DIFFUSION TENSOR IMAGING REVEALS MICRO-STRUCTURAL ALTERATIONS IN BRAIN WHITE MATTER OF ADULT CHRONIC ACTIVE MARIJUANA USERS

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List of Abbreviations

AIR = Automatic Image Registration

AD = Axial Diffusivity

ANCOVA = Analysis of Covariance

ANOVA = Analysis of Variance

BET = Brain Extraction Tool

CB = Cannabinoid

DSM = Diagnostic and Statistical Manual of Mental Disorders IV

DTI = Diffusion Tensor Imaging

FA = Fractional Anisotropy

GM = Gray Matter

GM/WM = Gray Matter/ White Matter

JHU-MNI-SS = John Hopkins University Montreal Neurological Institute Single Subject

LDDMM = Large Deformation Diffeomorphic Metric Mapping

MJ = Marijuana

MRI = Magnetic Resonance Imaging

PCR = Posterior Corona Radiata

RD = Radial Diffusivity

SLF = Superior Longitudinal Fasciculus

THC = Delta-9 Tetrahydrocannabinol

Trace = Trace or Total Diffusivity

WM = White Matter

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INTRODUCTION

Marijuana (MJ) is the most commonly used illicit drug in the U.S., with 4 million persons 12 years or older using MJ on a daily basis in 2009 (SAMHSA 2010). Additionally, the rate of MJ use and the number of MJ users has been increasing since 2007 (SAMHSA 2010). However, despite these increases, the long-term effects of chronic MJ use on brain tissue microstructure (e.g., fiber bundle coherence, myelin and axonal integrity) are still unclear (Gruber and Yurgelun-Todd 2005; Delisi, Bertisch et al. 2006; Arnone 2006b; Arnone, Barrick et al. 2008; Ashtari, Cervellione et al. 2009; Gruber, Silveri et al. 2011).

The consumption of MJ leads to a variety of acute effects due to binding of the main psychoactive cannabinoid in MJ, delta-9-tetrahydrocannabinol (THC) (Gaoni 1964; Hirst 1998), to endogenous cannabinoid (CB1) receptors in the brain (Freund, Katona et al. 2003). These receptors are expressed within white matter tissue and myelin-producing oligodendrocytes (Molina-Holgado, Vela et al. 2002; Bava, Frank et al. 2009; Mato, Alberdi et al. 2009). CB1 receptors influence brain white matter development in early and late gestational periods (Berrendero, Garcia-Gil et al. 1998). While many of the processes involved in brain white matter development begin in utero (i.e., myelin formation, neuronal migration, axonal formation and elongation), they often continue into at least the 3rd decade of life (Pfefferbaum, Mathalon et al. 1994; Courchesne, Chisum et al. 2000). Thus, the age of MJ initiation may be important in understanding its effects in brain white matter as certain regions may be more sensitive to chronic MJ exposure than others. In fact, the age of MJ initiation is especially relevant as more than half (55% to 90%) of long-term, MJ-using adults begin smoking before the age of 18 (SAMHSA 2005; UNODC 2011). For these reasons, we hypothesize that long-term, daily MJ use in adults will be associated with measurable alterations in white matter microstructure.

Diffusion tensor imaging (DTI) can be used to non-invasively evaluate the integrity of brain white matter microstructure (Le Bihan, Mangin et al. 2001; Jiang, van Zijl et al. 2006), by measuring the motion of water molecules (Jiang, van Zijl et al. 2006). Fractional Anisotropy (FA) reflects the organization or coherence of axonal fibers (lower values may indicate lower coherence), Trace represents the total diffusivity (Trace/3 =

Mean), Axial Diffusivity (AD) is water movement measured along the longitudinal axis and is a putative axonal marker (lower values may indicate axonal degeneration), and Radial Diffusivity (RD) is water movement along the perpendicular axis and is a putative marker of myelin integrity (higher values may indicate loss of myelin) (Le Bihan, Mangin et al. 2001; Song, Sun et al. 2002; Song, Sun et al. 2003; Alexander, Lee et al. 2007). To date, a limited number of DTI studies evaluated white matter microstructure in long-term, daily MJ using adults, and these studies showed conflicting results (Gruber and Yurgelun-Todd 2005; Delisi, Bertisch et al. 2006; Arnone 2006b; Ashtari, Cervellione et al. 2009; Gruber, Silveri et al. 2011). One study reported no significant differences in brain microstructure between adults who used heavily (at least 4,000 times) versus non-drug using controls (Gruber and Yurgelun-Todd 2005). In contrast, five other studies found differences in microstructure between heavy MJ users and non-drug using controls (Delisi, Bertisch et al. 2006; Arnone 2006b; Arnone, Barrick et al. 2008; Ashtari, Cervellione et al. 2009). Additionally, the locations of diffusivity changes are not consistent across studies (Delisi, Bertisch et al. 2006; Arnone 2006b; Arnone, Barrick et al. 2008; Ashtari, Cervellione et al. 2009) with some finding alterations in the frontal and parietal lobes and the cingulate region (Gruber and Yurgelun-Todd 2005; Delisi, Bertisch et al. 2006; Arnone 2006b; Gruber, Silveri et al. 2011), as well as in connective tracts such as the fronto-temporal connections (Ashtari, Cervellione et al. 2009) and the corpus callosum (Gruber and Yurgelun-Todd 2005; Arnone, Abou-Saleh et al. 2006a; Arnone 2006b; Gruber, Silveri et al. 2011).

