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An MRI study of white matter tract integrity in regular cannabis users: effects of cannabis use and age

David D. Jakabek
University of Wollongong, djakabek@uow.edu.au

Murat Yücel
Monash University, murat@unimelb.edu.au

Valentina Lorenzetti
Monash University

Nadia Solowij
University of Wollongong, nadia@uow.edu.au

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Abstract

Rationale Conflicting evidence exists on the effects of cannabis use on brain white matter integrity. The extant literature has exclusively focused on younger cannabis users, with no studies sampling older cannabis users. Objectives We recruited a sample with a broad age range to examine the integrity of major white matter tracts in association with cannabis use parameters and neurodevelopmental stage. Methods Regular cannabis users (n = 56) and non-users (n = 20) with a mean age of 32 (range 18-55 years) underwent structural and diffusion MRI scans. White matter was examined using voxel-based statistics and via probabilistic tract reconstruction. The integrity of tracts was assessed using average fractional anisotropy, axial diffusivity and radial diffusivity. Diffusion measures were compared between users and non-users and as group-by-age interactions. Correlations between diffusion measures and age of onset, duration, frequency and dose of current cannabis use were examined. Results Cannabis users overall had lower fractional anisotropy than healthy non-users in the forceps minor tract only (p = .015, partial eta = 0.07), with no voxel-wise differences observed. Younger users showed predominantly reduced axial diffusivity, whereas older users had higher radial diffusivity in widespread tracts. Higher axial diffusivity was associated with duration of cannabis use in the cingulum angular bundle (beta = 5.00 x 10−5, p = .003). Isolated higher AD in older cannabis users was also observed. Conclusions The findings suggest that exogenous cannabinoids alter normal brain maturation, with differing effects at various neurodevelopmental stages of life. These age-related differences are posited to account for the disparate results described in the literature.

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An MRI study of white matter tract integrity in regular cannabis users: effects of cannabis use and age

David Jakabek [1], Murat Yücel [2], Valentina Lorenzetti [2] and Nadia Solowij [3]

1 Graduate School of Medicine, University of Wollongong, Wollongong, NSW 2522, Australia

2 Brain & Mental Health Laboratory, Monash Institute of Cognitive and Clinical Neurosciences, School of Psychological Sciences, Monash University, Clayton, VIC, Australia

3 School of Psychology, Centre for Health Initiatives and Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, NSW 2522, Australia

Communicating author: Associate Professor Nadia Solowij, School of Psychology, University of Wollongong, Northfields Avenue, Wollongong, NSW 2522, Australia. Tel: +61 2 4221 3732, Fax: +61 2 4221 4163, E-mail: nadia@uow.edu.au

Acknowledgements & Conflicts of Interests

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Conflicting evidence exists on the effects of cannabis use on brain white matter integrity. The extant literature has exclusively focused on younger cannabis users, with no studies sampling older cannabis users.

Objectives

We recruited a sample with a broad age range to examine the integrity of major white matter tracts in association with cannabis use parameters and neurodevelopmental stage.

Methods

Regular cannabis users (n=56) and non-users (n=20) with a mean age of 32 (range 18–55 years) underwent structural and diffusion MRI scans. White matter was examined using voxel-based statistics and via probabilistic tract reconstruction. The integrity of tracts was assessed using average fractional anisotropy, axial diffusivity and radial diffusivity. Diffusion measures were compared between users and non-users and as group-by-age interactions. Correlations between diffusion measures and age of onset, duration, frequency and dose of current cannabis use were examined.

Results

Cannabis users overall had lower fractional anisotropy than healthy non-users in the forceps minor tract only (p=.015, partial eta =0.07), with no voxel-wise differences observed. Younger users showed predominantly reduced axial diffusivity, whereas older users had higher radial diffusivity in widespread tracts. Higher axial diffusivity was associated with duration of cannabis use in the cingulum angular bundle (Beta=$5.00x10^{-5}$, p=.003). Isolated higher AD in older cannabis users was also observed.

Conclusions

The findings suggest that exogenous cannabinoids alter normal brain maturation, with differing effects at various neurodevelopmental stages of life. These age-related differences are posited to account for the disparate results described in the literature.

Keywords: Cannabis; Brain; Diffusion tensor imaging; Magnetic resonance imaging; Tractography; White matter.

Introduction

With the increasing legalisation of cannabis for medicinal (Martin 2014) and recreational (Room 2014) purposes there is a greater need than ever to better characterise the long-term consequences of cannabis use on the brain. Evidence suggests that the endocannabinoid system is critically involved in regulating neural development through activation of neurotrophic factors (Díaz-Alonso et al. 2012; Bilkei-Gorzo 2012) and promoting oligodendrocyte maturation (Molina-Holgado et al. 2002). Given this regulatory role in white matter growth, it follows that perturbation of this system by chronic exposure to exogenous cannabinoids may alter the development of white matter tracts (Hirvonen et al. 2012).

The current literature on white matter in cannabis users has generally focused on adolescents and young adults, utilising samples with a mean age less than 25 (DeLisi et al. 2006; Arnone et al. 2008; Gruber et al. 2011; Gruber et al. 2014; Shollenbarger et al. 2015) or 20 (Ashtari et al. 2009; Jacobus et al. 2009; Bava et al. 2010; Becker et al. 2015), with only two studies utilising slightly older participants (mean 27 years of age, Rigucci et al. 2015, and mean 28 years of age, Filbey et al. 2014). While this focus may be justified by concerns regarding the critical brain development occurring during this period (Bossong and Niesink 2010), restriction to young samples may impose major limitations to understanding the impact of cannabis exposure on white matter given its prolonged developmental trajectory. While grey matter continues to be refined until approximately the late 20's (Giedd et al. 1999), white matter development is thought to peak in the 30's and 40's (Westlye et al. 2010; Peters et al. 2014). Importantly, the average age of cannabis users has increased over the last decade, with approximately one quarter of cannabis users now comprising older individuals (defined as over 35 years of age) (Burns et al. 2013). Harm from cannabis use (as measured by hospital Emergency Department attendance) is also increasing in older adults (Kaar et al. 2015).

