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I, Jenessa S Price, hereby submit this original work as part of the requirements for the degree of Doctor of Philosophy in Psychology.

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Effects of Marijuana Use on Prefrontal and Parietal Volumes and Cognition in Emerging Adults

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Effects of Marijuana Use on Prefrontal and Parietal Volumes and Cognition in Emerging Adults

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ABSTRACT

Introduction: Initiation of marijuana (MJ) use typically coincides with continuing brain neuromaturation, particularly in frontal and parietal areas. Chronic MJ use has been associated with disruption of frontoparietal pathways and dose-dependent deficits in attention and executive function among young users. Imaging studies reveal evidence of white matter microstructural abnormalities and indications of disruption of pruning processes among MJ-using adolescents. This study investigated past year MJ use and its interaction with gender on prefrontal cortex (PFC) and parietal cortex volumes and executive function in a sample of healthy emerging adults.

Method: Participants were 27 MJ users (>25 past year MJ joints) and 32 controls (<5 past year and <50 lifetime MJ joints), balanced for gender, and meeting eligibility requirements (ages 18-25 and no history of medical/neurologic illness, DSM-IV Axis I diagnosis, or heavy other drug use). Urine toxicology testing assessed abstinence. Self-report measures included the Frontal Systems Behavior Scale (FRSBE) and the BIS/BAS. Cognitive variables included tasks measuring complex attention and cognitive inhibition. FreeSurfer pre-processed and obtained PFC and parietal cortex volumes on T-1 weighted 3D anatomical brain images, and the author performed blind inspection and editing when necessary. A series of multiple regressions examined whether MJ use status predicted PFC and parietal volumes after controlling for ICV, body mass index, verbal IQ, gender, alcohol, hallucinogen and nicotine use; a second block examined the interaction between MJ use and gender. A second series of regressions examined impact of past year MJ use on PFC and parietal volumes utilizing the same covariates. Post-hoc analyses consisted of brain-behavior correlations between ROI findings and cognitive variables.
Fisher’s z tests assessed whether brain-behavior relationships differed between MJ users and controls.

**Results:** On self-report measures, past year MJ users endorsed significantly more symptoms of executive dysfunction and disinhibition versus controls on the FRSBE. After controlling for gender, BMI, verbal IQ estimate, and alcohol, nicotine and hallucinogen use, MJ users demonstrated significantly smaller medial orbitofrontal cortex (mOFC; \( p = .004; f^2 = .18 \)) and inferior parietal volumes (\( p = .04; f^2 = .09 \)); increased past year MJ use *dose-dependently* predicted smaller mOFC volume (\( p = .05; f^2 = .09 \)). There were no significant group-by-gender interactions. Post-hoc analyses yielded a significant brain-behavior difference by group; smaller mOFC volumes were associated with poorer WAIS-III Letter Number Sequencing, with the opposite pattern seen in controls (\( z = 1.87, p < .05 \)). Overall, smaller mOFC volumes were associated with poorer complex attention and inhibitory processing among MJ users.

**Discussion:** Among emerging adult men and women, MJ users demonstrated significantly smaller mOFC and inferior parietal volumes compared to controls, and smaller mOFC volumes were *dose-dependently* associated with past year MJ use. In MJ users, smaller volumes were associated with poorer complex attention and inhibitory processing. Regional findings suggest ongoing disruption to normal neurodevelopmental processes associated with regular MJ use. Group differences in the inferior parietal region may reflect premorbid factors that differ among youth who initiate use. Future research should incorporate multi-modal imaging, genotyping, and longitudinal design in examining the neurocognitive effects of MJ use, towards the goal of characterizing at-risk populations and creating optimal interventions.
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I. INTRODUCTION

Prevalence

Marijuana (MJ), containing delta$^9$-tetrahydrocannabinol (THC), is the most popular illicit drug among adolescents and emerging adults (Martin et al., 1999; Johnston et al., 2013). According to the 2010 National Institute on Drug Abuse’s (NIDA) Monitoring the Future survey, MJ has recently surpassed nicotine cigarette use among high school seniors (Johnston et al., 2011; 2013). Researchers recorded the highest levels of daily MJ use among high school seniors in 2010 (6.1%); use continued to increase in 2011 and leveled-off in 2012 at 6.5%, suggesting that 1 in 15 high school seniors smoke daily or near daily. Among young adults (ages 19-28), nearly one third reported past year use. On balance, the Substance Abuse and Mental Health Services Administration (SAMHSA, 2013) reported MJ to have the highest rate of dependence or abuse of illicit drugs in 2012, affecting 4.3 million individuals.

Brain Development

Given that the average first MJ use occurs at age 17 (SAMHSA, 2013), effects of use on brain development and brain function is a major public health concern. Indeed, neuromaturation continues in several areas of the brain during adolescence and emerging adulthood, including the prefrontal cortex (PFC), parietal lobes, cerebellum, and subcortical regions (Yakovlev & Lecours, 1967; Huttenlocher, 1990; Giedd et al., 1999; Gogtay & Thompson, 2010). A significant amount of neural pruning occurs into emerging adulthood, resulting in reduction in gray matter volume, while white matter volumes demonstrate significant proliferation during this time (Sowell et al., 2002; Jernigan et al., 1991; Pfefferbaum et al., 1994; Giedd et al., 1999). Structural brain imaging studies have documented patterns of development that progress from
posterior to anterior and inferior to superior regions, with frontal and parietal areas being the last to mature (Sowell et al., 1999; Jernigan et al., 1991). It has also been theorized that increased risk-taking during adolescence may be related to developmental trajectories of the PFC and the limbic system as well as their complex interactions with each other (see Casey et al., 2008). Specifically, they posit that as the PFC develops, it may impart increased top-down control of the limbic system, which completes development earlier (Galvan et al., 2006; Giedd et al., 1996). In turn, this is associated with optimized inhibitory control and affective processing (Lisdahl et al., 2013; Casey et al., 2008, 2005; 1997; Liston et al., 2006; Monk et al., 2003).

Gender differences have also been noted in the developmental trajectory. Generally, males tend to have larger brain volumes than same-aged females throughout childhood and into adulthood. Females demonstrate earlier gray matter pruning, while males have greater increases in myelination (Giedd et al., 1996; Lenroot & Giedd, 2010). To summarize, brain maturation is still occurring in emerging adult populations, especially in anterior and superior regions such as frontal and parietal lobes. Further, gender differences occur in the timing and trajectory of neurodevelopment, especially in areas underlying higher order cognitive functions (Lenroot & Giedd, 2010).

*Cannabinoid System and Neurodevelopment*

Developmental patterns have also been identified within the cannabinoid system in the brain. Two kinds of G-coupled cannabinoid receptors exist: CB₁ receptors located in the brain are involved in central nervous system function, and CB₂ receptors located peripherally are primarily involved in immune system function (Alger, 2002). CB₁ receptors are usually located on presynaptic sites but have also been found on neuronal cell bodies, axons, and dendrites as
well as glial cells (Tsou et al., 1998). Endogenous endocannabinoids, such as anandamide and 2-arachidonylglycerol, act as postsynaptic messengers (Terry et al., 2009). Such retrograde signaling serves as a means of synaptic regulation (Alger, 2002). As a result, stimulation of CB₁ receptors by endocannabinoids can inhibit the release of GABA, glutamate, and dopamine (Terry et al., 2009; Alger, 2002). Exogenous MJ use disrupts this process; THC binds to CB₁ receptors, acutely causing psychotropic effects (Ameri, 1999; Freund et al., 2003; Westlake et al., 1994) and with chronic use causing downregulation of CB₁ receptors in both adolescent and adult rats (Burston et al., 2010).

A handful of studies have examined CB₁ receptor binding using autoradiographic techniques as well as positron emission tomography (PET) scans in an effort to confirm receptor location in the brain. Early autoradiographic studies showed extensive binding in the frontal cortex of rats, with greater densities in cortical layers I and IV (Hajos and Freund, 2002; Herkenham et al., 1990; Herkenham et al., 1991). In a biodistribution study in mice, greater in-vivo uptake of a radioligand was found in brain areas responsible for higher cognition, such as the striatum, hippocampus, cortex, and cerebellum, as compared to the thalamus and brain stem; and in a similar study in baboons, high concentration of CB₁ receptors was noted in the putamen (Horti, 2006). Consistent with these results and with prior autoradiographic studies in humans (Glass et al., 1997; Mato & Pazos, 2004), a PET study in humans found the greatest uptake of a CB₁ receptor-selective inverse agonist in the putamen, prefrontal cortex, and cerebellum, with lesser uptake in the pons and white matter (Terry et al., 2009).

