Diffusion tensor imaging in the early phase of schizophrenia
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Abstract

Adolescent-onset cannabis use, compared with adult-onset use, has been associated with a higher risk for developing symptoms of schizophrenia-like psychotic disorders. To test the hypothesis that onset of cannabis use in early adolescence in male schizophrenia patients is associated with abnormalities in white matter structure and integrity, we used high resolution structural and diffusion tensor brain images to compare three groups of patients: those who started regular use of cannabis (1) before the age of 15 years (early-onset cannabis users, n=10) or (2) at the age of 17 years or later (late-onset cannabis users, n=8), and (3) those who were cannabis naïve (n=8). To verify patient findings, we also compared white matter integrity of the three patient groups with that of a healthy control group (n=10). Cannabis naïve patients showed reduced white matter density and reduced fractional anisotropy, an indicator for white matter integrity, in the splenium of the corpus callosum compared with patients with early-onset cannabis use. In the same brain area, cannabis naïve patients showed reduced fractional anisotropy compared with healthy controls. Our results suggest that the age of onset of cannabis use is not an identifying characteristic for white matter abnormalities in schizophrenia patients; however, our results might indicate a more vulnerable brain structure in cannabis naïve schizophrenia patients.
3.2.1 Introduction

Cannabis is one of the most commonly used substances in patients presenting to psychiatric services with their first episode of schizophrenia (Hambrecht and Hafner, 1996; VanMastrigt et al., 2004; Barnes et al., 2006). Cannabis use is known to be related to an earlier age of onset of psychotic disorders (Veen et al., 2004; Barnes et al., 2006; Gonzalez-Pinto et al., 2008), and to an increased risk of developing psychotic outcomes independently of confounding and transient intoxication effects (Moore et al., 2007). Stefanis et al. (2004) have shown that early-onset cannabis use conferred the greatest risk for developing positive and negative dimensions of psychosis. Caspi et al. (2005) reported that adolescent-onset cannabis use, compared with later use, is associated with a higher risk of developing symptoms of schizophrenia in carriers of the COMTval158 allele. One explanation for this increased risk could be that individuals who start using cannabis during pubertal brain development are most vulnerable to its deleterious effects (Ehrenreich et al., 1999; Pope et al., 2003; Schneider and Koch, 2003; Schneider, 2008). Schneider and Koch (2003) suggested that the endogenous cannabinoid system is highly susceptible to the effects of cannabinoid administration during pubertal development. In addition to this, there is evidence that a dysregulation in the endogenous cannabinoid system is associated with the pathogenesis of schizophrenia (Dean et al., 2001; Leweke et al., 1999; Giuffrida et al., 2004). However, a clear biological explanation for the increased risk for psychosis in individuals who use cannabis in adolescence is not yet available (DeLisi, 2008). Some MRI studies in non-psychotic cannabis users have examined the effect of early-onset cannabis use on brain structure. DeLisi et al. (2006) suggested that the establishment of new cortical connections and growth of axons that normally occur during adolescence could be disrupted by frequent cannabis use; however, diffusion tensor imaging (DTI) findings in non-psychotic individuals were not conclusive.

In line with DeLisi’s study, Tzilos et al. (2005) investigated the effects of early cannabis use on brain morphology in non-psychotic individuals and reported no significant differences in measures of brain volume between groups with different ages of onset of cannabis use. However, Wilson et al. (2000) reported early-onset cannabis users to have smaller whole brain, smaller percent gray matter (GM) and larger percent white matter (WM) volumes compared with late-onset cannabis users.

Some MRI studies investigated the effects of cannabis use on brain morphology in patients with schizophrenia (Cahn et al., 2004; Szaszko et
In schizophrenia, abnormalities in WM connectivity have been reported as a crucial neurobiological marker, which is thought to arise from myelin-related and oligodendroglia dysfunction (Davis et al., 2003). In schizophrenia patients, compared with healthy controls, several DTI studies reported reduced fractional anisotropy (FA) in prefrontal and temporal lobes, connecting fibres, and the corpus callosum. However, results from DTI studies are not conclusive (Kanaan et al., 2005). To date, no DTI study reported on schizophrenia patients using cannabis. To test the hypothesis that cannabis use during adolescence may disrupt WM integrity in patients with schizophrenia, we compared WM structure and FA – employing optimized voxel-based morphometry (VBM) – in early and late-onset cannabis users, and cannabis naïve patients with recent-onset schizophrenia. Cannabis use before the age of 15 was considered early-onset cannabis use. Cannabis use at age 15 or later was considered as late-onset cannabis use, as neurodevelopment, including development of the dopamine and endocannabinoid system, undergoes major maturation in puberty and is largely concluded by the age of 16 (Sundram, 2006; Shaw et al., 2006; Schneider, 2008). Cannabis use before 15 years of age might therefore have a different impact on brain structure and function than cannabis use at age 17 or later. Adverse cognitive consequences of cannabis use in humans before the age of 17 have already been reported (Ehrenreich et al., 1999; Pope et al., 2003).