Given the changing cannabis use epidemiology there is a clear need to characterise white matter integrity in older cannabis users.

Diffusion tensor imaging (DTI) has been used in the last decade to probe the extent of white matter abnormalities in cannabis users. Briefly, DTI exploits the properties of water when confined in nerve sheaths to infer the physical properties of white matter. White matter integrity can be derived by examining the overall directions of water displacement (fractional anisotropy; FA), which is sensitive, but not specific, to aberrant cellularity such as decreased myelination or reduced axonal numbers (Alexander et al. 2007). Additional detail on white matter integrity can be obtained from axial (AD) and radial (RD) diffusivity metrics (Alexander et al. 2007). Decreased AD and increased RD are generally posited to describe reduced axonal volume and reduced myelination, respectively (Song et al. 2002).

Previous research of white matter in younger cannabis users has generally found inconsistent results. Lower FA has been demonstrated in cannabis users in the anterior corpus callosum (Gruber and Yurgelun-Todd 2005; Arnone et al. 2008; Gruber et al. 2011; Gruber et al. 2014; Epstein and Kumra 2015), fronto-thalamic connections (Ashtari et al. 2009), uncinate fasciculus (Shollenbarger et al. 2015), as well as temporal and parietal brain regions, including the left superior longitudinal fasciculus and inferior longitudinal fasciculus (Bava et al. 2010; Epstein and Kumra 2015). Additionally, using newer whole-brain network analysis methods, we have previously reported that cannabis users had fewer streamlines in the right fimbria of the hippocampus and in hippocampal commissural fibres connecting the splenium of the corpus callosum and the right precuneus (Zalesky et al. 2012), with lower FA in the hippocampal commissural fibre bundle (Solowij et al. in press). Contrary to these findings, other studies noted *higher* FA in cannabis users in widely distributed cortical areas (DeLisi et al. 2006), forceps minor (Filbey et al. 2014, Becker et al. 2015), left superior longitudinal fasciculus, left superior corona radiata (Jacobus et al. 2009) and the right superior

longitudinal fasciculus (Bava et al. 2010). Moreover, longitudinal studies have demonstrated cannabis use has complex alterations to white matter integrity over time (Becker et al. 2015, Jacobus et al. 2013). In summary, the studies to date have implicated cannabis effects on multiple tracts, however the direction of change remains equivocal.

This inconsistency has been attributed to low sample sizes (Gruber and Yurgelun-Todd 2005; DeLisi et al. 2006) or less detailed scanning parameters in earlier studies (as described by Ashtari et al. 2009) which may have overlooked smaller, more subtle, or regionally-specific changes. Additionally, some studies have been confounded by samples with concomitant cannabis and alcohol abuse, preventing the detection of cannabis specific effects (Jacobus et al. 2009; Bava et al. 2010). The methods of imaging analysis have also yielded different findings. The majority of studies have manually described ROIs in the frontal lobes, whilst the few studies using whole-brain analysis have primarily relied on Tract-Based Spatial Statistics (TBSS) with the exception of Zalesky and colleagues (Zalesky et al. 2012). ROI analyses are more time consuming to trace than the more automated methods, and thus provide only a limited picture of changes within the brain. While the whole-brain TBSS approach may be sufficient for exploratory analyses, it does not inform the extent to which a localized difference in white matter integrity may influence the integrity of a tract as a whole. Our previous network analysis (Zalesky et al. 2012) was limited to equally spaced $5mm^3$ voxel connections, which may overlap or underestimate the anatomical locations of defined white matter tracts. An alternative method is to apply expert neuroanatomists' segmentations of major white matter tracts to individual participants (TRActs Constrained by UnderLying Anatomy, TRACULA, (Yendiki et al. 2011)) and calculate average white matter integrity over the entire tract. In combining automated methodologies, we utilised the exploratory voxel-based comparison of TBSS together with the overall tract integrity assessment of

TRACULA. Moreover, we sought to expand our search beyond just anterior tracts (Shollenbarger et al. 2015) to all major defined tracts in the brain.

Thus, the aim of the current study was to apply, for the first time, these combined analysis methods to an older sample of well-matched cannabis users and healthy non-users to examine group differences in the integrity of major white matter tracts. Additionally, we investigated whether changes between groups were different at different ages, and we sought to determine the impact of age of onset, duration of regular cannabis use, and current cannabis dose and frequency, on white matter integrity in these tracts. It was hypothesised that cannabis use would be associated with decreased white matter integrity in regionally-select tracts, and that this decrease would be greater in older users. Furthermore, we hypothesised that white matter integrity would be would be diminished by an earlier age of onset of regular use, longer duration of use and higher current dose and frequency.

Materials and Methods

Participants

Cannabis users and non-using healthy controls were recruited from the general community by means of local advertisements. The sample ranged in age from 18 to 55 years; demographic and substance use characteristics are presented in Table 1. A portion of this overall sample was reported in Zalesky and colleagues (Zalesky et al. 2012), with further details provided there.

Table 1. Sample characteristics.

	Cannabis	Control	*p* value
N	56	20	
Age	32.3 (10.8)	30.0 (10.6)	0.12
Gender (% male)	42	40	0.83
Education (years)	12.7 (2.3)	13.6 (1.4)	0.11
IQ	102.4 (12.0)	107.1 (11.2)	0.13
Alcohol (drinks/weekly)	5.4 (6.7)	5.6 (6.9)	0.93
Tobacco (cigarettes/daily)	9.3 (7.4)	1.4 (3.5)	< 0.01
Age of first cannabis use (years)	15.1 (2.3)		
Age of onset regular cannabis use (years)	16.3 (2.6)		
Duration of regular cannabis use (years)	15.5 (9.7)		
Current cannabis dose (cones/month)	460.7 (350.1)		
Current cannabis frequency of use (days/month)	25.5 (8.0)		

Values are mean (SD); IQ assessed using the Wechsler Abbreviated Scale of Intelligence. Regular use defined as ≥ twice monthly. *p* value represents the *t*/Chi-squared test between cannabis users and controls on demographic variables.