The endogenous cannabinoid system also undergoes developmental changes during the teenage and emerging adult years, with peak increases in the number of cannabinoid receptors in the cortex, cerebellum, striatum, and hippocampus (Belue et al., 1995; Howlett et al., 2002).
Further, research has suggested that the endocannabinoid system plays a direct role in brain development, moderating neurotransmitter release and neurogenesis (Viveros et al., 2005). Gender may also moderate these effects in the developing brain. Following exposure to THC, female adolescent rats displayed greater CB\textsubscript{1} desensitization in the prefrontal cortex, hippocampus, periaqueductal gray matter, and ventral midbrain compared to males; no gender effect was noted among adult rats in the same conditions (Burston et al., 2010). Results suggest a vulnerability of the female adolescent rat brain to THC-associated CB\textsubscript{1} receptor signaling changes; however, the authors posited that perhaps such increased desensitization could reflect protective adaptation. Thus, MJ exposure may have a neurotoxic effect on theannabinoid system, resulting in brain structural abnormalities that may differ according to gender and developmental period.

**MJ Exposure: Cognitive and Functional Findings**

Animal studies have demonstrated additional cellular changes, including downregulation of CB\textsubscript{1} receptors, following THC exposure among adolescents compared to adults. It is reasonable then to assume that there may be functional abnormalities among adolescent MJ-using humans that parallel these anatomic alterations. Indeed, subtle cognitive deficits have been noted in teenage and young adult chronic MJ users in attention, processing speed, executive ability (including cognitive disinhibition and perseveration), and learning and memory (Fried et al., 2005; Gonzalez et al., 2012; Grant et al., 2012; Hanson et al., 2007, 2010; Harvey et al., 2007, Medina et al., 2007a; Piechatzek et al., 2009; Scholes & Martin-Iverson, 2009; Schwartz et al., 1989, Tait et al., 2011; Tapert et al., 2002; Thoma et al., 2011). A review by Schweinsburg, Brown, and Tapert (2008a) suggests that while neuropsychological functioning may return after
approximately one month of abstinence, adolescent users show deficits up to six weeks after last use. Consistent with a prior study in teens (Medina et al., 2007a), our lab recently reported a dose-dependent association between past year MJ exposure and poorer sustained attention, psychomotor speed, and cognitive inhibition in emerging adult users who had been abstinent for an average of 50 days (Lisdahl & Price, 2012).

Therefore, in youth it appears that MJ may have a particular impact on cortical areas associated with working memory/processing speed and executive function, such as the PFC and parietal cortex, as well as the cerebellum (Medina et al., 2010) and subcortical regions (including the amygdala and hippocampus). In general, MJ exposure has been shown to increase PFC cerebral blood flow and metabolism in abstinent users, especially to frontal areas (Volkow et al., 1996; O’Leary et al., 2000, 2002; Lundqvist et al., 2001). Tapert and colleagues (2007) found over-activation of cortical control regions (dorsolateral PFC and parietal cortex) among abstinent adolescent MJ-users, indicative of altered, compensatory functioning. A more recent study from the same group reported reduced cerebral blood flow in temporal, insular, and PFC regions among adolescent heavy MJ users with an average of 5.1 days of abstinence; following 4 weeks of monitored abstinence, there were no differences in activation patterns between MJ and control groups (Jacobus et al., 2012). During a novel working memory task, increased PFC activity was found in a sample of MJ-using adolescents and young adults (Jager et al., 2010), but decreased activity in the PFC and anterior cingulate (ACC) has been found among adult heavy MJ users (Kanayama et al., 2004). On a spatial working memory task, adolescent MJ users demonstrated increased activation in spatial rehearsal and visual areas (posterior parietal and occipital cortices) but decreased activation in the dorsolateral PFC, suggesting overcompensation in areas necessary to complete the task (Schweinsburg et al., 2008b; Padula et al., 2007). Increased activation, again
most notably for early-onset adolescent users, has also been found in the left superior parietal lobe during a verbal working memory task (Becker et al., 2010). In another study examining verbal working memory, nicotine withdrawal among adolescent MJ users was predictive of increased posterior activity and disruption of frontoparietal connectivity as well as poorer recall (Jacobsen et al., 2007). Further, there was stronger functional activity noted in the right prefrontal cortex of users compared to nonusers for this task. Alternatively, Sneider et al. (2013) found poorer recall and decreased activation in the right parahippocampal gyrus and cingulate gyrus among MJ users during a visuospatial memory task. Other research has noted significantly decreased activity in the ACC and insular cortex, areas related to drug craving and reward, on inhibitory tasks (Hester et al., 2009; Brody et al., 2002; Naqvi & Bechara, 2009). In sum, it appears that adolescent and emerging adult MJ users have altered functioning in cortical areas associated with complex, higher-order cognitive processes, especially the PFC and parietal cortex. Deficits in these areas may reflect less efficient processing related to disrupted frontoparietal pathways, resulting in poorer complex attention and executive functioning performances. However, examination of chronicity of MJ use, length of MJ abstinence, and recent nicotine use, are important in interpreting findings.

MJ Exposure: Structural findings

MJ exposure among adolescents has primarily been associated with cognitive deficits in areas underlying frontal, parietal, hippocampal and cerebellar functioning, areas that have dense CB1 receptor availability. However, there are relatively few studies of brain morphology in adolescent and emerging adult users, and because of these individuals’ continuing neurodevelopment, adult results may not generalize. Among adult addiction populations, there is
evidence of PFC connectivity and structural changes associated with longstanding histories of chronic use (Goldstein & Volkow, 2011). Some examinations have noted no differences between adult MJ users and controls in white matter integrity (Delisi et al., 2006; Gruber and Yurgelun-Todd, 2005); however, Arnone et al. (2008) found reduced white matter integrity in prefrontal fiber bundles and the right genu among those who initiated use early, and these abnormalities in the genu were related to increased impulsivity (Gruber et al., 2011). In MJ-using adolescents and young adults, MJ use has been associated with white matter microstructural abnormalities in areas of the corpus callosum (De Bellis et al., 2008), left corona radiata and superior longitudinal fasciculus (Jacobus et al., 2009), and arcuate fasciculus (fronto-temporal connection; Ashtari et al., 2009). Additionally, magnetic resonance spectroscopy (MRS) studies have shown neurochemical alterations among marijuana users. Silveri and colleagues (2011) found decreased distribution of the glial metabolite myo-inositol (mI) in white matter in several tissue areas that related to poorer mood state among young adult MJ-using men. Among adolescent MJ users, Prescot et al. (2011) reported reduced anterior cingulate glutamate, N-acetyl aspartate, total creatine, and myo-inositol levels.

In adults, although some studies have found no differences between MJ users or controls in regional or global gray matter volumes or cortical shape (Tzilos et al., 2005; Hannerz et al., 1983), others have noted reduced volumes in the hippocampus, amygdala, and total gray matter (Lorenzetti et al., 2010; Yucel et al., 2008; Wilson et al., 2000). Our laboratory has found that adolescent MJ users demonstrate a pattern suggesting a disruption of the gray matter pruning process that may be exaggerated in females (Medina et al., 2010; Medina et al., 2009; McQueeny et al., 2011). For example, Medina et al. (2009) found that gender marginally moderated the effects of MJ use on PFC structure in adolescents, such that female users had larger volumes
compared to control females and this was in turn associated with poorer executive functioning. While volumes were not significantly different between MJ users and controls, larger volumes among female users, suggestive of disrupted gray matter pruning, indicated that such structural effects were exacerbated by MJ in females and may represent inefficiency in functioning. In addition, Medina and colleagues noted a negative relationship between total white matter volume and non-clinical depressive symptoms in MJ-using adolescents, suggesting possible disruption of frontal and limbic pathways associated with mood regulation among users (Medina et al., 2007b).

