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Prefrontal cortex morphometry in abstinent adolescent marijuana users: Subtle gender effects

Krista Lisdahl Medina, Ph.D.[3], **Tim McQueeny**[2], **Bonnie J. Nagel, Ph.D.**[4], **Karen L. Hanson, Ph.D.**[1,2], **Tony T. Yang, M.D.**[1], and **Susan F. Tapert, Ph.D.**[1,2,*]

[1]Department of Psychiatry, University of California, San Diego, California

[2]VA San Diego Healthcare System, San Diego, California

[3]Department of Psychology, University of Cincinnati, Ohio

[4]Department of Psychiatry, Oregon Health and Science University, Portland, Oregon

Abstract

Introduction—Adult human studies suggest frontal dysfunction associated with chronic marijuana (MJ) use, but due to continued neuromaturation, adult studies may not generalize to adolescents. This study characterized prefrontal cortex (PFC) morphometry in chronic MJ-using adolescents following one month of monitored abstinence.

Methods—Data were collected from MJ users (n=16) and controls (n=16) aged 16-18. Extensive exclusionary criteria included comorbid psychiatric and neurologic disorders. Substance use and anatomical measures were collected after 28 days of monitored abstinence. PFC volumes were ascertained from manual tracing by reliable raters on high-resolution magnetic resonance images.

Results—After controlling for lifetime alcohol use, gender, and intracranial volume, MJ users did not differ from controls in PFC volume. However, marginal group-by-gender interactions were observed (p<.09): female MJ users demonstrated comparatively larger PFC volumes while male MJ users had smaller volumes compared to same-gender controls. Further, group status and total PFC volume interacted in predicting executive functioning (p<.05). Among MJ users, smaller PFC total volume was associated with better executive functioning while the opposite pattern was seen among the controls.

Conclusions—These preliminary results indicate that gender may moderate the relationship between MJ use and PFC morphometry. Given the relationship between larger PFC total volumes and poorer executive functioning among MJ users, female MJ users may be at increased risk for neurocognitive consequences. Future research will measure PFC gray and white matter separately and follow boys and girls over adolescence to examine the influence of MJ use on neurodevelopment.

Keywords

Adolescents; Cannabis; Drug Effects; Executive Functioning; Gender; MRI

INTRODUCTION

Marijuana continues to be the most popular illicit drug used among adolescents. Almost half of 12th graders have used marijuana and 5% use daily (Johnston et al., 2005). Given the high

*Correspondence concerning the paper should be addressed to Susan F. Tapert, Ph.D., 3350 La Jolla Village Drive (151B), San Diego, CA 92161, Phone: (858) 552-8585. stapert@ucsd.edu.

prevalence of marijuana use during adolescence, a time of continued neurodevelopment (e.g. Giedd et al., 1996b; Lenroot and Giedd, 2006; Nagel et al., 2006; Sowell et al., 2004), the impact of chronic use on brain morphometry among teens is of great interest.

Animal and human research have suggested that the prefrontal cortex (PFC), the last brain region to undergo maturation during adolescence (Gogtay et al., 2004; Lenroot and Giedd, 2006; Sowell et al., 2004), may be particularly vulnerable to the effects of heavy marijuana exposure (see Egerton et al., 2006; Loeber, 1999). Recent research has found particularly high cannabinoid receptor type 1 (CB1) density in the PFC region among humans compared to other species (Eggan and Lewis, 2007; Herkenham et al., 1990). *In vivo* measurement of CB1 binding, completed through simultaneous administration of a high-affinity CB1 radioligand ([^{18}F]MK-9470) and a positron emission tomography brain scan, confirmed higher CB1 density in the PFC in healthy adult humans compared to other species, especially in the orbitofrontal cortex (see Van Laere et al., 2008a for details regarding this novel methodology).

Although onset of marijuana use is typically during adolescence, a time when the brain is still maturing, few brain structure studies have been conducted with adolescent users. Adult human neuroimaging studies have also suggested vulnerability of the PFC to chronic marijuana exposure (Loeber, 1999). In general, fMRI and PET studies have found differential activation in the PFC among MJ users compared to controls, including the dorsolateral and orbitofrontal PFC (Amen and Waugh, 1998; Block et al., 2000; Block et al., 2002; Chang et al., 2006; Eldreth et al., 2004; Gruber and Yurgelun-Todd, 2005; Jager et al., 2007; Kanayama et al., 2004; Lundqvist et al., 2001; Volkow et al., 1996). Despite these functional abnormalities, few studies have examined structural brain changes as a result of marijuana exposure during adulthood, and none have specifically examined PFC structure. The existing studies utilizing high-resolution magnetic resonance imaging (MRI) technology have yielded conflicting results. One study found reduced global brain volume, smaller cerebellar vermis, and focal white matter abnormalities in young adult marijuana users with a history of polysubstance abuse (Aasly et al., 1993). However, this may be due to alcohol use in the sample, as alcohol has been independently associated with smaller brain volumes (e.g., Deshmukh et al., 2002; Medina et al., 2008). Matochik and colleagues (Matochik et al., 2005) reported decreased gray matter and increased white matter density in hippocampal regions among young adult marijuana users compared to controls. Yücel and colleagues (2008) found smaller hippocampal and amygdala volumes in chronic marijuana using adults than in non-using controls. In contrast, two studies examining overall white and gray matter volumes (Block et al., 2000) and hippocampal volumes (Tzilos et al., 2005) in adult marijuana users did not find structural abnormalities. These studies conducted with adults cannot necessarily generalize to adolescent marijuana users.