Cannabis use was assessed using a structured interview incorporating a timeline follow-back procedure (Sobell and Sobell 1992). Use was quantified as "cones" which is approximately 0.1 grams of cannabis smoked through a water pipe (3 cones are roughly equivalent to one joint; see https://ncpic.org.au/media/1593/timeline-followback.pdf). The minimum use for inclusion in the study was twice a month for at least three years; the majority of the sample recruited were using near daily (Table 1). Participants were not formally assessed for any cannabis use disorder. All participants were right-handed, except for one left-handed cannabis user. Exclusion criteria for the study included any neurological conditions, head injuries with concussion or requiring hospitalisation, or psychiatric illness (defined by the

Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders-IV Axis I disorders (First et al. 2002). There was minimal reported other illicit drug use in the cannabis users (amphetamines, benzodiazepines, cocaine, ecstasy, hallucinogens, inhalants and opiates); median lifetime use was between 0 to 4 occasions for any other drug. Cannabis users were asked to abstain from using cannabis for at least 12 hours before imaging due to additional neuropsychological and functional MRI studies being conducted on the same day (not reported here; median self-reported abstinence 15 hours). Urinary metabolites were positive for most users; this was not an exclusion criterion for the study.

Alcohol use was quantified as standard drinks using the Alcohol Use Disorders Identification Test and structured interview. IQ was assessed using the Wechsler Abbreviated Scale of Intelligence (Wechsler 1999). Ethical approval was provided by the Melbourne Health Research Ethics Committee and informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

MRI acquisition

Both structural and diffusion MRI images were obtained using a Siemens Magnetom Trio 3T scanner with a 32-channel head coil at the Murdoch Children's Research Institute, Melbourne, Australia. Structural images were acquired using a T1 weighted magnetisation prepared rapid acquisition gradient recalled echo sequence (TR = 1900ms, TE = 2.19ms, FOV = 256mm, $1mm^3$ voxel resolution with 176 sagittal slices). Diffusion images were obtained using a spin-echo echo-planar imaging sequence (TR = 7000ms, TE = 96ms, FOV = 24x24cm, 2.3x2.3mm in-plane voxel resolution with 54 axial slices of 2.3mm thickness). Five non-diffusion weighted images were acquired initially before 42 images with uniform diffusion weighting (b = 2000s/mm).

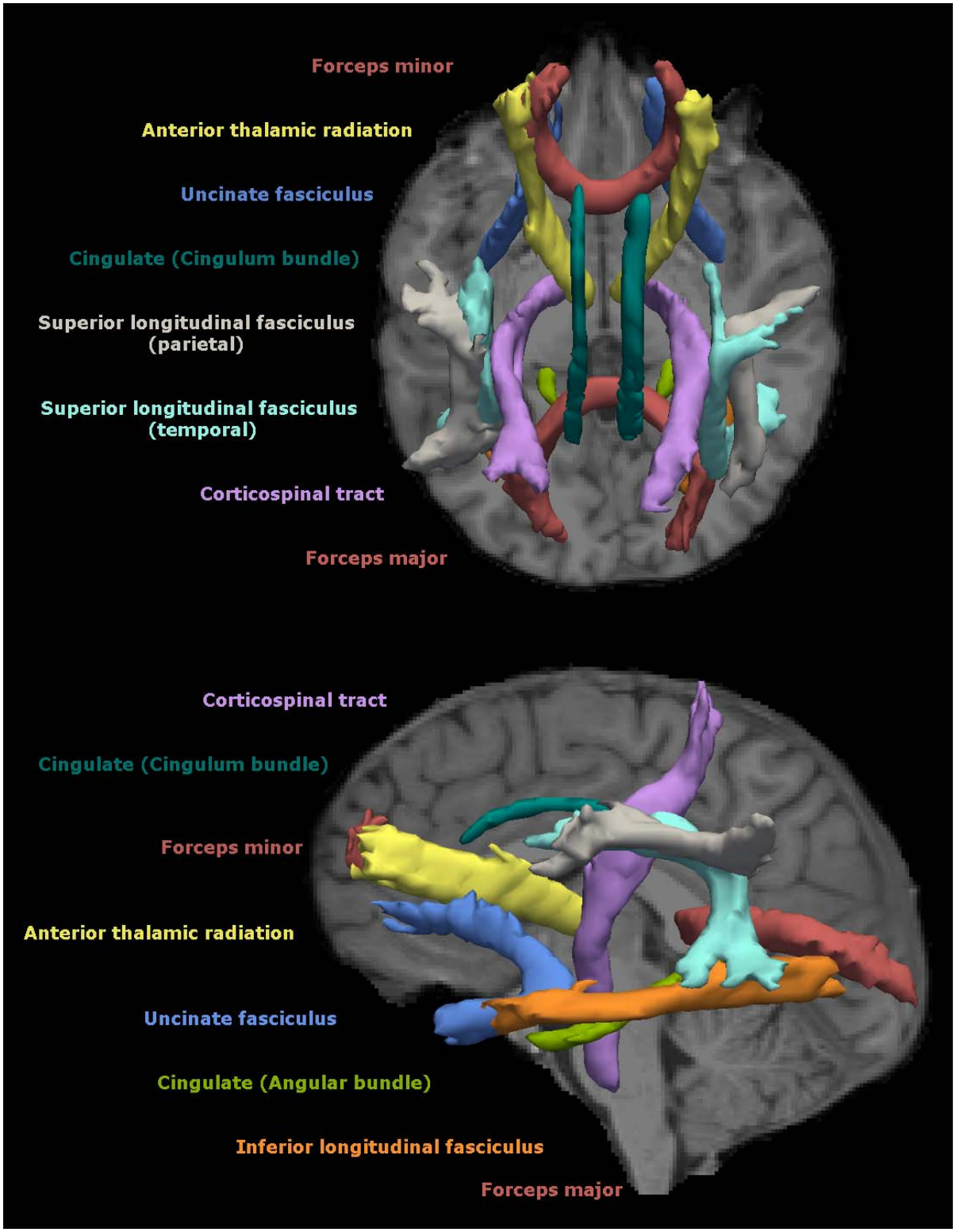
MRI data processing

Structural imaging data were processed using Freesurfer 5.3 software (http://surfer.nmr.mgh.harvard.edu/) for cortical reconstruction and volumetric segmentation. Output was manually checked for errors and corrected according to the user manual, which primarily involved removal of the superior sagittal sinus from inclusion in the pial segmentation.