To verify patient findings, we also compared white matter integrity of the patients groups with those of healthy controls.

### 3.2.2 Materials and methods

#### Subjects and clinical measures

We included 26 male patients with a Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV; American Psychiatric Association, 1994) diagnosis of schizophrenia from the open-ward inpatient and day-care units of the Adolescent Clinic of the Academic Medical Centre, University of Amsterdam. The clinic is specialized in the treatment...
Clinical diagnoses of patients were made according to DSM-IV criteria by two psychiatrists with the use of all available information, including a patient history provided by a close relative or friend (most often the parents) at admission (Longitudinal Expert Assessment of Diagnosis procedure, Spitzer and Williams, 1995). An estimate of the duration of illness, defined as the time between the start of the first psychotic episode (hallucinations, delusions and/or disorganisation) and MRI scanning, and estimates of total duration of antipsychotic medication were based on a detailed history taken from the patient and the parents, and all available clinical information (including case records and information from previous mental health professionals). The Clinical Global Impression-Severity of Illness (CGI-S; Guy, 1976) was used for assessing the severity of the disease (state), and the Global Assessment of Functioning (GAF; American Psychiatric Association, 1994) was used for assessing psychosocial functioning.

Information about substance use, including cannabis use, was retrieved with the use of a detailed history taken from the patient on present and lifetime substance use and frequency of use. Further, we used all available clinical information on substance use, such as case records, information from parents and information from previous mental health professionals.

Patients were included in the study if they had either started the regular use of cannabis before the age of 15 years (early-onset cannabis users, n=10), if they had started the regular use of cannabis at the age of 17 years or later (late-onset cannabis users, n=8), or if they had never used cannabis (cannabis naïve patients, n=8).

Patients eligible for the early and late-onset cannabis groups were included in the study if their cannabis use consisted of at least weekly use during at least 6 months of their lives. All patients were receiving antipsychotic neuroleptics and chlorpromazine equivalents were calculated (Woods, 2003). Male healthy control subjects were recruited through local advertisements and were matched for age.

Exclusion criteria for all subjects were a history of a demonstrable neurological or endocrine disease that might affect brain structure, and a history of a head trauma with loss of consciousness for more than 15 min. Additional exclusion criteria for healthy controls were as follows: a personal or family history of a major psychiatric illness, or a lifetime diagnosis of alcohol or other substance abuse or dependence. After complete
description of the study, written informed consent was obtained from all participants. The study was approved by the human subject review board of our institution.

For baseline demographics and clinical variables, see Table 3.2.1.

There were no significant patient group differences in age at onset of schizophrenia, age at time of scanning, duration of illness, duration of antipsychotic medication, previous hospitalization for psychosis, GAF and CGI score.

Of the 10 patients in the early-onset group, eight had used cannabis on a daily basis for 1 or more years during adolescence. The other two patients from the early-onset group had, respectively, used cannabis 2–3 times a week for several years during adolescence, and used cannabis daily to weekly for at least 6 months during adolescence. Of the eight patients in the late-onset group, six had used cannabis on a daily basis for 1 or more years. The other two patients from the late-onset group were daily heavy users for at least 6 months of their lives. All patients from the early and late-onset groups received a DSM-IV diagnosis of cannabis use disorder, of which some were in remission; in the early-onset group, three patients had a current diagnosis of cannabis abuse, four patients had a current diagnosis of cannabis dependence, and three patients had a cannabis use disorder in early remission; in the late-onset group, one patient had a current diagnosis of cannabis abuse, six patients had a cannabis use disorder in early remission, and one patient had a cannabis use disorder in sustained remission. Two patients in the early-onset group had a DSM-IV diagnosis of alcohol use disorder (alcohol abuse \(n=1\) and alcohol dependence \(n=1\)), and four patients in the late-onset group had a DSM-IV diagnosis of alcohol abuse disorder in remission. One patient in the early-onset group had a DSM-IV diagnosis of cocaine abuse.

