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Recent-Onset Schizophrenia and Adolescent Cannabis Use: MRI Evidence for Structural Hyperconnectivity?

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ABSTRACT *~There is growing evidence that brain white matter abnormalities are implicated in the pathophysiology of schizophrenia. Cannabis use is an independent risk factor for schizophrenia. We tested the hypothesis that cannabis use during early adolescence is associated with white matter abnormalities in schizophrenia patients. Thirty-five male recent-onset schizophrenia patients, with and without a history of cannabis use before age 17, and twenty-one matched healthy comparison men without illicit drug use were assessed with diffusion tensor imaging (DTI). White matter regions of interest were examined in co-registered DTI images. Compared to controls, patients with cannabis use before age 17 showed increased directional coherence in the bilateral uncinate fasciculus, anterior internal capsule and frontal white matter. These abnormalities were absent in patients without cannabis use before age 17. The abnormalities were not related to lifetime doses of cannabis or other illicit drugs. We could not exclude confounding effects of other illicit drugs. Recent-onset schizophrenia patients with start of cannabis use during early adolescence use may represent a subgroup of schizophrenia patients with increased white matter directional coherence, which may reflect structural hyperconnectivity. This is in contrast with most DTI studies in schizophrenia, which have produced evidence for hypoconnectivity. Further studies are necessary to assess the effect of adolescent cannabis and other illicit drug use on brain white matter in schizophrenia.* Psychopharmacology Bulletin. *2009;42(2):75–88.*

INTRODUCTION

It has been proposed that schizophrenia is a progressive neurodevelopmental disorder, which can be formulated in a 'three hit' model.[1,2] The 'first hit' may be

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abnormal brain development in utero caused by genetic vulnerability together with pre- or perinatal insults, and the 'second hit' may be altered brain development during adolescence caused by environmental factors such as cannabis use and/or psychosocial stress. These two hits would together cumulate into a first psychotic episode, most often in late adolescence or early adulthood. The third hit may be a neurotoxic process during recurrent psychotic episodes after illness onset. It has been hypothesized that this abnormal brain development may lead to 'dysconnectivity' between brain areas, and that from this dysconnectivity symptom dimensions and cognitive dysfunctioning in schizophrenia arise. The neuroanatomical basis for this dysconnectivity may be dysplastic white matter tracts as a result from abnormal development in utero,[3] demyelination during adolescence and adulthood,[4,5] or an arrest in the normal process of myelination during brain development in adolescence ('hypomyelination').[5] Post-mortem studies in healthy subjects have shown a great increase in myelin content during adolescence and further myelin content increase during adulthood.[6,7]

One environmental factor causing abnormal brain development in schizophrenia may be cannabis use. Cannabis use at age 18 has been found to be a risk factor for schizophrenia, even after correcting for important confounders.[8] In addition, there is evidence from studies in subjects without major psychiatric illness that cannabis use during adolescence has an effect on brain structure and function. One MRI study found that subjects who started using cannabis before age 17 had significantly larger percent white matter volume, and smaller whole brain and percent gray matter volumes, compared with cannabis users with start of use after age 17.[9] Another study found verbal memory dysfunction in cannabis users with start of use before age 17, even after a controlled abstinence period of 28 days.[10] Similarly, cannabis use before age 16 is associated with specific visual attention deficits.[11]

With a relatively new magnetic resonance imaging technique, diffusion tensor imaging (DTI), it is possible to assess microstructural features of brain white matter *in vivo*. In DTI the magnetic resonance signal is made sensitive to the movement (or diffusion) of water molecules. In white matter, water molecules can diffuse freely along the length of axons, but diffusion is restricted perpendicular to the length of axons, which is called anisotropy. Anisotropy is increased by myelination,[12,13] coherence of fiber tracts,[14] and by the structural integrity of fibers, their diameter and packing density.[15] Anisotropy is reduced in white matter diseases such as multiple sclerosis and amyotrophic lateral sclerosis.[16]