This heterogeneity in results may arise from several different factors. First, previous studies used manual region of interest analyses (Gruber and Yurgelun-Todd 2005; Arnone 2006b; Gruber, Silveri et al. 2011), which limit the number of regions that can be evaluated (Ashtari, Cervellione et al. 2009; Faria, Zhang et al. 2010) and reproducibility is low (Faria, Zhang et al. 2010). Second, the abuse of other drugs such as alcohol may have confounded the results (Delisi, Bertisch et al. 2006; Ashtari, Cervellione et al. 2009); for instance, excessive alcohol consumption has been associated with impaired myelination, which can be detected by DTI analysis (lower FA) (Arnone, Abou-Saleh et al. 2006a; Bava, Frank et al. 2009). Lastly, gender differences were not evaluated (groups were primarily male) and studies had small to moderate sample sizes

(between 9 to 30 subjects) (Gruber and Yurgelun-Todd 2005; Delisi, Bertisch et al. 2006; Arnone 2006b; Arnone, Barrick et al. 2008; Ashtari, Cervellione et al. 2009; Gruber, Silveri et al. 2011).

Since CB1 receptors are expressed within adult brain white matter (Molina-Holgado, Vela et al. 2002) and influence brain white matter development (Armstrong, Schleicher et al. 1995; Berrendero, Garcia-Gil et al. 1998), we hypothesized that long-term, daily MJ use in adults would result in lower FA and AD and higher Trace and RD in regions that continue to myelinate into adulthood such as the superior longitudinal fasciculus (Sowell, Thompson et al. 1999; Ashtari, Cervellione et al. 2007), temporal white matter (Ashtari, Cervellione et al. 2007), and frontal white matter (Ashtari, Cervellione et al. 2007). Conversely, we did not expect to see alterations in the corpus callosum or internal capsule as these structures complete myelination within the first year of life (Brubaker, Schmithorst et al. 2009). Additionally, we hypothesized that there would be a correlation between microstructural changes and age of MJ initiation.

MATERIALS AND METHODS

Subjects

27 chronic MJ users (15 men, 12 women) and 27 healthy comparison non-drug users (15 men, 12 women) were screened and enrolled in the study. Subjects were enrolled only if they fulfilled the following criteria: (1) male or female age 18 to 45 years; (2) residing on the island of O‘ahu, Hawai‘i; (3) willing and able to comply with study procedures; (4) able to verbalize understanding of the consent form and (5) right-handed. MJ users had to meet the additional criteria of: (1) using MJ 6-7 days per week for at least one year and (2) positive urine toxicology test for THC on each day of testing. Exclusion criteria for all subjects included: (1) confounding neurological or chronic psychiatric disorder (e.g., multiple sclerosis, stroke, schizophrenia, bipolar disorder); (2) chronic severe medical condition that can confound the analysis of the study (e.g., renal or liver failure, diabetes, or chronic hypertension); (3) on medications that may confound the analysis of the study; (4) pregnancy (excluded by urine pregnancy test) and (5) contraindication for MR studies (e.g., ferromagnetic metal implants or severe

claustrophobia). Additionally, all subjects were not currently or previously dependent on any illicit drug (except MJ for users) according to DSM-IV (APA 2000) and subjects were required to test negative on a urine toxicology screen for cocaine, amphetamine, methamphetamine, THC (except for MJ users), opiates, and benzodiazepines. MJ users were instructed to abstain from smoking MJ on day of testing.

The protocol and consent forms were approved by the University of Hawaii Co-Operative Institutional Review Board. Following verbal and written consent, all subjects were evaluated with detailed medical and drug use histories during face-to-face interviews by trained research staff, and by a physician, using structured physical and neurological evaluations.

Image acquisition

MR scans were performed on a 3 T Siemens TIM Trio scanner (Siemens Medical Solutions, Erlangen, Federal Republic of Germany). Two full brain axial diffusion scans were acquired with 4 mm slice thickness: (1) Transversal Diffusion Weighted Image (DWI) (TR/TE = 4000/80 ms, 128 x 128 x 28 matrix), b = ([0,1000] s/mm^2), (2) Transversal Diffusion Tensor Image (DTI) (TR/TE = 3700/88 ms, 128 x 128 x 28), b factor = ([0,1000] s/mm^2, 12 diffusion directions). All images were reviewed to ensure that there were no structural abnormalities, or DTI artifacts/dropouts.

Diffusion tensor imaging and LDDMM analyses

First, the Diffusion Tensor Imaging (DTI) datasets were processed using DtiStudio software version 3.0.1beta (H. Jiang and S. Mori, John Hopkins University, www.MriStudio.org) (Jiang, van Zijl et al. 2006). In DtiStudio, an map was created for fractional anisotropy (FA) (Pierpaoli and Basser 1996) and diffusivity maps were created for tensor trace (trace/3 = mean diffusion), axial (AD) and radial diffusivities (RD) (Jiang, van Zijl et al. 2006). The mean of all images without diffusion weighting (B=0) was used as a reference image (Faria, Zhang et al. 2010).