As the PFC continues to refine into late adolescence/early adulthood (e.g., Lenroot and Giedd, 2006), the introduction of exogenous cannabinoids may interrupt its development. This is of great concern given the high prevalence of marijuana use during adolescence, which may place teens at increased risk for neurocognitive deficits (Ehrenreich et al., 1999; Medina et al., 2007a; Pope et al., 2003; Wilson et al., 2000) or psychiatric disorders (Arseneault et al., 2002; Bovasso, 2001; Brook et al., 2002; Cohen, Solowij, & Carr, 2008; Fergusson et al., 2002; Green and Ritter, 2000; Medina et al., 2007b; Patton et al., 2002). Functional neuroimaging studies conducted with adolescents have found abnormal PFC activation patterns among adolescent marijuana users compared to controls in response to go/no-go (Tapert et al., 2007), verbal working memory (Jacobsen et al., 2007) and spatial working memory (Schweinsburg et al., 2005; Schweinsburg et al., 2008) tasks.

From a structural standpoint, our laboratory found that marijuana users did not significantly differ from controls in hippocampal volumes, although correlations between hippocampal size and verbal memory were abnormal when contrasted to non-users (Medina et al., 2007c). A recent study examining white matter integrity, utilizing diffusion tensor imaging, found reduced apparent diffusion coefficient values in the left middle frontal gyrus and posterior cingulate and increased fractional anisotropy values in the left anterior cingulate, right medial frontal gyrus, left precentral gyrus, right inferior parietal, right cingulate gyrus, and left superior frontal gyrus among adolescents and young adults with histories of marijuana use compared to controls (Delisi et al., 2006). Although no structural MRI studies in adolescent marijuana users have focused on the PFC to date, recent findings have reported abnormal N-acetylaspartate/total creatine ratio in the dorsolateral PFC among late adolescent and young adult male marijuana users (Hermann et al., 2007).

During adolescence, gender may moderate the effects of marijuana on the brain (Benes et al., 1994; Block et al., 2000; Giedd, 2004; Huttenlocher, 1979; Huttenlocher and Dabholkar, 1997; Spear, 2000). In healthy adolescents, on average, females' PFC gray matter volumes peak one to two years earlier than males (Giedd et al., 1996b). In contrast to prior studies of white matter neuromaturation suggesting increased myelination of the PFC among female adolescents (e.g., Giedd et al., 1999; Pfefferbaum et al., 1994; Reiss et al., 1996), our laboratory found that, among females, PFC white matter volume actually decreased from age 15-18, while males' PFC white matter remained relatively stable (Nagel et al., 2006). Given these gender differences in neuromaturation, chronic endogenous cannabinoid exposure may have disparate effects on PFC structure and function for boys and girls. Indeed, gender has been shown to moderate the relationship between neurocognitive functioning and drug effects in young adult polydrug (including marijuana) users (Bolla et al., 1998), young adult and adolescent alcohol users (Caldwell et al., 2005; Medina et al., 2008), and young adult marijuana users (Pope et al., 1997). In addition, our laboratory has reported a relationship between decreased white matter volume and depressive symptoms that was primarily driven by female adolescent marijuana users (Medina et al., 2007b).

As noted above, although animal and adult human studies have suggested marijuana-induced PFC alterations, no studies of adolescents have yet examined marijuana's effect on PFC morphometry and executive functioning, which is subserved by the PFC. Identifying whether boys and girls demonstrate different neurocognitive consequences of marijuana use may help clarify whether sex hormones and the endogenous cannabinoid system influence PFC neurodevelopment. The goal of the current study was to determine whether gender moderates the relationship between chronic marijuana use, PFC volume, and executive functioning in adolescent marijuana users without comorbid psychiatric disorders after approximately one month of abstinence.

METHODS

Participants

Adolescents were recruited from local high schools and colleges via flyers and advertisements. To assess eligibility, a comprehensive telephone screen was administered to both adolescents and parents/guardians. Inclusion criteria required that youth were age 16-18, fluent in English, and had a parent or legal guardian available to consent and provide historical data. Exclusionary criteria were: history of chronic medical illness, neurological condition (e.g., meningitis, migraine), or head trauma with loss of consciousness >2 minutes; history of DSM-IV Axis I disorder (other than substance use disorder) or use of psychoactive medications; prenatal alcohol (≥4 drinks in a day or ≥7 drinks in a week) or drug exposure; complicated delivery or premature birth (<33 weeks gestation); learning disability or mental retardation; first-degree relative with history of bipolar I or psychotic

disorders; left-handedness; non-correctable vision, colorblindness or hearing impairments; and MRI contraindications. If at any time during the 28-day monitored abstinence period preceding the brain scan, a participant reported or tested positive for any substance use, he/she was excluded from study and not included in any analyses.