As expected, the groups differed in education years \(F=16.65; \text{df}=3; P<0.0001\) with healthy controls having on average more years of education than the patient groups.

**Neuroimaging protocol**

All participants were scanned with a 3Tesla TMRI system (Philips Medical System, Best, The Netherlands). Individuals were introduced to the scanning procedure before assessment. All images were acquired in the same session.
<table>
<thead>
<tr>
<th></th>
<th>Schizophrenia Patients</th>
<th>Healthy Controls</th>
<th>$F$</th>
<th>$\chi^2$</th>
<th>$FE$</th>
<th>df</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early-onset cannabis use</td>
<td>Late-onset cannabis use</td>
<td>Cannabis naive</td>
<td>$F$</td>
<td>$\chi^2$</td>
<td>$FE$</td>
<td>df</td>
</tr>
<tr>
<td>Age (years)</td>
<td>20.9 (2.9)</td>
<td>22.2 (2.3)</td>
<td>20.4 (2.3)</td>
<td>21.1 (2.8)</td>
<td>0.62</td>
<td>3</td>
<td>0.61</td>
</tr>
<tr>
<td>Education (years)</td>
<td>10.1 (1.5)</td>
<td>10.5 (0.9)</td>
<td>10.8 (1.2)</td>
<td>13.8 (1.5)</td>
<td>16.65</td>
<td>3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age of disease onset (years)</td>
<td>18.6 (3.0)</td>
<td>20.2 (1.7)</td>
<td>19.3 (2.5)</td>
<td>-</td>
<td>0.93</td>
<td>2</td>
<td>0.41</td>
</tr>
<tr>
<td>Duration of illness (years)</td>
<td>2.3 (2.1)</td>
<td>1.9 (1.5)</td>
<td>1.2 (0.7)</td>
<td>-</td>
<td>1.17</td>
<td>2</td>
<td>0.33</td>
</tr>
<tr>
<td>Number of previous hospitalizations for psychosis</td>
<td>0.60 (0-2)</td>
<td>0.9 (0-1)</td>
<td>0.5 (0-2)</td>
<td>-</td>
<td>2.31</td>
<td>2</td>
<td>0.32</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>8 (80%)</td>
<td>7 (87.5%)</td>
<td>5 (62.5%)</td>
<td>-</td>
<td>4.54</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>Schizophrenia, paranoid type</td>
<td>1 (10%)</td>
<td>1 (12.5%)</td>
<td>3 (37.5%)</td>
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</tbody>
</table>
Table 3.2.1: continued

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Schizophrenia Patients</th>
<th>Healthy Controls</th>
<th>F</th>
<th>χ²</th>
<th>FE</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early-onset cannabis use (n=10)</td>
<td>Late-onset cannabis use (n=8)</td>
<td>Cannabis naive (n=8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Antipsychotic medication at time of scanning</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Atypical</td>
<td>7</td>
<td>6</td>
<td>8</td>
<td>-</td>
<td>2.79</td>
<td>2</td>
<td>0.32</td>
</tr>
<tr>
<td>Classical</td>
<td>3</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medication dose (chlorpromazine equivalents, mg/day)</td>
<td>260 (110)</td>
<td>336 (166)</td>
<td>355 (178)</td>
<td>-</td>
<td>1.02</td>
<td>2</td>
<td>0.38</td>
</tr>
<tr>
<td>Duration antipsychotic medication (weeks)</td>
<td>54.3 (3-208)</td>
<td>25.8 (16-39)</td>
<td>44.8 (8-103)</td>
<td>-</td>
<td>1.59</td>
<td>2</td>
<td>0.45</td>
</tr>
<tr>
<td>Characteristics positive symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delusions</td>
<td>10 (100%)</td>
<td>8 (100%)</td>
<td>6 (75%)</td>
<td>-</td>
<td>3.31</td>
<td>2</td>
<td>0.17</td>
</tr>
<tr>
<td>Hallucinations</td>
<td>6 (60%)</td>
<td>7 (87.5%)</td>
<td>5 (62.5%)</td>
<td>-</td>
<td>1.83</td>
<td>2</td>
<td>0.56</td>
</tr>
<tr>
<td>Global Assessment of Functioning</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Mean (sd)</td>
<td>49.5 (9.8)</td>
<td>48.1 (12.2)</td>
<td>40.6 (10.2)</td>
<td>-</td>
<td>1.68</td>
<td>2</td>
<td>0.21</td>
</tr>
<tr>
<td>Clinical Global Scale</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range)</td>
<td>4.9 (4-6)</td>
<td>5.0 (4-6)</td>
<td>5.5 (4-7)</td>
<td>-</td>
<td>2.15</td>
<td>2</td>
<td>0.34</td>
</tr>
<tr>
<td>Age of onset cannabis use (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (range)</td>
<td>12.9 (10-14)</td>
<td>18.3 (17-23)</td>
<td>-</td>
<td>-</td>
<td>-3.63&quot;</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