Second, non-brain tissue was deleted from diffusion tensor images using the Brain Extraction Tool (BET) in FSL 4.0. (Smith 2002). Third, the pre-processed diffusion tensor images were loaded into DiffeoMap software version 1.7.1 (X. Li, H. Jiang, L.

Yue, and S. Mori, John Hopkins University, www.MriStudio.org) for registration. The images were initially linearly aligned with the JHU-MNI-SS (John Hopkins University Montreal Neurological Institute Single Subject) template by automated image registration (AIR) (Mori, Oishi et al. 2008; Oishi, Faria et al. 2009; Faria, Zhang et al. 2010). After linear normalization, the images underwent nonlinear, dual-contrast LDDMM transformation (Miller, Beg et al. 2005; Faria, Zhang et al. 2010), using the remote LDDMM server (multi-channel; α/γ ratio of 0.005) (Ceritoglu, Oishi et al. 2009). After the completion of LDDMM, the linearly aligned subject images were then non-linearly registered to the atlas using the resultant transformation matrix (i.e., Kimap.vtk) (Faria, Zhang et al. 2010).

Finally, using the ROIEditor software 1.1 (X. Li, H. Jiang, L. Yue, and S. Mori, John Hopkins University, www.MriStudio.org), the LDDMM transformed brains were segmented into 34 labeled atlas regions using the JHU-MNI-SS-WMPM (White Matter Parcellation Map) Type II atlas (Oishi, Faria et al. 2009). Next, the brains were further segmented into 46 atlas regions by separating the white matter and the cortex using a FA threshold of ≥ 0.25 in each subject (Oishi, Faria et al. 2009; Faria, Zhang et al. 2010). Final segmentation resulted in a total of 80 regions (left and right hemispheres), whose diffusivity values (FA, trace, axial, and radial) provided main outcome measures (Figure 1).

Statistical analyses

A two-way ANOVA, with drug use and gender as the main effects, was used to compare demographic characteristics of MJ and comparison subjects. Statistical significance was defined as $p<0.05$. Using Systat version 10 (SAS Institute Inc., Cary, NC), white matter DTI data were analyzed by a mixed-model ANOVA, with drug use and gender as between-subjects factors and hemisphere as a within-subject factor. Age and predicted verbal IQ from the WTAR-Predicted WAIS-III Scores (U.S. Standardization) were used as covariates. Significant main effects for the 80 regions were analyzed further using Simes multiple testing procedure (4-levels). Measures of correlation were only analyzed for DTI variables that showed significant group

differences using the Pearson product-moment correlation coefficient (denoted by *r*) and a Bonferroni correction was used for multiple comparisons.

RESULTS

Subject demographics (Table 1)

All subject groups were well matched by age and predicted verbal IQ. Lifetime alcohol consumption was not different between male and female subjects, or MJ users and comparisons subjects. There were two active nicotine smokers (Pack Years: 0.64 $\pm$ 0.11) in the control group and fifteen active nicotine smokers (Pack Years: 1.12 $\pm$ 0.59) in the MJ group. Lastly, none of the MJ use characteristics were different between male and female users.

Marijuana (Figure 2 and Table 2)

Compared to controls, MJ users had lower trace diffusivity values in the posterior corona radiata (PCR) ($F(1,48) = 13.65$, $p=0.0006$, $t(52) = 3.31$, -4.0%) and superior longitudinal fasciculi (SLF) ($F(1,48) = 10.72$, $p=0.0020$, $t(52) = 2.97$, -2.3%). Along the axial direction, MJ users showed lower diffusion in SLF ($F(1,48) = 13.48$, $p=0.0006$, $t(52) = 3.47$, -2.6%) and middle temporal white matter ($F(1,48) = 11.29$, $p=0.0015$, $t(52) = 2.92$, -2.2%) compared to controls. MJ users exhibited lower radial diffusion than controls only in the PCR ($F(1,48) = 13.33$, $p=0.0006$, $t(52) = 3.28$, -5.3%). There were no other significant differences after correction for multiple comparisons. Also, none of the interactions were significant (e.g., group-by-gender, gender-by-hemisphere, group-by-gender-by-hemisphere).

Gender (Figure 3 and Table 2)

Compared to males, females had lower FA values in the bilateral supramarginal white matter ($F(1,48) = 11.11$, $p=0.0017$, $t(52) = 2.99$, -2.7%), inferior frontal white matter ($F(1,48) = 13.12$, $p=0.0007$, $t(52) = 3.36$, -3.0%), and middle frontal white matter ($F(1,48) = 11.25$, $p=0.0016$, $t(52) = 3.17$, -3.3%).

Hemisphere (Table 3)

After correction for multiple comparisons, there were significant hemispheric differences for at least one diffusivity measure for all except 10 of the 40 white matter regions. Table 3 presents those areas where at least three of four diffusivity measures were significantly different.

Drug use history correlations with DTI measures

After correction for multiple comparisons, there were no significant correlations between diffusivity measures and MJ usage characteristics for the combined MJ group or for male and female MJ users separately.