With respect to other indices of gray matter structure, a recent study measured cortical thickness in PFC regions among adolescent controls and MJ users (average use 10.4 smoking events per week; Lopez-Larson et al., 2011). Among users, there was reduced cortical thickness in the right caudal middle frontal, bilateral insular, and bilateral superior frontal cortices compared to controls. There was also a negative relationship between thickness and age of first regular MJ use, indicating a possible disruption of gray matter development related to early initiation and chronicity. Of note, one of the 18 MJ users had a past history of a major depressive episode and another a past history of heavy alcohol use; no other subjects including controls had any major psychiatric or other substance use disorders or use of psychotropic medication. Both depression and heavy alcohol use, including binge drinking, have been associated with reductions in volume and cortical thickness in prefrontal areas, and thus these results may have been related to such methodological concerns (Kroes et al., 2011; Drevets et al., 2008; McQueeny & Medina, 2011). Other limitations include the possibility that recent drug use may have affected results, as subjects were not required to remain abstinent prior to the study procedure, and poor generalization of results to females, as the majority of the sample was male. Churchwell et al., 2010 reported decreased right medial orbitofrontal cortex volumes among adolescent MJ users
compared to controls; however, the majority of this sample was also male. A similar study examining cortical surface structure in a sample of early adult users and controls also found cortical abnormalities (mean ages 25.7 and 25.8 years, respectively; Mata et al., 2010). Among MJ users, there were indications of reduced concavity of sulci bilaterally, especially for the right frontal lobe, even when controlling for cortical thickness and gyrification. In summary, thinning of the cortical architecture in PFC regions have been reported in MJ-using adolescents and emerging adults, although these findings may not generalize to females and the effects of MJ use on other cortical regions, such as the parietal cortex, have yet to be examined.

**GOALS OF CURRENT STUDY**

The purpose of this study was to investigate the effects of chronic MJ use on frontal and parietal volumes in emerging adults. Specifically, this project expanded upon research findings for adolescent users, as reported by Medina and colleagues (2009) in a separate sample of emerging adult users. Further, this project extended findings from a previous study with an overlapping but not identical sample, which found dose-dependent deficits in sustained attention, psychomotor speed/sequencing ability, and cognitive inhibition among emerging adults MJ users, especially males (Lisdahl & Price, 2012). The present study is unique in that it explored relationships between past year MJ use and prefrontal and parietal volumes in a sample of both male and female emerging adults (ages 18-25) with no current Axis I psychiatric diagnoses. Additionally, we examined whether gender moderates these relationships (Giedd et al., 1996; Lenroot & Giedd, 2010; Burston et al., 2010). Finally, brain-behavior relationships examined whether volume differences were associated with poorer cognitive functioning in emerging adults.
AIM 1a: To determine the relationship between MJ group status (i.e., user vs. nonuser) and regional volumes in prefrontal and parietal cortices. **Hypothesis 1a:** It is hypothesized that MJ users will have decreased volumes in the aforementioned areas compared to controls. **AIM 1b:** To determine the relationship between past year MJ use and regional volumes in prefrontal and parietal cortices. **Hypothesis 1b:** It is hypothesized that increased MJ use will be associated with decreased volumes in aforementioned areas. **AIM 1c:** To examine whether interactions between MJ use and gender are significant predictors of volumes in specified regions. **Hypothesis 1c:** It is hypothesized that female MJ users will have larger volumes than male MJ users, whereas there will be no difference between male and female controls.

**AIM 2:** To examine brain-behavior relationships between ROI volumes and complex attention and inhibitory processing in regions that significantly differed between the groups (MJ users and controls). **Hypothesis 2:** It is hypothesized that both MJ users and controls will demonstrate significant relationships between decreased volumes and poorer complex attention and inhibitory processing, suggesting that smaller volumes among the MJ users is detrimental to cognition.

**II. METHODS**

**PARTICIPANTS**

As part of a larger imaging genetics study (PI: Lisdahl, KM; 1R03 DA027457-01), participants were recruited through advertisements in a local free newspaper and fliers distributed throughout the community and local universities. Please see Lisdahl & Price (2012) and Price et al. (2013) for other investigations that included participants overlapping with the
present sample. Interested participants were screened by phone. Participants were required to be fluent in English, between the ages of 18-25 years, and right-handed. Exclusion criteria included MRI contraindications (pregnancy, claustrophobia, weight over 250 lbs., metal in body, pacemaker or other device in body); history of chronic medical or neurologic illness or injury (meningitis, HIV, epilepsy, brain tumor, traumatic brain injury, injury resulting in greater than two minutes of unconsciousness and concussion symptoms, stroke, cerebral palsy, Parkinson’s disease, Huntington’s disease, high blood pressure, diabetes, consistent migraines); history of a learning disability; substantial complications during birth or premature birth; known prenatal exposure to alcohol (>4 drinks/day or >7 drinks/week) or other drugs (>10); use of psychoactive medication; preexisting DSM-IV Axis I disorders independent of substance use (including major depressive disorder, bipolar disorder, attention deficit hyperactivity disorder, conduct disorder, social phobia, agoraphobia, panic disorder, generalized anxiety disorder, obsessive compulsive disorder, anorexia, and bulimia); and refusal to remain abstinent from all drugs and alcohol for at least seven days prior to participation. Eligible participants for this study were chosen from the existing database consisting of 131 completed subjects. Inclusion was based on whether they fit in one of two groups: current MJ users (>25 past year MJ joints; >50 joints lifetime) or controls (<5 past year and <50 lifetime MJ joints) and balanced for gender.

PROCEDURES

The Institutional Review Board at the University of Cincinnati approved all aspects of the larger study. After individuals provided oral informed consent, trained research assistants screened prospective participants over the phone for aforementioned inclusion/exclusion criteria, including questions regarding past year drug use in order to assess classification into a particular
drug use group. In addition, screeners utilized a semi-structured interview based on DSM-IV-TR criteria for Axis I psychotic, anxiety, and mood disorders. Those who had positive responses to the screening questions were discussed in committee; if clear decisions could not be reached then they were re-contacted and administered additional diagnostic questions based on the SCID I/P (determined by Lisdahl; First et al., 2001).

Following the phone screen, eligible participants completed either one or two sessions. Those with considerable drug or alcohol use completed a first session three to four days before the second session, in which they were informed of the purpose of the study, procedures, potential risks and benefits, and confidentiality before providing written informed consent. They then provided a urine sample for a drug toxicology screen and completed questionnaires for background and demographic information and trait-specific psychological measures. During the second session (lasting approximately five to six hours), abstinence was once again verified through a urine drug screen and breathalyzer test, psychological measures were completed, a drug use interview and neuropsychological battery were administered, and participants underwent a high-resolution MRI scan. Control participants with little drug use completed all tasks during one session (approximately four to five hours). Participants were paid $160 for two sessions or $110 for one session, reimbursed for parking, and received drug and alcohol treatment resources and images of their brain.

SCREENING INVENTORIES AND QUESTIONNAIRES

**Biological Samples.** Participants were administered a urine toxicology screen using the One Step Drug Screen Test, a breathalyzer test, and a pregnancy test. Participants in the MJ group were permitted to continue the session with positive THC toxicology results. Those who
tested positive for drugs and/or alcohol other than MJ were excluded, and pregnant women were excluded because of effects of MRI scans on fetuses is unknown. Two MJ users were excluded from analyses due to positive toxicology screen results for multiple substances. The urine toxicology test also assessed cotinine levels using NicAlert strips (cotinine is a nicotine metabolite and a proxy for recent nicotine exposure) as cigarette smoking has been linked to alterations in the prefrontal cortex and hippocampus in adolescents and young adults (Fried et al., 2006; Jacobsen et al., 2005; Musso et al., 2006). Additionally the sample was assessed using an iScreen Specimen Validity Test adulteration strip, which indicated whether the provided sample had been tampered with prior to testing. For MJ users with positive THC results, additional urine samples were sent to a laboratory specializing in testing biological samples; chosen tests for this population were more sensitive measures of THC metabolites (to corroborate length of abstinence reported) and creatinine levels (to examine whether the participant had either drank excessive water prior to the session or added water to the sample).

