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Cannabis use and progressive cortical thickness loss in areas rich in CB1 receptors during the first five years of schizophrenia

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Abstract

Cerebral grey matter volume reductions are progressive in schizophrenia, with larger grey matter volume decreases associated with cannabis use. It is unknown whether this grey matter loss is globally distributed over the entire brain or more pronounced in specific cortical brain regions. Fifty-one patients with recent-onset schizophrenia and 31 matched healthy subjects were included. For all subjects, magnetic resonance imaging scans were obtained at inclusion and at 5-year follow-up. Nineteen patients (ab-)used cannabis but no other illicit drugs; 32 patients and the healthy comparison subjects did not use any drugs during the 5-year follow-up. At follow-up, clinical outcome was measured. To evaluate the local differences in cortical thickness change over five years between the two groups regression analysis was carried out over the cortical surface. At inclusion cortical thickness did not differ between patients and controls and between cannabis-using and non-using patients. Over the follow-up period we found excessive thinning of the right supplementary motor cortex, inferior frontal cortex, superior temporal gyrus, angular gyrus, occipital and parietal lobe in patients relative to controls after controlling for cannabis use. Patients who used cannabis showed additional thinning in the left dorsolateral prefrontal cortex (DLPFC), left anterior cingulate cortex (ACC) and left occipital lobe as compared to those patients that did not use cannabis during the scan interval. First-episode schizophrenia patients who use cannabis show a more pronounced cortical thinning than non-using patients in areas known for their high density of CB1 receptors, such as the ACC and the DLPFC.

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1. Introduction

Structural brain imaging studies have consistently demonstrated brain volume abnormalities in schizophrenia, with increases in ventricular volumes as well as decreases in cortical grey and white matter volumes (for review see Honea et al., 2005 and Wright et al., 2000). Longitudinal studies show that brain volume diminishes more extensively in patients relative to controls, with most (van Haren et al., 2007, 2008; Rais et al., 2008; for a review see Pantelis et al., 2005), but not all (DeLisi et al., 2004), studies reporting the largest brain volume loss in patients with the poorest outcome.

Interestingly, cannabis use has been associated with poor clinical and functional outcome in schizophrenia. This finding is relevant since cannabis (ab-)use is common in schizophrenia, occurring in up to half of the patients (Boydell et al., 2006), with cannabis-using patients showing more positive (Bersani et al., 2002; Buhler et al., 2002; Dubertret et al., 2006; Grech et al., 2005; Mauri et al., 2006) (but not negative; Bersani et al., 2002; Compton et al., 2004 Dubertret et al., 2006; Grech et al., 2005; Peralta and Cuesta, 1992) symptoms, an earlier disease onset (Veen et al., 2004) and increased number of psychotic relapses or exacerbations (Caspari, 1999; Grech et al., 2005; Linszen et al., 1994) compared with non-using patients. In view of the reported relationship between poor outcome and progressive brain volume loss (van Haren et al., 2007, 2008; Rais et al., 2008; for a review see Pantelis et al., 2005), it could be expected that patients abusing cannabis show larger brain volume loss over time than patients who do not. Indeed, we recently reported more overall grey matter loss and excessive ventricle enlargement over five years in cannabis-using first-episode schizophrenia patients compared with non-using patients and healthy comparison subjects (Rais et al., 2008). However, since that study only examined global brain structures such as the cerebrum and ventricles, it is unknown whether this grey matter loss is globally distributed over the entire brain or more pronounced in specific brain regions. The grey matter volume is principally represented in the cerebral cortex. It can be defined as the product of the cortical thickness and the cortical surface. Therefore, to investigate whether it is indeed particular cortical areas that are vulnerable to the effects of cannabis use change in cortical thickness over five years was compared between cannabis-using and non-using schizophrenia patients.

To our knowledge this is the first longitudinal study analyzing cortical thickness in cannabis-using schizophrenia patients as compared to non-using patients and healthy comparison subjects.

2. Experimental procedures

2.1. Subjects

Patients with first-episode schizophrenia ($N=51$) recruited from the First-Episode Schizophrenia Research Program at the University Medical Center Utrecht, Utrecht, The Netherlands, and healthy comparison subjects ($N=31$) were included in the study. The study received approval of the local ethical committee. Two MRI scans were obtained with an interval of approximately 5 years. This group of subjects has been described in more detail previously (Rais et al., 2008).

In short, both at inclusion (T0) and follow-up (T5), the patients were assessed with the Comprehensive Assessment of Symptoms and History (Andreasen et al., 1992) by two trained raters who independently determined the diagnosis and achieved consensus afterward; severity of illness was measured with the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) and drug use was assessed with the Composite International Diagnostic Interview (CIDI) (Robins et al., 1988). Patients with a lifetime diagnosis of abuse or dependence of a substance other than cannabis (except nicotine) were excluded. The time interval between inclusion in the study at T0 and follow-up measurement at T5 is referred to as the scan interval. At T5, all 51 patients met DSM-IV criteria for schizophrenia. Nineteen patients used only cannabis and no other illicit drugs during the scan interval, and 32 patients did not use any illicit drugs during the scan interval. Of this latter group, 15 patients never used cannabis during their lifetime, and 17 patients stopped using cannabis before baseline.