DISCUSSION

Our main DTI findings in chronic MJ-using adults compared to healthy comparison subjects were lower diffusivity in projection and association fiber tracts and temporal white matter of the brain. Alterations in diffusivity represent a modification of normal white matter organization (Song, Sun et al. 2002; Song, Sun et al. 2003).

Chronic marijuana consumption and the superior longitudinal fasciculi

In the SLF, lower trace diffusivity in the MJ users may be due to an altered, more compact organization of axonal fibers (Song, Sun et al. 2002). Here, fiber compaction may be related to decreased axonal caliber of fibers as indicated by lower axial diffusivity in MJ users compared to controls (Song, Sun et al. 2002; Song, Sun et al. 2003). Additionally, lower axial diffusivity in the SLF may be ascribed to increased tortuosity of axonal fiber organization in MJ users compared to controls (Ashtari, Cervellione et al. 2007), which is different from normal neurodevelopment patterns in white matter fiber tracts in the human brain (i.e., less tortuous and more coherent) (Ashtari, Cervellione et al. 2007).

We suggest that an altered axonal fiber organization in the SLF of chronic, MJ users may be related to an altered cortical surface morphology (Mata, Perez-Iglesias et al. 2010). Sulcal curvature may provide information on the forces that influence the folding process such as white matter connectivity (Armstrong, Schleicher et al. 1995; Mata, Perez-Iglesias et al. 2010). A macro-structural study evaluating cortical gyrification in MJ users reported significantly greater flattening of the frontal, temporal, and parietal sulci (Mata, Perez-Iglesias et al. 2010); which are the lobules that the SLF connects (Makris, Kennedy et al. 2005; Mata, Perez-Iglesias et al. 2010). Interestingly, both studies evaluated adult MJ users who began MJ use in adolescence (Mata, Perez-Iglesias et al. 2010). In agreement with the current study's hypothesis, the authors of the cortical gyrification study also speculate that MJ use in adolescence may be associated with disruption of normal neurodevelopment (Mata, Perez-Iglesias et al. 2010).

A previous study by Ashtari and colleagues (2009) evaluated the white matter organization of the SLF in abstinent, MJ using young adults using DTI fiber tractography, and found higher trace and radial diffusivities in the left and right SLF of MJ users (Ashtari, Cervellione et al. 2009). Conversely, we found lower trace and axial diffusivity and no differences in radial diffusivity in the SLF of MJ users. The difference in findings between the previous study and the current study may be due to different study populations: in the prior study, subjects were abstinent young adult MJ users (mean ages = 19.3 years, mean time since last MJ use = 6.7 months), with co-morbid alcohol abuse (Ashtari, Cervellione et al. 2009), whereas our study evaluated active, adult MJ users with no history of abuse or dependence on other drugs.

Chronic marijuana consumption and the middle temporal white matter

In the middle temporal (MT) white matter, lower axial diffusivity in chronic MJ users compared to controls may reflect axonal degeneration (Song, Sun et al. 2002; Alexander, Lee et al. 2007) and/or increased tortuosity of axonal fibers (Ashtari, Cervellione et al. 2007). Similar to findings in the SLF, increased tortuosity of axonal fiber organization suggests abnormal neurodevelopment patterns of white matter in the human brain (Ashtari, Cervellione et al. 2007). To the best of our knowledge, no other studies have specifically evaluated the middle temporal white matter in chronic adult MJ

users. However, other studies have evaluated brain structures that reside within the middle temporal lobes, such as the hippocampus (Ashtari, Avants et al. 2011). For instance, in a second study by Ashtari and colleagues (2011) hippocampal morphology was compared between abstinent, MJ using young adults and controls (Ashtari, Avants et al. 2011), and smaller hippocampi was found in abstinent MJ users (Ashtari, Avants et al. 2011). While the previous study and the current study had different study populations (i.e., abstinent versus active MJ use, mean time since last use = 6.7 months), these studies did share the similarity that the MJ users evaluated began MJ use in adolescence (Ashtari, Avants et al. 2011). Together, these data may suggest that the middle temporal region is susceptible to structural alterations related to chronic, MJ use beginning in adolescence.

Chronic marijuana consumption and the posterior corona radiata

In the posterior corona radiata (PCR), chronic MJ users compared to controls had lower trace and radial diffusivities. While higher radial diffusivity is often associated with myelin loss (Song, Sun et al. 2002; Alexander, Lee et al. 2007), the PCR findings might be due to more compact axonal fiber packing, an increase in protein content (i.e., microtubules and neurofilaments), and axonal degeneration (Song, Sun et al. 2002).

While we are not aware of prior DTI findings in the PCR of chronic adult MJ users, one previous DTI study found lower fractional anisotropy (FA) in the superior region of the corona radiata (SCR) in young adults who co-abused alcohol and MJ compared to controls (Jacobus, McQueeny et al. 2009). Additionally, FA values in the SCR increased with increasing MJ use (Jacobus, McQueeny et al. 2009). Overall, the diffusivity alterations in the corona radiata of MJ users further support our hypothesis that regions still developing in adolescence may be more susceptible to concurrent MJ use.

