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Longitudinal changes in white matter microstructure after heavy cannabis use

Mary P. Becker[a,b,*], Paul F. Collins[a,b], Kelvin O. Lim[c], R.L. Muetzel[a], M. Luciana[a,b]

[a] Department of Psychology, University of Minnesota, 75 East River Road, Minneapolis, MN 55455, United States
[b] Center for Neurobehavioral Development, University of Minnesota, 717 Delaware Street SE, Ste. 333, Minneapolis, MN 55414, United States
[c] Department of Psychiatry, University of Minnesota, 2450 Riverside Avenue South, Minneapolis, MN 55454, United States

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ABSTRACT

Diffusion tensor imaging (DTI) studies of cannabis users report alterations in brain white matter microstructure, primarily based on cross-sectional research, and etiology of the alterations remains unclear. We report findings from longitudinal voxelwise analyses of DTI data collected at baseline and at a 2-year follow-up on 23 young adult (18–20 years old at baseline) regular cannabis users and 23 age-, sex-, and IQ-matched non-using controls with limited substance use histories. Onset of cannabis use was prior to age 17. Cannabis users displayed reduced longitudinal growth in fractional anisotropy in the central and parietal regions of the right and left superior longitudinal fasciculus, in white matter adjacent to the left superior frontal gyrus, in the left corticospinal tract, and in the right anterior thalamic radiation lateral to the genu of the corpus callosum, along with less longitudinal reduction of radial diffusion in the right central/posterior superior longitudinal fasciculus, corticospinal tract, and posterior cingulum. Greater amounts of cannabis use were correlated with reduced longitudinal growth in FA as was relatively impaired performance on a measure of verbal learning. These findings suggest that continued heavy cannabis use during adolescence and young adulthood alters ongoing development of white matter microstructure, contributing to functional impairment.

Cannabis is experiencing its moment in the spotlight in the United States. Currently, 23 states and the District of Columbia have legalized cannabis for medical use, and 4 states have legalized it for both medical and recreational use. In the context of its changing legal status, cannabis has been the most commonly used "illicit" substance in the United States (Substance Abuse and Mental Health Services Administration, 2014), with 14% of 11th and 12th grade students reporting lifetime use (Falck et al., 2012), and 6.5% of 12th grade students reporting daily use (Johnston et al., 2013). Adolescent use may be problematic.

Adolescence is a critical period of neural maturation, during which there is reorganization of cortical connections, an increase in the fidelity of corticolimbic interactions, and neurochemical changes that promote adaptive behavioral regulation (Colby et al., 2011; Giedd, 2004; O'Hare and Sowell, 2008; Wahlstrom et al., 2010). Gray matter develops along a nonlinear trajectory that varies by region, with peak proliferation prior to puberty, followed by a gradual decline and pruning during adolescence and young adulthood, extending into the 30s (Giedd, 2004; Giedd et al., 1999; Gogtay et al., 2004; Pfefferbaum et al., 1994; Sowell et al., 2001, 2003). In contrast, white matter volume increases linearly in a less regionally-variant manner from childhood to adolescence (Giedd et al., 1999; Paus et al., 2001), following a posterior–inferior to anterior–superior developmental trajectory (Colby et al., 2011; Sowell et al., 1999). White matter volume does not reach its peak until adulthood, between the mid-30s and 40s (Bartzokis et al., 2001; Sowell et al., 2003; Westlye et al., 2010). White matter is critical for efficiency of signal conduction between brain regions, and its development is associated with improved cognitive control and executive functioning during adolescence (Peters et al., 2014; Treit et al., 2013).

Diffusion tensor imaging (DTI) is a sensitive neuroimaging technique that characterizes parameters of white matter microstructure, yielding measures of its directional organization (fractional anisotropy: FA), averaged water diffusion in all directions (mean diffusivity: MD), and water diffusion perpendicular to the primary fiber orientation (radial diffusivity: RD). Although several eigenvalues of the diffusion tensor contribute to the calculation of FA and are averaged to yield MD, the reduction in RD

* Corresponding author at: Department of Psychology, University of Minnesota, 75 East River Road, Minneapolis, MN 55455, United States.
E-mail address: petr0308@umn.edu (M.P. Becker).

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has been specifically associated with increased myelination and axonal packing in animal models (Budde et al., 2007; Song et al., 2003, 2005), and age-related changes in FA in humans are often attributed to decreased RD (Giorgio et al., 2008; Lebel and Beaulieu, 2011; Lebel et al., 2008). In general, increases in FA and decreases in MD as well as RD are observed in major white matter fiber tracts throughout adolescence and extending into adulthood. The biological mechanisms that drive MD changes are less clear given that MD is an imprecise measure.

Estimates of peak development of white matter microstructure vary between ages 20 and 42 (Kochunov et al., 2012; Lebel et al., 2012; Schmithorst and Yuan, 2010; Westlye et al., 2010). Unlike studies of white matter volume, there is considerable regional variability in the maturational timing of white matter microstructure, with earlier maturation in commissural and projection white matter tracts, and later development during adolescence and young adulthood in the association tracts (Ashtari et al., 2007; Kochunov et al., 2012; Lebel and Beaulieu, 2011; Lebel et al., 2008).

As is the case with other active periods of brain development (Fride, 2008; Huizink, 2014; Linnet et al., 2003), substance use during adolescence and young adulthood may interfere with normative development, altering the structural integrity and function of the adult brain. A growing literature suggests neurocognitive deficits and neuroanatomical alterations among adolescent and young adult cannabis users (CUs) relative to non-users. Behaviorally, cannabis use in adolescence and young adulthood is most commonly associated with relative deficits in verbal learning and memory, decision-making, abstract reasoning, and complex spatial working memory (Becker et al., 2014; Bolla et al., 2002; Dougherty et al., 2013; Fontes et al., 2011; Gruber et al., 2012; Hanson et al., 2010; Lisdahl and Price, 2012; Medina et al., 2007) with deficits in verbal learning and memory representing the most replicated finding.

Neuroimaging studies find alterations in brain structure and function associated with use. Reduced hippocampal (Ashtari et al., 2011; Demirakca et al., 2011; Lorenzetti et al., 2015; Schacht et al., 2012; Yücel et al., 2008), parahippocampal gyrus (Battistella et al., 2014; Matochik et al., 2005), orbital frontal (Battistella et al., 2014; Churchwell et al., 2010; Filbey et al., 2014), and increased cerebellum (Battistella et al., 2014; Cousijn et al., 2012; Medina et al., 2010) gray matter volumes are among the more consistent findings within CUs. Alterations in amygdala gray matter are reported, but the direction of alterations varies between reports (Gilman et al., 2014; Lorenzetti et al., 2015; Schacht et al., 2012; Yücel et al., 2008). The degree to which groups are matched on potential confounds of sex, age, and other substance use varies between studies. One notable recent study found no group differences between CUs and controls, who were matched on alcohol use, on measures of gray matter morphology in the nucleus accumbens, amygdala, hippocampus, and cerebellum, suggesting alcohol use or other confounds may significantly contribute to reported group differences (Weiland et al., 2015). Further, findings across studies suggest cannabis use likely exerts only a modest effect on gray matter volume, indicating that large sample sizes may be needed to detect a true effect (Weiland et al., 2015).