Demographic Information. Participants completed a Background Questionnaire outlining demographic variables including age; gender; ethnicity; self and biological parents’ educations, incomes, and employments; marital status; number of biological and/or step/half siblings; history of medical or neurologic illness, psychological disorders or use of psychiatric medication, and learning disability; involvement in extracurricular activities or hobbies, gambling frequency, and smoker status. Height and weight were also collected to calculate body mass index (BMI; (weight in kilograms/(height in meters)$^2$). Higher BMI has been associated with structural abnormalities in several regions, including frontal, in older adults (Ho et al., 2010; Gustafson et al., 2004; Pannaccuilli et al., 2006; Raji et al., 2010; Taki et al., 2008) and poorer executive ability in youth and adults (Bauer et al., 2010, Gunstad et al., 2007; Volkow & Wise,
2005; Hillman et al., 2006; Themanson & Hillman, 2006; Themanson et al., 2006; Lisdahl et al., under review), but its relationship with MJ use remains unclear (Foltin et al., 1988; Smit & Crespo, 2001; Warren et al., 2005).

**Psychological Tests.** Relevant self-administration questionnaires included in the current study include the Frontal Systems Behavior Scale (FrSBe) and the Behavioural Inhibition and Behavioural Activation Scales (BIS/BAS). The FrSBe (Grace & Malloy, 2002) is a 46-item questionnaire assessing three areas of frontal function, apathy, disinhibition, and executive function ability. It can also capture changes in behavior over time using self-ratings for an earlier time point and at the present time; participants were asked to complete ratings for only the present time. Variables examined in this study will be the three areas and total score. The BIS/BAS (Carver & White, 1994) contains 24 items and results in three BAS scales measuring appetitive motives (Drive, Fun Seeking, and Reward Responsiveness) and a BIS scale measuring aversive motives. In addition, the Beck Depression Inventory – Second Edition (BDI-II) assessed for mood (Beck et al., 1996).

**Drug Use.** Drug use frequency was recorded to exclude very heavy users as well as to control for possible variance in cognition based on amount used. A modified version of the Time-Line Follow-Back (Sobell et al., 1979) interview was conducted, using memory cues such as holidays and personal events recorded on a calendar to measure past year drug use. Average use of each substance in a given month was recorded in addition to use during special events. To measure lifetime drug use frequency, the researcher performed a semi-structured interview, in which average weekly use for each year of use was discussed in the context of memory cues such as developmental milestones, year in school, and job history. Drug categories assessed were as follows: nicotine cigarettes, chewing tobacco/snuff/pipe, cigars/hookah, alcohol, MJ,
ecstasy, sedatives (barbiturates, valium, Xanax, Ativan, ketamine, GHB), stimulants (cocaine, crack cocaine, amphetamine, and methamphetamine), hallucinogens (PCP, LSD, DMT, peyote, acid, mushrooms), opioids (heroin, opium, pain pills), inhalants (paint, glue, household cleaners, nitrous oxide, gas), and other (anything else not mentioned). The participant’s drug use was measured by the number of standard units (cigarettes, hits, cigars for nicotine; standard drinks for alcohol; joints for MJ; tablets for ecstasy; grams for stimulants; number of hits or pills for inhalants, hallucinogens, and opioids; and pills or hits for sedatives). Participants were administered the Customary Drinking and Drug Use Record (CDDR) to assess withdrawal symptoms, DSM-IV abuse and dependence criteria, and substance-related difficulties (Brown et al., 1998; Stewart & Brown, 1995). In addition, relevant drug use information obtained from the more sensitive toxicology results sent out after the initial screen was examined as potential covariates when available; specifically, THC-COOH metabolite levels served as a proxy for recency of use and creatinine levels provided information about validity of the urine test.

NEUROPSYCHOLOGICAL ASSESSMENTS

A battery of neuropsychological tasks was administered to participants. Described below were tasks used in the present study, including three variables representing cognitive inhibition and complex attention, consistent with findings from Lisdahl & Price (2012).

Premorbid Intelligence. The Wide Range Achievement Test-4th edition (WRAT-4) Reading subtest (Wilkinson, 2006) estimates intelligence and quality of education for group comparison purposes (see Manly, 2002). Early-onset MJ users and current heavy users have been shown to have poorer Verbal IQ performances, and thus it was examined in this study as a
potential confounding variable (Pope et al., 2003; Fried et al., 2002; Fontes et al., 2011; Meier et al., 2012).

**Complex Attention.** The Wechsler Adult Intelligence Scale – Third Edition (WAIS-III) Letter Number Sequencing (LNS) total correct and the Paced Auditory Serial Attention Test (PASAT) total correct assessed complex attention. The LNS contributes to the WAIS-III Working Memory Index and requires the participant to hold auditory information in mind and accurately manipulate it in correct order (Wechsler, 1997). The examiner reads aloud jumbled groupings of numbers and letters ranging from two to eight items, and the participant is asked to immediately order the information numerically then alphabetically over a series of several trials (maximum 21). The PASAT is a working memory task in which single digit numbers are presented at the rate of one every two seconds (Gronwall, 1977). Participants must add each number to the preceding one, providing totals for the last two numbers presented across the presentation of 60 numbers.

**Cognitive Inhibition.** The D-KEFS Color Word Interference Test Inhibition total time to complete task assessed this domain. For the D-KEFS Color Word Interference Test, the participant is presented with stimuli cards containing colors and words that he or she must identify. During the Inhibition condition, color words are printed in different colored ink (i.e., “red” is printed in green ink), and the participant must correctly name the color of the ink as quickly as possible without making mistakes (Delis et al., 2001).

**MRI DATA ACQUISITION**

High-resolution anatomical images were optimized on a 4T Varian MRI scanner at the Center for Imaging Research (CIR). T1-weighted, 3-D anatomical brain scan was obtained using
A modified driven equilibrium Fourier transform (MDEFT) sequence ($T_{MD}=1.1$ s, $TR=13$ ms, $TE=6$ ms, $FOV=25.6 \times 19.2 \times 19.2$ cm, matrix $256 \times 192 \times 96$ pixels, flip angle=20 degrees; 15 min; Lee, et al., 1995). A neuroradiologist at the CIR reviewed anatomical scans and reported neurologic abnormalities. For the present study, no participants from the larger sample with clinical abnormalities on their scans will be included in this sample.

**IMAGE PROCESSING**

**Structural (Region of Interest) ROI Analysis.** All T-1 weighted 3D anatomical datasets underwent automatic alignment, removal of non-brain materials, and skull-stripping using the FreeSurfer software program. Following these pre-processing steps, the program automatically performed whole brain segmentation of white and gray matter as well as registration of anatomical brain regions (http://surfer.nmr.mgh.harvard.edu; Fischl et al., 2002). This was accomplished through a series statistical techniques that distinguish both tissue type (i.e., gray matter, white matter, cerebrospinal fluid) as well as anatomical class. First, image intensity was assessed to assign tissue type. Next, a probabilistic atlas evaluated the likelihood of particular anatomical region relative to spatial position within the brain as well as to other nearby regions (Markov random fields; MRVs). Additionally, subcortical structures were calculated separately from cortical structures, in order to restrict anatomical probability and increase likelihood of accurate registration. Cortical structures were calculated using a gyral-based automated method resulting in 37 separate anatomical labels (see Figures 1 and 2) and is comparable to manual labeling methods (see results of Fischl et al., 2002; Desikan et al., 2006). As defined by FreeSurfer, regions of interest (ROIs) were prefrontal and parietal. Regional cortex volumes included in this analysis include the following: lateral orbitofrontal, medial
orbitofrontal, superior frontal, rostral middle frontal (which corresponds to the dorsolateral PFC), and inferior parietal. Left and right hemispheres were combined for increased power. All automated steps were checked for processing errors and re-processed if necessary by trained imaging personnel. Additionally, this author, blind to participant group status and gender, utilized a randomized subject list, inspected automatic segmentation/parcellation masks, and manually edited the mask using the FreeSurfer editing program to ensure accurate segmentation for each case, using visual inspection in multiple views (Fischl et al., 2002).

Figure 1. Taken from FreeSurfer Analysis Pipeline Overview [FreeSurfer Wiki].

Figure 2. Taken from Desikan et al. (2006).
DATA ANALYSIS

**Preliminary Analysis.** ANOVAs and Chi-square tests were run to examine potential demographic differences as well as differences in past year drug use histories between MJ users and controls by gender.