Diffusion sequences were pre-processed using the TRACULA pipeline (Yendiki et al. 2011), which involved eddy-current correction and rotation of b-vectors, followed by brain extraction and co-registration between structures and diffusion (b=0) volumes. Fractional anisotropy (FA), axial diffusivity (AD), and radial diffusivity (RD) volumes were created using the DTIFIT command from FSL (Smith et al. 2004) utilising ordinary least squares tensor fitting. Rotational and translational measures of head motion were computed and no participant exceeded 2mm in either measure, nor were there statistically significant differences between groups on these measures (Yendiki et al. 2014). Individual structural volumes, diffusion volumes, and template volumes were co-registered. Probabilistic tractography was performed using FSL software Bedpostx ball-and-stick model (Jbabdi et al. 2007). Prior expert segmentation of major white matter pathway seed points was applied to each participant's diffusion volume to recreate major white matter tracts for each participant. Pathways reconstructed were: forceps major, forceps minor, bilateral anterior thalamic radiation, cingulum (angular bundle & cingulate gyrus), corticospinal tract, inferior longitudinal fasciculus, superior longitudinal fasciculus (parietal and temporal divisions), and the uncinate fasciculus (Fig. 1). Tracts were manually inspected to ensure valid reconstructions. The average measures of FA, AD, and RD within each pathway were calculated for each participant.

For TBSS analysis (Smith et al. 2006), the FA, AD, and RD maps from the TRACULA pre-processing stages were used. The FA volumes were subsequently aligned to the FMRIB58 FA 1mm image using non-linear registration. These realigned images were used to generate a mean, shared, "skeleton" tract, for which each participant's FA map was projected onto to create the final skeletonized FA data. The registration warps from the FA process were subsequently utilized for the AD and RD images to create skeletonized AD and RD volumes for subsequent analysis.

Fig. 1 Reconstructed major white matter tracts



Legend: Superior (top) and lateral (bottom) views of the major white matter tracts superimposed on a sample T1 image.

Statistical analysis

Group (controls and cannabis users) characteristics were compared using independent sample *t*-tests or Chi-squared tests as appropriate. The white matter integrity measures FA, AD and RD were examined for a main effect of group status (users and non-users) and a group-by-age interaction effect. The effect of age alone (adjusted for covariates) is included in the supplemental Table S1 for reference. Lastly, DTI measures were correlated with cannabis use measures in the cannabis user group only. All white matter integrity analyses included covariates of age, gender, education, IQ, alcohol and cigarette consumption since these variables have known associations with white matter integrity (Szeszko et al. 2003; Lebel et al. 2008; Pfefferbaum et al. 2009; Hudkins et al. 2012). Given the unequal tobacco use between groups, we conducted additional partial analysis of models to assess for confounding of tobacco use which is presented in the supplemental information.

For voxel-wise analysis of TBSS skeletonised data, permutation tests with 1000 replications were performed using the FSL randomise tool with family-wise correction for multiple comparisons (Smith et al. 2004) and cluster formation using Threshold-Free Cluster Enhancement (Nichols and Holmes 2002). Significance was set at $p < .05$. To further clarify the group-by-age interaction effect, the voxel with the maximal significance value was identified for each DTI metric (i.e. FA, AD, and RD) and the DTI values were plotted across ages between groups.

For the tract reconstructions, analyses were performed in R version 3.1.2 (R Development Core Team 2008) using linear models. Assumptions of normality and homogeneity of variances were met through visual inspection of histograms and residual plots. Where significant group-by-age interactions were present then the Johnson-Neyman test (D'Alonzo

2004) was performed to identify age ranges where significant group differences were observed.