Numerous DTI studies found reduced anisotropy in different brain regions in schizophrenia patients,[17] lending support for the

dysconnectivity hypothesis. Some DTI studies found increased anisotropy in patients with auditory hallucinations.[18,19,20] No DTI study examined whether adolescent cannabis use had an effect on anisotropy in schizophrenia patients. De Lisi et al. studied white matter anisotropy in nonpsychotic young-adults with frequent cannabis use during adolescence and found increased fractional anisotropy in several white matter regions.[21] It was concluded that adolescent cannabis use is unlikely to be neurotoxic to the normal developing adolescent brain. In accordance with this, one conventional MRI study found no differences in white matter volume in non-psychotic long-term cannabis users, and no effect of age of onset of cannabis use on hippocampal volume.[22] Conventional MRI studies in schizophrenia patients with cannabis abuse or dependence found no differences in brain white matter volumes.[23,24]

In the present study, we examined whether schizophrenia patients with adolescent cannabis use have specific DTI abnormalities compared to schizophrenia patients without adolescent cannabis use and compared to healthy controls.

It is important to recognize that there is evidence that schizophrenia patients with a history of cannabis use perform better than patients without such history in a number of domains: they are found to have better cognitive functioning early in the course of the illness,[25] less negative symptoms,[26,27,28] better premorbid adjustment,[29] less incoherent speech,[30] fewer neurological soft signs,[27] and less qualitative MRI abnormalities.[31]

These findings may suggest that schizophrenia patients who use cannabis are a subgroup of patients with distinct pathophysiological mechanisms. In this subgroup, cannabis use may interact with a vulnerability for psychosis to cause a functional derailment of the brain, while individuals developing a psychosis without cannabis use may have more severe early-developmental, structural brain abnormalities and cognitive deficits.[32]

Considering the above, we hypothesized that patients with adolescent cannabis use would differ from patients without adolescent cannabis use in comparison to healthy controls without a history of cannabis use, but we did not assume *a priori* that patients with adolescent cannabis use would have more abnormalities than patients without adolescent cannabis use.

Furthermore, we explored whether any effect on anisotropy was specific for adolescent cannabis use or reflected an effect of illicit drug use in general. Lim et al. found that cocaine-dependent patients without major psychiatric illness had significantly reduced fractional anisotropy in frontal white matter.[33]

MATERIALS AND METHODS

Patients

This study was approved by the local and national medical ethics committees. Patients were recruited from the inpatient and day-care units of the Adolescentclinic of the Academic Medical Center, University of Amsterdam, who had a diagnosis of schizophrenia, schizo-affective disorder or schizophreniform disorder according to DSM-IV criteria. Only patients with a recent-onset of illness were included in this study to minimize the effects of treatment and illness chronicity. Healthy control subjects were recruited through local advertisements. Only males were included to increase subject homogeneity, as post-mortem research has shown that white matter abnormalities in schizophrenia patients are gender-related.[34,35]

Exclusion criteria were: history of a neurological or endocrine disease which may affect brain structure, history of head trauma with loss of consciousness for more than 15 minutes, mental retardation according to DSM-IV criteria (prior to onset of the first psychotic episode for patients), gross brain abnormalities on conventional MRI other than atrophy or ventricular enlargement, and any standard MRI scanning exclusion criterion. Additional exclusion criteria for healthy controls were: a personal or family history of a major psychiatric illness, lifetime use of more than 25 cannabis joints or any harddrugs more than 5 times, or a lifetime diagnosis of alcohol abuse or dependence. After complete description of the study, written informed consent was obtained from all subjects.

Assessments

Clinical discharge diagnoses of patients, including alcohol dependence, were made according to DSM-IV criteria with the use of all available diagnostic information, including a history taken from a close relative or friend at admission, by two clinical psychiatrists and two residents (Longitudinal Expert Assessment of Diagnosis procedure).[36] Healthy control subjects were matched to patients for age, educational level and handedness. Handedness was determined with the Annett Handedness Questionnaire.[37]

An estimate of patients' lifetime substance use was based on a detailed history taken in an interview and medical records. The number of cannabis joints used per age year, and the total number of times use of harddrugs were estimated.