**Primary Analysis.** In order to test whether MJ impacts PFC and inferior parietal brain structure, two series of regressions were run. The first examined whether MJ group status predicted ROI volume after controlling for gender, WRAT-4 Reading Standard Score, intracranial volume (ICV), body mass index (BMI), and past year alcohol, nicotine and hallucinogen use. The interaction term (MJ group-by-gender) was entered in the second block. The same series of regressions was then run to assess whether past year frequency of MJ use was significantly associated with ROI volume after controlling for the same covariates. The findings of this analysis were similar when age was included as a covariate in the regression models, and thus it was excluded in these analyses to conserve degrees of freedom.

**Post-hoc Brain-Behavior Analysis.** In order to examine the second aim, a series of correlations to examine the bivariate relationships between ROIs that significantly differed by group and complex attention (LNS and PASAT total scores) and cognitive inhibition (completion time on D-KEFS Color Word Interference Test). Next, Fisher Z tests were calculated to examine whether brain-behavior relationships were significantly different according to group.

For all analyses, interpretations about statistical significance were made if $p<.05$. Trends were reported for results of $p=.06-.15$. Additionally, $f^2$ was used to examine effect sizes for
multiple regression analyses. Effect sizes were defined as: small=.02-.14, medium=.15-.34, and large=>.35 (Cohen, 1988).

III. RESULTS

DEMOGRAPHIC, SELF-REPORT, BMI, AND NEUROPSYCHOLOGICAL PERFORMANCE INFORMATION ACCORDING TO GROUP

Participants were 59 right-handed emerging adults, 27 MJ users (12 female) and 32 controls (18 female). ANOVA and chi-square tests were conducted to determine whether the MJ users and controls differed demographically (see Table 1). There were no group differences according to age \([F(1,58)=.28, p=.60]\), gender composition [30 females, 29 males; \(x^2(1)=.82, p=.37\)], ethnicity [64.41% Caucasian, \(x^2(4)=2.55, p=.64\)], WRAT-4 Reading standard score \([F(1,58)=2.65, p=.11]\), or education \([F(1,58)=1.08, p=.30]\), or BMI \([F(1,58)=0.01, p=.93]\).

With respect to self-report measures, there were no group differences noted for BDI-II depressive symptoms \([F(1,58)=2.65, p=.11]\), FRSBE Apathy \([F(1,58)=2.13, p=.15]\), BIS/BAS Drive Motives \([F(1,58)=1.21, p=.28]\), BIS/BAS Reward Responsiveness Motives \([F(1,58)=.61, p=43.]}\), or BAS System Total \([F(1,58)=2.07, p=.16]\). However, there were significant differences between groups for FRSBE Executive Dysfunction \([F(1,58)=5.72, p=.02]\) and FRSBE Disinhibition \([F(1,58)=7.01, p=.01]\); while their mean scores were not within a clinically-significant range (i.e., \(T\geq65\)), MJ users reported increased symptoms related to difficulty planning and completing tasks, disorganization, and spontaneous behaviors versus their control counterparts. There were marginally significant findings for BIS/BAS Fun-Seeking Motives \([F(1,58)=3.18, p=.08]\), and BIS/BAS Aversive Motives \([F(1,58)=3.16, p=.08]\).
Interestingly, there was a trend towards increased self-report of BIS/BAS Fun-Seeking Motives
among controls compared to MJ users (i.e., openness to new experience, behaving hastily, seeking excitement). Additionally, MJ users exhibited a slight trend toward increased self-report of aversive motives on the BIS/BAS (i.e., nervousness, sensitivity to criticism, wariness of interpersonal conflict, perceived fears).

For the neuropsychological variables, there were no significant differences between groups for cognitive inhibition \([F(1, 58)=1.03, p=.31]\), WAIS-III Letter Number Sequencing \([F(1, 58)=1.85, p=.18]\), or PASAT Total Correct \([F(1, 58)=0.35, p=.54]\).

### Table 1. Demographic, Self-Report, BMI and Neuropsychological Performance Information According to Group.

<table>
<thead>
<tr>
<th></th>
<th>MJ users (n = 27)</th>
<th>Controls (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>21.41 (2.21) [18 – 25]</td>
<td>21.09 (2.32) [18 – 25]</td>
</tr>
<tr>
<td>Gender (% Female)</td>
<td>44.44%</td>
<td>56.3%</td>
</tr>
<tr>
<td>Ethnicity (% Caucasian)</td>
<td>62.97%</td>
<td>66.67%</td>
</tr>
<tr>
<td>WRAT-4 Reading Standard Score</td>
<td>106.81 (13.87) [85 – 134]</td>
<td>101.72 (10.14) [81 – 120]</td>
</tr>
<tr>
<td>Years of Education</td>
<td>13.48 (1.81) [9 – 17]</td>
<td>13.97 (1.79) [11 – 18]</td>
</tr>
<tr>
<td>BDI-II Total Score</td>
<td>5.67 (5.29) [0 – 25]</td>
<td>3.78 (3.55) [0 – 14]</td>
</tr>
<tr>
<td>FRSBE Apathy (T)</td>
<td>56.44 (14.64) [28 – 102]</td>
<td>51.16 (13.18) [25 – 87]</td>
</tr>
<tr>
<td>FRSBE Executive Dysfunction (T) *</td>
<td>58.52 (12.53) [38 – 98]</td>
<td>50.78 (12.25) [31 – 86]</td>
</tr>
<tr>
<td>FRSBE Disinhibition (T)**</td>
<td>57.82 (10.64) [31 – 81]</td>
<td>49.56 (12.91) [28 – 84]</td>
</tr>
<tr>
<td>BIS/BAS Drive</td>
<td>8.00 (2.68) [4 – 15]</td>
<td>8.75 (2.55) [4 – 14]</td>
</tr>
<tr>
<td>BIS/BAS Fun-Seeking ^</td>
<td>6.48 (2.44) [4 – 14]</td>
<td>7.68 (2.63) [4 – 15]</td>
</tr>
<tr>
<td>BIS/BAS Reward Responsiveness</td>
<td>6.89 (1.78) [5 – 11]</td>
<td>7.37 (2.76) [5 – 15]</td>
</tr>
<tr>
<td>BAS System Total</td>
<td>21.37 (5.30) [14 – 35]</td>
<td>23.74 (6.99) [14 – 43]</td>
</tr>
<tr>
<td>BIS/BAS Aversive ^</td>
<td>15.67 (3.45) [8 – 21]</td>
<td>14.28 (2.52) [9 – 19]</td>
</tr>
<tr>
<td>Body Mass Index (BMI)</td>
<td>24.31 (4.30) [19.37 – 36.61]</td>
<td>24.43 (5.58) [18.90 – 40.23]</td>
</tr>
</tbody>
</table>
DRUG USE INFORMATION

All participants reported abstinence from all drugs and alcohol for at least six days (one MJ user reported use of alcohol and MJ six days prior to the cognitive testing session. Urine toxicology results were negative for all illicit substances with the exception of THC (including cocaine and other amphetamines, benzodiazepines, opiates, barbiturates, hallucinogens, and ecstasy). Measured blood alcohol content (BAC) levels were 0.00. 19 MJ users yielded positive urine toxicology results for THC; 14 of those participants underwent protocol with a second toxicology session and yielded positive THC toxicology results as well. Additionally, 2 MJ users who tested negative for THC during the first session but positive during the second session were included in the analysis. (For these two subjects, THC levels as assessed by an outside toxicology lab were only available for one subject’s second session, and results were 0.00. The first subject reported abstinence from MJ for 9 days whereas the second reported abstinence for 36 days). One control tested positive for THC and was excluded from the analyses. Thus, the final sample contained 59 subjects: 27 MJ users and 32 controls.

Groups did not differ in past year stimulant \([F(1, 58) = 2.35, p = .13]\), ecstasy \([F(1, 58) = 2.47, p = .12]\), inhalant \([F(1, 58) = 1.19, p = .28]\), sedative \([F(1, 58) = 1.28, p = .26]\), or opioid use \([F(1, 58) = 1.90, p = .17]\). There were significant differences between groups for cotinine level \([F(1,
58) = 28.50, p = .000] and past year nicotine [F(1, 58) = 9.12, p = .004], alcohol [F(1, 58) = 7.26, 
p = .009], MJ [F(1, 58) = 29.01, p = .000], and hallucinogen [F(1, 58) = 5.36, p = .02] use (see Table 2. 
Overall, MJ users used more substances, especially nicotine, alcohol, MJ, and hallucinogens, 
within the past year versus controls. As such, past year nicotine, alcohol, and hallucinogen use 
were included in all regressions as covariates.