All participants and their parents/guardians (if teen was a minor) underwent written informed consent (written assent for minors) in accordance with the UCSD Human Research Protections Program. Teens were classified as a marijuana user ("MJ-user") or as a drug-free ("control") group. MJ-users had >60 lifetime marijuana experiences; past month marijuana use; <25 lifetime uses of drugs other than marijuana, alcohol, or nicotine; and did not meet Cahalan criteria for heavy drinking status (Cahalan et al., 1969). Controls had <5 lifetime experiences with marijuana (none in the past month), no previous use of any other drug except nicotine or alcohol, and did not meet criteria for heavy drinking status.

Measures

Detailed Screening Interview—The computerized *NIMH Diagnostic Interview Schedule for Children* (C-DISC-4.0; (Shaffer et al., 2000)) was administered to exclude participants with major psychiatric disorders, including DSM-IV Axis I mood, anxiety, attention deficit hyperactivity and conduct disorders. Parallel modules of the computerized *Diagnostic Interview Schedule* (C-DIS-IV;(Robins et al., 1996) were used for 18-year-olds. The *Structured Clinical Interview* (*SCI*) measured psychosocial functioning, activities, last menstruation (for females), health history, and handedness, and family history of psychiatric and substance use disorders was assessed (Rice et al., 1995).

Adolescents were administered the *Customary Drinking and Drug Use Record (CDDR)* to assess lifetime and past 3-month marijuana, alcohol, nicotine, and other drug use, withdrawal symptoms, DSM-IV abuse and dependence criteria, and substance-related life problems (Brown et al., 1998; Stewart and Brown, 1995). The modified *Timeline Followback* (TLFB; Sobell and Sobell, 1992) was administered to obtain detailed information regarding type, quantity, and frequency of substance use during the month prior to the monitored abstinence period. Teens were asked about frequency of use for each of the following drugs: marijuana, alcohol, nicotine, stimulants (cocaine, amphetamine, methamphetamine, MDMA/ecstasy), opiates (heroin, narcotic pain relievers other than as prescribed), dissociatives/hallucinogens (PCP, mushrooms, LSD, ketamine), sedatives (GHB, barbiturates, benzodiazepines), and misuse of other prescription or over-the-counter medications.

Parent Interview—If the teen continued to be eligible, their parent or guardian underwent a detailed screening interview using the parent version of the *SCI*, including information on prenatal/infant development, childhood behavior, age of developmental milestones, parental socioeconomic status (Hollingshead, 1965), family history of psychiatric and substance use disorders (Rice et al., 1995), and youth medical and psychiatric history. Parents/guardians were also administered the parent version of the *C-DISC-4.0* and the *TLFB* on youth behaviors.

Executive Functioning—Participants completed a neuropsychological evaluation. As the focus of the current study was on the relationship between PFC morphometry and executive functioning, a composite variable from a principal components analysis comprised of executive functioning variables (Medina et al., 2007a) was calculated using scores from the Delis-Kaplan Executive Function System (D-KEFS; Delis et al., 2001) Verbal Fluency total correct, Tower total achievement score, and Tower error scores.

Procedures

Eligible teens were scheduled to begin a monitored abstinence protocol, followed by neuropsychological testing and structural brain imaging. Adolescents were monitored with supervised urine and Breathalyzer tests every 3-4 days for 4 weeks. Youths with positive urine samples or breath alcohol concentrations (Intoximeter AlcoSensor IV, St. Louis, MO) or who appeared intoxicated were offered the option of restarting the toxicology screen procedure at a later time or to discontinue the study. If toxicology results indicated cessation and maintenance of abstinence, the adolescent completed the research battery between Day 23 and 27. Youth who did not maintain abstinence were discontinued and compensated for their time. Upon completion of the study, youth and parents/guardians received financial compensation for participation.

High-resolution anatomical magnetic resonance images were collected for all teens on a 1.5 Tesla GE Signa LX system using a sagittally acquired inversion recovery prepared T1-weighted 3D spiral fast spin echo sequence (TR = 2000 ms, TE = 16 ms, FOV = 240 mm, voxel dimensions = 0.9375 × 0.9375 × 1.328 mm, 128 continuous slices, acquisition time = 8:36) (Wong, 2000). Each participant's high resolution anatomical image was AC-PC aligned and skull-stripped using a combination of a hybrid watershed and deformable surface semi-automated skull-stripping program (Segonne et al., 2004), and manual editing to remove any non-brain matter and CSF beyond the dura matter. Intracranial volume (ICV) was determined from this hand-edited skull-stripped brain, and PFC regions of interest (ROI) were defined manually utilizing the below described protocol in AFNI (Cox, 1996) by raters blind to participant characteristics who attained high levels of inter-rater reliability (intraclass correlation coefficients >.90) prior to data collection.