Among magnetic resonance imaging (MRI) and positron emission tomography (PET) studies of functional relationships among brain regions, CUs demonstrate altered patterns of brain activity when compared to non-using controls, particularly within the prefrontal cortex (Batalla et al., 2013), orbitofrontal network (Filbey et al., 2014), prefrontal and occipitoparietal cortex (Harding et al., 2012), and frontal-subcortical network (Filbey and Yezhuvath, 2013). A potential mechanism driving these differences may be disruption of white matter fiber tracts that support signaling between neurons within and across neural nodes. The direction of alterations in white matter volume in a variety of cortical and subcortical regions has been inconsistently reported in CUs, with one group finding increased density of white matter associated with the left parahippocampal and fusiform gyri and lower density in left parietal lobe white matter (Matochik et al., 2005), and a second group noting cerebellar white matter volume reduction in CUs (Solowij et al., 2011b). However, other studies find no association between cannabis use and white matter extent or volume (Block et al., 2000; Cousijn et al., 2012; Jager et al., 2007), making it difficult to draw general conclusions from the existing studies. It appears that measures of white matter volume or extent derived from T_1 weighted structural MRI scans may not be sufficiently sensitive to subtle alterations in white matter microstructure that impact information processing.

DTI has the potential to address this limitation given that white matter organization can be examined at the microstructural level. DTI studies indicate that CUs demonstrate altered white matter organization, although again there are inconsistencies across studies in the direction of MRI-measured changes. Most studies indicate that FA is lower in various white matter regions in CUs. Some DTI reports include findings of increased CU RD and MD, also indicative of reduced white matter organization in CUs. DTI findings for CUs have involved widely scattered brain regions, including the superior longitudinal fasciculus (Ashtari et al., 2009; Bava et al., 2009; Thatcher et al., 2010; Yücel et al., 2010), arcuate fasciculus (Ashtari et al., 2009), frontal white matter adjacent to the anterior cingulate cortex (Gruber et al., 2011) and hippocampus (Zalesky et al., 2012), internal capsule (Gruber et al., 2014), and the rostrum (Arnone et al., 2008), genu (Gruber et al., 2014), and splenium (Zalesky et al., 2012) of the corpus callosum. Cannabis use may disrupt the developmental trajectory of white matter organization since lower FA levels have been associated with an earlier age of cannabis use onset (Gruber et al., 2011, 2014).

Not all DTI studies have found evidence for lower FA and/or greater RD and MD in CUs. Greater FA and lower RD have been reported in the forceps minor of the corpus callosum, a tract that connects the orbitofrontal cortices (Filbey et al., 2014), although years of use within the CU group showed a curvilinear (quadratic) association with declining FA levels and rising RD levels. One early study found increased FA in CUs in white matter associated with medial frontal, cingulate, precentral, and parietal cortex, as well as decreased MD in cingulate and medial frontal white matter (Delisi et al., 2006).

Though findings are compelling, this literature is comprised of cross-sectional designs, limiting interpretation of group-based differences. Longitudinal research may clarify some interpretative complexities, by assessing neural changes over time in relation to ongoing substance use. Recent longitudinal studies have explored white matter development associated with alcohol initiation (Luciana et al., 2013), binge alcohol use with comorbid cannabis use (Jacobus et al., 2013a), and polysubstance use (Bava et al., 2013). No longitudinal study to date has explored white matter microstructure specifically related to sustained cannabis use during young adulthood.

This study examines change in measures of axonal fiber organization across time as a function of cannabis use. Young adult, regular CUs and control participants were assessed at two time points, with a two-year time interval between assessments. It was predicted that ongoing development of axonal fiber organization, as measured by increasing FA and decreasing RD, would be relatively diminished among CUs after multiple years of chronic use. Given the broadly distributed DTI findings to date, we expected alterations of fiber organization in frontal white matter as well as fiber tracts connecting frontal and posterior regions; hippocampal white matter; the corpus callosum; and cortical–subcortical projection fibers such as the corticospinal tract. We predicted that these alterations in fiber organization would be correlated with

amount of cannabis use in the follow-up interval in the CU group. To facilitate interpretation of the DTI findings in terms of behavioral functioning, we include a correlational analysis of scores on a test of auditory verbal learning and memory. CUs have consistently shown impairments in this domain (e.g., Becker et al., 2014) and we predicted that test performance would correlate positively with FA changes in task-relevant brain regions.

1. Methods

1.1. Sample

Thirty-seven CUs were initially recruited into this longitudinal study through university advertisements. CUs were recruited if they reported using cannabis at least 5 times per week for at least 1 year. Cannabis use onset was required to be before age 17 so that length of use across study participants would be relatively uniform and to index adolescent use onset. CUs were excluded if they were daily cigarette smokers or if alcohol use exceeded 4 drinks for females and 5 drinks for males on more than 2 occasions per week. Participants were initially tested on a comprehensive behavioral and imaging battery (Becker et al., 2014; Muetzel et al., 2013). Of those who initially participated, 27 CUs returned for follow-up assessment after a two-year interval. Of these, 2 CUs were excluded from the current analysis because they reported cannabis use patterns markedly lower than the majority of the CU sample (≤5 times in past 12 months) at follow-up. Twenty-three of the remaining 25 subjects (16 males, 7 females), aged 18–20 years (M = 19.45, SD = 0.66) generated artifact-free scanning data. Twenty-three controls (16 males, 7 females), aged 15–23 years old (M = 19.19, SD = 2.31), were selected from a larger longitudinal study of adolescent brain development (described in more detail in Luciana et al., 2013; Urošević et al., 2012) to match the CUs on age and sex and scanning parameters.

General inclusion criteria for all participants included being a native English speaker, right-handed, and with normal/corrected-to-normal vision and hearing. Exclusion criteria included any contraindications to MRI scanning, a reported history of neurological problems or significant head injury, intellectual disability, or current pregnancy. Control subjects were excluded at study enrollment for evidence of Axis I DSM-IV psychopathology. Adult participants and parents of minors provided informed consent, and minors (those under age 18) assented to participate in the study. The University of Minnesota's Institutional Review Board approved the protocol. Inclusion criteria were verified during an initial visit by a demographic and health interview questionnaire, the Edinburgh Handedness Inventory (Oldfield, 1971), the Wechsler Abbreviated Scale of Intelligence (WASI: Wechsler, 1999), and by structured diagnostic interviews (the Kiddie Schedule for Affective Disorders and Schizophrenia; Kaufman et al., 1997) administered to the participant as well as (for those under age 18) a parent. Participants who met inclusion criteria returned for a second assessment, which included MRI scans as well as a battery of neuropsychological tasks. Cannabis users were asked to refrain from drug use for at least 24 h before testing so as not to be acutely high during the assessment. Longer periods of abstinence were not required, because we did not wish to study individuals in the midst of drug withdrawal and because a goal of the study was to capture functional capacities in the context of active use (Becker et al., 2014; Muetzel et al., 2013).

Participants completed MRI scanning and the neuropsychological battery at study enrollment (Time 1) and at a follow-up assessment (Time 2), approximately 2 years after study enrollment (M = 2.23 years, SD = 0.52).

1.2. Verbal learning and memory

We selected the *Rey Auditory Verbal Learning Test* (RAVLT; Lezak et al., 2004; Rey, 1993) to assess verbal learning and memory performance among the sample. This task distinguished CU from controls in a prior comprehensive assessment of neuropsychological performance at baseline (Becker et al., 2014). It is a robust measure of effortful performance, and represents a cognitive domain that is commonly reported to be diminished among CUs (Bolla et al., 2002; Gonzalez et al., 2012; Grant et al., 2003; Hanson and Luciana, 2010; Harvey et al., 2007; Solowij et al., 2011a). The RAVLT measures acquisition, storage, and retrieval of verbal information. During the learning phase, participants are first read and asked to recall a list of 15 words. This procedure is repeated four additional times to yield the number of items correctly recalled. This five-trial learning phase assesses the participant's immediate learning and temporary storage of verbal information. Following the learning trials, participants are then read and asked to recall a new list of 15 words (interference trial). The interference trial assesses immediate learning of new information, and is presented only once. Following the interference trial, participants are then asked to freely recall as many words as they can from the first list (immediate recall) and again following a 30-min delay (delayed recall). Statistical analyses were conducted using the total number of words recalled during the learning trials, interference trial, immediate recall, and delayed recall trials.