With respect to lifetime use frequencies, there were no significant differences between 
groups for inhalant [F(1, 58) = 2.13, p = .15] or sedative [F(1, 58) = 1.98, p = .16] use. There were 
significant differences between groups for lifetime nicotine [F(1, 58) = 8.73, p = .005], alcohol 
[F(1, 58) = 11.97, p = .001], MJ [F(1, 58) = 9.08, p = .004], stimulant [F(1, 58) = 3.86, p = .05], ecstasy 
[F(1, 58) = 6.51, p = .013], and hallucinogen [F(1, 58) = 4.21, p = .05] use. In addition, there was a 
marginally significant difference between groups for lifetime opioid use [F(1, 58) = 3.40, p = .07] 
(see Table 2). There was also a trend towards significance for age of first MJ use [F(1, 58) = 3.29, 
p = .078]. MJ users not only tended to try more substances and accumulate higher frequencies 
over their lifetimes, but they also appeared to initiate MJ use slightly earlier than controls.

<table>
<thead>
<tr>
<th>Table 2. Substance Use Information According to Group.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MJ users (n = 27)</td>
</tr>
<tr>
<td>Past year nicotine use**</td>
</tr>
<tr>
<td>Cotinine levels**</td>
</tr>
<tr>
<td>Past year alcohol (# drinks)**</td>
</tr>
<tr>
<td>Past year marijuana (# joints) **</td>
</tr>
<tr>
<td>Past year stimulants (# grams)</td>
</tr>
<tr>
<td>Past year ecstasy (# tablets)</td>
</tr>
<tr>
<td>Past year inhalants (# hits/pills)</td>
</tr>
<tr>
<td>Past year hallucinogens (# hits/pills) *</td>
</tr>
<tr>
<td>Past year sedatives (# hits/pills)</td>
</tr>
<tr>
<td>----------------------------------</td>
</tr>
<tr>
<td>Past year opioids (# hits/pills)</td>
</tr>
<tr>
<td>Lifetime nicotine use**</td>
</tr>
<tr>
<td>Lifetime alcohol (# drinks) **</td>
</tr>
<tr>
<td>Lifetime marijuana (# joints) **</td>
</tr>
<tr>
<td>Lifetime stimulants (# grams) *</td>
</tr>
<tr>
<td>Lifetime ecstasy (# tablets) **</td>
</tr>
<tr>
<td>Lifetime inhalants (# hits/pills)</td>
</tr>
<tr>
<td>Lifetime hallucinogens (# hits/pills) *</td>
</tr>
<tr>
<td>Lifetime sedatives (# hits/pills)</td>
</tr>
<tr>
<td>Lifetime opioids (# hits/pills) ^</td>
</tr>
<tr>
<td>Length of abstinence from MJ (days)</td>
</tr>
<tr>
<td>Age first used marijuana ^</td>
</tr>
</tbody>
</table>

Notes: **p<.01; *p<.05; ^p<.10.

PREFRONTAL AND PARIETAL CORTEX VOLUMES

The FreeSurfer program automatically extracted ROI volumes separately for each hemisphere and reported in mm$^3$; hemispheres were combined for increased power. Consistent with Churchwell et al. (2010), ROI volumes for the present study were calculated as ratios to intracranial volume, also extracted by FreeSurfer and reported in mm$^3$, and multiplied 100 [i.e. (ROI/ICV)*100]. These obtained ratio values are reported as ROI volumes in Table 3.

Table 3. Prefrontal and Parietal Volumes Expressed as a Percentage of ICV$^1$ by Group.

<table>
<thead>
<tr>
<th>MJ users (n = 27)</th>
<th>Controls (n = 32)</th>
<th>Regression values (beta; p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M (SD)</td>
<td>Range</td>
<td>M (SD)</td>
</tr>
<tr>
<td></td>
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<td></td>
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</tbody>
</table>

24
<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>95% CI</th>
<th>Mean (SD)</th>
<th>95% CI</th>
<th>Mean (SD)</th>
<th>95% CI</th>
<th>Mean (SD)</th>
<th>95% CI</th>
<th>Mean (SD)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral Orbitofrontal</td>
<td>1.04 (0.28)</td>
<td>0.80–1.52</td>
<td>1.13 (0.22)</td>
<td>0.71–1.61</td>
<td>-0.18; 0.12</td>
<td>-0.04; 0.776</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial Orbitofrontal**</td>
<td>0.65 (0.13)</td>
<td>0.45–0.99</td>
<td>0.76 (0.16)</td>
<td>0.44–1.15</td>
<td>-0.36; 0.004**</td>
<td>-0.27; 0.05*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rostral Middle Frontal</td>
<td>2.55 (0.49)</td>
<td>1.83–3.60</td>
<td>2.72 (0.42)</td>
<td>2.15–3.60</td>
<td>-0.14; 0.17</td>
<td>-0.05; 0.65</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior Frontal</td>
<td>3.52 (0.87)</td>
<td>2.21–6.22</td>
<td>3.49 (0.70)</td>
<td>2.34–4.89</td>
<td>-0.01; 0.92</td>
<td>0.11; 0.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total PFC Volume</td>
<td>7.77 (1.60)</td>
<td>5.42–12.15</td>
<td>8.10 (1.24)</td>
<td>6.15–10.25</td>
<td>-0.13; 0.17</td>
<td>0.002; 0.99</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inferior Parietal Cortex</td>
<td>2.13 (0.50)</td>
<td>1.41–3.29</td>
<td>2.25 (0.38)</td>
<td>1.60–3.08</td>
<td>-0.24; 0.04*</td>
<td>-0.08; 0.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: *Prefrontal and parietal volumes totaled across left and right hemispheres; Volume/ICV ratios multiplied by 100. **p<.01; *p<.05.

MULTIVARIATE RELATIONSHIPS

Dependent variables were raw ROI volumes. All regression analyses controlled for verbal intellectual ability (WRAT-4 Reading), gender, ICV, BMI, and past year alcohol, nicotine and hallucinogen use.

**Primary Regression Analyses: MJ Group Status & ROI Volume.** After controlling for the aforementioned covariates, MJ users demonstrated significantly smaller medial orbitofrontal cortex (mOFC) \([beta=-.36, p=.004, f^2=.18]\) and inferior parietal \([beta=-.24, p=.04, f^2=.09]\) volumes (see Figure 1) and marginally smaller lateral orbitofrontal volume \([beta=-.18, p=.12, f^2=.05]\). There were no significant group-by-gender interactions found.

**Dose-Dependent Relationships between Past year MJ use & ROI Volume.** There was a significant dose-dependent relationship present, with increased past year MJ predicting smaller mOFC volume \([beta=-.27, p=.05, f^2=.09]\) (see Figure 2). Past year MJ use did not significantly predict any other ROI volumes \((ps=.35-.98)\).
**Past year MJ use*Gender & ROI Volume.** Past year MJ use did not interact with gender in significantly predicting any ROI volume ($ps=.31-.87$).

**BMI, Comorbid Drug Use, Gender & ROI Volume.** Higher WRAT-4 Reading score significantly predicted larger superior frontal ($t(52)=2.13$, $beta=0.25$, $p=.04$) and inferior parietal volumes ($t(52)=2.41$, $beta=0.30$, $p=.02$) and marginally predicted larger total PFC volume ($t(52)=1.81$, $beta=0.18$, $p=.08$). Increased past year hallucinogen use significantly predicted larger superior frontal ($t(52)=3.05$, $beta=0.39$, $p=.004$) and total PFC volumes ($t(52)=4.80$, $beta=0.62$, $p=.000$); however, it should be noted that these findings were driven by the 4 MJ participants who endorsed past year hallucinogen use. Additionally, ICV predicted all ROI volumes ($p=.000-.05$), and male gender marginally predicted larger rostral medial frontal volumes ($t(52)=-1.92$, $beta=-.26$, $p=.06$). Further analysis revealed a significant difference between genders for ICV [$F(1, 58)=49.83$, $eta^2=.47$, $p=.000$], such that males had significantly larger total brain volume (mean ICV = 162623.03 mm$^3$; SD= 275075.85 mm$^3$) than women (mean ICV = 1181009.07 mm$^3$; SD = 205551.32 mm$^3$).
Figure 1: Mean Medial Orbitofrontal Cortex Volume by Group According to Gender.
Figure 2: Scatterplot of Medial Orbitofrontal Cortex Volume by Past Year MJ Use According to Gender.