Gender and white matter

Compared to men, women had lower fractional anisotropy (FA) in bilateral supramarginal white matter, middle frontal white matter and inferior frontal white matter. The current findings of sexual dimorphism in brain white matter are consistent with previous findings evaluating gray/white matter volumes in a cohort of healthy right-

handed male and female adults (Allen, Damasio et al. 2003). In this study, for all major lobes of the cerebrum, male volumes were greater compared to females, but the gray matter/white matter (GM/WM) ratio was consistently and significantly higher for females compared to males (Allen, Damasio et al. 2003). The authors of that study suggest that the higher GM/WM ratio in females is associated with a lower WM volume (Allen, Damasio et al. 2003), and not a higher GM volume as suggested by others (Gur, Turetsky et al. 1999). Lower WM volume is suggested because GM shows a less profound degree of sexual dimorphism relative to WM and the authors used an ANCOVA to parcel the effects (Allen, Damasio et al. 2003). Lower WM volume is consistent with our findings of lower FA in females, as this diffusion metric may reflect a more compact organization of white matter fibers present (Alexander, Lee et al. 2007). There was not an increase in total diffusivity for these regions, thus our results do not reflect a lower number of fibers (Sullivan, Adalsteinsson et al. 2006). That study by Allen and colleagues (2003) is similar to the current study as both evaluated right-handed adults with a similar range of ages (Allen, Damasio et al. 2003).

Correlations between group diffusivity differences and marijuana usage characteristics

After correction for multiple comparisons, we did not observe any significant correlations for any diffusivity measure with any MJ usage characteristic. Previous studies demonstrated (1) a trend towards a positive correlation between mean diffusion with longer duration of MJ consumption in the corpus callosum ($r = 0.66$, $p = 0.053$) (Arnone, Barrick et al. 2008) and (2) a negative correlation ($r = -0.57$, $p = 0.03$) between the right hippocampus volume and higher amount of MJ use (total number of joints smoked) (Ashtari, Avants et al. 2011). However, in both previous studies, analysis was performed only with male adults, thus gender differences where not evaluated (Arnone, Barrick et al. 2008). Overall, there is a paucity of information on gender differences in chronic MJ abuse. Future studies should include comparable numbers of males and females, because white matter has the most profound degree of sexual dimorphism in the healthy adult (Allen, Damasio et al. 2003), and CB1 receptors are expressed within adult

brain white matter (Molina-Holgado, Vela et al. 2002) and influence brain white matter development (Armstrong, Schleicher et al. 1995; Berrendero, Garcia-Gil et al. 1998).

Chronic marijuana consumption and white matter

Overall, our chronic MJ-using adults compared to controls had lower trace, axial, and radial diffusivities in in several white matter regions. Alterations in diffusivity represent a modification of normal white matter organization (Song, Sun et al. 2002; Song, Sun et al. 2003). Lower axial diffusivity, but not lower trace and radial diffusivity is consistent with our hypothesis that regions that continue to mature into early adulthood would be more susceptible to microstructural alterations related to chronic MJ use beginning in adolescence and continuing into adulthood. Such regions include the SLF and middle temporal white matter (Sowell, Thompson et al. 1999; Ashtari, Cervellione et al. 2007; Bava, Thayer et al. 2010). Likewise, regions continuous with the PCR, such as the superior corona radiata and posterior limb of the internal capsule, all continue the myelination and brain maturation processes into late adolescence and early adulthood (Lazar, Weinstein et al. 2003; Dougherty, Ben-Shachar et al. 2007; Bava, Thayer et al. 2010).

The current results of alterations in brain white matter of chronic MJ users are consistent with previous literature highlighting the expression and influence of CB1 receptors, the main site of action of THC (Freund, Katona et al. 2003), in oligodendrocyte development and in brain white matter maturation (Pfefferbaum, Mathalon et al. 1994; Berrendero, Garcia-Gil et al. 1998; Courchesne, Chisum et al. 2000; Molina-Holgado, Vela et al. 2002; Bava, Frank et al. 2009; Mato, Alberdi et al. 2009). Oligodendrocytes are cells responsible for production and maintenance of the myelin sheath, the insulating material that allows proper conduction of axon potentials (Mato, Alberdi et al. 2009). In the endogenous cannabinoid system, activation of CB1 receptors promotes oligodendrocyte survival in unfavorable conditions, drives progenitor proliferation, and promotes differentiation into myelinating cells (Molina-Holgado, Vela et al. 2002; Mato, Alberdi et al. 2009). However, chronic MJ exposure may cause a down-regulation of CB1 receptors and suppress oligodendrocyte function (Bava, Frank et al. 2009). Additionally, chronic MJ exposure may adversely impact oligodendrocyte development

leading to aberrant cell differentiation and migratory processes (Bava, Frank et al. 2009). Reductions in myelin initiation, deposition, compaction, and maintenance can be a result of poor oligodendrocyte differentiation or death (Davis, Stewart et al. 2003). Other studies have also shown that chronic MJ exposure can alter the expression of myelin proteolipid protein, a protein necessary for normal myelin structure (Grigorenko, Kittler et al. 2002).