F = F score ANOVA, χ² = chi-square in Kruskal Wallis test, FE = Fisher’s Exact value

" Z score of Mann-Whitney U test.
**Structural imaging**
3D T1-weighted gradient-echo images served (TR/TE 9.8/4.6 ms; axial orientation; 120 continuous slices; slice-thickness 1.2 mm; flip angle 8°; 224 mm FOV; acquisition matrix 256×256; voxel size 1.20×0.8×0.8 mm) to identify morphometric differences of WM density on a voxel-by-voxel basis and for anatomical localization of FA maps in standard space.

**Diffusion tensor imaging**
DTI data were acquired using 3D multi-slice spin echo single shot echo-planar imaging (EPI) with the following parameters: TR/TE 4831–6248/94 ms, diffusion sensitivities of b=0 and b=1000 s/mm²; and 16 non-collinear directions, each direction being scanned twice. FOV was 230–256 mm (depending on head size), acquisition matrix 256×256, and slice thickness 3 mm no gap. The DTI data were post-processed to create FA value maps. FA maps are a representation of the directionality and density of WM fibre tracts and are an indicator of WM integrity. Additionally, 3D T2-weighted turbo-spin-echo images (TR/TE: 7117/80 ms; axial orientation; 48 continuous (no inter-slice gap) slices; slice thickness 3 mm, 224 mm FOV; acquisition matrix 448×448; acquisition voxel size 0.5×0.5×3 mm) were acquired for an anatomical FA map coregistration and template creation.

**Quantitative neuroimaging analysis and voxel-based comparisons**
All data were processed using SPM2 (Wellcome Department of Cognitive Neurology, London, UK) modified for optimized VBM on a MATLAB platform (The MathWorks Inc., USA; version 7.4). All images were pre-processed and checked for artefacts and image corruption before entering statistical analysis.

We used optimized VBM (Good et al., 2001) implemented in SPM2 (Institute of Neurology, Queen's Square, London (www.fil.ion.ac.uk)), to identify regional differences in WM concentration (density). Automated optimizations (Department of Psychiatry, University of Jena, Germany) in SPM2 were used to spatially normalize and segment all T1-weighted images, based on the customized T1-template. The prior images of GM, WM and cerebrospinal fluid (CSF) were used for segmentation. All standard presets in SPM2 were maintained. For statistical comparison WM segments were smoothed with a 10 mm FWHM isotropic Gaussian ker-
nel, which renders the data more normally distributed to achieve optimal outcome in parametric statistical comparisons.

Also, we used a modified optimized VBM procedure to analyse FA images: We matched structural to DTI images by using the contrast level of the images’ WM map; hence, individual 3D T2-weighted images were used in the process to create a customized template of the b=0 images. The T2-weighted template was created to fit the same standard space as the T1-weighted template, with voxel dimensions of $1 \times 1 \times 1$ mm. All b=0 images were thereafter spatially normalized using the customized (DTI) template. A T2-weighted WM mask was created to remove non-brain tissue from the FA images. The FA images were co-registered (write normalized) to the spatially normalized b=0 image of the corresponding subject. The images were then smoothed with 10 mm FWHM preceding statistical analyses (Eriksson et al., 2001).