POST-HOC BRAIN-BEHAVIOR RELATIONSHIPS

In order to examine whether brain-behavior relationships differed between MJ users and controls, bivariate correlations between ROIs that significantly differed between the groups (mOFC and inferior parietal volumes) and complex attention (LNS and PASAT total score) and inhibitory processing variables were run (see Table 5). Fisher’s z tests were then conducted to determine whether brain-behavior correlations significantly differed by group status.
**Marijuana Users.** In the MJ users, smaller mOFC and inferior parietal cortex volumes were associated with poorer performance on inhibitory control and complex attention tasks. Specifically, smaller mOFC volumes were associated with poorer PASAT scores \((r = .44, p = .02)\), and there were trends towards associations with poorer LNS \((r = .35, p = .08)\) and inhibitory control \((r = -.24, p = .23)\). Similarly, smaller inferior parietal volumes were associated with poorer PASAT \((r = .39, p = .05)\) and marginally poorer LNS \((r = .30, p = .13)\) and inhibitory control \((r = -.18, p = .36)\).

**Controls.** In general, in the controls, smaller mOFC and inferior parietal volumes were associated with complex attention. Specifically, smaller mOFC volumes were marginally associated with poorer PASAT performance \((r = .31, p = .09)\). Similar findings were seen between smaller inferior parietal volumes and poorer PASAT \((r = .60, p = .00)\) and marginally poorer LNS \((r = .22, p = .23)\) performances.

**Group Differences.** There was a significant difference between the brain-behavior correlation for mOFC and LNS by group. Specifically, for MJ users, smaller volumes were associated with poorer complex attention as measured by LNS, whereas the controls demonstrated the opposite relationship \((z = 1.87, p < .05)\). There were no other significant differences in brain-behavior correlations between MJ users and controls.
Table 4. Bivariate Correlations and Fisher’s z between ROI Volumes and Cognitive Variables by Group.

<table>
<thead>
<tr>
<th></th>
<th>MJ Users (n = 27)</th>
<th>Controls (n = 32)</th>
<th>Fisher’s z</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medial Orbitofrontal Cortex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-KEFS Color Word Interference Test (time in seconds)</td>
<td>-.24</td>
<td>.06</td>
<td>-1.10</td>
</tr>
<tr>
<td>PASAT Total Score</td>
<td>.44*</td>
<td>.31 ‾</td>
<td>.55</td>
</tr>
<tr>
<td>WAIS-III Letter Number Sequencing</td>
<td>.35 ‾</td>
<td>-.15</td>
<td>1.87</td>
</tr>
<tr>
<td><strong>Inferior Parietal Cortex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-KEFS Color Word Interference Test (time in seconds)</td>
<td>-.18</td>
<td>-.27</td>
<td>.34</td>
</tr>
<tr>
<td>PASAT Total Score</td>
<td>.39*</td>
<td>.60**</td>
<td>-1.02</td>
</tr>
<tr>
<td>WAIS-III Letter Number Sequencing</td>
<td>.30</td>
<td>.22</td>
<td>.31</td>
</tr>
</tbody>
</table>

Note: Correlations are Pearson Product Moment Correlations. *p<.05; **p<.01; ‾ p<.10.

**Correlations in bold** signify significantly different brain-behavior relationships between MJ and control groups.

**DISCUSSION**

This study examined whether MJ users demonstrated different PFC and parietal cortex volumes in a sample of healthy adolescents and emerging adults without independent Axis-I diagnoses. Secondary analysis examined dose-dependent and brain-behavior relationships. Primary results were consistent with hypotheses: MJ user group status significantly predicted smaller medial orbitofrontal (mOFC) and inferior parietal cortex volumes and marginally predicted smaller lateral orbitofrontal volumes after controlling for intracranial volume (ICV), gender, premorbid IQ and comorbid nicotine, alcohol and hallucinogen use. Increased past year MJ use also significantly predicted smaller mOFC volumes, although dose-dependent
relationships were not seen in the inferior parietal cortex. Effect size was medium for the association between MJ group status and smaller mOFC volume; effect sizes for all other findings were small. Examination of brain-behavior correlations was also consistent with hypotheses and revealed that in the MJ users, smaller mOFC and inferior parietal cortices were associated with poorer inhibitory control and complex attention.

These regional findings are consistent with results from separate samples from Medina et al. (2009) and Churchwell et al. (2010). Medina and colleagues (2009) reported that gender might moderate the relationship between MJ use and PFC volume among adolescent MJ users (ages 16-18). Female users demonstrated larger PFC volumes versus male users and controls; additionally, there was a significant group-by-volume interaction, such that smaller volumes in MJ users were associated with better executive function, and vice-versa in controls. Churchwell et al. (2010) reported smaller right mOFC volumes among adolescent MJ users aged 16-19, and total mOFC volume significantly related to age of first use. Participants in the present study represented an older cohort aged 18-25, and results showed evidence of smaller total mOFC volumes associated with poorer complex attention and inhibitory processing among MJ users. In addition, the hypothesis regarding an interaction between MJ use and gender was not supported; there were no differential findings by gender. Participants were approximately 3 years older than those in Medina’s (2009) study (mean ages = 21 years and 18 years, respectively). Additionally, the average age of onset for MJ users in our sample was 16 years old, slightly later but mostly consistent with the approximate age of onset around 15 years old reported by both Medina et al. (2009; see Results section for mean ages and length of MJ use) and Churchwell et al. (2010). If considered together, results of all studies provide evidence of neurocognitive abnormalities associated with chronic MJ exposure among youth. Further examinations of findings along a
developmental trajectory indicate disruption of typical brain development patterns. Medina et al. (2009) suggested that larger volumes were not advantageous, and that adolescent female MJ users may be at increased risk for neurocognitive changes associated with use. It would be expected that females would have undergone neural pruning processes earlier than males, as girls exhibit smaller gray matter volumes compared to same-aged boys in normal adolescent development (Giedd et al., 1996). The pattern of smaller mOFC volumes for emerging adults may demonstrate continued disruption of developmental processes associated with approximately 4 years of exposure to MJ. For this older cohort, considerable gray matter pruning in addition to white matter proliferation would be expected with increased age; thus, the smaller volumes noted may reflect a sensitive developmental time point during which gray matter pruning has occurred without progression of white matter proliferation, perhaps due to inadequate feedback from regions densely populated with cannabinoid receptors and heavily impacted by MJ exposure (i.e., PFC regions). It is notable that automated image processing methods in this study demonstrated difficulty accurately delineating gray matter from white matter, and further examination of white matter pathways in this sample is warranted. Finally, subtle neuropsychological deficits among MJ users were generally consistent with a separate report based on an overlapping but not identical sample from the same dataset, which examined relationships between past year MJ use and attention/executive function in emerging adults (Lisdahl & Price, 2012).

These findings lend additional evidence of inferior parietal abnormalities in adolescent and emerging adult MJ users, including increased cortical thickness (Lopez-Larson et al., 2011) and poorer white matter integrity (i.e., lower fractional anisotropy; FA; Bava et al., 2009). In addition, inefficient activation patterns have been identified among young users, during
inhibitory processing (Tapert et al., 2007), spatial working memory (Schweinsburg et al., 2008b), and verbal working memory tasks (while also undergoing nicotine withdrawal; Jacobsen et al., 2007). Interestingly, in the present study dose-dependent relationships between past year MJ use and parietal volumes were not seen. This may suggest that parietal abnormalities may be associated with premorbid factors that lead individuals to use MJ, or that structural abnormalities are due to lifetime exposure, rather than recent use. Indeed, several authors have discussed the difficulty of determining whether neurocognitive differences between MJ users and nonusers result from recent or chronic use or whether it predicts initiation of substance use (Lisdahl et al., 2013). There is evidence that early onset of use (prior to age 18) is related to poorer cognition, including attention, executive ability, and verbal IQ (Tamm et al., 2013; Pope et al., 2003; Fontes et al., 2011). Meier et al. (2012) followed over 1,000 participants from birth through adulthood and reported that those who used MJ as adolescents demonstrated at least one standard deviation difference on full-scale IQ when examined as adults, even after prolonged abstinence during adulthood. Work from Gruber et al. (2012; 2013) reported that age of onset as well as frequency were related to poorer cognition, decreased FA, and increased impulsivity; further, among users with early onset, significant correlations between FA and impulsivity were noted. As previously mentioned, decreased superior PFC cortical thickness (Lopez-Larson et al., 2011) as well as total mOFC volume (Churchwell et al., 2010) were also associated with earlier onset of use. On balance, increased risk-taking behaviors during adolescence, including substance use, have been also been related to structural and cognitive abnormalities (Hanson et al., 2010; Gruber et al., 2011; 2012). Of note, there were no significant predictions in the present sample of inferior parietal volumes as well as other ROI volumes by either lifetime MJ use or age of first use; however, MJ users and controls significantly differed in age of first MJ use. Thus, further
examination of age of onset of regular use in addition to frequency of recent use is suggested by recent literature, as it may identify individuals at risk for negative neurocognitive consequences.