At follow-up, information was obtained on the average number of alcohol consumptions per week. Two patients in the cannabis-using group and two patients in the non-using group met the DSM-IV criteria for alcohol abuse, whereas no healthy comparison subjects met these criteria. The three groups did not differ significantly on average number of alcohol consumptions per week at follow-up. To calculate the cumulative dose of typical antipsychotic medication, a table from the Dutch National Health Service was used to derive haloperidol equivalents. The patients used only one antipsychotic at a time. For atypical antipsychotics, the respective pharmaceutical companies suggested how to convert the dose into haloperidol equivalents (clozapine, 40:1; olanzapine, 2.5:1; risperidone, 1:1; sulpiride, 170:1; quetiapine, 50:1; and sertindole, 2:1).

The healthy comparison subjects fulfilled criteria for "never mentally ill" both at baseline and follow-up. The healthy comparison subjects did not use any illicit substances before or during the study. The groups were matched for sex, age, handedness, and socioeconomic status of their parents (expressed as the highest level of education completed by one of the parents). After a complete description of the study to the subjects, written informed consent was obtained.

3. MRI procedures and measurements

Brain scans were acquired on a Philips NT (Best, The Netherlands) scanner operating at 1.5 T for all subjects. A three-dimensional fast field echo (TE = 4.6 ms, TR = 30 ms, flip angle = 30°, field of view = 256 × 256 mm^2) scan with 160–180 contiguous coronal 1.2-mm slices and a T2-weighted dual-echo turbo spin echo (TE1 = 14 ms, TE2 = 80 ms, TR = 6350 ms, flip angle = 90°, field of view = 256 × 256 mm^2) scan with 120 contiguous coronal 1.6-mm slices of the whole head were used for the quantitative measurements (Hulshoff Pol et al., 2001).

Processing was done on the neuroimaging computer network of the Department of Psychiatry at the University Medical Center Utrecht. Processing procedures have been described before (Hulshoff Pol et al., 2002; van Haren et al., 2003).

In short, all images were coded to ensure investigator blindness to subject identification and diagnosis; scans were put into Talairach orientation without scaling and corrected for intensity non-uniformity artifacts (Sled et al., 1998). Intensity histogram analysis on the T1 image yielded thresholds for separating brain tissue from cerebrospinal fluid and, within the brain, grey matter from white matter. Grey and

white matter segments were created by applying these thresholds to the images (Schnack et al., 2001). These segments were used as input for an advanced neural net classifier (Zijdenbos et al., 2002).

To analyze the cortical thickness, the CLASP algorithm designed at the McConnell Brain Imaging Centre of the Montreal Neurological Institute was employed (Kabani et al., 2001; Kim et al., 2005; MacDonald et al., 2000).

A 3D surface comprising 81,920 polygons per hemisphere was fitted to the white matter/grey matter intersection, which created the inner surface of the cortex which was then expanded out to fit the grey matter/cerebrospinal fluid intersection, thereby creating the outer cortical surface. Cortical thickness was estimated by taking the distance between the two surfaces such that each of the 81,924 vertices of the polygons on the outer surface had a counterpart vertex on the inner surface. Each subject's thickness measurements were smoothed across the surface using a 20 mm surface-based blurring kernel (Chung and Taylor, 2004).This method of blurring improves the chances of detecting population differences, but also follows the curvature of the surface to preserve any anatomical boundaries within the cortex.

For each subject, change in cortical thickness (T5−T0) was calculated for every vertex in individual space, then transformed to the ICBM template. The surfaces of each subject were registered to an average surface created from 152 healthy subjects aged 18−40 years (ICBM 152) (Lyttelton et al., 2007), allowing comparison of cortical thickness locally between subjects.

4. Statistical analysis

4.1. Demographic and clinical data

Data were examined for outliers and normality of the distribution.

To assess whether the groups differed on demographic or clinical variables, multiple analyses of variance were conducted for non-categorical variables and chi-square analyses for categorical variables (Table 1).

To estimate clinical outcome in the different groups of patients, change in positive and negative symptoms over 5 years was quantified by subtracting baseline PANSS scores from follow-up PANSS scores (PANSS T5−PANSS T0).

4.2. Group differences in cortical thickness

To evaluate the differences in cortical thickness change over five years between the two groups a vertex-by-vertex analysis was carried out.

In each vertex group differences in baseline cortical thickness and in cortical thickness change were calculated by using regression analyses with diagnosis (patient−control), cannabis use (yes−no), age and sex as covariates. Baseline cortical thickness and cortical thickness change respectively were included in the analysis as dependent variable. This produced *F*-statistics at each vertex, one for the effect of diagnosis (thus corrected for the effect of cannabis use), one for the effect of cannabis use (in patients only), one for the effect of age, and one for the effect of sex. In our previous paper (Rais et al., 2008) we showed decreased grey matter volumes in patients in general relative to controls and in cannabis-using patients relative to non-using patients. Therefore, we adjusted for multiple comparisons using a False Discovery Rate (FDR) of alpha=0.10 (one-tailed). Statistical maps were created showing significant differences in cortical thickness (change) between patients and healthy comparison subjects and between cannabis-using patients and non-using patients.