A couple of limitations in this study should be noted. First, the accuracy of the self-reported drug history data may be constrained. We did perform urine toxicology on MJ subjects to verify recent usage of MJ, but more detailed MJ usage history with timeline-follow back interviews (Huestegge, Radach et al. 2009) and hair analyses may also be useful. Additionally, while there were no significant differences between groups for drug use other than MJ (e.g., alcohol, nicotine). If self-reported data underestimated other drug use, such alcohol consumption for example, excessive drinking could have confounded our study results (lower FA) (Arnone, Abou-Saleh et al. 2006a; Bava, Frank et al. 2009). Second, the current study used 12 diffusion directions for DTI analysis, but more directions may have provided more information on microstructure integrity. Finally, the current study results observed might reflect genetic differences that make users more likely to chronically abuse MJ (Wang, Yuan et al. 2011). In conclusion, chronic MJ-using adults compared to healthy comparison subjects had lower diffusivity in projection/association fiber tracts and temporal white matter of the brain, brain regions that continue neurodevelopment into early adulthood (Lazar, Weinstein et al. 2003; Dougherty, Ben-Shachar et al. 2007; Bava, Thayer et al. 2010). Therefore, the current findings support our hypothesis that brain regions still developing in adolescence may be sensitive to chronic MJ exposure. Consequently, chronic MJ use in adolescence and continuation into adulthood may adversely impact normal brain white matter development.

Table 1. Demographics and MJ use characteristics (means and standard error).

	Controls (n= 27)		MJ Users (n= 27)		
	Males (n=15)	Females (n=12)	Males (n=15)	Females (n=12)	Two-Way ANOVA F(1,50) =
Age (years)	27.5 (2.7)	26.2 (2.0)	26.1 (2.1)	23.4 (1.3)	Gender: 0.74, p = 0.39 Group: 0.86, p = 0.36; Group-by-Gender: 0.10, p = 0.76
Predicted Verbal IQ	111.4 (1.8)	109.0 (2.0)	106.5 (1.9)	107.2 (2.6)	Gender: 0.18, p = 0.67 Group: 2.63, p = 0.11 Group-by-Gender: 0.54, p = 0.47
Alcohol (lifetime exposure grams) x 10^2	315.2 (280.8)	122.7 (56.8)	358.4 (114.3)	135.2 (38.8)	Gender: 1.45, p = 0.24 Group: 0.02, p = 0.87; Group-by-Gender: 0.008, p = 0.93
Nicotine (pack years for current smokers)	0.53 (0.00)*	0.75 (0.00)*	1.23 (0.98)**	0.97 (0.36)**	
MJ Use Characteristics					
Age of First Use (years)			15.5 $\pm$ 0.8	16.3 $\pm$ 0.5	t(25) = 0.66, p = 0.52
Mean Daily Use (grams/day)			4.8 $\pm$ 1.9	2.8 $\pm$ 0.8	t(25) = 0.90, p = 0.38
Duration of Use (years)			10.1 $\pm$ 2.3	6.9 $\pm$ 1.6	t(25) = 0.65, p = 0.52
Lifetime exposure (grams)			16,683 $\pm$ 7360	4,947 $\pm$ 1197	t(25) = 0.94, p = 0.36
No significant differences. *Nicotine Mean Pack Years (SEM) for Controls (Males, n=1; Females, n=1). **Nicotine Mean Pack Years (SEM) MJ Users (Males, n = 9; Females, n = 6).					

Table 2. Mean DTI values and SEM (arbitrary units) in the bilateral white matter for group and gender. After correction for multiple comparisons, there were no significant interactions (group-by-gender) in the white matter (WM).

Region	Controls (n=27)	MJ Users (n=27)	Males (n=30)	Females (n = 24)
Tensor Trace x 10^{-3}				
Posterior Corona Radiata	2.33 ± 0.016**	2.24 ± 0.022**	2.29 ± 0.021	2.27 ± 0.019
Superior Longitudinal Fasciculi	2.19 ± 0.011*	2.14 ± 0.013*	2.17 ± 0.011	2.17 ± 0.014
Fractional anisotropy				
Supramarginal WM	0.35 ± 0.002	0.36 ± 0.003	0.36 ± 0.002*	0.35 ± 0.003*
Inferior Frontal WM	0.37 ± 0.002	0.37 ± 0.003	0.37 ± 0.002**	0.36 ± 0.003**
Middle Frontal WM	0.37 ± 0.003	0.37 ± 0.003	0.37 ± 0.002*	0.36 ± 0.003*
Axial Diffusivity x 10^{-4}				
Middle Temporal WM	10.9 ± 0.06*	11.2 ± 0.06*	11.1 ± 0.06	11.0 ± 0.06
Superior Longitudinal Fasciculi	10.7 ± 0.06 **	10.4 ± 0.06**	10.6 ± 0.06	10.5 ± 0.07
Radial Diffusivity x 10^{-4}				
Posterior Corona Radiata	5.9 ± 0.05**	5.6 ± 0.07**	5.7 ± 0.07	5.7 ± 0.07

* $p \leq 0.0020$
** $p \leq 0.0007$
Simes (4-Level) Corrected P-Values.

Table 3. Mean DTI values and SEM (arbitrary units) in white matter (WM) for each hemisphere, where at least three of four diffusivity measures were significantly different after correction for multiple comparisons. All corrected p-values were less than 0.0001, unless otherwise noted.