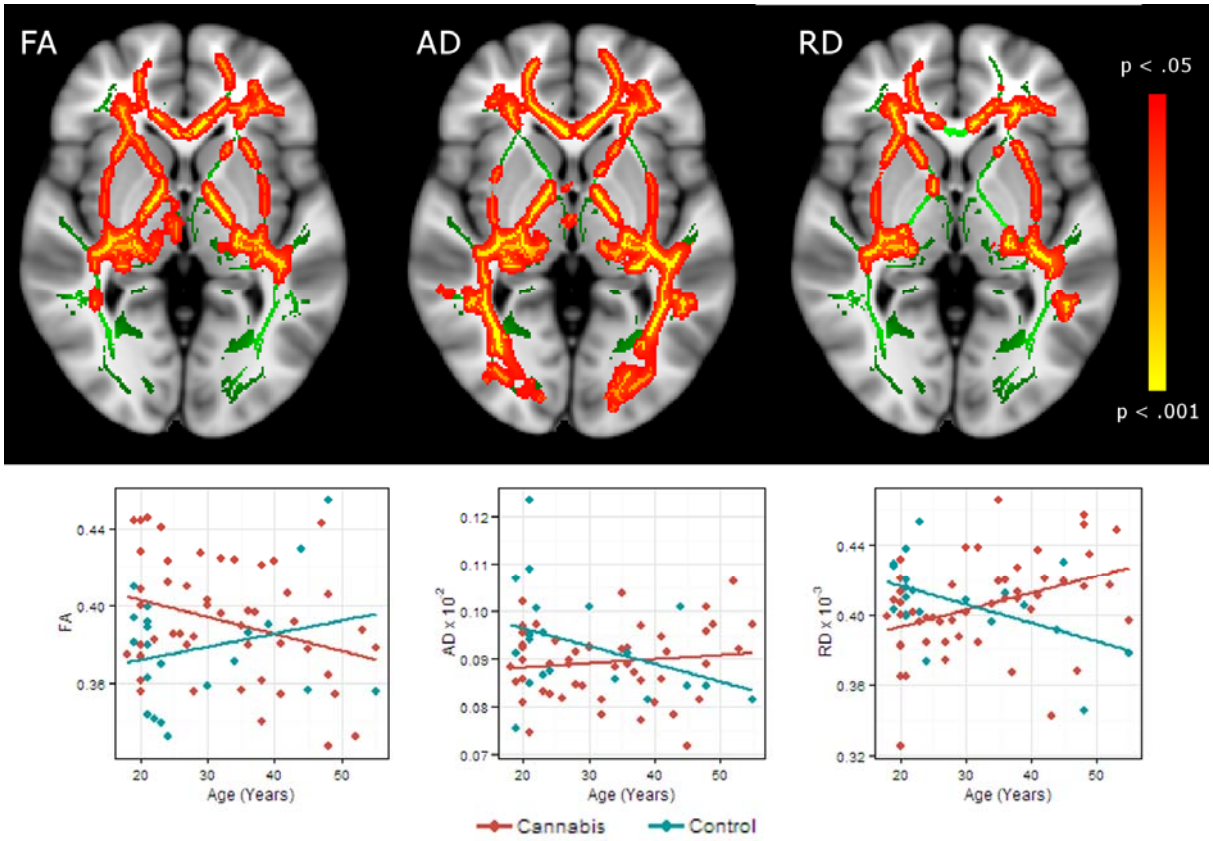
Previous studies have examined only one, or at most three (Filbey et al. 2014), specific tracts at any one time due to the laborious process of manual segmentation. To ensure comparable error rates with previous research on fewer tracts, we considered *p* values significant at the .05 level. For definitive conclusions about differences in tracts, we lowered the significance level to .003 to take into account the 18 different tracts being compared (i.e. .05/18), which is an approach utilised by prior studies (e.g. Hatton et al. 2014). All reported *p* values are uncorrected. We also attempted False Discovery Rate (FDR) correction across the entire sample, although this is problematic with correlated independent variables (Schwartzman and Lin 2011). No *p* values survived the FDR correction.

Results

Tract-Based Spatial Statistics

TBSS analysis found no significant differences between groups. However, widespread significant interactions between cannabis use status and age were noted across predominantly frontal and central tracts for all white matter integrity indices (FA, AD and RD; Fig. 2). The interaction effect was demonstrated by plotting the regression slopes of the maximally significant voxel, showing that there are significantly different values of FA, AD, and RD measures depending on both cannabis use status and age at the time of assessment.

Fig. 2. Significance maps of the interaction between cannabis use status and age across DTI metrics (top) and regression slopes for the maximally significant voxel (bottom).



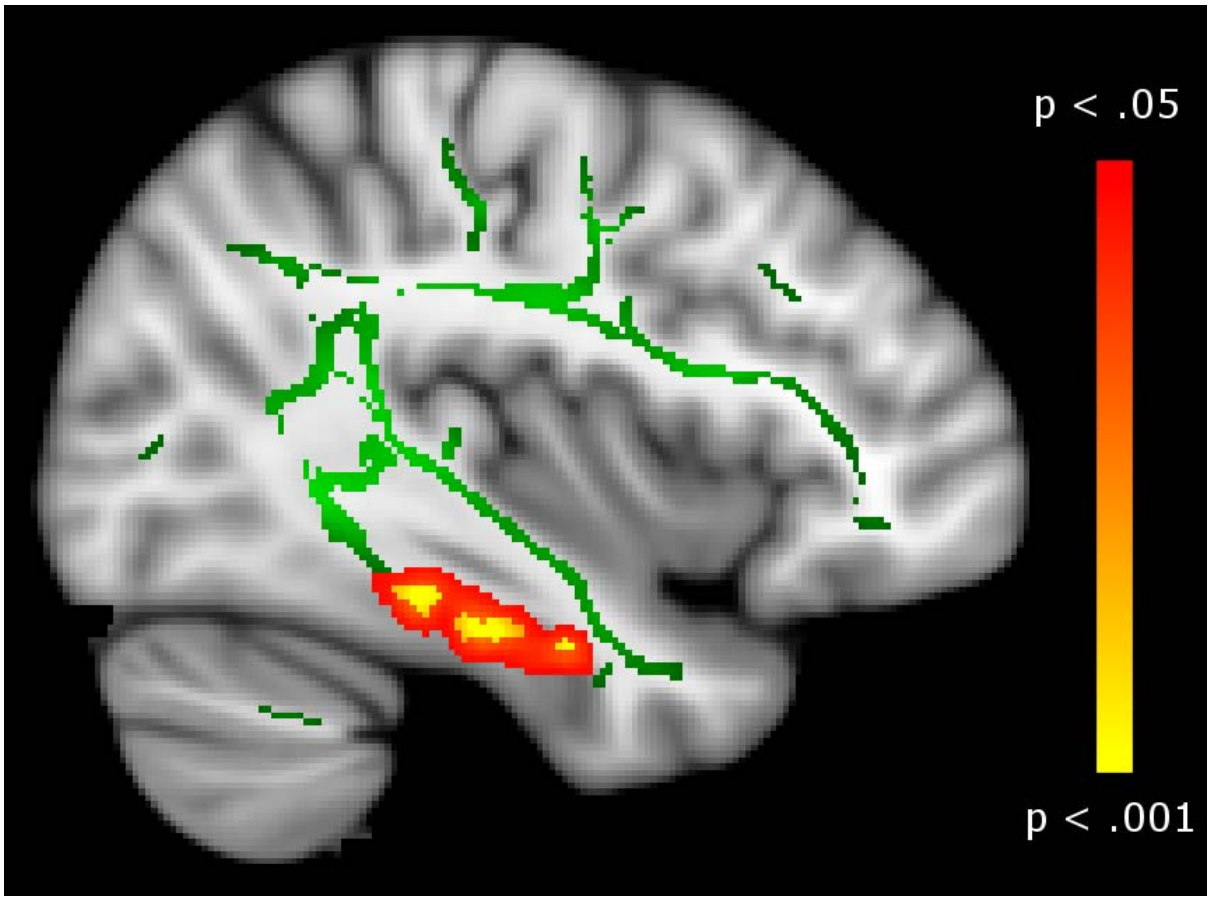
Top panel: Significance maps displayed for the interaction between cannabis use status and age on FA, AD, and RD skeletons. All significance maps overlaid over mean FA skeleton (green) and standard MNI T1 brain image at Z=0 slice.