MR imaging was performed on a 1.5T Siemens Vision, and included 3-dimensional (1 mm^3) T1-weighted imaging. The MRI images were evaluated by a neuroradiologist to rule out gross brain abnormalities. For DTI imaging, a spin-echo EPI sequence was used, with an extra

180° pulse and balanced diffusion sensitizing gradients to minimize artefacts induced by eddy currents.[38] Other imaging parameters were b = 0 and b = 1000 s/mm^2, voxelsize 6.5 × 2 × 2 mm, slice thickness 5 mm with 1.5 mm interslice gap, TE 109 ms, 2 acquisitions with six icosahedric diffusion directions.[39] Fractional anisotropy (FA) images were calculated as previously described.[39] A linear spatial normalization was used for each subject to register the FA images to the T1-weighted images with FMRIB's Linear Image Registration Tool (FLIRT) (http://www.fmrib.ox.ac.uk/fsl/). The T1 weighted image was non-interactively segmented into gray and white matter. Next, a non-linear spatial normalization procedure was applied to the T1-images with Automated Image Registration (AIR) software (version 5.2.5; http://bishopw.loni.ucla.edu/AIR5/)[40] to register them to the Montreal Neurological Institute MNI 152 T1-template in MNI co-ordinate space. The algorithms for these transformations were then applied to create co-registered FA images to create one group-average FA image of all subjects in MNI coordinate space. Regions of interest (ROIs) were manually defined relative to this average image. We studied ROIs found abnormal in previous DTI studies: (1) splenium of the corpus callosum (2) frontal white matter (3) parieto-occipital white matter (adjacent to the splenium of the corpus callosum) (4) anterior limb of the internal capsule (5) uncinate fasciculus (6) arcuate fasciculus (7) dorsal cingulum. All ROIs were bilateral except the splenium of the corpus callosum. The ROIs in the uncinate and arcuate fasciculi were placed at the MNI coordinates also used by Burns et al.[41] Frontal white matter was measured at the medial side of the frontal lobe, measured in one slice through the anterior and posterior commissures (ac-pc line) and one slice 5 mm above the ac-pc line). The dorsal cingulum was defined according to Kubicki et al.[42] The ROIs for the other three structures were placed where the structure was visualized to be of maximal thickness. After smoothing with a 10 mm kernel, the average FA within each of the ROIs was calculated for each of the subjects.

Data Analyses

The effect of adolescent cannabis use and the effect of harddrug use on FA were tested correlational and categorical. For the present investigation adolescent cannabis use was defined as start of use before the age of 17. Spearman's rho (two-tailed) was used to correlate FA in each of the six ROIs with 1) total number of cannabis joints used before the age of 17 and 2) total number of times of harddrug use. For the categorical analysis, repeated-measures analyses of variance (ANOVA) for between-group differences in FA were performed for each of the six ROIs. Drug use data were simplified into just two bands: 'no use' and

'some use' to compare 1) patients with cannabis use before age 17 vs. patients without cannabis use before age 17 vs. controls, 2) patients with harddrug use vs. patients without harddrug use vs. controls). Due to our sample size there was insufficient statistical power to test the two drug use measures in one statistical model. Follow-up tests of significant repeated-measures ANOVAs ($p < 0.05$) were performed with univariate ANOVAs. ROIs showing significant differences ($p < 0.05$) in the univariate ANOVAs were tested post-hoc with the Bonferroni test (two-tailed) to evaluate pairwise differences between groups.

RESULTS

Twenty-nine patients with schizophrenia, two with schizophreniform disorder, and four with schizo-affective disorder (mean age 22.4 years, SD = 2.6) were included. All patients received antipsychotic medication. Mean duration of antipsychotic medication use was 54 weeks (SD = 103). Mean duration of illness from the first psychotic episode to time of MRI scanning was 97 weeks (SD = 129). Twenty-one healthy control subjects (mean age 22.6 years, SD = 3.4) were included. Patients and controls were matched for handedness and educational level (see Table 1). Eleven patients had never used cannabis before age 17. Thirteen patients had never used other illicit drugs.