For those cortical areas that showed significant differences between cannabis-using patients, non-using patients or between patients and controls the most significant vertex was identified visually using the cortical surface viewer Brain-view developed at the Montreal Neurological Institute.

To evaluate the differences in mean cortical thickness change over the whole cortex between the patients and healthy comparison subjects and between cannabis-using and non-using patients a linear regression analysis was carried out with diagnosis (patient−control), cannabis use (yes−no), age and sex as covariates.

To assess the correlation between cortical thickness change (in peak vertices only) and change in positive and negative symptoms in first-episode patients with schizophrenia multiple regressions were performed with cortical thickness change (most significant vertex) as dependent variable and age, sex and change in positive and negative symptoms as independent variable.

Furthermore, to exclude possible effect of alcohol abuse, the main analysis was repeated excluding the four patients with alcohol abuse during the interval.

5. Results

Demographic and clinical data have been described previously (Rais et al., 2008) and are briefly reported in Table 1. The groups did not significantly differ with regard to sex, handedness, age, level of parental education, and duration of scan interval.

At inclusion and follow-up, the cannabis-using patients and those not using cannabis did not differ significantly on positive and negative symptoms and type and cumulative amount of medication during the scan interval. Cumulative duration of hospitalization during the scan interval did not differ significantly between the two patient groups. However, the subjects not using cannabis showed a small but significant improvement in positive and negative symptoms compared with the cannabis-using group over the follow-up interval.

Mean cortical thickness decrease over the whole cortex was significantly more pronounced in patients as compared to healthy subjects over both the right (mean [sd]: controls: −0.0028 [0.014] mm; patients: −0.014 [0.014] mm; $t=-2.74$; $df=4$, 81; $p=0.008$.) and left (mean: controls: −0.003 [0.014] mm; patients: −0.016 [0.015] mm; $t=-2.5$; $df=4,81$; $p=0.014$) hemisphere. No significant differences in mean global cortical thickness change were found between cannabis-using and non-using patients. No significant increases in cortical thickness were found between patients and healthy subjects and between cannabis-using and non-using patients.

At inclusion, focal cortical thickness did not differ significantly between schizophrenia patients and healthy comparison subjects and between cannabis-using and non-using patients.

Table 1 Demographic and clinical data of the cannabis-using and non-using schizophrenia patients and healthy comparison subjects.

	Cannabis− $N=32$				Cannabis+ $N=19$				Controls $N=31$				Sig.
	Mean/N	SD	Range		Mean/N	SD	Range		Mean/N	SD	Range		
Sex, No. of subjects m/f	26/6				19/0				25/6				NS
Handedness, No. of subjects r/l/a	27/2/3				18/0/1				26/5/0				NS
Age, years	23.28	5.10	15.70	37.03	21.83	3.91	16.73	31	24.72	6.66	16.74	40.21	NS
Parental education level, years	12.77	6.66	6	17	13.32	3.59	6	17	13.68	2.83	10	17	NS
MRI interval, years	5.28	0.50	4.13	6.39	5.35	0.64	4.54	7.08	5.21	0.18	4.78	5.50	NS
Age first psychosis, years	22.54	4.94	14.24	36.63	20.53	3.93	16.45	29.64					NS
Duration of illness, days	350.61	388.07	9	1408	429	631.65	66	2866					NS
PANSS T0 positive	18.82	5.19	9	28.00	15.63	5.73	7	25					NS
PANSS T0 negative	19.29	4.90	10	30	17.06	4.75	8	24					NS
PANSS change positive *	−5.59	4.98	−17.00	4.00	−1.94	6.43	−15.00	12.00					0.043
PANSS change negative *	−6.15	6.25	−15.00	10.00	−1.00	7.21	−16.00	11.00					0.018
CAN T5 total — staff	8.87	5.34	0	20	11.94	7.60	0	24					NS
GAF T5	52.62	17.90	30	90	53.94	20.37	15	90					NS
Days of hospitalization	120.48	104.24	0	430	171.26	254.83	0	1062					NS
Cumulative antipsychotic medication T5 (mg eq. Haloperidol)	13,711	6968	2966	30,873	12,302	5767	1511	20,810					NS
Type antipsychotic medication before T0 (naive/typ/atyp/both)	14/13/1/4				7/6/2/4								NS
Type antipsychotic medication T0T5 (typ/atyp/both/missing)	0/13/18/1				0/7/11/1								NS
Duration of treatment at T0 (days)	77	126	0	483	119	142	0	413					NS

PANSS: Positive And Negative Syndrome Scale, CAN: Camberwell Assessment of Needs, GAF: Global Assessment of Functioning.
* Significant differences were found between cannabis-using and non-using patients in change in positive symptoms ($F=4.35$; $df=42, 1$; $p=0.043$) and change in negative symptoms ($F=6.08$; $df=42, 1$; $p=0.018$) over five years.