**Statistical analysis**

Analysis of variance (ANOVA) was carried out using the SPM2 platform to investigate group differences on a voxel-by-voxel basis for WM segments and FA value maps of the three patient groups. Twoway ANOVA for group comparisons was thresholded in a successive order, starting at (i) $P<0.001$, uncorrected for multiple comparisons with height threshold (t) at $zt=4.02$ at voxel level, with a minimal cluster size (cluster extend threshold at $P<0.001$) of 50 voxels; then, (ii) individual significant clusters ($P<0.05$ (at cluster level, $P_c$)); and (iii) false discovery rate (FDR) and family wise error (FWE) corrections of multiple voxel comparisons were applied. Voxels and clusters were localized using the Montreal Neurological Institute (MNI) space and transformed into Talairach and Tournoux (T&T) co-ordinates. For results in the WM segments, T&T* co-ordinates are given as an indication of the voxel location in a standardized brain. Additionally, resulting voxel and cluster maps of FA images were overlaid on corresponding T1-weighted images for anatomical assessment. To verify our patient findings, we compared FA values of schizophrenia patients in the cannabis naïve, early-onset, and late onset cannabis subgroups with those of healthy controls using the same procedure as above.
3.2.3 Results

**Neuroimaging findings**

**Neuroimaging findings in patient comparisons**

We identified reduced FA values (\(P_{c} < 0.005\), corrected) and WM density (\(P_{c} < 0.009\), corrected) in the left posterior corpus callosum (splenium) in cannabis naïve individuals with schizophrenia, compared with early-onset cannabis users with schizophrenia (Figure 3.2.1, Table 3.2.2). Additionally, cannabis naïve schizophrenia patients, compared with early cannabis users, show significant WM density reduction in the right occipital lobe (\(P_{c} < 0.008\), corrected) and the left temporal (limbic) lobe (\(P_{c} < 0.001\), corrected).

We did not find increased WM density and FA values. We also did not find structural or diffusion differences that survived correction for multiple comparisons in any of the other patient group comparisons.

**Neuroimaging findings in patient-control comparisons**

Cannabis naïve schizophrenia patients, compared with healthy controls, showed significantly (\(P_{c} < 0.03\), corrected) reduced FA values of the left posterior corpus callosum (Table 3.2.2, Figure 3.2.2) and the frontotemporal junction (\(P > 0.001\), uncorrected) (Table 3.2.2).

Early-onset and late-onset cannabis using schizophrenia patients, compared with healthy controls showed significantly (\(P < 0.001\), uncorrected) reduced FA values in the left temporal lobe. These findings are not corrected for multiple comparisons and need to be considered as preliminary (Table 3.2.2).

We did not find increased FA values.

3.2.4 Discussion

Our main findings were that cannabis naïve schizophrenia patients showed brain abnormalities in the splenium of the corpus callosum, compared with early-onset cannabis users. No morphological differences were found between early-onset cannabis users and late-onset cannabis users. Compared with healthy controls, cannabis naïve patients also showed reduced FA values in the splenium. Furthermore, all patient
Chapter 3

Figure 3.2.1: FA differences in cannabis naïve patients, compared to patients with early-onset cannabis use. Coronal view: FA reduction in the splenium of the corpus callosum (Talairach and Tournoux (T&T) co-ordinates: -14, -46, 27).

Figure 3.2.2: Fractional anisotropy value differences in cannabis naïve patients, compared to healthy controls Coronal view: FA reduction in the splenium of the corpus callosum, bilaterally (Talairach and Tournoux (T&T) co-ordinates: -13, -42, 21/ 12, -47, 10).
groups, compared with control subjects, showed reduced temporal lobe FA values; however these temporal lobe findings did not survive a correction for multiple comparisons and should therefore be considered as preliminary findings.

Our findings are partly contrasting with findings of Bangalore et al. (2008). The authors reported brain abnormalities of reduced GM density in the posterior cingulate cortex (PCC) in cannabis using schizophrenia patients compared with cannabis naïve patients. We identified congruent anatomical and diffusion abnormalities manifesting in the posterior corpus callosum, a brain area adjacent to the PCC in cannabis naïve schizophrenia patients. Other MRI studies also investigated the effects of cannabis use on brain morphology in patients with schizophrenia (Cahn et al., 2004; Potvin et al. 2007; Szeszko et al., 2007; Rais et al., 2008). They revealed that in cannabis using schizophrenia patients, GM volume of the anterior cingulate cortex was decreased (Szeszko et al., 2007), and brain volume reduction was more pronounced over a 5-year follow-up (Rais et al., 2008) in comparison with patients with no cannabis use. Potvin et al. (2007) found increased striatal GM densities in schizophrenia patients with substance use disorder (most of them used cannabis) compared with patients without substance abuse and compared with controls. However, Cahn et al. (2004) found no differences in brain structure between schizophrenia patients who did and did not use cannabis. These studies did not specifically focus on patients who used cannabis in adolescence. DeLisi et al. (2006) did focus on adolescent cannabis users, and their findings are in line with our results. The authors reported higher FA values in frequent adolescent cannabis users compared with cannabis naïve individuals. However, FA differences were found in parts of the brain other than the corpus callosum, and they conducted their study in non-psychotic individuals.