The repeated-measures ANOVA for cannabis use before age 17 showed a significant effect of group ($F = 6.0$, $df = 2$, $p = 0.005$). Follow-up ANOVA's revealed significant differences between groups in the anterior internal capsule ($F = 5.0$, $df = 2$, $p = 0.01$), fasciculus uncinatus ($F = 11.1$, $df = 2$, $p < 0.001$) and frontal white matter ($F = 5.2$, $df = 2$, $p = 0.009$). Post-hoc tests showed that FA in these ROIs was significantly higher in patients who had used cannabis before age 17 compared to controls (see Table 2). The patients without

TABLE 1

DEMOGRAPHIC FEATURES OF SCHIZOPHRENIA PATIENTS AND CONTROLS

	PATIENTS n = 35	CONTROLS n = 21	P-VALUE[a]
Age, yrs, m ± sd	22.4 ± 2.6	22.6 ± 3.4	N.S.
Right-Handed, %	29 (83)	19 (90)	N.S.
Education, %			
Lower Professional Training	2 (6)	0 (0)	N.S.
Middle Professional Training	10 (29)	5 (24)	
Bachelor Level	11 (31)	6 (29)	
Master Level	12 (34)	10 (48)	

[a]Calculated using *t*-test, Fisher's Exact Test and Pearson Chi-Square (all three two-tailed).

cannabis use before age 17 did not differ significantly from the controls, and the patient groups did not differ significantly from each other.

The repeated-measures ANOVA for harddrug use showed a significant effect of group as well ($F = 6.1$, $df = 2$, $p = 0.004$). Follow-up ANOVAs revealed significant differences between groups in the anterior internal capsule ($F = 5.7$, $df = 2$, $p = 0.006$), fasciculus uncinatus ($F = 11.7$, $df = 2$, $p < 0.001$) and frontal white matter ($F = 4.9$, $df = 2$, $p = 0.011$) (see Table 3). Post-hoc tests showed that FA in these ROIs was significantly higher in patients who had used harddrugs compared to controls (see Table 3). The patient group without harddrug use did not differ significantly from the controls, and the patients groups did not differ significantly from each other.

No significant correlations were found in the patient group between the two drug use measures (number of cannabis joints used before age 17, and lifetime number of times of harddrug use) and FA in any of the six ROIs.

There was no significant correlation between total duration of antipsychotic use, or dose of antipsychotic medication in haloperidol equivalents at time of MRI scanning and FA in any of the ROIs.

Of note, there was high overlap between the patient groups. Of the 24 patients who had used cannabis before age 17, 18 patients had used harddrugs. Of the 22 patients who had used harddrugs, 18 had used cannabis before age 17. Furthermore, of the eleven patients who had not used cannabis before age 17, seven patients had not used harddrugs, and five had not started cannabis use after age 17. Of the thirteen patients who had not used harddrugs, seven patients had not used cannabis

TABLE 2

MEAN FRACTIONAL ANISOTROPY IN RECENT-ONSET PATIENTS WITH AND WITHOUT CANNABIS USE BEFORE AGE 17 AND HEALTHY CONTROLS WITHOUT DRUG USE

REGIONS OF INTEREST	MEAN FA ± sd CONTROLS n = 21	PATIENTS −[a] n = 11	PATIENTS +[b] n = 24	SIGNIFICANT PAIRWISE DIFFERENCES	95% C.I.	P-VALUE[c]
Anterior Internal Capsule	417 ± 38	437 ± 25	453 ± 42	Patients + > Controls	8–64	0.008
Uncinate Fasciculus	319 ± 16	338 ± 16	350 ± 29	Patients + > Controls	15–49	<0.001
Frontal	381 ± 19	399 ± 17	403 ± 32	Patients + > Controls	5–43	0.008

[a]Patients − = patients without cannabis use before age 17.
[b]Patients + = patients with cannabis use before age 17.
[c]Pair-wise differences evaluated with the Bonferroni test (two-tailed).