Bottom panel: Interaction effect between DTI measures (FA, fractional anisotropy at X=34, Y=-22, Z=-6; AD, axial diffusivity at X=42, Y=-7, Z=-31; and RD, radial diffusivity at X=-26, Y=-21, Z=28) for cannabis users and control participants across the age range.

The only significant association between cannabis exposure measures (i.e. age of regular use, duration of use, current dose and current frequency of use) and DTI metrics was between

greater duration of cannabis use and lower FA in an area corresponding to the left inferior longitudinal fasciculus (Fig. 3).

Fig. 3 Significance map of the association between duration of cannabis use and FA



Significance map overlaid over mean FA skeleton (green) and standard MNI T1 brain image at X = -38 slice.

Tract group differences

Comparison of cannabis users and controls revealed significant differences between groups for FA in the forceps minor only (cannabis users FA = 0.472, SD = 0.052; controls FA = 0.488, SD = 0.026; p = .015).

The group-by-age interaction was significant across a number of tracts and is demonstrated in Table 2. The interaction pattern was similar to the TBSS interaction pattern demonstrated in Fig 2. Together with the Johnson-Neyman technique, tract-based analysis revealed lower FA in older users, lower AD and RD in younger users, and higher AD and RD in older users.

Table 2. Beta-values and p values for group by age interactions, and age cut-offs where significant differences in white matter integrity measures were found between cannabis users and controls.

	FA			AD			RD		
	Beta $x10^4$	p value	age	Beta $x10^6$	p value	age	Beta $x10^6$	p value	age
Forceps major	-1.4	0.874		13.5	0.215		8.10	0.292	
Forceps minor	-0.3	0.968		26.4	0.004	>34.8	10.05	0.204	
L anterior thalamic radiation	-10.7	0.083		14.0	0.064		16.16	0.014	<18.9 >49.4
L cingulum (angular bundle)	-7.1	0.364		8.0	0.415		13.29	0.113	
L cingulum (cingulate gyrus)	-21.7	0.077		-27.4	0.059		9.35	0.326	
L corticospinal tract	-13.7	0.024	>36.3	4.9	0.488		14.18	0.005	>34.7
L inferior longitudinal fasciculus	-11.5	0.240		15.4	0.201		17.50	0.033	>31.9
L parietal superior longitudinal fasciculus	-7.8	0.320		14.4	0.035	<18.0	13.32	0.041	
L temporal superior longitudinal fasciculus	-10.7	0.174		11.2	0.136		13.54	0.041	
L uncinate fasciculus	-1.9	0.767		16.4	0.057		9.11	0.214	
R anterior thalamic radiation	-6.5	0.339		21.1	0.007	<18.6 >44.3	15.81	0.013	>39.7
R cingulum (angular bundle)	-7.2	0.399		15.6	0.158		18.05	0.030	>53.1

R cingulum (cingulate gyrus)	-14.2	0.196		11.9	0.327		20.12	0.026	<23.7
R corticospinal tract	-14.9	0.043	>34.6	-4.5	0.541		12.22	0.044	>49.0
R inferior longitudinal fasciculus	-9.8	0.221		12.1	0.269		16.44	0.039	>53.1
R parietal superior longitudinal fasciculus	0.4	0.957		19.1	0.026	<18.6 >53.7	10.45	0.139	
R temporal superior longitudinal fasciculus	-1.6	0.796		10.5	0.197		8.09	0.203	
R uncinate fasciculus	-13.5	0.017	>41.8	11.3	0.176		18.90	0.004	<19.7 >41.5

Standardised Beta values of the interaction effect between groups and the p value of the interaction effect. Age refers to the cut off in years beyond which significant differences between cannabis users and controls emerge, determined using the Johnson-Neyman statistical method (D'Alonzo 2004). FA, fractional anisotropy; AD, axial diffusivity; RD, radial diffusivity.

Tract associations with cannabis use

Within the right cingulate gyrus, AD was negatively associated with the age of onset of regular cannabis use (Beta = -1.4×10^{-5}, p = .027) and positively associated with the duration of cannabis use (Beta = 5.00×10^{-5}, p = .003). For the same tract, FA had a positive association with current dose (Beta = .013, p = .044).

Longer duration of regular use was also associated with decreased in RD in the left angular bundle (Beta = -0.15×10^{-5}, p = .020). Current dose was also associated with lower FA in the forceps minor (Beta = 8.01, p= .045) and left anterior thalamic radiation (Beta = -8.37, p = .017). There were no significant associations with frequency of current use.

Discussion

In this study we examined the integrity of major white matter tracts between cannabis users and non-user healthy controls and evaluated age-related white matter integrity associations with the extent of cannabis exposure. Our hypothesis of worsening DTI measures in cannabis users overall was only partially supported, with FA in cannabis users being significantly lower in the forceps minor only. Interaction effects between group and age, however, demonstrated widespread altered DTI metrics in the cannabis users across different analysis methodologies, primarily located within frontal, parietal and motor tracts. In particular, both localised and widespread changes in RD were observed, with younger users having lower RD and older users having higher RD than non-using controls, consistent with our hypothesis. As further hypothesised, lifetime cannabis exposure also had isolated deleterious effects, primarily in the right cingulate gyrus, whereas current cannabis dose was associated with lower FA in frontal tracts.