1.3. Substance use assessment

Substance use frequency and amount were assessed with the K-SADS interview, and substance use frequency was further assessed with the self-reported Personal Experience Inventory (PEI; Henley and Winters, 1989). Within the PEI, participants reported the number of times they used substances (alcohol, cannabis, and non-cannabis illicit substances) within the last 12 months on a 5-point scale (never, 1–5 times, 6–20 times, 21–49 times, 50–99 times, 100+ times). The adolescent version of the PEI was used with participants under the age of 18, and the adult version of the PEI was used with participants 18 years and older. Scoring was harmonized to create comparable metrics across the two versions. An in-house interview questionnaire, based on guidelines provided by the National Institute on Alcohol Abuse and Alcoholism, was also implemented to assess alcohol and cannabis use characteristics in the sample. Participants reported their typical use patterns, as indexed by frequency and quantity of use, as well as heavier use patterns, defined as the number of occasions CUs used their self-defined maximum number of cannabis hits.

The Achenbach's Adult Self-Report (ASR; Achenbach and Rescorla, 2003) questionnaire assessed self-reported daily tobacco use during the previous 6 months for subjects who endorsed tobacco use.

1.4. MRI data acquisition and processing

MRI data were collected on a Siemens 3T Tim Trio scanner (Siemens Medical Systems, Erlangen, Germany) using a 12-channel array head coil at the University of Minnesota Center for Magnetic Resonance Research. Diffusion weighted data were acquired in the axial plane using a dual spin echo, single-shot, pulsed-gradient, echo-planar imaging (EPI) sequence (TR = 8500 ms, TE = 90 ms, 64 slices, no gap, FOV = 256 mm, voxel size = 2.0 mm × 2.0 mm × 2.0 mm, b value = 1000 s/mm^2, GRAPPA iPAT = 2). The acquisition box was positioned to cover the cerebrum and as much of the cerebellum as possible. Thirty-six volumes were acquired in the diffusion scan: 6 non-diffusion-weighted volumes (b = 0) and 30 diffusion-weighted volumes (b = 1000 s/mm^2),

using gradient vectors distributed uniformly in 3-dimensional space according to an electrostatic repulsion algorithm (i.e., the "Jones30"; Jones et al., 1999). For off-line EPI geometric distortion correction, *b*0 field maps were constructed from gradient-echo images acquired using different echo times (TE = 4.62 ms and 7.08 ms; TR = 700 ms, flip angle = 90°, 64 slices, no gap, voxel size = 2.0 mm × 2.0 mm × 2.0 mm, FOV = 256 mm).

Diffusion MRI data were processed using the FDT and TBSS packages in FSL (FMRIB Software Library v4.0.1, http://www.fmrib.ox.ac.uk/fsl, Smith et al., 2004). Each diffusion-weighted volume was corrected for head motion and eddy current distortions using an affine registration to the first *b*0 reference volume. The diffusion series was corrected for geometric distortion caused by magnetic field inhomogeneity using PRELUDE (Phase Region Expanding Labeler for Unwrapping Discrete Estimates) and FUGUE (FMRIB's Utility for Geometrically Unwarping EPIs) in conjunction with the b0 field maps. Brain tissue was extracted using BET (Brain Extraction Tool). The diffusion tensor was fit at each voxel by submitting the preprocessed data from the *b*0 reference volume and the 30 diffusion-weighted volumes to FSL's DTIFIT. Diagonalization yields the eigenvectors (V1, V2, V3) and corresponding eigenvalues (L1, L2, L3) of the diffusion tensor, which describe the directions and apparent magnitudes of water diffusion within each voxel. Two scalar variables were computed from these tensor components: radial diffusivity (RD), which is the average of L2 and L3 and reflects the magnitude of diffusion perpendicular to white matter tracts; and fractional anisotropy (FA), a variance measure that reflects how strongly water diffusion is restricted to the principle eigenvector and ranges from 0 (equivalent diffusion along V1, V2, and V3) to 1 (no diffusion along V2 and V3). Given our focus on the development of fiber organization, we did not analyze mean diffusivity, which is less interpretable than RD in regions with complex fiber orientations (Vos et al., 2012), or axial diffusivity, which is more informative in the context of axonal injury and degeneration (e.g., Budde et al., 2009).

Tract-Based Spatial Statistics (TBSS; Smith et al., 2006) was used to align the DTI scalar volumes so that voxelwise statistical analysis could be performed across participants and between time points. All FA volumes were aligned to a common space template using the nonlinear registration IRTK (Rueckert et al., 1999; www.doc.ic.ac.uk/~dr/software). The template was an average FA volume constructed from 72 participants drawn from our full research sample, aligned to 2 mm isotropic MNI voxel space (Luciana et al., 2013; Olson et al., 2009). RD volumes were aligned to common space by applying the transformation matrices previously computed during the FA volume alignments. FA volumes were masked at a threshold of FA ≥ 0.15 to reduce partial volume effects, and the same voxel masks were applied to RD volumes. To assess changes over time while controlling for baseline differences, common space DTI scalar volumes at follow-up were regressed on baseline volumes, yielding follow-up vs. baseline regression residuals for FA and RD. These residualized volumes were smoothed at 4 mm FWHM prior to statistical analysis. In longitudinal data analysis using this regressor variable method (Allison, 1990), the variance in time 2 performance, residualized for prediction based on time 1 variables, reflects change over time that was not predicted by baseline levels (see Cohen and Cohen, 1983, pp. 402–422; Cronbach and Furby, 1970; Metalsky et al., 1987). Baseline data were separately examined.

1.5. Statistical approach

Distributions of variables were examined, and variables that did not meet the assumptions for parametric analysis were square root transformed, including cannabis use variables of total number of hits for the past 12 months and maximum cannabis hits frequency in the last 12 months. Demographic data as well as behavioral characteristics related to substance use were compared between groups using analyses of covariance, Chi-square analysis of independence, and the Mann–Whitney *U* test at baseline and follow-up (computed in SPSS, version 22).

SPM8 (www.fil.ion.ucl.ac.uk/spm/software/spm8) was used to perform voxelwise multiple regression analysis of the FA and RD follow-up vs. baseline residuals. Predictors in the regression equations included group, sex, age at baseline, time interval between baseline and follow-up assessments, and average alcohol use (the mean of baseline and follow-up PEI ratings for use in the past 12 months). Preliminary analyses were conducted using both baseline and follow-up PEI ratings for alcohol use in the past 12 months, but no supra-threshold clusters were produced for the alcohol predictors. Accordingly, the final regression models were simplified to include a single alcohol covariate representing averaged use levels across baseline and follow-up. To assess group differences at baseline, multiple regressions were performed on baseline FA and RD volumes with group, sex, age at baseline, and alcohol use at baseline (past 12 months) as predictors in the model. These analyses yield the relationship between a predictor variable and criterion variable while regressing out the variance accounted for by other predictors in the model (i.e., age at baseline, sex, interval between assessments, average alcohol use).

All regression analyses were evaluated according to cluster-level statistical significance after family-wise error correction for multiple comparisons based on random field theory (Worsley et al., 1992), using an input voxelwise height threshold of $p < 0.01$.