TABLE 3

MEAN FRACTIONAL ANISOTROPY IN RECENT-ONSET PATIENTS WITH AND WITHOUT HARDDRUG USE AND HEALTHY CONTROLS WITHOUT DRUG USE

REGIONS OF INTEREST	CONTROLS n = 21	MEAN FA ± sd PATIENTS –[a] n = 13	PATIENTS +[b] n = 22	SIGNIFICANT PAIRWISE DIFFERENCES	95% C.I.	P-VALUE[c]
Anterior Internal Capsule	417 ± 38	434 ± 28	456 ± 41	Patients + > Controls	10–67	0.004
Uncinate Fasciculus	319 ± 16	338 ± 18	352 ± 29	Patients + > Controls	16–50	<0.001
Frontal	381 ± 19	402 ± 22	403 ± 32	Patients + > Controls	3–42	0.011

[a]Patients − = patients without harddrug use.
[b]Patients + = patients with harddrug use.
[c]Pair-wise differences evaluated with the Bonferroni test (two-tailed).

before age 17, and six never used cannabis after age 17. Five patients had never used any illicit drugs. Only two patients had a history of alcohol dependence; one had never used cannabis or harddrugs and one had used both cannabis before age 17 and harddrugs.

DISCUSSION

In this DTI study increased anisotropy was found in frontal white matter, the uncinate fasciculus and the anterior internal capsule of recent-onset schizophrenia patients who had used cannabis before age 17 or who had used harddrugs, compared to controls. We could not determine whether the results were specifically related to cannabis use before age 17 or harddrug use, because there was high overlap between these two patient groups. Furthermore, almost half of the patients without cannabis use before age 17 had not used cannabis after age 17. Therefore, we can not exclude that our results apply to cannabis use in general, instead of specifically to adolescent cannabis use.

Increased anisotropy in our young-adult patient group may reflect an effect of cannabis on brain development during adolescence or a direct effect of illicit drugs on the brain. However, the lack of correlation between FA and number of cannabis joints used before the age of 17 or lifetime number of times of harddrug use argues against a neurodevelopmental or direct effect. It is more likely that young-adult patients with early cannabis use or harddrug use represent a distinct subgroup of patients. This is in accordance with findings of differences between schizophrenia patients with and without cannabis use summarized in the introduction of this paper. Characteristics of this group may increase their urge and ability to acquire drugs.[43]

Our findings only partly confirm our hypothesis, as the patients with and without illicit drug use (including adolescent cannabis use) did not differ significantly from each other. Patients without adolescent cannabis use or harddrug use had higher FA than controls, but not significantly. The fact that these comparisons were not significant may be due to lack of statistical power. Other factors than adolescent cannabis use or harddrug use may contribute to increased FA in both groups of our patient population.

Our findings are in line with DeLisi et al. who found increased white matter anisotropy in non-psychotic young-adults with frequent cannabis use during adolescence.[21] The biological substrate of increased anisotropy may be increased fiber number or diameter, increased fiber coherence, greater fiber packing density and/or enhanced myelination. To our knowledge, there is little post-mortem evidence for any of such abnormalities in schizophrenia. Chance et al. found increased fiber density in the fornix of male schizophrenia patients, and suggested this may stem from delayed myelination.[44] However, a study by Highley et al. showed no altered fiber density of the uncinate fasciculus in schizophrenia patients.[45] We propose two possible mechanisms for increased anisotropy in some patients with recent-onset schizophrenia: 1) reduced elimination of excess axonal fibers during post-natal development[46] or 2) increased myelination during childhood and adolescence, perhaps to compensate for gray matter reductions.[47]

Our finding of increased anisotropy was unexpected as it is in contrast with previous findings of reduced FA in patients with chronic schizophrenia[17] and first-episode patients.[48–54] Moreover, anisotropy in white matter diseases and in other neuropsychiatric illnesses has been found without exception to be decreased, not increased.[16,55] It is therefore of interest that increased FA values have been found in schizophrenia patients with auditory hallucinations.[18,19,20] Hubl et al. hypothesized that 'hyperconnectivity between frontal speech production areas and auditory perception areas may cause co-activation of these areas during inner speech resulting in auditory hallucinations'.[18]

These results in patients with auditory hallucinations and our results may be considered as evidence for 'hyperconnectivity' in some schizophrenia patients. We define connectivity here as the structural connection between brain areas that is directly related to the neural communication between these areas. 'Hyperconnectivity' then means more efficient 'hardwiring', which results in increased functional communication. It must be noted here that, to our knowledge, there is no direct evidence that increased anisotropy reflects increased connectivity. However, it is generally assumed that reduced anisotropy is related to hypoconnectivity, because 1) diseases, in which disruption of white

matter is believed to be the primary cause of symptoms (multiple sclerosis, amyotrophic lateral sclerosis), show reduced anisotropy[16] and 2) lower anisotropy has been related to lower functional performance.[56,57] We assume here that the reverse is true, i.e., that increased anisotropy reflects increased connectivity, or 'hyperconnectivity'.