Unique to this study is the consideration of white matter integrity in a wide age range of cannabis users. Our finding of lower FA in the forceps minor tract has been demonstrated in the literature for younger users (Ashtari et al. 2009; Filbey et al. 2014), although Becker et al. (2015) did note higher FA in cannabis users than non-users. This effect on the forceps minor is congruent with the known increased density of cannabinoid type 1 receptors (which are activated by the main psychoactive constituent of cannabis, delta-9-tetrahydrocannabinol (THC)) in frontal brain regions (Svíženská et al. 2008). Our finding of reduced FA in a wide-ranging in age sample provides evidence of a negative impact of cannabis on frontal white matter integrity not just in younger users, but across older users as well.

Utilising this older sample, we found for the first time, significantly lower FA in older cannabis users in bilateral corticospinal tracts and higher RD of bilateral corticospinal,

bilateral inferior longitudinal, right anterior thalamic radiation, right angular bundle and right uncinate fasciculus tracts. These differences were significant from approximately age 32 onwards, highlighting the limitations of past research that typically assessed samples with a mean age below 25 (DeLisi et al. 2006; Arnone et al. 2008; Ashtari et al. 2009; Jacobus et al. 2009; Bava et al. 2010; Gruber et al. 2011; Gruber et al. 2014; Becker et al. 2015; Shollenbarger et al. 2015). Our findings provide a preliminary extension for longitudinal studies in younger users which noted altered FA in the frontal regions (Becker et al. 2015) and the left inferior longitudinal fasciculus (Epstein and Kumra 2015) to additional tracts within the brain. Our finding of significant voxel-wise associations between duration of use and FA in the left inferior longitudinal fasciculus is consistent with that of Epstein and Kumra (2015); it is unclear why this tract in particular is vulnerable to cumulative dose-dependent effects of cannabis. We encourage further histological analysis of this tract in long-term cannabis users.

Although decreased FA was observed in the forceps minor when considering the tract as a whole, we did not replicate previous findings of reduced FA values in this tract in cannabis users using TBSS (Arnone et al. 2008). Notably, previous research only examined younger cannabis users. Combined with the substantially increased AD in this tract only in older users (>34.8 years; Beta = 26.4 p = .004), this may indicate that variable insults are more distributed, and may involve increased axon density, in older users. Additionally, the general paucity of significant voxel-based differences observed using TBSS suggests that the effect of cannabis is evident only when considering the tract as a whole. Notably, recent research has generally moved away from voxel-based comparisons (DeLisi et al. 2006; Arnone et al. 2008) to considering tracts as a whole (Filbey et al. 2014; Epstein and Kumra 2015; Shollenbarger et al. 2015). We have demonstrated somewhat consistent effects on RD

utilising both approaches, however we note that different methods have isolated different tracts of interest. Multi-modal approaches are encouraged for future research.

Paradoxically, our findings also suggested that younger and older users had improved metrics of white matter integrity in certain tracts. Older users had an increased AD in forceps minor, whereas younger users had increased RD in the left anterior thalamic radiation, right cingulate gyrus and right uncinate fasciculus) compared to non-using controls. These findings are corroborated in the literature, with increased measures of integrity reported in frontal and parietal tracts in cannabis users (DeLisi et al. 2006; Jacobus et al. 2009; Bava et al. 2010; Filbey et al. 2014).

A potential mechanism to explain these paradoxical results is that cannabis use in critical periods of development may accelerate aspects of brain maturation in young adulthood but in later years lead to potentially toxic effects (Bilkei-Gorzo 2012). Importantly, our results for healthy control participants demonstrate a gradual decline in both AD and RD into middle age, which is consistent with previous studies of healthy controls and likely reflects improvements in efficiency rather than decreased integrity per-se (Westlye et al. 2010). Animal models indicate that the endocannabinoid signaling system, particularly in the dorsolateral prefrontal cortex, peaks in early childhood and progressively declines into adulthood (Long et al. 2012). Therefore, the potential for exogenous cannabis use to interact with the endogenous system is greater the earlier that regular cannabis use is commenced. Given that our findings primarily involve increased RD in cannabis users (reflecting decreased myelination (Song et al. 2002)) and given the known role of endogenous cannabinoids on promoting oligodendrocyte maturation (Molina-Holgado et al. 2002), our results suggest that cannabis use predominantly alters myelination in the brain. Moreover, exogenous cannabis use has been shown to interfere with normal pruning processes (Rubino et al. 2015), thus possibly elevating measures of white matter connectivity. Alternatively,

these increases in white matter may arise as a reactive response to reduced gray matter (Yücel et al. 2008; Lorenzetti et al. 2010) or be an imaging artefact due to smaller axons leading to falsely elevated axon density (Beaulieu 2002). Additionally, differentially altered white matter integrity across age groups, which to our knowledge has not been previously examined, may explain the seemingly disparate reports of both increases (DeLisi et al. 2006; Jacobus et al. 2009; Bava et al. 2010; Filbey et al. 2014) and decreases (Gruber and Yurgelun-Todd 2005; Arnone et al. 2008; Ashtari et al. 2009; Gruber et al. 2011; Gruber et al. 2014) in white matter integrity in cannabis users. Lastly, cannabinoid neurotoxicity may be mediated by immune processes (Molina-Holgado et al. 2003), or be compounded by direct toxicity from hydrocarbons and carbon monoxide produced by combustion during cannabis smoking (Prockop and Naidu 1999; Abrams et al. 2007). Given the imaging evidence of differential effects of cannabis on white matter tracts, further histopathological research on the effects on cannabis on axonal development is encouraged.

After correcting for multiple comparisons, there remained a strong positive association between the AD of the right angular bundle and duration of regular cannabis use. The right cingulum angular bundle runs along the inferior cingulum and connects frontal to hippocampal and para-hippocampal regions (Schmahmann et al. 2007). Given that chronic cannabis use is associated with worsening verbal memory (Solowij and Battisti 2008; Broyd et al. 2015), and poorer integrity of the cingulum angular bundle is also associated with poorer memory in both younger and older adults (Fjell et al. 2015; Ezzati et al. 2015), our findings may provide further evidence for impairment in the memory systems of chronic cannabis users.