Mean values were computed within clusters from the FA-change regression analyses and then correlated with cannabis use variables within the CU group, while controlling for variables of no interest. Specifically, follow-up cannabis use measures were regressed on baseline cannabis use measures, along with sex, age at baseline, time interval between baseline and follow-up assessments, and average 12-month alcohol use. Residuals from these regressions were used as the outcome use variables. Measures of cannabis use examined included: age of regular cannabis use, total number of hits for the past 12 months, and maximum cannabis hits frequency in the past 12 months. For comparison, correlations were performed on follow-up measures of alcohol use (i.e., total number of drinks, binge-drinking occasions in the last 12 months, and maximum drinks frequency in the past 12 months, controlling for interval time and sex as described above) and tobacco use, controlling for sex, time interval between baseline and follow-up, and average 12-month alcohol use.

Additionally, partial correlations were computed over the entire sample (both CUs and controls) between cluster mean values and total scores on the RAVLT, controlling for sex, age at baseline, time interval between baseline and follow-up assessments, average 12-month alcohol use, and WASI IQ scores. This correlational analysis was conducted to clarify the direction of functional associations for the longitudinal FA-change clusters, i.e., to determine for each cluster whether increasing or decreasing FA over the follow-up period was associated with better cognitive performance. A repeated measures analysis of covariance (ANCOVA) was performed to assess the temporal stability of RAVLT scores over the follow-up interval as well as group differences in performance at both time points. More comprehensive assessment of the neuropsychological functioning of this CU sample at baseline can be found in Becker et al. (2014).

2. Results

2.1. Demographic and substance use characteristics

Groups were matched on sex distribution, ethnic and racial background, years of education, and estimated IQ (see Table 1). The study participants were largely Caucasian and had above average

Table 1
Demographic and substance use characteristics of cannabis users (CUs) and healthy controls. Values represent means and standard deviation units, unless otherwise specified. Group comparisons were conducted using Chi-square, Mann–Whitney *U*, and one-way analysis of variance.

Variable	Control	CU	F, U, χ^2	p
n	23	23		
Time 1: Baseline characteristics				
Age	19.19 (2.31)	19.45 (0.66)	0.27	0.61
#Male/#Female	16/7	16/7	$\chi^2 = 0.00$	1.00
#Caucasian/#Other Ethnicity	21/2	19/4	$\chi^2 = 0.77$	0.38
Years of education	13.09 (2.07)	13.26 (0.92)	0.14	0.71
Estimated Full Scale IQ	115.65 (9.46)	115.17 (11.02)	0.03	0.88
Vocabulary T-Score	62.09 (5.43)	61.83 (8.34)	0.02	0.90
Matrix reasoning T-Score	55.65 (7.06)	55.35 (5.58)	0.03	0.87
Alcohol use, past 12 months *(Median PEI rating)*	1	4	$U = 54.00$	< 0.00
0: Never (*n*)	9	1	–	–
1: 1–5 times (*n*)	7	0	–	–
2: 6–20 times (*n*)	3	3	–	–
3: 21–49 times (*n*)	3	6	–	–
4: 50–99 times (*n*)	1	8	–	–
5: 100+ times (*n*)	0	5	–	–
Cannabis use, past 12 months	0	5	$U = 0.00$	< 0.00
0: Never (*n*)	21	0	–	–
1: 1–5 times (*n*)	2	0	–	–
2: 6–20 times (*n*)	0	0	–	–
3: 21–49 times (*n*)	0	0	–	–
4: 50–99 times (*n*)	0	0	–	–
5: 100+ times (*n*)	0	23	–	–
Age of regular cannabis use onset	–	15.35 (1.16)	–	–
Total # hits, past 12 months	–	3032.55 (2395.31)	–	–
Maximum cannabis hits frequency, past 12 months	–	11.21 (13.77)		
Time 2: Follow-up characteristics				
Years between assessments	2.12 (0.66)	2.34 (0.31)	2.18	0.15
Age at follow-up	21.31 (2.43)	21.79 (0.82)	0.82	0.37
Age range at follow-up	17.2–26.0	20.6–23.3	–	–
Alcohol use, past 12 months *(Median PEI rating)*	3	4	$U = 84.5$	< 0.00
0: Never (*n*)	2	1	–	–
1: 1–5 times (*n*)	3	0	–	–
2: 6–20 times (*n*)	6	1	–	–
3: 21–49 times (*n*)	8	2	–	–
4: 50–99 times (*n*)	3	11	–	–
5: 100+ times (*n*)	1	8	–	–
Cannabis use, past 12 months	0	5	$U = 0.00$	< 0.00
0: Never (*n*)	14	0	–	–
1: 1–5 times (*n*)	7	0	–	–
2: 6–20 times (*n*)	2	0	–	–
3: 21–49 times (*n*)	0	1	–	–
4: 50–99 times (*n*)	0	6	–	–
5: 100+ times (*n*)	0	16	–	–
Total # hits, past 12 months	–	2637.92 (2203.77)	–	–
Maximum cannabis hits frequency, past 12 months	–	16.20 (28.66)	–	–

IQ estimates. Groups were matched in terms of mean age, though a larger age range characterized the control sample to balance the sex distribution across both groups. Time to follow-up assessment was matched between groups, with an overall average of 2.23 years ($SD = 0.52$).

All CUs reported consistent cannabis use prior to both the Time 1 and Time 2 assessments (see Table 1). Given the high prevalence of cannabis use in the general population, controls who had minimally experimented with cannabis use (that is, use that did not exceed 5 times in the past year) were included. Minimal experimentation with cannabis use was reported by 2 healthy controls at the Time 1 assessment (both reported using cannabis 1–5 times in the past 12 months); at Time 2, $n = 7$ reported using 1–5 times in the past 12 months, $n = 2$ reported having transitioned into using 6–20 times in the past 12 months. Additionally, CUs reported greater alcohol use (Time 1 & Time 2 CU *Mdn* use = 50–99 times in past 12 months) than healthy controls (Time 1 *Mdn* use = never in the past 12 months; Time 2 *Mdn* use = 21–49 times in past 12 months). Minimal non-cannabis drug use was reported among CUs (use fewer than 20 times), and controls (Time 1: no reported use; Time 2: fewer than 5 times, see Table 1). As indicated in our prior publications (Becker et al., 2014; Muetzel et al., 2013), CUs largely met criteria for DSM-IV cannabis dependence at baseline without evidence of comorbid psychopathology.

2.2. FA development

Significant differences in two-year FA change between CUs and non-using controls were identified in five voxel clusters (see Table 2) with trend-level differences in two additional clusters. In five of these seven clusters, the Controls had more positive FA change than CUs over the two-year follow-up interval (Fig. 1), while the converse held in the remaining two clusters (Fig. 2). The largest Controls > CU FA-change cluster (3864 mm^3) was located in the right hemisphere primarily along the superior longitudinal fasciculus (SLF) adjacent to the precentral and postcentral gyri. It extended anteriorly into the junction between the SLF and the corticospinal tract (CST). The next largest FA cluster (1632 mm^3) had its peak adjacent to the parietal operculum in the left hemisphere. The cluster followed the SLF adjacent to the supramarginal and angular gyri, extending into the forceps major of the corpus callosum (CC) near the anterior portion of the lateral occipital cortex; above the forceps major, it overlapped a caudodorsal portion of the inferior longitudinal fasciculus (ILF). The posterior portion of the third FA

Table 2
Analysis of group differences in 2-year change in fractional anisotropy (FA) and radial diffusivity (RD). Results from voxelwise multiple regression analysis of FA and RD 2-year change values (i.e., residuals from regression of each participant's follow-up FA or RD volume on the corresponding baseline FA or RD volume). Multiple regression covariates were group, sex, age at baseline, time interval between baseline and follow-up assessments, and average alcohol use (mean of baseline and follow-up PEI ratings for use in past 12 months). All regression analyses were evaluated according to cluster-level statistical significance after family-wise error (FWE) correction for multiple comparisons based on random field theory, using an input voxelwise height threshold of $p < 0.01$; trend-level results (FWE $p \leq 0.10$) are included as well. Abbreviations: ATR = anterior thalamic radiation; CC = corpus callosum; CST = corticospinal tract; CWP = cluster-wise FWE-corrected p-value; FOF = fronto-occipital fasciculus; SLF = superior longitudinal fasciculus.