Innocenti et al reviewed morphological, neurophysiological and neuropsychological evidence in relation to different theories of connectivity in schizophrenia.[58] They concluded that although most evidence favours a global hypoconnectivity in patients, there may be subgroups of patients with hyperconnectivity. One neuropsychological study found evidence for interhemispheric hyperconnectivity: an increased Stroop effect in schizophrenia patients in a divided visual field set-up.[59] In this study, the Stroop effect in controls was smaller when the incongruent stimuli were each presented separately to one of the hemispheres, compared to when they were both presented to one hemisphere. This was reversed in the patients. In another study, tactile evoked potentials, after contralateral stimulation of the arm, showed reduced latency and increased amplitude of activation in the right hemisphere of patients. This suggests increased left to right transcallosal conduction.[60] Finally, in a SPECT study diffuse bilateral hyperactivation was found in unmedicated patients with positive symptoms but without negative symptoms (referred to as type I patients) compared with nonreactivity in patients with marked negative symptoms (type II patients).[61] MRI measurement of the corpus callosum in these patients revealed increased relative callosal area in type I as compared with type II patients. The authors proposed 'diffuse hyperactivation and increased callosal size to be connected with positive symptomatology/good prognosis schizophrenia and vice versa'. They also suggested that in the course of the illness brain structure and functioning of type I patients may be replaced by a type II pattern.

Our findings can be related to the findings of Gunther et al.[61]: cannabis using patients are found to have less negative symptoms,[26,27,28] and more positive symptoms.[62,63]

There are several methodological limitations to our study. First, we could not determine in this study whether the results were a specific effect of cannabis use during adolescence or an effect of harddrug use. This limitation may be overcome in future studies by studying larger and more homogeneous groups of patients, such as patients with (adolescent) cannabis use but without harddrug use. The effect of alcohol dependence on anisotropy could not be assessed due to a low number of patients with this variable (n = 2). Second, it can not be excluded that the lack of correlation between FA and number of cannabis joints used or number of times of harddrug use is due to inaccuracy of the

estimates of these amounts. Third, some of our ROIs lacked anatomical specificity of white matter tracts. FA images are limited by their low anatomical resolution. This may be improved with fibertacking of individual fiber bundles.[64]

We do not believe that partial volume effects had a significant effect on our results. A partial volume effect would very likely decrease FA in the patient group, as schizophrenia patients are found to have a greater heterogeneity of spatial distribution of brain structures.[65]

In conclusion, we found increased anisotropy in recent-onset schizophrenia patients with start of cannabis use before age 17 and patients with harddrug use. We conclude that this increased anisotropy is probably not secondary to the use of these drugs. Our findings support the hypothesis that patients with schizophrenia and (early) illicit drug use have specific clinical characteristics (e.g., less negative symptoms), and probably different pathophysiological mechanisms, leading both to illicit drug use and schizophrenia. Increased FA may reflect increased connectivity between brain areas. Functional neuroimaging data support the hypothesis of structural and functional hyperconnectivity in some subgroups of patients.[61] Further DTI studies with higher image resolution and larger sample sizes, in schizophrenia patients and subjects without a major psychiatric illness, with and without adolescent cannabis use or harddrug use, are required to assess the relation between early cannabis and harddrug use and white matter microstructure. Postmortem studies are required to assess the neuropathology of increased or decreased FA. DTI may be combined with other neuroimaging modalities, clinical, neuropsychological, neurophysiological and postmortem studies to further define which subgroups of schizophrenia patients are characterized by hyperconnectivity.✤

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