Data Processing

All PFC ROIs were manually delineated on coronal 3D images (perpendicular to the AC-PC plane). The PFC ROI protocol (Medina et al., 2008) was a modified version based on Nagel and colleagues (2006). The *posterior PFC* ROI included all cortical area (gray matter, white matter, and sulcal CSF) anterior to the anterior commissure and posterior to the anterior edge of the genu, and excluded the corpus callosum, optic tracts, insula, subcortical regions (caudate, putamen, globus palladus, internal capsule), and lateral ventricles. The anterior PFC ROIs (dorsal and ventral) included all cortical areas anterior to the anterior edge of the genu. The *anterior dorsal PFC* ROI included all cortex superior to the midline of the most anterior portion of the genu, while the *anterior ventral PFC* ROI included cortex inferior to the midline of the genu. Tracing continued until the most anterior slice where cortex was still visible and these ROIs excluded the lateral ventricles and the corpus callosum (see Figure I).

For each ROI, white matter was segmented from gray and sulcal CSF compartments by processing skull-stripped T1 images with the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain's FAST automated segmentation tool (Zhang et al., 2001). Based on visual inspection of each subject, it was found that the automated segmentation process did not adequately separate gray matter from sulcal CSF, but correctly identified white matter (WM) within each of the ROIs. To calculate regional WM volume, each PFC ROI mask was applied to the WM compartment. All total and WM volumes were analyzed as a ratio to overall ICV to control for individual variability in brain size (Giedd et al., 1996b).

Data Analysis

ANOVAs and chi-square tests compared groups on important demographic and drug use variables. Bivariate correlations between drug use variables, executive functioning, and PFC volumes were run. Interpretations of statistical significance were made if $p<.05$.

To assess the relationships between group status, gender-by-group interactions, and PFC volumes, ordinary least squares multiple regressions were run ($N = 32$) with each of the eight PFC variables (total, anterior dorsal, anterior ventral, and posterior volumes; total WM, anterior dorsal WM, anterior ventral WM, and posterior WM volumes). The first step entered the following independent variables: gender, lifetime alcohol use, and group status (MJ-user vs. control). An interaction between group and gender was entered on the second step. As a follow-up analysis, regressions were run to assess the relationship between PFC volumes, group, and PFC-by-group interactions in predicting executive functioning after controlling for gender and lifetime alcohol use.

To improve power to detect a medium effect size in a relatively small sample, alpha was set at .10 for statistical significance in both analyses (primary analysis: medium effect size $f^2=.15$, $N=32$, numerator $df=1$, predictors=4; power=.69; follow-up analysis: $f^2=.15$, $N=32$, numerator $df=1$, predictors=5; power=.68, respectively) (Faul et al., 2007).

RESULTS

Descriptive Comparisons

ANOVAs and chi-squares assessed whether MJ-users and controls differed demographically ($n=16$/group). Groups differed in ethnic identification [$x^2(4)=10.18$, $p=.04$]: MJ-users were 75% Caucasian, 13% multiple ethnicities, 6% Pacific Islander, and 6% “other,” while controls were 63% Caucasian and 37% Asian-American (see Table I). The MJ-users and controls did not differ on age [average=18.0 years, range 16.0-18.9; $F(1,31)=.12$, $p=.74$]; WRAT-3 (Wilkinson, 1993) Reading standard score [MJ-users 106.7 mean±6.4 SD, range 98-119; controls 106.0±8.6, range 85-116; $F(1,31)=.14$, $p=.71$], or gender composition [MJ-users 4 females, 12 males; controls 6 females, 10 males; $x^2(1)=.52$, $p=.45$]. The MJ-users and controls did not significantly differ in ICV [$F(1,31)=.15$, $p=.71$] or PFC/ICV volumes (see Table I for raw values by group and gender).

Abstinence was monitored with urine toxicology screens and Breathalyzer tests that occurred for a minimum of 23 days, and in all, participants were abstinent from all drugs for at least 30 days (light to moderate alcohol use may have occurred; participants with self-reported binge drinking or biological evidence of alcohol use during this time were excluded). MJ-users reported more lifetime episodes of MJ use than controls [MJ-users 475.6±268.5, range 60-1000; controls 0.6±1.4, range 0-5; $F(1,31)=50.0$, $p=.0001$]. On average, MJ-users had used marijuana for 3.4 years (±1.7, range= 0.8-6.7), and had more lifetime episodes of alcohol intake than controls [MJ-users 194.5±136.8, range 20-420; controls 22.6±46.9, range 0-160; $F(1,31)=22.6$, $p=.0001$] (see Table 1). No control had used any drug besides alcohol or marijuana, but MJ-users had used other drugs an average of 6.9 times in their lives [±8.6, range 0-25; $F(1,31)=10.42$, $p=.003$]. The average abstinence from any alcohol use for MJ-users was 44 days (±61, range=9-270 days) and 132 days (±130, range=30-365 days) for controls. Average abstinence from drugs for the MJ-users was 107 days (±33, range=30-300 days).