Measure	Contrast	Peak *Z*-stat	Peak partial *r*	Cluster size (mm^3)	CWP	X	Y	Z	Hemisphere	Anatomical region
Fractional anisotropy	Controls > CU	5.80	0.76	3864	0.001	32	−32	40	Right	SLF, extending to junction with CST
Fractional anisotropy	Controls > CU	4.12	0.59	1632	0.017	−38	−44	24	Left	SLF, extending to CC forceps major
Fractional anisotropy	Controls > CU	3.88	0.56	1464	0.030	−18	16	42	Left	White matter adjacent to superior frontal gyrus
Fractional anisotropy	Controls > CU	4.46	0.63	1232	0.065	−16	−28	50	Left	CST, adjacent to pre- and postcentral gyri
Fractional anisotropy	Controls > CU	4.25	0.61	1208	0.071	26	16	12	Right	ATR; superior FOF; adjacent to frontal operculum
Fractional anisotropy	Controls < CU	4.49	0.63	1808	0.010	−14	16	26	Left	Anterior CC
Fractional anisotropy	Controls < CU	3.75	0.55	1696	0.014	−10	−24	−6	Left	White matter adjacent to posterior thalamus
Radial diffusivity	Controls < CU	4.54	0.64	6848	0.001	16	−36	38	Right	CST; SLF; posterior cingulum
Radial diffusivity	Controls > CU	3.97	0.57	2368	0.006	−24	−18	4	Left	CST

cluster (1464 mm^3) was located in the white matter of the middle region of the superior frontal gyrus in the left hemisphere, while the anterior portion extended into the lateral anterior cingulum and anterior thalamic radiation (ATR) adjacent to the anterior superior frontal gyrus. Most of the fourth FA cluster (1232 mm^3; trend level at clusterwise FWE $p = 0.065$) was located within the CST adjacent to the precentral and postcentral gyri in the left hemisphere, with the lateral aspect of the cluster extending into the SLF. The fifth Controls > CU FA-change cluster (1208 mm^3; trend level at clusterwise FWE $p = 0.071$) was adjacent to the inferior frontal gyrus (specifically, the frontal operculum) in the right hemisphere, with the medial aspect of the cluster overlapping the anterior thalamic radiation and the lateral aspect extending into the superior portion of the FOF. As will be discussed, these trend level findings are mentioned because they replicate findings reported in the extant literature.

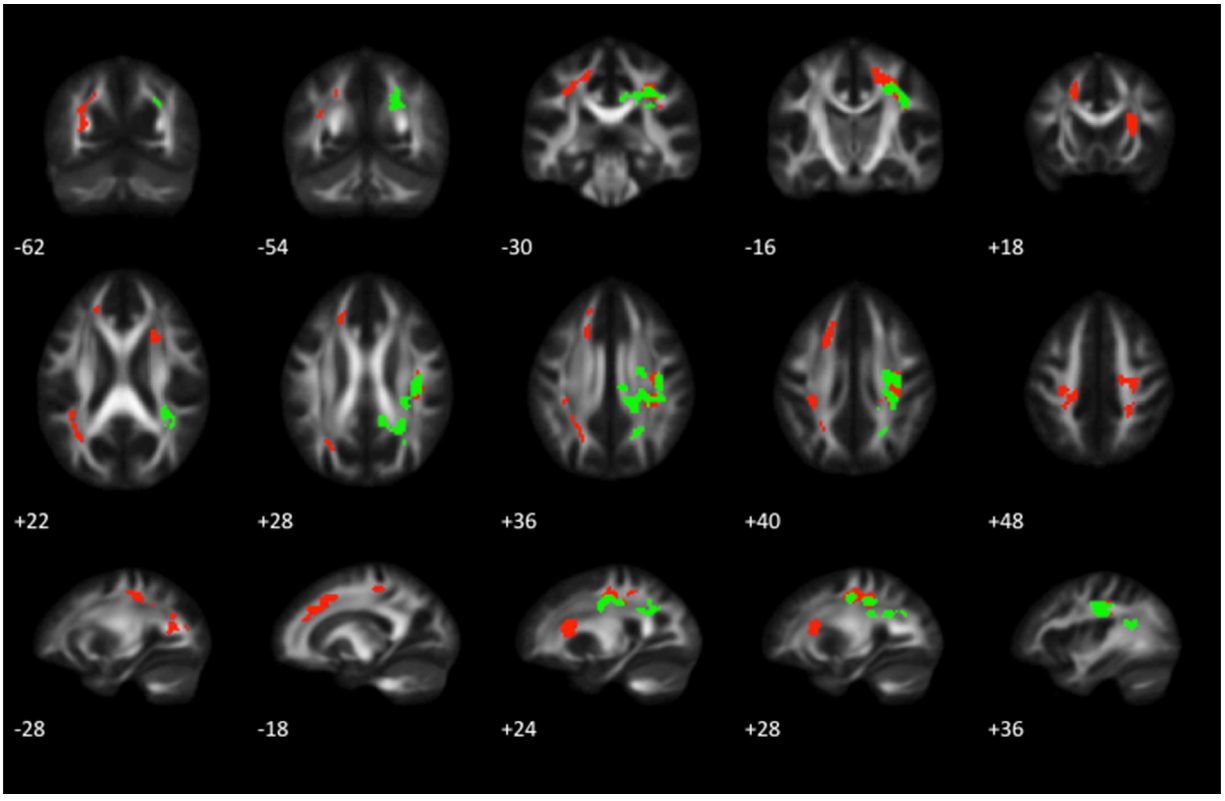

Fig. 1. Voxelwise analysis of group differences in 2-year change in fractional anisotropy and radial diffusivity. Multiple regression analysis as described for Table 2. Color coding indicates contrasts for multiple regression effects: red = fractional anisotropy, Controls > cannabis users; green = radial diffusivity, Controls < cannabis users. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