Despite evidence for altered white matter integrity at various ages, the current cross-sectional design precludes making definitive conclusions about causality. Our findings may be confounded with cohort effects resulting from, for example, the increasing THC

concentrations in cannabis over time (Cascini et al. 2012). However, the lower potency exposed to early in the drug using career of our older cohort would arguably lead to fewer observed group differences, yet, this group still showed poorer white matter integrity indices. This suggests that exposure even to lower potency cannabis during adolescence/early adulthood in combination with the effects of prolonged exposure to cannabis over many years, manifests most prominently as white matter integrity group differences from non-users in older age. Of concern is the current increased potency and earlier onset regular use in younger cannabis users; as these users age there may appear even greater disparity in white matter integrity between users and non-users.

Further consideration should be given to a reported decrease in orbitofrontal grey matter volume predating cannabis use (Cheetham et al. 2012), which may lead to connectivity alterations that precede the commencement of cannabis use and may differentially interact with subsequent exposure to cannabis. Despite this, in recent longitudinal studies of white matter and cannabis use, white matter changes were found to develop subsequent to the commencement of regular cannabis use (Bava et al. 2010; Becker et al. 2015; Epstein and Kumra 2015). The current trend for longitudinal studies of cannabis users is encouraged, particularly into older age.

There are a number of limitations to this study which deserve acknowledgement. Although we revised our significance level downward to account for the multiple comparisons (as in previous research, e.g. Hatton et al. 2014), we also considered results at a $p < .05$ where the results were consistent, since this is the first study, to our knowledge, of older cannabis users. Although this may inflate the Type I error rate, our findings are generally consistent with previous research. Additionally, given the number of covariates included in our analyses, our study may be potentially underpowered to detect smaller effects. Further research into the effects of cannabis use on white matter in older cannabis users is encouraged to verify our

findings. Furthermore, both tobacco use and the duration of cannabis use are both potential confounds in our sample. Tobacco use was significantly greater in users than non-users, and thus may have confounded the group effect, although there was limited evidence of this in an additive model (Supplemental Table 2). Further confounding in our study occurs with participants commencing regular cannabis use at a similar age, and so the effects of aging and duration of cannabis use are closely integrated. More heterogenous cannabis users, and well-matched groups of users and non-users, would allow better quantification of effects due to aging or cannabis exposure. Lastly, the sub-acute effects of recent cannabis use on DTI measures require further investigation. Participants self-reported a median 15 hours abstinence from cannabis and urinalysis was performed. We assume that residual cannabinoids are unlikely to influence brain microstructure at the level detectable by DTI, however further research to clarify any such effects would be warranted.

In conclusion, there have been inconsistent reports of both decreased and increased white matter microstructure in previous DTI studies of cannabis users. This study is the first to capture both trends in the same sample by assessing a sample with a wider age range than previous studies. Our findings provide evidence of predominantly degraded white matter integrity with increased cannabis use, with most prominent effects on indices of myelination. Replication of these findings in prospective longitudinal research in recreational cannabis users is encouraged to further elucidate the effects of cannabis across the lifespan.

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Supplemental Information

An MRI study of white matter tract integrity in regular cannabis users: effects of age and cannabis use

Jakabek, Yücel, Lorenzetti and Solowij

The main effect of age (adjusted for covariates) on DTI metrics across both users and non-users is presented in Table S1. Results indicate that age is an important predictor of white matter integrity and thus supports the investigation of cannabis use across the lifespan.

Table S1. Main effect of aging on DTI metrics, corrected for cannabis use, gender, IQ, education, tobacco and alcohol use.

	FA		AD		RD	
	Beta $x10^4$	p value	Beta $x10^6$	p value	Beta $x10^6$	p value
Forceps major	-10.91	0.008	-1.00	0.040	0.77	0.026
Forceps minor	-12.01	0.000	-0.59	0.170	0.83	0.018
L anterior thalamic radiation	-8.80	0.002	-0.74	0.033	0.51	0.085
L cingulum (angular bundle)	-7.35	0.036	-1.21	0.006	-0.01	0.985
L cingulum (cingulate gyrus)	-8.90	0.100	-0.35	0.577	0.83	0.046
L corticospinal tract	-6.63	0.020	-1.44	0.000	0.10	0.664
L inferior longitudinal fasciculus	-12.79	0.005	-1.45	0.008	0.44	0.235
L parietal superior longitudinal fasciculus	-8.55	0.016	-0.55	0.084	0.46	0.104
L temporal superior longitudinal fasciculus	-6.96	0.054	-0.68	0.049	0.32	0.280
L uncinate fasciculus	-4.19	0.138	-1.38	0.000	-0.29	0.370
R anterior thalamic radiation	-0.23	0.941	0.15	0.686	0.16	0.586

R cingulum (angular bundle)	-4.74	0.214	-1.02	0.038	-0.14	0.718
R cingulum (cingulate gyrus)	2.83	0.606	-0.19	0.740	-0.05	0.911
R corticospinal tract	-9.05	0.007	-1.06	0.001	0.43	0.112
R inferior longitudinal fasciculus	-13.58	0.001	-1.47	0.006	0.51	0.155
R parietal superior longitudinal fasciculus	-14.23	0.000	-0.60	0.112	0.83	0.008
R temporal superior longitudinal fasciculus	-7.64	0.007	-0.32	0.371	0.45	0.106
R uncinate fasciculus	-5.84	0.025	-0.80	0.026	0.13	0.661

A partial model analysis is presented for our only significant overall group difference (FA of the forceps minor) in Table S2. Results indicate generally stable parameter estimates and significance values, which suggest that the effect of tobacco use (which significantly differed between groups) does not substantially confound the results.

Table S2. Partial model analysis of the FA of the forceps minor

Model	Beta (Group)	*p* value (Group)
Age + Gender + Group	0.014	0.012
Age + Gender + Cig + Group	0.017	0.012
Age + Gender + Cig + Education + IQ + Alcohol + Group	0.017	0.015