Because the study examined gender-by-group interactions, drug use variables were examined between the male and female MJ users (see Table I for values by gender and group); they did not differ in MJ dependence symptoms, duration of regular MJ use, lifetime uses of other illicit drugs, recent MJ use, lifetime MJ use, years of regular drinking, length

of abstinence from either alcohol or other drugs, or ICV. However, female MJ users (M=301±132) had marginally more lifetime drinking episodes compared to male MJ users (M=158±123) [$F(1,15)=3.87$, $p=.07$] and greater alcohol dependence symptoms [female MJ users M=3.5±1.3; male MJ users M=1.4±1.3; $F(1,15)=7.6$, $p=.02$]. Control females and males did not differ on any substance use variable.

Correlations

See Table II for bivariate relationships by group. Among controls, greater alcohol use (recent use and number of alcohol dependence symptoms) was marginally associated with smaller posterior PFC volumes ($p<.09$) and significantly associated with smaller posterior PFC WM volumes ($p<.05$). Recent alcohol use, lifetime alcohol use, and number of dependence symptoms were also significantly associated with poorer executive functioning ($p<.05$). Finally, superior executive functioning was associated with larger total PFC volumes ($p<.05$).

In contrast, weak relationships were observed between alcohol use variables and PFC volumes among the MJ users; although there were marginal negative relationships between number of alcohol dependence symptoms and anterior dorsal PFC total and WM volumes (ps<.09). Increased recent MJ use was associated with *larger* anterior ventral PFC total and WM volumes (ps<.05).

Executive functioning did not correlate with drug use among the MJ users. Finally, relative to controls, the opposite relationship was observed between PFC volume and executive functioning; among the MJ users, superior executive functioning was associated with *smaller* total PFC volumes ($p<.05$). Relationships between PFC volume and executive functioning were similar for males and females, therefore correlations for all marijuana users are reported here.

Multivariate Relationships: Predicting PFC Volumes

Group effects—After controlling for gender and lifetime alcohol use, group status (MJ users vs. controls) was not associated with any of the PFC volumes. Calculated effect sizes for the addition of MJ group status to the model were all small (f^2 range .00-.03; Cohen, 1988).

Group x gender interactions—After controlling for group, gender, and lifetime alcohol use, gender and group interacted in predicting total PFC volume/ICV [*beta* = -.68, $p < .09$]. This interaction had a medium effect size ($f^2= .11$) while the interactions between gender and group and the other measures of PFC structure were small (f^2 range .006-.08) (Cohen, 1988; Faul et al., 2007). Visual inspection of the data revealed that female MJ users demonstrated *larger* PFC total volumes compared to female controls, while male MJ users had *smaller* PFC total volumes compared to male controls (see Figure II).

Multivariate Relationships: Predicting Executive Functioning

PFC volume effects—After controlling for gender, group, and lifetime alcohol use, larger anterior ventral PFC WM volume predicted superior executive functioning in both groups [*beta* = .32, $p < .09$; $f^2= .13$].

Group x PFC volume interactions—After controlling for group, gender, total PFC volume, and lifetime alcohol use, group and total PFC volume significantly interacted in predicting executive functioning [*beta* = -.44, $p < .05$; $f^2= .13$]; inspection of the scatter plots (see Figure 3) and correlations (Table 2) revealed that among the controls, increased total PFC volume was associated with improved executive functioning while the opposite was

true for the MJ users. Marginal interactions were also found between posterior PFC volume and group [*beta* = -.40 $p < .09$] and posterior PFC WM volume and group [*beta* = -.40, $p < .09$] and executive functioning, although the effect sizes were relatively small ($f^2 = .07$). Among controls, larger PFC volumes were associated with better executive functioning, while MJ users showed negative relationships between these PFC volumes and executive functioning. The remaining non-significant relationships also had small effect sizes (f^2 range .00-.05).