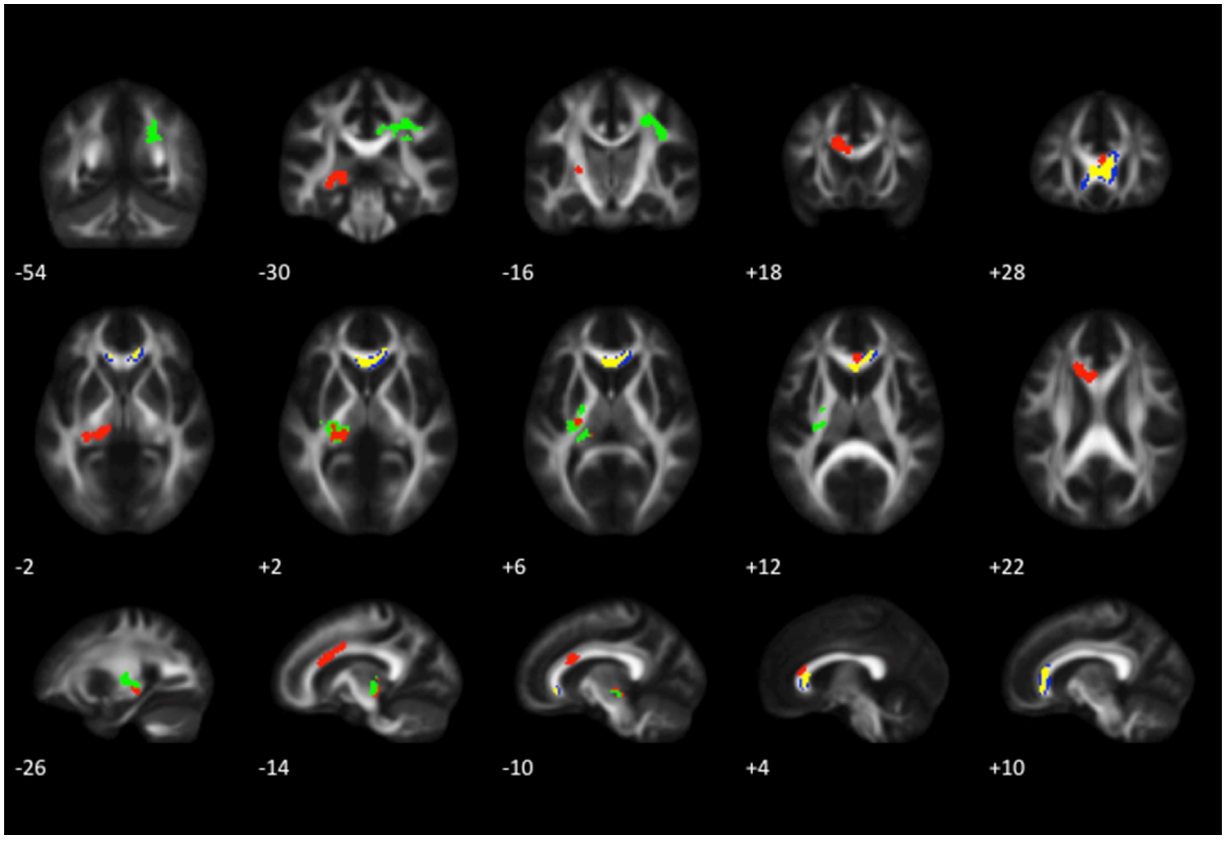

Fig. 2. Voxelwise analysis of group differences in 2-year change in fractional anisotropy and radial diffusivity. Multiple regression analysis as described for Table 2. Color coding indicates contrasts for multiple regression effects: red = fractional anisotropy, Controls < cannabis users; green = radial diffusivity, Controls > cannabis users; blue = baseline fractional anisotropy, Controls < cannabis users; yellow = baseline radial diffusivity, Controls > cannabis users. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

More positive two-year FA change was observed for CUs than Controls within two clusters in the left hemisphere. One cluster (1808 mm^3) was located primarily in anterior corpus callosum (posterior genu, rostral body), with a small overlap into the caudal anterior cingulum. The other cluster (1696 mm^3) was located in posterior thalamus white matter. The medial aspect of this cluster overlapped the CST and the ATR, while the lateral aspect extended into the acoustic and optic radiations.

Baseline differences between groups did not fully account for these findings. Analysis of FA group differences at baseline yielded one significant voxel cluster (2584 mm^3), in which FA levels were greater for CUs than Controls (Table 3). This cluster covered the right side of the genu of the CC, crossing over the CC midline to extend into the inferior part of the left genu. In terms of fiber tracts, the cluster overlapped the caudal forceps minor, with a much greater extension into the right hemisphere than the left.

2.3. RD development

In addition to the FA results, significant differences in two-year DTI change between CUs and Controls were identified in the analysis of RD data. It was generally expected that RD would decrease over time. RD change was more positive for CUs than Controls in a very large voxel cluster (6848 mm^3) that extended over the posterior two-thirds of the right hemisphere, in white matter adjacent to precentral, postcentral, supramarginal, inferior parietal, precuneus, and posterior cingulate cortex (Table 2). The cluster peak was located in the medial aspect of the cluster, which overlapped the posterior cingulum. The middle section of the cluster overlapped the CST, while the lateral aspect overlapped the SLF. Both of these cluster segments had substantial overlap with a similarly located Controls > CU FA-change cluster (note that increased FA often is accompanied by decreased RD; see Fig. 1). RD change was more positive for Controls than CUs in a left hemisphere voxel cluster (2368 mm^3) located along the CST, with a peak in the posterior limb of the internal capsule.

Analysis of RD group differences at baseline yielded one trend-level voxel cluster (1640 mm^3; trend level at clusterwise FWE $p = 0.10$; Table 3), in which RD levels were greater for Controls than CUs. The Controls > CUs baseline RD cluster was located in the same CC region as the Controls < CUs baseline FA cluster, but was smaller in size (see Fig. 2).

2.4. Brain–behavior associations

Cannabis use characteristics. Within CUs, extent of cannabis use was negatively associated with cluster mean FA-change values, for three Controls > CU FA-change clusters (see Table 4) such that higher levels of reported use were associated with lower magnitudes of change over time. Specifically, the total number of hits during the past 12 months was significantly negatively associated with cluster mean FA-change in the left CST ($r = -0.259$, $p < 0.05$) and left SLF/CC forceps major junction ($r = -0.525$, $p < 0.01$). The maximum cannabis hits frequency during the past year was significantly negatively associated with cluster mean FA-change in the right SLF/CST junction ($r = -0.514$, $p < 0.05$). Age of cannabis use onset was not significantly correlated with cluster mean FA-change values. Analogous correlations were examined in relation to measures of alcohol and tobacco use at follow-up but were non-significant.

Table 3
Baseline group differences in fractional anisotropy (FA) and radial diffusivity (RD). Results from voxelwise multiple regression analysis of FA and RD at baseline. Multiple regression covariates included group, sex, age at baseline, and alcohol use. All regression analyses were evaluated according to cluster-level statistical significance after family-wise error (FWE) correction for multiple comparisons based on random field theory, using an input voxelwise height threshold of $p < 0.01$; trend-level result (FWE $p \leq 0.10$) is included as well. Abbreviations: CC = corpus callosum; CWP = cluster-wise FWE-corrected p-value.

Measure	Contrast	Peak Z-stat	Peak partial r	Cluster size (mm^3)	CWP	X	Y	Z	Hemisphere	Anatomical region
Fractional anisotropy	Controls < CU	4.18	0.60	2584	0.012	4	26	4	Right	Genu, forceps minor of CC
Radial diffusivity	Controls > CU	3.86	0.56	1640	0.102	2	26	6	Right	Genu, forceps minor of CC

Table 4
Partial correlations of cluster mean fractional anisotropy (FA) change values with cannabis use. Partial correlations between cluster mean values and measures of cannabis use over the past 12 months, controlling for interval, sex, age at baseline, and average alcohol use ($n = 23$). Abbreviations: ATR = anterior thalamic radiation; CC = corpus callosum; CST = corticospinal tract; FOF = fronto-occipital fasciculus; SFG = superior frontal gyrus; SLF = superior longitudinal fasciculus; CU = cannabis user; L = left hemisphere; R = right hemisphere.

FA cluster	Total # hits[a]	Maximum cannabis hits frequency[a]	Age of regular cannabis use
CU > Controls			
L anterior CC (−14,16,26)	0.209	−0.325	−0.313
L thalamic white matter (−10,−24,−6)	−0.004	0.335	−0.087
Controls > CU			
R SLF/CST (32,−32,40)	0.115	−0.514*	0.281
L CST (−16,−28,50)	−0.259*	0.354	−0.016
R ATR (26,16,12)	−0.152	0.321	−0.010
L SLF/CC forceps major (−38,−44,24)	−0.525*	0.345	0.074
L SFG white matter (−18,16,42)	−0.230	−0.061	0.065

[a] Square root transformed.
* $p \leq 0.05$.