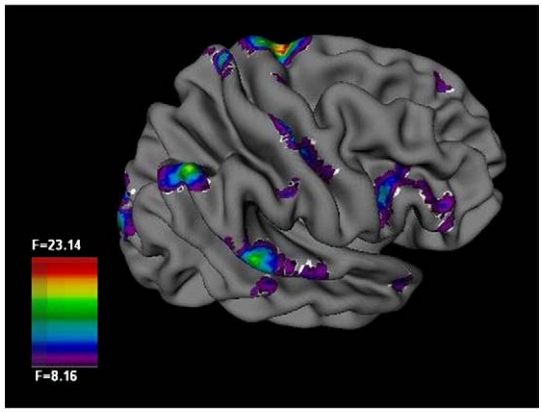

Figure 1 Lateral view of the right hemisphere of the statistical map comparing patients with schizophrenia and healthy comparison subjects on cortical thickness change over five years after FDR correction. Colored areas indicate the areas where the cortical thickness was significantly decreased in first-episode patients with schizophrenia (in red see right supplementary motor cortex).

Fig. 1 shows the statistical difference map of the cortical thickness change over time in the patient group, corrected for the effect of cannabis use, as compared to the healthy comparison subjects at a corrected threshold of $F>8.16$ ($p<0.005$; FDR corrected at alpha=0.10). Cortical thinning in patients was most apparent in the right supplementary motor cortex (SMC), right inferior frontal cortex, right superior temporal gyrus and angular gyrus, right occipital lobe (cuneus) and right parietal lobe (postcentral gyrus) (see Table 2).

Fig. 2 shows statistical difference maps of the cortical thickness change over five years in the cannabis-using patients as compared to the non-using patients at a corrected threshold of $F>9.2$ ($p<0.003$; FDR corrected at alpha=0.10). Cortical thinning was most prominent in patients who used cannabis during the scan interval compared with patients who did not use cannabis in the left dorsolateral prefrontal cortex (DLPFC); in the left anterior cingulate cortex (ACC) and in the left occipital lobe (see Table 2). Figs. 3 and 4 show cortical thickness changes (10^{-2} mm) over time, over the right (A) and left (B) hemisphere respectively in first-episode schizophrenia patient vs. healthy comparison subjects (Fig. 3A and B) and in cannabis-using patients vs. non-using patients (Fig. 4A and B).

Table 2 Difference in cortical thickness change in the peak (most significant) vertex over five years in patients vs. healthy comparison subjects and in cannabis-using schizophrenia patients vs. non-using patients.

	Difference (in mm)	*df*	*F*	*p*
Right SMC	−0.0622	81, 4	23.14	7.3×10^{-6}
Right IFC	−0.0203		14.18	3.2×10^{-4}
Right OL	−0.0146		18.06	5.8×10^{-5}
Right STG	−0.0335		19.32	3.4×10^{-5}
Right AG	−0.0271		17.44	7.6×10^{-5}
Right PL	−0.033		18.44	5.1×10^{-5}
Left DLPFC	−0.0292		17.6	7.2×10^{-5}
Left ACC	−0.0221		18.78	4.4×10^{-5}
Left OL	−0.0256		18.79	4.4×10^{-5}

SMC: supplementary motor cortex; IFC: inferior frontal cortex; OL: occipital lobe; STG: superior temporal gyrus; AG: angular gyrus; PL: parietal lobe; DLPFC: dorsolateral prefrontal cortex, ACC: anterior cingulate cortex, OL: occipital lobe.

In the patient group, a negative correlation was found between change in the negative symptoms and changes in the DLPFC ($B=-0.17$; $t=-2.06$; $p=0.05$) and in the occipital lobe ($B=-0.167$; $t=-2.78$; $p=0.01$).

Finally, two patients with alcohol abuse during follow-up were included in each patient group (can+ and can−). The exclusion of these patients did not influence the results of the main analysis.

6. Discussion

This five year longitudinal study investigated differences in cortical thickness change in 19 first-episode schizophrenia patients who used cannabis during the scan interval, 32 first-episode schizophrenia patients who did not and 31 cannabis-naïve healthy comparison subjects. We found that while the three groups did not differ in cortical thickness at baseline, after controlling for cannabis use, relative to controls schizophrenia patients showed excessive thinning of the right supplementary motor cortex (SMC), right inferior frontal cortex, right superior temporal gyrus and angular gyrus, right occipital lobe (cuneus) and parietal lobe (postcentral gyrus). In patients who used cannabis additional excessive thinning was found in the left dorsolateral prefrontal cortex (DLPFC), left anterior cingulate cortex (ACC) and left occipital lobe as compared to those patients that did not use cannabis during the scan interval. These findings suggest that the excessive thinning of the left DLPFC, ACC and the cortex of the occipital lobe are probably related to the use of cannabis.

Importantly, these findings could not be explained by differences in cortical thickness at baseline. As mean cortical thinning was significantly more pronounced in patients as compared to controls, this might explain the excessive loss of gray matter volume in these patients. However, no mean cortical thinning was found in cannabis-using patients as compared to non-using patients.

