1999), and the results of the most recent treatment trial targeting the ECS (Leweke et al., 2012; Meltzer et al., 2004), the cannabinoid hypothesis of SCZ deserves further investigation, to gain further insight in the role of the ECS in this complex disorder and to provide additional proof-of-mechanism for cannabinoid-related therapy.

Limitations

Some limitations should be considered in the interpretation of our findings. First, due to the small cohort of SCZ-F patients compared to the SCZ-T group, the inverse correlation between the ventral striatal CB₁ receptor binding and negative symptoms in antipsychotic-free SCZ from the current study needs independent replication in a larger SCZ-F sample. Second, the lack of a control group with a lifetime history of cannabis use might complicate the delineation of the effects of diagnosis and history of cannabis use on the observed group differences in CB₁ receptor availability. However, a major impact on this study is not expected, as we did not see any CB₁ receptor difference between patients with and without past lifetime cannabis use. Thirdly, the control group consisted of only 12 subjects, which were age, gender and education level matched. This size was taken as a reasonable number compared to the size of the various subgroups (monotherapy subgroups, antipsychotic-free and antipsychotic-naïve). Sensitivity to detect further subtle differences between groups may be enhanced by having considered larger control population. Finally, as the mSUV parameter is correlated to total distribution volume (V₅) and as such an index of receptor availability, there is a significant component of non-specific binding in these measures that can reduce sensitivity of differences in or correlations with receptor-availability. Even though simple activity determinations can lead to improved precision (Terry et al., 2009), this approach may have led to a slight bias in specific group differences or suboptimal correlations.

Conclusions

In conclusion, both medicated and antipsychotic-free patients show increased CB₁ receptor binding compared to controls, particularly pronounced in the NuAc, cingulate and insular cortex. Moreover, the increased CB₁ receptor binding is negatively associated with negative symptoms and depression in antipsychotic-free patients. The current in vivo data strengthens the hypothesis that the endogenous cannabinoid system of the brain is involved in the pathology of SCZ.

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Conflicts of interest

The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2013.04.052.

References