Table 5
Partial correlations of cluster mean fractional anisotropy (FA) change values with RAVLT scores. Partial correlations between cluster mean values and total scores on the Rey Auditory Verbal Learning Test (RAVLT), controlling for sex, age at baseline, time interval between baseline and follow-up assessments, average 12-month alcohol use, and IQ. Abbreviations: ATR = anterior thalamic radiation; CC = corpus callosum; CST = corticospinal tract; FOF = fronto-occipital fasciculus; SFG = superior frontal gyrus; SLF = superior longitudinal fasciculus; CU = cannabis user; L = left hemisphere; R = right hemisphere.

FA cluster	RAVLT: Baseline	RAVLT: Follow-up
CU > Controls		
L anterior CC (−14,16,26)	−0.226	−0.504**
L thalamic white matter (−10,−24,−6)	−0.407**	−0.342*
Controls > CU		
R SLF/CST (32,−32,40)	0.350*	0.364*
L CST (−16,−28,50)	0.360*	0.334*
R ATR (26,16,12)	0.288	0.210
L SLF/CC forceps major (−38,−44,24)	0.319*	0.317*
L SFG white matter (−18,16,42)	0.315*	0.357*

* $p \leq 0.05$.
** $p \leq 0.01$.

Verbal learning and memory. Partial correlations of cluster mean FA-change values and total RAVLT scores, computed over the entire sample of controls and CUs, are listed in Table 5. For both baseline and follow-up RAVLT total scores, when examined separately, negative partial correlations were obtained for clusters derived from the CUs > Controls contrast in the voxelwise analysis, while positive partial correlations were obtained for clusters from the Controls > CUs contrast. No significant partial correlations were observed between cluster mean FA-change values and RAVLT *difference scores* (follow-up score minus baseline score), as RAVLT scores showed little change during the longitudinal testing interval ($F = 0.972$ in a repeated measures ANCOVA that controlled for the same nuisance variables as in the partial correlations). It should be noted that CUs performed significantly more poorly than Controls at both time points ($F = 8.18$, $p = 0.007$, partial eta squared = 0.173 for the main effect of group in the repeated measures ANCOVA).

3. Discussion

This study assessed changes in axonal fiber organization over a 2-year period of continued heavy cannabis use in young adults who initiated use during adolescence. White matter microstructure is known to show continued developmental changes during this age period (Lebel et al., 2012). As predicted, FA change was generally lower and RD change was generally higher for CUs as compared to controls. Specifically, CUs showed less FA change in the central and parietal regions of the right and left SLF (extending into the temporal/parietal/occipital white matter junction region on the left), in white matter adjacent to the left superior frontal gyrus, in the left CST just medial to the SLF, and in the right anterior thalamic radiation lateral to the genu of the corpus callosum. A corresponding finding of more RD change for CUs overlapped the FA cluster in the right SLF, but encompassed a much larger white matter region that included adjacent medial structures such as the CST and posterior cingulum.

These deviations in the development of white matter microstructure were observed primarily in widely distributed cortical association fibers, which is consistent with findings from functional MRI and behavioral studies of cannabis users. Cognitive studies consistently find diminished CU performance on tasks that require more effortful performance, such as executive functioning, verbal free recall of previously learned information, decision-making, abstract reasoning, and complex spatial working memory (Becker et al., 2014; Bolla et al., 2002; Dougherty et al., 2013; Fontes et al., 2011; Gruber et al., 2012; Hanson et al., 2010; Lisdahl and Price, 2012; Medina et al., 2007). In functional MRI studies, CUs tend to show a broader pattern of cortical activation and recruit alternative brain networks during task performance, as compared to non-using controls (Block et al., 2002; Chang et al., 2006; Harding et al., 2012; Jacobsen et al., 2004; Kanayama et al., 2004; Padula et al., 2007; Schweinsburg et al., 2010; Tapert et al., 2007). Increased activation and recruitment of alternative information processing pathways by CUs may reflect imperfect functional compensation for subtle degradations in structural connectivity, such as those reported here.

Some of the changes in CUs white matter microstructure were dose-dependent, i.e., correlated with self-reported quantity of typical and heavier cannabis use patterns, again in accord with prediction. The total number of cannabis hits in the past 12 months, as assessed at follow-up, demonstrated strong negative correlations with mean FA change in the left SLF (−38,−44,24) and CST (−16,−28,50) clusters, as did the maximum cannabis hits frequency with the right SLF (32,−32,40) cluster. Similar measures of alcohol use and a measure of tobacco use did not correlate with FA change. Thus, although the CU group used more alcohol and tobacco than controls, only their cannabis use was associated in a continuous quantitative manner with FA change, thereby strengthening the inference that cannabis use itself was a primary factor underlying the observed group differences. This finding contradicts existing longitudinal DTI studies that have not documented changes over time attributable to amount of cannabis use. Bava et al. (2013) reported a large number of DTI voxel clusters differentiating a group of alcohol-plus-cannabis users from non-users but found that results were driven by alcohol use over the 18-month follow-up period rather than cannabis use. Similarly, Jacobus et al. (2013a) found that over a 3-year interval, a group of binge alcohol users and a group of combined binge alcohol and cannabis users differed from non-using controls in 15 voxel clusters, but with only one exception, the clusters did not differentiate the two user groups. However, a smaller longitudinal DTI study ($n = 16$) on the initiation (rather than continuation) of heavy alcohol and cannabis use reported 20 clusters distributed throughout the brain that differentiated participants who initiated combined alcohol and cannabis use vs. alcohol use alone, with decreased FA shown almost exclusively by the combined alcohol and cannabis users over the 3-year follow-up period (Jacobus et al., 2013b). Our results are consistent with Jacobus et al. (2013b) in suggesting that brain white matter changes driven by heavy cannabis use during late adolescence and early adulthood can be detected by DTI despite the presence of concurrent alcohol use. This is an important empirical demonstration, given that white matter development extends into the third decade of life (e.g., Lebel et al., 2012) and that cannabis use during adolescence and early adulthood has been found to have long-term impacts on cognitive functioning and brain structure and organization, both in animal models (e.g., Bambico et al., 2010; Gleason et al., 2012; Pistis et al., 2004; Raver et al., 2013; Rubino et al., 2009; Schneider and Koch, 2003, 2007; Schneider et al., 2008; Stopponi et al., 2014) and in humans (e.g., Battisti et al., 2010; Ehrenreich et al., 1999; Fontes et al., 2011; Lisdahl et al., 2013; Meier et al., 2012; Pope et al., 2003; Solowij et al., 2012; Wagner et al., 2010).

Contrary to our predictions, several findings for the CU group involved DTI changes generally associated with what might be interpreted as better white matter organization, i.e., increased FA and decreased RD. In the left hemisphere, more positive FA change was observed for CUs in the anterior CC (posterior genu, rostral body) and posterior thalamic white matter. A corresponding decreased-RD cluster overlapped the posterior thalamic area and also extended medially into the CST. In analyses of baseline data, the medial CC genu region (caudal forceps minor tract) showed higher FA values, and there was a trend effect of lower RD values in the CU group. Filbey et al. (2014) also reported higher FA and lower RD for CUs in the forceps minor of the CC, and more generally an early study reported a similar pattern in several regions including the medial frontal white matter (Delisi et al., 2006).