Although our study is the first to examine the relationship between cannabis use and cortical thickness change over time in schizophrenia, our results are consistent with two previous cross-sectional MRI studies, in a comparable number of subjects as our sample, reporting grey matter deficits in the posterior (Bangalore et al., 2008) and anterior cingulate cortex (Szeszko et al., 2007) in cannabis-using first-episode schizophrenia patients as compared to non-using patients and healthy subjects. In addition, functional MRI studies have reported an association between exposure to cannabis and changes in brain activity in the prefrontal cortex and anterior cingulate in healthy subjects (for review see Martin-Santos et al., 2009; Quickfall and Crockford, 2006). Also consistent with our findings, exposure to cannabis has been associated with cognitive impairments via altered neural transmission in the prefrontal cortex (for a review see Egerton et al., 2006). Since dysfunction of the DLPFC (Baare et al., 1999; Gur et al., 2000; Kuperberg and Heckers, 2000; Wible et al.,

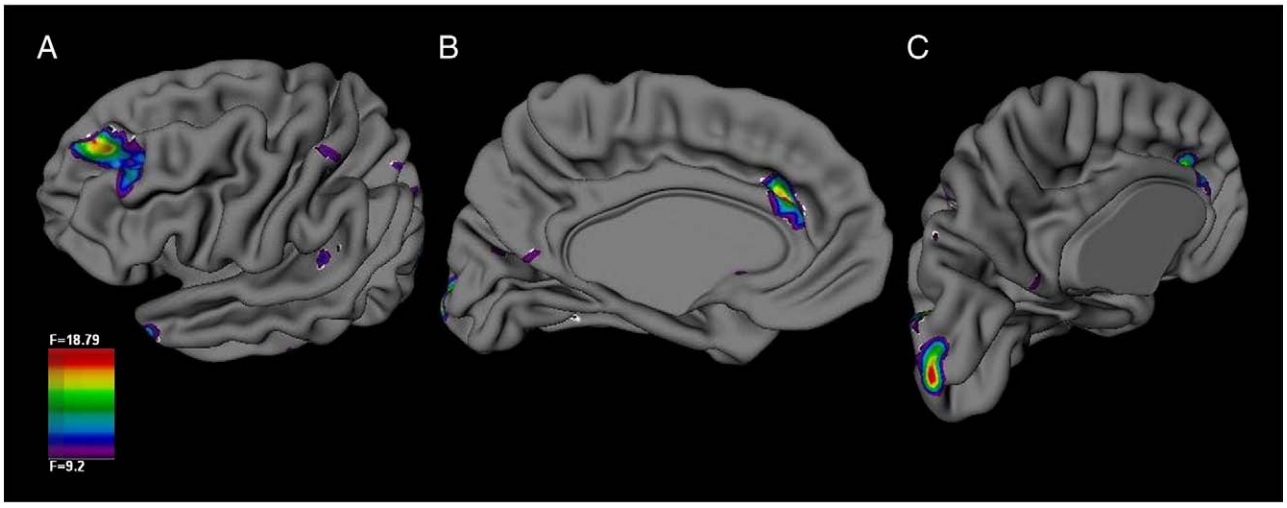

Figure 2 View of the left hemisphere of the statistical map comparing cannabis-using patients and non-using patients with schizophrenia on cortical thickness change over five years after FDR correction. Colored areas indicate the areas where cortical thickness was significantly decreased in cannabis-using patients. A) Lateral view: left dorsolateral prefrontal cortex; B) medial view: left anterior cingulate cortex; C) posterior view: left occipital lobe.

2001) and ACC (Szeszko et al., 2000) have been found to be related to the negative symptoms and cognitive impairment in schizophrenia, the cortical thinning in the DLPFC and ACC in the cannabis-using patients may be functionally relevant. Indeed, we found less improvement in the negative symptoms in the cannabis-using patients as compared to those who did not use cannabis. Moreover, change in the negative symptoms was associated with changes in the dorsolateral prefrontal cortex and in the occipital lobe suggesting that improvement of the negative symptoms over time was associated less loss of thickness in the DLPFC and occipital lobe. These data suggest an association between