Our combined findings of WM density and FA reduction in the splenium of cannabis naïve schizophrenia patients are an indicator for reduced WM integrity in this part of the corpus callosum, maybe due to a disruption of axonal integrity, or myelin dysfunction (Beaulieu, 2002). Lower FA values in the splenium may have a neurodevelopmental origin. In our group of cannabis naïve patients we also found reduced WM density in the occipital and temporal lobes compared with early-onset cannabis use patients. Inter-hemispheric fibres from the temporal and occipital lobes transverse the splenium (Hofer and Frahm, 2006). It is therefore possible that the lower FA values in the splenium may have resulted from a focal disruption of the cortical neurons or axons of adjacent brain areas.
Table 3.2.2: Neuroimaging findings in cannabis naïve, early onset and late onset cannabis use schizophrenia patients and control subjects.

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>Brain area</th>
<th>Talairach and Tournoux (T&amp;T) Coordinates</th>
<th>T value</th>
<th>P value at voxel level</th>
<th>Pc (corrected for multiple comparisons at cluster level)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients:</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1) cannabis naïve (CN) vs early onset (EO) cannabis users</td>
<td></td>
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</tr>
<tr>
<td>FA value differences (FA)</td>
<td>Left posterior corpus callosum</td>
<td>-14 -46 27</td>
<td>5.53</td>
<td>P &lt; 0.00001</td>
<td>Pc &lt; 0.005</td>
</tr>
<tr>
<td>WM density differences (WM)</td>
<td>Left posterior corpus callosum</td>
<td>-13 -46 28</td>
<td>5.34</td>
<td>P &lt; 0.00001</td>
<td>Pc &lt; 0.009</td>
</tr>
<tr>
<td></td>
<td>Right occipital lobe</td>
<td>20 -77 39</td>
<td>5.25</td>
<td>P &lt; 0.00001</td>
<td>Pc &lt; 0.008</td>
</tr>
<tr>
<td></td>
<td>Left temporal lobe</td>
<td>-30 -1 -10</td>
<td>5.21</td>
<td>P &lt; 0.00001</td>
<td>Pc &lt; 0.001</td>
</tr>
<tr>
<td>2) CN vs late onset (LO) cannabis users</td>
<td></td>
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<tr>
<td>FA</td>
<td></td>
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<td>WM</td>
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<td>3) EO vs LO</td>
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<td>FA</td>
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<td>WM</td>
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<tr>
<td><strong>Patients vs controls:</strong></td>
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</tr>
<tr>
<td>1) CN vs Controls (FA)</td>
<td>Left posterior corpus callosum</td>
<td>-13 -42 21</td>
<td>4.98</td>
<td>P &lt; 0.0002</td>
<td>Pc &lt; 0.03</td>
</tr>
<tr>
<td></td>
<td>Right posterior corpus callosum</td>
<td>12 -47 10</td>
<td>4.27</td>
<td>P &lt; 0.0001</td>
<td>Pc &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Left temporal lobe</td>
<td>-27 -14 17</td>
<td>3.59</td>
<td>P &lt; 0.001</td>
<td>-</td>
</tr>
<tr>
<td>2) EO vs Controls (FA)</td>
<td>Left temporal lobe</td>
<td>-31 -3 3</td>
<td>3.67</td>
<td>P &lt; 0.001</td>
<td>-</td>
</tr>
<tr>
<td>3) LO vs Controls (FA)</td>
<td>Left temporal lobe</td>
<td>-59 -39 11</td>
<td>3.69</td>
<td>P &lt; 0.001</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: Fractional anisotropy (FA), White matter (WM), cannabis naïve (CN), early onset cannabis use (EO), late onset cannabis use (LO), P = P value at < 0.001, Pc = P values corrected for multiple comparisons at cluster level, Pc < 0.05, T value = standard deviation score, T&T= Talairach and Tournoux co-ordinates (estimated for WM).
Furthermore, reduced FA values in the splenium of the corpus callosum have been reported in a number of DTI studies in patients with schizophrenia (Foong et al., 2000; Agartz et al., 2001; Ardekani et al., 2003; Rotarska-Jagiela et al., 2008). In line with these findings, morphological, electrophysiological and neuropsychological studies suggest that callosal connections are altered in patients with schizophrenia (Innocenti et al., 2003; Arnone et al., 2008).