Differences observed at baseline may reflect areas of relative strength in the CU group, consistent with our previous behavioral report that described increased processing speed in CUs in the context of numerous relative cognitive deficits (Becker et al., 2014). Interpretation of FA increases and RD decreases in the CU group over the 2-year follow-up period is more complex, as the functional implications depend on more nuanced aspects of the development of axonal fiber organization that can drive DTI metrics in opposing directions. For example, FA increases may be observed over time when axonal connections align more cohesively in a single fiber population, i.e., during development within a parallel bundled tract, while the converse (FA decreases) may be observed when neural development occurs within multiple fiber populations in the same region, i.e., areas where fibers cross or fan out into gray matter (e.g., Douaud et al., 2011). Thus, although both group contrasts, Controls > CUs and CUs > Controls, generated significant clusters in the voxelwise analysis, it does not necessarily follow that the CUs > Controls contrast clusters represent regions of enhanced white matter development in CUs. Such determinations ultimately depend on correlations with behavioral measures. Although mapping the DTI results to longitudinal changes in performance on our full neuropsychological battery, which includes nearly a hundred variables, was beyond the scope of this report, an informative correlational pattern emerged when we analyzed the direction of associations between the mean FA-change cluster values and performance on a demanding test of learning and memory, the RAVLT. Total RAVLT scores correlated positively with FA change within five of the seven clusters, namely those generated by the Controls > CUs contrast. Conversely, total RAVLT scores correlated negatively with FA change within the remaining two clusters generated by the CUs > Controls contrast, i.e., better performance was associated with more negative, rather than positive, FA change during the follow-up period within the thalamic/CST and anterior CC regions. Thus, it is possible that all of our longitudinal DTI results reflect deleterious effects of continued heavy cannabis use, as expressed in white matter microstructure in brain regions spanning different forms of fiber tract development, and in continuing functional decrements in learning and memory. Interpretation is limited, though, by the fact that CUs demonstrated consistently poor performance relative to controls in their verbal learning and memory performance and did not show a performance decline over time.

Notably, our findings are also informative regarding the timing of use-related impacts on brain structure. Neurocognitive deficits (Fontes et al., 2011; Gruber et al., 2012) as well as functional alterations observed in task-based fMRI paradigms (Tapert et al., 2007) in cannabis users have been attributed to early onset of use, most typically operationalized as a pattern of regular use beginning prior to the age of 16–17 years. Because early onset users are likely to use in large quantities and with higher frequencies than later onset users (Sagar et al., 2015), it is unclear whether age of onset, frequency, and magnitude of use show independent effects on brain structure and function. Indeed, early onset use, in combination with the high frequency of use and use in large amounts that tends to co-occur, may represent additive vulnerabilities as suggested by Sagar et al. (2015). Heavy chronic use has been associated with structural brain deviations (Battistella et al., 2014; Filbey et al., 2014) and neurocognitive changes (Bolla et al., 2002). Our findings suggest that impacts of heavy cannabis use on white matter microstructure do not necessarily depend on age of onset. Furthermore, such impacts may not be reliably detectable until after at least 3–4 years of heavy use beginning in the adolescent period given that few differences at our baseline assessment (after 1–2 years of heavy use) were observed. This patterning is consistent with the idea that enduring changes in brain structure, particularly non-lesion-based alterations that can be detected with MRI, emerge gradually due to the impacts of exogenous cannabinoid exposure on neurochemical substrates that ultimately impact axonal integrity. Larger studies are needed to disentangle individual differences in the effects of early exposure and levels of use on later neural and behavioral disruptions.

Unlike previous cross-sectional studies, we did not find DTI differences between CUs and non-using controls in white matter

tracts associated with core limbic structures such as the hippocampus (e.g., fimbria; Zalesky et al., 2012; Yücel et al., 2010) and amygdala (e.g., uncinate fasciculus; Jacobus et al., 2013a,b; Shollenbarger et al., 2015), either at baseline or follow-up. These limbic structures are rich in endocannabinoid receptors, and cannabis exposure has been linked with hippocampal damage in animal models (Rubino et al., 2009). Other studies may have had greater sensitivity to detect effects in these smaller tortuous white matter tracts due to the use of fiber tractography (Shollenbarger et al., 2015; Zalesky et al., 2012) and incorporation of substantially lower cluster-size thresholds in voxel-based analysis (Jacobus et al., 2013a,b).

Limitations of our study must be noted. The sample was relatively small and comprised mostly of Caucasian participants. While the DTI results were similar to those reported in other samples, additional research is needed among more racially and ethnically diverse samples to improve generalizability of the findings. Similarly, our sample is characterized by high average IQ estimates, which again limits the generalizability of findings and perhaps skews overall FA levels toward higher values (Navas-Sánchez et al., 2014). That said, these findings are sobering regarding potential impacts of chronic cannabis use across late adolescent development in an otherwise low-risk sample. An additional limitation with our sample is the greater range of ages in the control group relative to CUs. Groups were matched on mean age, and we took the additional step of controlling for age at baseline in statistical analyses to mitigate potential confounding by age variance. Baseline age did not produce supra-threshold clusters in analyses of FA and RD change over the two-year follow-up period, which indicated that the group-wise DTI findings were not confounded significantly by the age range difference between groups.

Perhaps more importantly, CUs and controls differed substantially in alcohol use, which is commonly observed in studies of adolescent and young adult cannabis users (e.g., Arnone et al., 2008; Bava et al., 2009, 2013; Gruber et al., 2014; Jacobus et al., 2013a,b; Shollenbarger et al., 2015; Thatcher et al., 2010). To assess this sampling confound, past 12-month alcohol use at both baseline and follow-up were included in preliminary analyses of FA and RD change, which produced no supra-threshold clusters for the two alcohol use variables. To provide statistical control for sub-threshold differences, we included the average of the baseline and follow-up alcohol use variables in the final DTI analyses. The dose-dependent findings also indicated that cannabis use was the primary factor in our DTI cluster results, as mean values from several of the FA change clusters correlated with past 12-month use variables for cannabis but not alcohol. Nevertheless, the optimal design to detect and isolate longitudinal cannabis effects would employ contrasting groups that used alcohol at equivalent levels.

While we requested that participants be free of substance use for at least 24 h prior to scanning, we did not assess for recency of cannabis, alcohol, or other drug use and did not employ drug testing to verify reported levels of cannabis and other substance use among CUs or abstinence in both groups. CU and control participants completed multiple self-report and interview measures of substance use, and responses across measures were compared for reliability of information. While it is possible that participants endorsed false use levels, this appears unlikely given consistency of reports across measures within time points and across the follow-up interval. Further, CUs' self-reported level of cannabis use was supported by evidence of DSM-IV cannabis dependence in the sample as well as the neurocognitive impairments noted at baseline for these participants (see Becker et al., 2014).

Finally, while reports have described alterations in cerebellar white matter in CUs, our field-of-view placement did not allow for equivalent examination of this region across participants. Therefore, the current study could not address the impact of cannabis use on cerebellar white matter development.

4. Conclusions

Our findings are consistent with cross-sectional DTI research indicating that heavy cannabis use is associated with deleterious alterations in white matter microstructure within major fiber tracts, such as the superior longitudinal fasciculus, corticospinal tract, and corpus callosum. Our longitudinal study design extends the existing literature by assessing white matter microstructural change in the context of continuing regular cannabis use by high-functioning, non-treatment-seeking young adults who were free of comorbidities other than alcohol use. This design allowed us to distinguish a small number of baseline DTI differences between CUs and non-using controls from a larger number of group differences in FA and RD change over time. These differences suggest aberrant patterns of neurodevelopment as a consequence of heavy cannabis use given that several DTI indicators of disrupted white matter development were correlated in a dose-dependent pattern with the quantity and frequency of cannabis use. These distributed alterations in white matter fiber development may underlie the inefficient functional MRI activation patterns shown by heavy cannabis users, as well as their deficits in complex cognition and effortful processing.

Conflict of interest

The authors report no conflicts of interest.

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