Region	Left Hemisphere (n=54)			Right Hemisphere (n=54)		
	FA (SEM) x 10^{-1}	Axial (SEM) x 10^{-3}	Radial (SEM) x 10^{-4}	FA (SEM) x 10^{-1}	Axial (SEM) x 10^{-3}	Radial (SEM) x 10^{-4}
Body of Corpus Callosum	5.14 (0.07)	1.70 (0.02)	7.31 (0.15)	5.00 (0.08)	1.78 (0.02)	7.95 (0.16)
Splenium of Corpus Callosum	5.67 (0.07)	1.63** (0.01)	6.01 (0.11)	5.80 (0.08)	1.60** (0.01)	5.64 (0.10)
Angular WM	3.74 (0.02)	1.08 (0.005)	6.00 (0.04)	3.68 (0.2)	1.10 (0.005)	6.13 (0.03)
Cingulate WM	3.58 (0.04)	1.13* (0.008)	6.31 (0.04)	3.51 (0.04)	1.14* (0.007)	6.48 (0.05)
Middle Frontal WM	3.62 (0.02)	1.06 (0.004)	6.05 (0.03)	3.68 (0.02)	1.05 (0.004)	5.92 (0.03)

* p = 0.0008, ** p = 0.0003
Simes (4-Level) Corrected P-Values

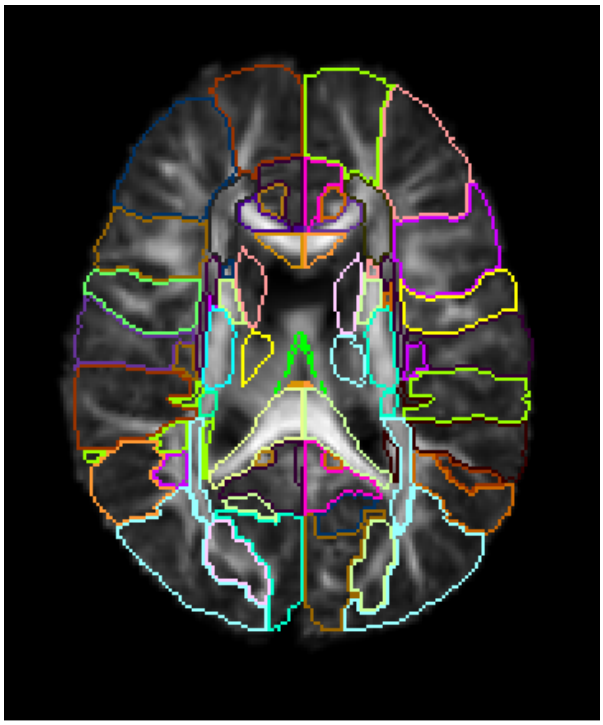

Figure 1. Final result of LDDMM transformation. Detailed region-of-interest analyses were performed on each subject brain using Large Deformation Diffeomorphic Metric Mapping (LDDMM). LDDMM extracted 80 white matter regions.