DISCUSSION

Marijuana (MJ) is the most commonly used illicit substance among adolescents (Johnston et al., 2005). Moreover, due to continued neurodevelopment (e.g., Giedd et al., 1996b; Sowell et al., 2002), adolescence may be a period of increased risk of MJ-induced neurocognitive deficits (Cha et al., 2006; Medina et al., 2007a). Prior animal (Diana et al., 1998a; Egerton et al., 2006; Jentsch et al., 1997b; Pistis et al., 2002; Verrico et al., 2003) and human (Amen and Waugh, 1998; Block et al., 2000; Block et al., 2002; Chang et al., 2006; Eldreth et al., 2004; Gruber and Yurgelun-Todd, 2005; Jager et al., 2007; Kanayama et al., 2004; Loeber, 1999; Lundqvist et al., 2001; McPartland et al., 2007; Van Laere et al., 2008a; Volkow et al., 1996) research has demonstrated that the prefrontal cortex (PFC) may be particularly vulnerable to MJ effects, and gender differences exist in PFC development and neurocognitive consequences of drug use during adolescence and young adulthood (Medina et al., 2008; Medina et al., 2007b; Pope et al., 1997). Therefore, the goal of the current study was to examine whether group status or interactions between MJ use and gender were associated with PFC volumes in a sample of marijuana using adolescents who demonstrated approximately one month of abstinence.

The primary finding was that after controlling for alcohol consumption, gender, and intracranial volume, MJ users had similar PFC volumes as controls. However, group-by-gender interactions were observed in the total PFC volume ($p<.09$; medium effect size $f^2 = .11$). It is important to note that although provocative, these preliminary findings should be viewed with caution due to the low sample of females (MJ users $n=4$, controls $n=6$). Female MJ users demonstrated comparatively larger and male MJ users had smaller PFC volumes compared to their same-gender controls. Put another way, MJ use may moderate the effects of gender on PFC structure. On average, females' PFC gray matter volume peaks one to two years earlier than males (Giedd et al., 1996b). Given that the adolescents from the current study are on average 18 years old, the girls should have undergone substantial pruning and show smaller PFC volumes compared to males, which is consistent with the findings seen in the healthy non-drug using adolescents in this study. However, this gender difference in PFC volume was different in the MJ users; female MJ users actually had somewhat larger PFC volumes compared to male users. Further, when examining the functional relationship between PFC volume and executive functioning ability, we found that among MJ users, larger PFC total volume was associated with worse executive functioning while the opposite pattern was seen among the controls ($p<.05$; medium effect size $f^2 = .13$). As stated above, because the study included relatively few female MJ users, findings need to be replicated in larger samples.

If replicated, larger PFC volumes in the female MJ users compared to female controls may indicate an interruption in the healthy pruning process. The mechanism underlying a possible disruption in healthy pruning is unknown. Recent animal findings found that astrocytes sparked neurons to release a complement protein, C1q, that in turn tagged neurons with weak synaptic connections; this protein peaked during the adolescent pruning process in rats (Stevens et al., 2007). Notably, exogenous cannabinoid exposure has been shown to affect normal astrocytic function (Bindukumar et al., 2008); therefore, repeated exposure of

cannabinoids during adolescence may directly affect the elimination of weak synapses. Chronic exposure may also alter brain-derived neurotrophic factor (BDNF) expression (Berghuis et al., 2005; D'Souza et al., 2008; Jockers-Scherubl et al., 2004), resulting in non-physiological increases in BDNF over time that may interfere with synaptic pruning. Both of these possibilities would lead to inefficient neural networks, which is consistent with our structural findings of larger volumes being associated with poorer executive functioning.

Other mechanisms that may interrupt healthy pruning are also possible. Administration of exogenous cannabinoids in animals has increased mRNA encoding of immediate early gene proteins, including zif-268, c-fos, c-jns, and FosB in the cingulate (Mailleux et al., 1994; Porcella et al., 1998) and PFC (Egerton et al., 2006), which may also alter neuronal functioning and result in morphometric changes over time. Structural abnormalities could also be due to marijuana-induced alteration of neurotransmitters such as dopamine and norepinepherine (Diana et al., 1998a; Egerton et al., 2006; Oropeza et al., 2007; Pistis et al., 2002; Verrico et al., 2003), an effect that has been associated with poorer executive functioning in animals (Jentsch et al., 1997a), reduced cerebral metabolism and regional blood flow in the PFC (Amen and Waugh, 1998; Block et al., 2002; Lundqvist et al., 2001; Tunving, 1985; Volkow et al., 1996), or vasoconstriction resulting in ischemic changes (Herning et al., 2001).

Possible reasons for gender differences in marijuana effects include differential hormone and receptor distributions that may interact with the cannabinoid system (Ahmed et al., 2008; Coddington, et al., 2007; Emanuele et al., 2001; Kim et al., 2003; Mani et al., 2000; Ogilvie and Rivier, 1997). Among young adults, increased age is associated with higher CB1 density in PFC regions among females, but not males (Van Laere et al., 2008a). Increases in CB1 availability and introduction of chronic MJ use may cause greater disruption of the above outlined cannabis-related neuromodulation in girls. It is also notable that the female MJ users demonstrated marginally higher lifetime drinking episodes and significantly greater alcohol dependence symptoms compared to male MJ users. However, alcohol was statistically controlled for and female MJ users demonstrated larger PFC volumes compared to female controls, which is inconsistent with recent research from our laboratory showing significantly *smaller* PFC volumes among females with alcohol use disorders compared to female controls (Medina et al., 2008).