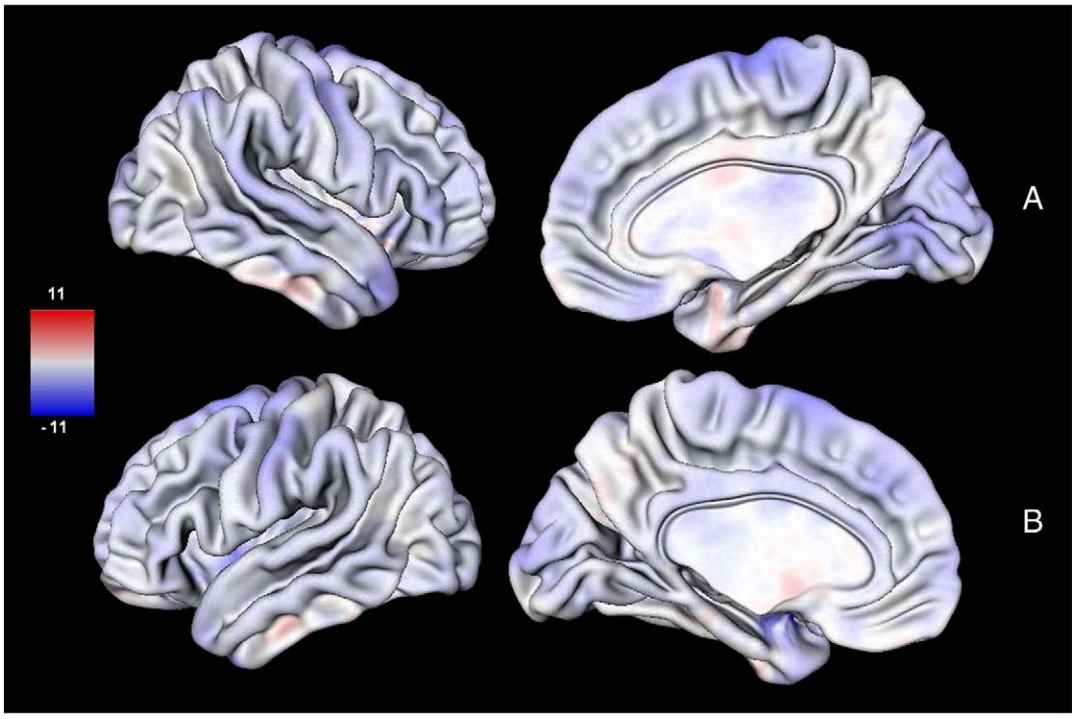

Figure 3 View of the right (A) and left (B) hemisphere comparing patients with schizophrenia and healthy comparison subjects on cortical thickness change (10^{-2} mm) over five years. Blue areas indicate the areas of cortical thinning over time in first-episode patients with schizophrenia relative to controls. Red areas are areas showing excessive thickening in patients relative to controls.

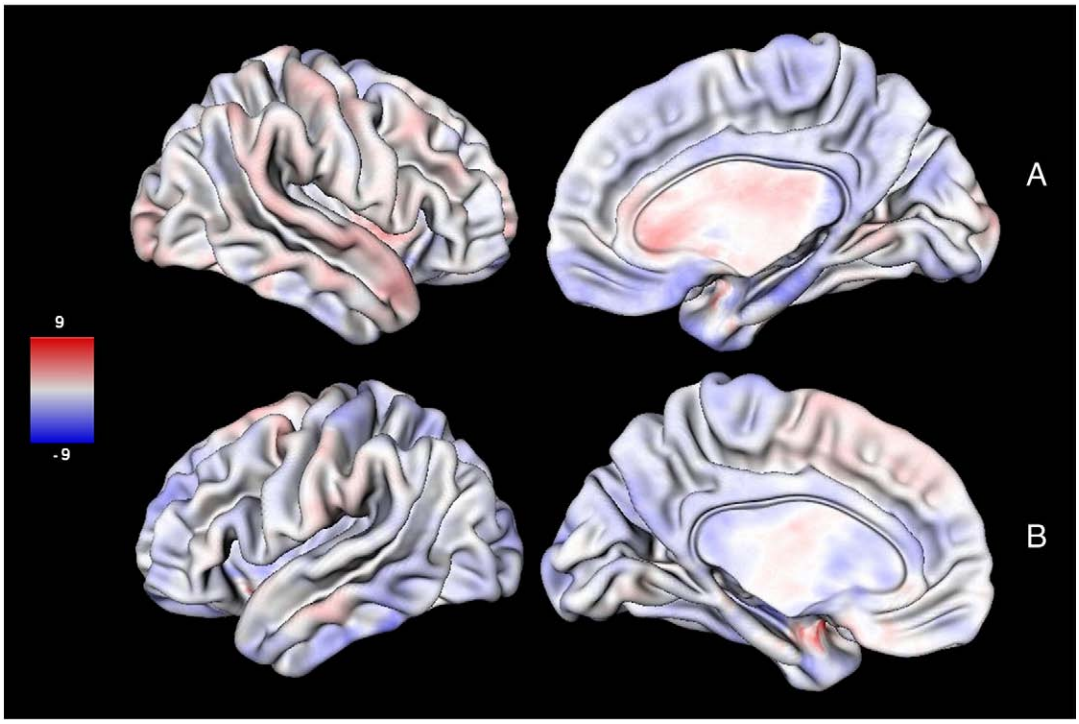

Figure 4 View of the right (A) and left (B) hemisphere comparing cannabis-using patients and non-using patients with schizophrenia on cortical thickness change (10^{-2} mm) over five years. Blue areas indicate the areas of cortical thinning over time in cannabis-using patients relative to non-using patients with schizophrenia. Red areas are areas showing excessive thickening in cannabis-using compared to non-using patients.

more pronounced negative symptoms and thinner cortex in these areas, irrespective of the use of cannabis. Unfortunately, we did not examine cognitive function in this sample.