Increased FA values have been found in schizophrenia patients who experience auditory hallucinations (Hubl et al., 2004; Shergill et al., 2007; Seok et al., 2007). Regions with higher FA were the anterior corpus callosum, the arcuate fasciculus, the superior longitudinal fasciculus, and the anterior cingulum. However, in our study auditory hallucinations were experienced by an equal amount of patients across the patient groups.

The results presented here are preliminary in nature since our sample size is limited and the study has an exploratory character; however, our findings may indicate that cannabis naïve individuals who develop schizophrenia have a more vulnerable brain structure compared with that of cannabis users who develop the disease. Cannabis use before the age of 15 may be an environmental trigger and interact as a partial causal risk factor with a less vulnerable brain. Early cannabis users who develop schizophrenia might belong to a patient subgroup that is (in someways) different from a patient group that never used cannabis and developed schizophrenia. Some reports have already suggested that cannabis abusers belong to a patient subgroup with better pre-morbid adjustment (Arndt et al., 1992), fewer negative symptoms (Bersani et al., 2002), and better neurocognitive function (Stirling et al., 2005; Coulston et al., 2007). On the other hand, several studies reported that co-morbid cannabis abuse was associated with poor outcome of schizophrenia with respect to relapse and exacerbation (Linszen et al., 1994; Martinez-Arevalo et al., 1994; Grech et al., 2005), poorer compliance with antipsychotic medication and rate of employment (Buhler et al., 2002), and poorer psychosocial functioning (Caspari, 1999).

Future studies relating neuroimaging findings to variation in susceptibility risk for schizophrenia are needed to test our hypothesis.

When interpreting our findings, certain limitations have to be considered: we only included male individuals in our study, and our sample size was relatively small. However, our group was homogeneous for age at time of MRI scanning, diagnosis, and disease duration. Another limitation is that we had to rely on a cross-sectional study design. It would be of interest to investigate the development of white matter structure
and integrity differences in cannabis naïve patients and those who use cannabis.

Furthermore, we had to rely on the patient’s self-reports of cannabis use. Nevertheless, these self-reports are likely to be a valid since the Dutch drug policy is liberal and cannabis use is discussed freely. However, the reports of cannabis use may have been prone to recall bias.

Another shortcoming of this study is that we have not included subjects with cannabis use disorder who do not have schizophrenia. The question whether WM structure and integrity in patients with cannabis use is changed because of cannabis use or because of having a dual diagnosis remains unanswered. Future brain-imaging studies focussing on substance use in schizophrenia patients should consider this issue. Lastly, since some patients in the early-onset group had a current DSM-IV diagnosis of alcohol abuse (n=1), alcohol dependence (n=1), or cocaine misuse (n=1), and four patients in the late onset group had a diagnosis of alcohol abuse disorder in remission, it is possible that substance use other than cannabis use could have accounted for part of the findings. However, both alcohol and cocaine use have been associated with decreased FA values (Sullivan and Pfefferbaum, 2005; Moeller et al., 2007). In our study, we did not find evidence for decreased FA values in the group of early-onset cannabis users, so it is unlikely that alcohol and cocaine use can explain the difference in FA between cannabis naïve patients and early-onset cannabis use patients. Still, for a better understanding of the effects of cannabis use on WM structure, future studies should preferably exclude patients with other substance abuse.

In conclusion, our results may suggest that the age of onset of cannabis use is not identifying for WM abnormalities in schizophrenia patients, or otherwise our results might indicate a more vulnerable brain structure in cannabis naïve schizophrenia patients. Further studies with a longitudinal design and studies integrating neuroimaging and gene-environment interaction are necessary to put our results in perspective.

### 3.2.5 References