Male MJ users had relatively smaller total PFC volumes compared to gender-matched controls, and smaller PFC volumes were associated with improved executive functioning among the MJ users. This may indicate that male MJ users may be less sensitive to the neurocognitive effects of marijuana than females. However, it is important to note that the male MJ users demonstrated an abnormal relationship between PFC volume and executive functioning compared to controls, for whom increased PFC volume was associated with improved executive functioning. The lack of white matter differences in the male MJ users is consistent with DeLisi et al (2006), who found no evidence of reduced white matter integrity among a primarily male (90%) young adult sample of previous marijuana users who began heavy use during adolescence. Although this preliminary evidence suggests that the structural effects of chronic marijuana use on the brain among male adolescents may be subtle compared to females, additional studies combining neuroimaging techniques are necessary. For example, one study reported abnormal NAA/tCR ratios in the dorsolateral PFC among male adolescent and young adult MJ users compared to controls (Hermann et al., 2007). Recently, our group found reduced white matter integrity in fronto-parietal tracks in adolescent marijuana users (Bava et al., under review). Therefore, macromorphic measures of PFC structure may not be sufficient to measure the effects of MJ on PFC development in male adolescents. Further, because the study is cross-sectional and males and females undergo neuromaturation at separate rates, it is possible that the gender

differences are not due to gender per se, but instead represents differential trajectories based on stage of neuronal maturation. Male MJ users could show the same patterns as the females, but at different times in adolescence. To further examine the effects of chronic MJ use on PFC integrity, longitudinal studies that investigate the interactions between gender (including hormone levels), CB1 density, markers of PFC integrity (including structure and function), and chronic MJ use during adolescence are needed.

Some methodological limitations of the current study should be considered. Preexisting differences in PFC morphometry and executive functioning (Aytaclar et al., 1999; Nigg et al., 2004) cannot be ruled out in this cross-sectional study. Still, it is of note that the current sample excluded individuals with Axis I comorbid disorders, including conduct disorder, and the groups were matched on family history of SUD, education, SES, and reading ability. The internal consistency of the sample may actually limit generalizalization of results, as the MJ using teens had above average verbal ability, high SES, and were able to complete the challenging month of abstinence protocol. Future studies are needed to examine PFC morphometry in more heterogeneous samples. Second, although comparable to other neuroimaging studies, the sample was relatively small and disproportionately male; therefore, the gender findings were based on a small sample of female users. This limited our power to detect significant differences, although the observed effect sizes were medium (f^2=.11 and .13). Because of low power, we did not perform correction for multiple analyses, so the correlation and multivariate analyses need to be replicated in samples with larger numbers of male and female MJ adolescent users. Third, although white matter was reliably separable from gray matter and CSF, consistent parcellation of sulcal CSF from gray matter was not possible. Although we believe the contribution of CSF on these findings are minimal due to hand editing of CSF from the vast majority of the PFC ROIs, we cannot definitively state that it did not have an impact. Therefore, additional research examining gray matter separated from CSF is needed to confirm that the results are driven by gray matter differences. Given the cross-sectional nature of the current study, it is unknown whether continued abstinence from MJ results in neurocognitive recovery or subsequent healthy neurodevelopment among adolescents. Therefore, longitudinal studies are necessary to investigate the long-term trajectory of PFC morphometry and executive functioning in adolescent MJ users.

In conclusion, the general pattern of results suggests that even after a month of abstinence, adolescent marijuana users demonstrate subtle PFC morphometry abnormalities that may be moderated by gender. No differences in PFC white matter volume were seen between the groups. Female MJ users demonstrated comparatively larger PFC volumes and male MJ users had comparatively smaller PFC volumes compared to their same-gender controls. Unlike controls, increased PFC volume was associated with poorer executive functioning among the MJ users, suggesting that female MJ users may be at increased risk for marijuana-induced PFC abnormalities. Given the preliminary nature of these results, additional preclinical and human studies are needed to examine PFC integrity among male and female adolescents following chronic marijuana exposure. Further studies examining the interaction between the endocannabinoid system and sex hormones on PFC development are also warranted. Given the current findings and prior results showing detrimental cognitive and mood consequences of adolescent marijuana use (Medina et al., 2007a; 2007b), additional research focused on pharmacological interventions in marijuana users are also needed.

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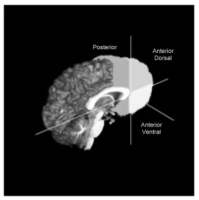

Figure I.
3D sagittal view of prefrontal cortex (PFC) boundary delineation.

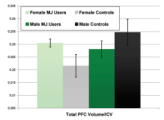

Figure II.
Total PFC volume/ICV by group and gender (multivariate interaction $p<.09$).

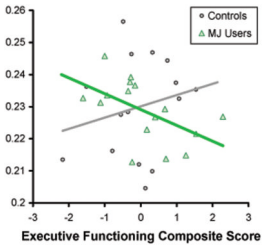

Figure III.
Bivariate scatterplot between the total PFC volume/ICV and executive functioning composite score by group (multivariate interaction $p<.05$).