The mechanism by which cannabis might be related to excessive cortical thinning in schizophrenia patients remains unclear. It could either be a direct consequence of cannabis intake or occur as a consequence of (psychotic) symptoms that have been found to be associated with cannabis use (Bersani et al., 2002; Buhler et al., 2002; Dubertret et al., 2006; Grech et al., 2005; Mauri et al., 2006).

Interestingly, increased cerebrospinal fluid (CSF) levels of endogenous cannabinoids have been reported in patients with schizophrenia suggesting a possible role for (changes in the) endocannabinoid signaling system in the pathogenesis of schizophrenia (Koethe et al., 2009; Leweke et al., 1999, 2007). Moreover, in post-mortem studies both DLPFC and ACC have not only been identified as being rich in cannabinoid (CB1) receptors in the brains of healthy individuals (Eggan and Lewis, 2007; Freund et al., 2003; Glass et al., 1997; Iversen, 2003) but also show increased density of these receptors in brain tissue of schizophrenia patients, irrespective of cannabis use (Dean et al., 2001; Zavitsanou et al., 2004). It has previously been hypothesized (Freedman, 2008) that the brain tissue loss due to cannabis use in schizophrenia patients (Rais et al., 2008) is a consequence of the CB1 receptors no longer protecting the brain against excitotoxic events. Indeed, CB1 receptors, when physiologically activated via endogenous cannabinoids are thought to protect the brain from excitotoxic injuries (Kim et al., 2006; Marsicano et al., 2003). However, while endogenous cannabinoids play a role in the physiological regulation of the neural activity in the PFC, exogenous cannabinoids might disrupt the physiological neural transmission in the PFC via the non-specific activation of the CB1 receptors (for a review see Egerton et al., 2006). Thus, a desensitization of the CB1 receptor by exogenous cannabinoids might lead to further loss of inhibition and consequently impair the neuroprotective effect of the endocannabinoid system. In fact, recent studies in animals demonstrated that stimulation of CB1 receptors enhance the glutamatergic and dopaminergic transmission in the prefrontal cortex via the reduction of GABA transmission (Pistis et al., 2002; for a review see Egerton et al., 2006), thereby increasing brain activation of glutamate and dopamine. Interestingly, individuals with schizophrenia might be particularly vulnerable to excitotoxic damage, especially in the DLPFC and ACC. Not only are these regions particularly rich in CB1 receptors in individuals with schizophrenia (Dean et al., 2001; Zavitsanou et al., 2004), a diminished inhibitory function of the GABAergic system in the DLPFC and ACC (Hashimoto et al., 2008) and a higher level of baseline activation in the DLPFC have also been reported in schizophrenia patients as compared to healthy controls (Tregellas et al., 2007, 2009).

Evidence for a direct effect of cannabis on the brain is also provided by studies reporting raised serum concentrations of Nerve Growth Factor (NGF) (Jockers-Scherubl et al., 2003) and Brain Derived Neurotrophic Factor (BDNF) (Jockers-Scherubl et al., 2004) in cannabis-using schizophrenia patients. Since NGF and BDNF are released as a consequence of neuronal damage, it was speculated that the higher levels

of these neurotrophins were a sign of cannabis-induced neurotoxicity in schizophrenia patients.

Alternatively, the excessive thinning in the cannabis-using patients could be explained as an indirect consequence of cannabis use. It has been suggested that brain changes in the early stages of schizophrenia are the result of the "toxic" effect of the psychotic state (Lieberman et al., 2001), and it is well known that cannabis-using patients have a poorer clinical outcome as compared to non-using patients (Bersani et al., 2002; Buhler et al., 2002; Caspari, 1999; Dubertret et al., 2006; Grech et al., 2005; Linszen et al., 1994; Mauri et al., 2006 Baeza et al., 2009; Gonzalez-Pinto et al., 2009). Evidence that this is possibly causally related to the effects of cannabis is provided by the finding that the clinical and functional outcome improves in those patients who cease cannabis use after illness onset (Baeza et al., 2009; Gonzalez-Pinto et al., 2009). Indeed, in our study the cannabis-using patients showed less improvement of positive and negative symptoms over five years as compared to the non-using patients. In other words, during the scan interval cannabis-using patients probably have been in a psychotic state longer than non-using patients and consequently may show larger decreases in brain volume over time.

Whether or not brain volume abnormalities are the consequence of antipsychotic medication intake is controversial (for a review see Navari and Dazzan, 2009). In our study both patient groups were matched on amount and type of medication used during the scan interval. Thus, it is unlikely that the cortical thinning in the cannabis-using patients might be related to the effect of antipsychotic medication.

Finding cortical thinning in the occipital lobe in cannabis-using patients as compared to non-using patients was unexpected and it has not been reported in previous studies on cannabis-using subjects. Nevertheless two previous cross-sectional studies reported cortical thinning of occipital regions in chronic (Kuperberg et al., 2003) and first-episode (Narr et al., 2005b) schizophrenia patients.