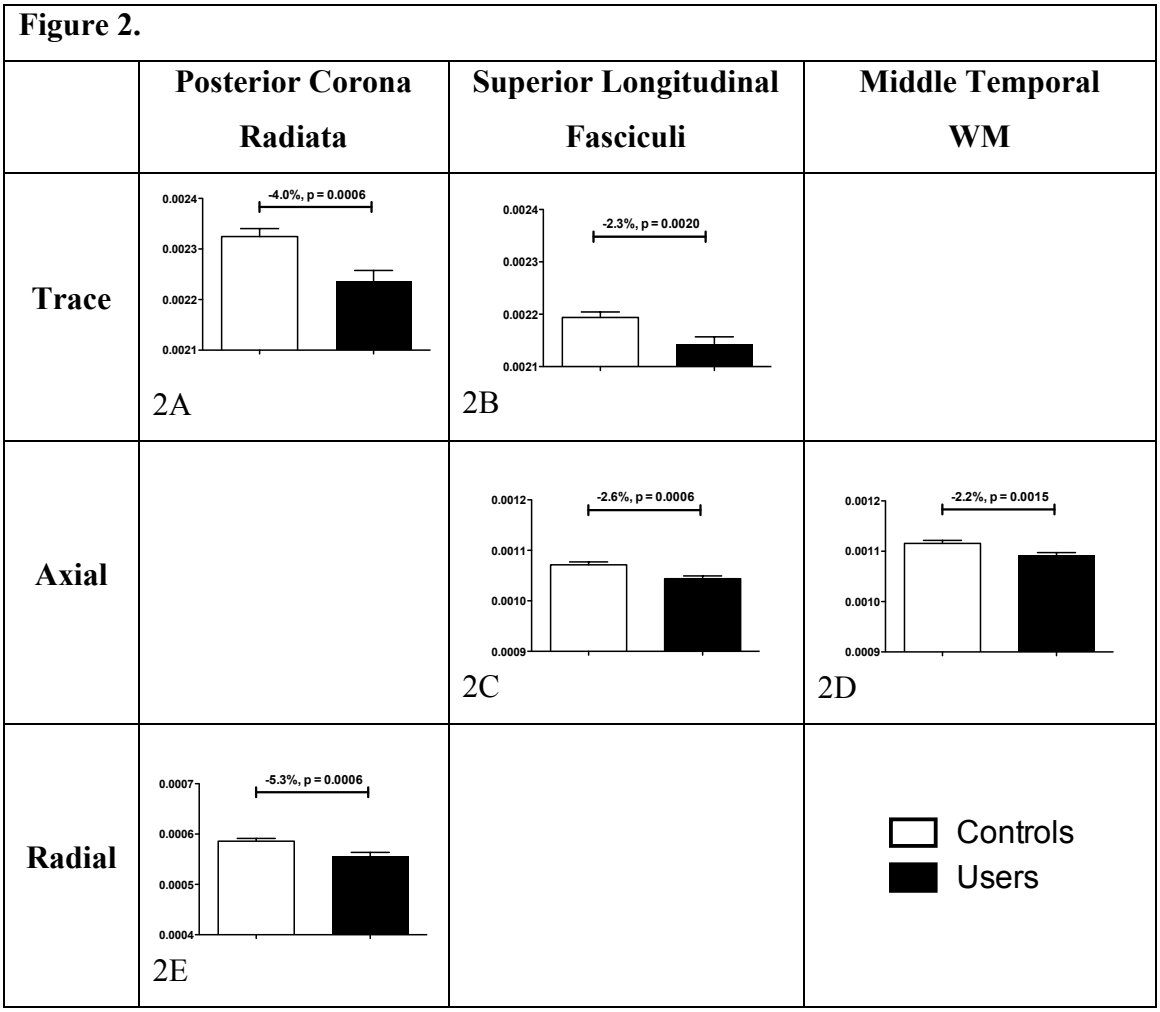

Figure 2. Summary of results for group. Group differences for total diffusion (trace) were observed in the (a) posterior corona radiata and (b) superior longitudinal fasciculi. Significant decreases in axial diffusion were observed in (c) superior longitudinal fasciculi and (d) middle temporal white matter. A significant decrease in radial diffusion in the (e) posterior corona radiata was also observed in MJ users compared to non-drug using controls. All diffusion data were corrected for multiple comparisons using a 4-level Simes test.

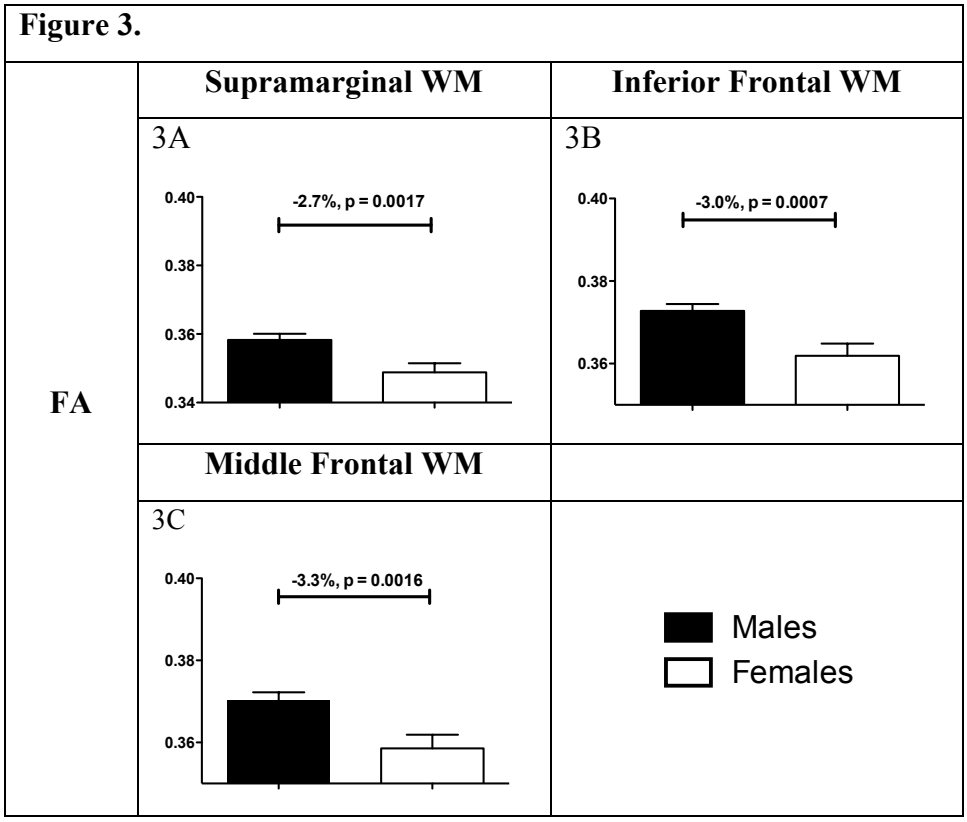

Figure 3. Summary of results for gender. Gender differences were observed for fractional anisotropy (FA) in (a) supramarginal white matter, (b) inferior frontal white matter, and (c) middle frontal white matter regions. All diffusion data were corrected for multiple comparisons using a 4-level Simes test.

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