Table I

Demographic, substance use, and PFC volume measures by group and gender

	Female MJ Users (*n*=4) % or M±SD	Female Controls (*n*=6) % or M±SD	Male MJ Users (*n*=12) % or M±SD	Male Controls (*n*=10) % or M±SD
Ethnicity (Caucasian) *	75%	83%	75%	50%
Age	18.2±0.6	18.5±0.5	18.1±0.8	17.7±1.1
Lifetime alcohol use (episodes) *	301±132	32±64	158±124	17±37
Lifetime marijuana use (episodes) *	515±392	0±.8	463±236	0.8±1.6
ICV (cc^3)	1427±119	1363±118	1515±100	1544±101
Total PFC (cc^3)	329±27	304±38	345±22	363±29
Total PFC /ICV (cc^3)	.2306 ±.0031	.2227 ±.0109	.2282 ±.0111	.2354 ±.0162
Anterior dorsal PFC /ICV (cc^3)	.0677±.0036	.0703 ±.0084	.0687 ±.0084	.0699 ±.0102
Anterior ventral PFC /ICV (cc^3)	.0557±.0005	.0502 ±.0046	.0559 ±.0058	.0582 ±.0091
Posterior PFC /ICV (cc^3)	.1072±.0057	.1021 ±.0084	.1036 ±.0130	.1072 ±.0135
Total PFC WM /ICV (cc^3)	.0662±.0019	.0665 ±.0099	.0682±.0033	.0714 ±.0058
Anterior dorsal PFC WM /ICV (cc^3)	.0158±.0010	.0174 ±.0039	.0172 ±.0027	.0178 ±.0038
Anterior ventral PFC WM /ICV (cc^3)	.0135±.0006	.0124 ±.0033	.0138 ±.0014	.0150 ±.0030
Posterior PFC WM /ICV (cc^3)	.0369±.0022	.0367 ±.0058	.0372 ±.0050	.0385 ±.0049

Notes: PFC=Prefrontal cortex volume; ICV=Intracranial Volume; WM=White matter. There were no within-gender differences by group in ICV.

* $p<.05$ between MJ users vs. controls.

Table II

Correlations between drug use, executive functioning, and PFC variables by group.

	Marijuana Users (*n*=16)†						
	Recent MJ Use	**Lifetime MJ Use**	**#MJ Symptoms**	**Recent Alc Use**	**Lifetime Alc Use**	**#Alc Symptoms**	**Exec Func**
Total PFC/ICV	.03	.12	-.24	-.20	-.13	-.10	-.53*
Anterior dorsal PFC/ICV	-.07	.01	-.36	-.12	.06	-.43	-.06
Anterior ventral PFC/ICV	.60*	.34	-.09	-.24	.06	-.17	.16
Posterior PFC/ICV	-.19	-.04	.06	.01	-.12	.27	-.47
Total PFC WM/ICV	-.10	-.05	-.32	.01	-.25	-.14	-.12
Anterior dorsal PFC WM/ICV	-.04	.02	-.39	-.08	.01	-.48	.12
Anterior ventral PFC WM/ICV	.56*	.21	-.10	-.19	-.11	-.17	.39
Posterior PFC WM/ICV	.23	-.10	.02	.10	-.15	.22	-.26
Executive functioning	.23	-.11	-.23	-.08	-.09	-.07	-
	Controls (*n*=16)						
	Recent MJ Use	**Lifetime MJ Use**	**# MJ Symptoms**	**Recent Alc Use**	**Lifetime Alc Use**	**# Alc Symptoms**	**Exec Func**
Total PFC/ICV	-	-	-	-.35	-.16	-.30	.22
Anterior dorsal PFC/ICV	-	-	-	.31	.30	.36	-.20
Anterior ventral PFC/ICV	-	-	-	-.22	-.11	-.19	.35
Posterior PFC/ICV	-	-	-	-.48	-.32	-.47	.17
Total PFC WM/ICV	-	-	-	-.40	-.41	-.37	.55*
Anterior dorsal PFC/ICV	-	-	-	.10	-.01	.14	.17
Anterior ventral PFC WM/ICV	-	-	-	-.21	-.20	-.19	.48
Posterior PFC WM/ICV	-	-	-	-.53*	-.48	-.53*	.39
Executive functioning	-	-	-	-.59*	-.52*	-.59*	-

Notes: MJ=marijuana; Alc=alcohol; # MJ/Alc Symptoms= denotes the number of DSM-IV marijuana or alcohol abuse or dependence symptoms met; Recent MJ/Alc Use= past 3 month in joints (MJ) or standard drinks (alcohol); Exec Func= Executive functioning composite score; PFC=Prefrontal cortex; ICV=Intracranial Volume; WM=White matter.

† Correlations are consistent for both male and female MJ users.

* $p<.05$