The excessive cortical thinning in the supplementary motor cortex, inferior frontal cortex, parietal, temporal and occipital lobe of schizophrenia patients relative to controls are in line with previous reports of cortical thinning in childhood onset (Greenstein et al., 2006; White et al., 2003), first-episode (Narr et al., 2005a, 2005b), and chronic (Kuperberg et al., 2003; Nesvag et al., 2008) schizophrenia patients. Also, previous cross-sectional volumetric studies report reduced SMC volume in schizophrenia patients as compared to normal controls (Exner et al., 2006; Suzuki et al., 2005).

Nevertheless, unlike the results reported in most cross-sectional studies in first-episode schizophrenia patients showing cortical thinning in prefrontal, temporal, parietal, occipital and cingulate cortices (Narr et al., 2005a,b), we could not demonstrate cortical thinning at baseline in patients as compared to healthy subjects. However, these differences in the results might probably be attributed to differences in sample size.

Some limitations need to be addressed. First, the number of subjects included was limited as a consequence of including only first-episode schizophrenia patients who used only cannabis and no other drugs. Secondly, based on our previous findings (Rais et al., 2008) of global loss of grey matter volume in the same sample, we chose to adjust for multiple comparisons with a one-tailed test. However, a global loss of grey matter volume does not completely exclude the possibility of finding local cortical thickness increases. Nevertheless, in our sample, there were no significant focal cortical thickness increases neither in patients as compared with healthy subjects neither in cannabis-using patients as compared with non-using patients. Thirdly, our study could not address the direction of causality and cannot therefore show whether a direct effect of cannabis use is causing the excessive cortical thinning or if those patients with excessive cortical thinning are more vulnerable to continue cannabis use. Moreover, since a healthy comparison cannabis-using group was not included, it remains unclear whether the cortical thinning in the DLPFC and ACC is a consequence of cannabis use per se or of the interaction between cannabis and schizophrenia. However, the higher density of CB1 receptors in the DLPFC and ACC reported in schizophrenia patients (Dean et al., 2001; Zavitsanou et al., 2004) might suggest that these regions might be particularly vulnerable to the effect of cannabis. Moreover, in our sample, the areas showing cortical thinning in schizophrenia patients irrespective of cannabis use are dissimilar to those that are related to the use of cannabis. This suggests that the effect of cannabis on the brain of schizophrenia patients is distinct from that of the illness itself.

Although results of previous sMRI studies in healthy subjects using cannabis have been contradictory (for review see Martin-Santos et al., 2009; Quickfall and Crockford, 2006) a recent region of interest study reported dose-related hippocampal and amygdala structural abnormalities in long-term healthy cannabis users, suggesting a possible direct neurotoxic effect of cannabis on the healthy human brain (Yucel et al., 2008). However, it cannot be excluded that the subjects with lower grey matter volume in these areas were also those more prone to use cannabis. Finally, no information could be provided regarding a dose–response relationship between delta-9-tetrahydrocannabinol (THC) intake and thinning of the cerebral cortex as this information was not available.

In conclusion, this study found progressive cortical thinning in the DLPFC and ACC, areas rich in CB1 receptors, in cannabis-using schizophrenia patients, but not in patients who did not use cannabis. Our results suggest that in the first-episode schizophrenia patients who continue to use cannabis after illness onset, it is particularly those cortical regions that are rich in CB1 receptors that are vulnerable to excessive cortical thinning. Interestingly, it is also these areas that are related to the negative symptoms and to poorer cognitive functioning in schizophrenia, providing a morphological explanation for the detrimental effects of cannabis in schizophrenia.

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None.

Contributors

Dr. M. Rais was involved in the design of the study, managed literature searches participated in the data analysis and wrote the first draft of the paper. Dr. N. van Haren was involved in the design

of the study, supervised the data analysis and assisted in the writing of the paper. Dr. W. Cahn was involved in the design of the study, and assisted in the writing of the paper. Dr. H. Schnack participated in the study's planning, analysis and supervised and participated in the developing of part of the brain imaging techniques. Dr. C. Lepage participated in the developing of part of the brain imaging techniques. Dr. L. Collins participated in the developing of part of the brain imaging techniques. Prof. A. Evans participated in the developing of part of the brain imaging techniques. Prof. H. Hulshoff Pol participated in the design of the study and supervised the statistical analysis. Prof. R. Kahn participated in the design of the study, supervised the statistical analysis and the writing of the paper. All authors contributed to the study and have approved the final draft of the manuscript.

Conflict of interest

Dr. N. Van Haren has received honoraria for education programmes for AstraZeneca, Eli Lilly, Janssen-Cilag. Dr. W. Cahn has received grants, honoraria for education programmes or served as consultant for: Eli Lilly, AstraZeneca, Bristol-Myers Squibb, Janssen-Cilag, Sanofi-Aventis, Lundbeck, Schering-Plough. Prof. H. Hulshoff Pol has received honoraria for education programmes for Ferris and Lundbeck. Prof. R. Kahn has received grants, honoraria for education programmes or served as consultant for Astellas, AstraZeneca, BMS, Dainippur, Eli Lilly, GSK, Johnson & Johnson, Janssen-Cilag, Pfizer, Roche and Sanofi-Aventis. All other authors declare that, except for income received from their primary employer, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional service and there are no personal financial holdings that could be perceived as consulting a potential conflict of interest.

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