Among MJ users, smaller mOFC volumes were associated with poorer complex attention, as measured by total score on WAIS-III Letter Number Sequencing (LNS) and PASAT total score, and inhibitory processing, as measured by time to complete the D-KEFS Color Word Interference Inhibition condition. The orbitofrontal cortex has been associated with cognitive functions including reward and value-based decision-making, (Glascher et al., 2012) and is also significant in addiction literature. ERP and fMRI studies have demonstrated increased activity in the ventromedial PFC/medial orbitofrontal cortex related to marijuana-cue reactivity and craving (Asmaro, Carolan, & Liotti, 2013, Goldman et al., 2013). Further, the orbitofrontal cortex is located in close proximity to subcortical networks involved in reward and emotion and is involved in the dopaminergic mesolimbic pathway, a key circuit in addiction. According to Koob and Volkow (2010) neural circuitry is associated with all 3 stages of the addiction cycle for all drugs of abuse, including MJ. The rewarding effects during binge/intoxication stage activate the nucleus accumbens which projects outward via the dorsal striatum. Subcortical regions including the amygdala, basal forebrain structures, and hippocampus interplay during the withdrawal/negative affect stage. Finally, during the preoccupation/anticipation (craving) stage, cortical structures including multiple regions of the PFC process higher-order cognitions related to abuse (i.e., consequences of use, emotions associated with use).

Given the relationship between PFC regions and later stages of abuse, cognitive deficits among those with DSM-IV cannabis use disorder (CUD) would not be unexpected. A study by Gonzalez and colleagues (2012) found evidence of poorer performance on the Iowa Gambling Task (IGT) among young adult MJ users with CUD. MJ users were noted to engage in current
and chronic long-term use and reported MJ as their drug of choice, >200 lifetime uses, at least four uses per week during peak use, and use within the last 45 days prior to the session. For our sample, 5% of participants met criteria for MJ abuse and 25% for MJ dependence; however, generally the MJ group had comparatively lower lifetime MJ exposure relative to other study samples. Nonetheless, examination of structural and cognitive differences according to presence of CUD may further explain dose-dependent findings. In addition, it would also be likely that those groups would perform more poorly on the IGT given relative deficits in inhibitory processing versus controls. Certainly, smaller mOFC volumes would be expected to confer less efficiency in top-down executive control for complex attention and inhibitory processing tasks, suggesting that subcortical regions involved in the reward circuit may instead demonstrate increased activity. It is important to note that in this study, raw scores were converted to z-scores for all measures (Delis et al., 2003), and mean z-scores were found to be within normal limits not suggestive of clinically significant findings (i.e., within 1 standard deviation). However, MJ users endorsed more symptoms of executive dyscontrol than controls, suggesting insight into their difficulties and potential for everyday, functional symptoms related to poor cognitive control. In addition, they noted anxiety regarding their achievement and increased fears (BIS/BAS). Kenneson et al. (2013) reported increased risk for a secondary mood disorder among those with adolescent and emerging adult onset of substance use disorder, especially for those meeting criteria for dependence vs. abuse. In addition, recent data suggest that variables including support network, coping, frequency of stressful events, and number of CUD symptoms significantly impacted transition from heavy MJ user to MJ dependence over a 3-year period among individuals aged 18-30 (van der Po et al., 2013). Thus, examination of magnitude of adverse consequences associated with use (i.e., severity of addiction) is important in
characterizing neurocognition among MJ-using emerging adults; further, measurement of variables reflecting coping strategies may help identify MJ users at greater risk for CUD and related functional deficits.

No treatment has yet been identified for cannabis use disorder, let alone specifically for adolescent or emerging adult populations. Typically, motivational interviewing (MI) techniques may enhance entry into addiction treatment, and contingency management (CM) has been discussed as a promising alternative for adolescents (Stanger & Budney, 2010). While CM has been shown to promote abstinence, limitations include costs associated with implementing the system and continued abstinence following termination. Among alcohol users across the lifespan, abstinence has been shown to predict improved executive functioning (Hanson et al., 2010), larger bilateral cerebellar volume (Lisdahl, et al., 2013) and larger volumes, surface area and cortical thickness for regions within the brain reward system (i.e., PFC, amygdala, and hippocampus; Durazzo et al., 2011). However, findings are mixed regarding whether abstinence alone predicts improved function (Hanson et al., 2010; Fried et al., 2005). In contrast, a recent prospective study by Cousjin and colleagues found that among heavy users, abnormal response in the working-memory network predicted an increase in MJ use 6 months later, independent of baseline MJ use and performance on the N-back task. Thus, potential considerations for treatment would be those that target regions including the PFC and amygdala.

In a sample of abstinent, alcohol-dependent adults, cue-exposure treatment was found to reduce activity in the anterior cingulate gyrus and insula as compared to increased activity in limbic regions noted during baseline (Klein et al., 2011). Chiesa and colleagues (2010) reviewed the literature regarding neurobiological results of mindfulness meditation practice, and reported increased activation in PFC and anterior cingulate cortical regions. Physical activity has also
been associated with improved neurocognition, although the majority of research has been conducted in older populations (Waters et al., 1995, Field et al., 2001). Lisdahl et al.’s review (2013) summarized preliminary findings among adolescents and emerging adults, suggesting that acute effects of exercise are associated with improved efficiency, as evidenced by cerebral blood flow (Pereira et al., 2007; Timinkul et al., 2008), white matter integrity (Marks et al., 2007), and executive control (Dustman et al., 1990; Hillman et al., 2003; Themanson and Hillman, 2006; Themanson et al., 2006; Ferris et al., 2007). Youth who exercise also demonstrate improved mood, better grades, and less drug use (Winnail et al., 1995; Field et al. 2001). In addition, much of the cognitive rehabilitation research has been performed in populations with brain injury (Cicerone et al., 2000). Little research exists regarding cognitive training techniques for relapse prevention (see Wiers et al., 2013) or improved cognitive performance (Macher & Earleywine, 2012; Sofuoglu et al., 2010). However, consistent with goals set forth by the Brain Injury Interdisciplinary Special Interest Group of the American Congress of Rehabilitation Medicine (ACRM), future interventions should be informed by neurocognitive findings and target deficits associated with known negative outcomes (i.e., relapse, cognitive dyscontrol). Finally, Lisdahl et al. (2013) discussed the importance of providing individualized feedback to young MJ users, incorporating neurocognitive data from same-aged youth with similar use patterns and frequency (see Feldstein-Ewing et al., 2012; Larimer and Cronce, 2007).

There are several limitations of the current study. As previously mentioned, there were identified challenges in accurately delineating white matter from gray matter using FreeSurfer automated methods, making examination of cortical architecture unreliable at the present time. However, several steps were taken to ensure accurate measurement of regional volumes, utilizing techniques applied at all stages of image processing and consistent with verification techniques.
typical for all neuroimaging research. This included: manual checks at each step in processing (with re-processing if indicated); individual examination of each brain for topological errors (with appropriate semi-automated edits and re-processing if indicated); and manual examination and intervention of brain masks, including addition and deletion of voxels if indicated. Thus, despite these issues, every attempt was made to ensure accuracy and results are considered to be valid. We also did not assess differences between hemispheres in the analyses, and both hemispheres were combined in order to improve detection. Most MJ users engaged in relatively low use compared to samples from other studies, and we were unable to corroborate reported last use with expanded toxicology reports (including THC and creatinine levels) for all MJ users. In addition, several of the heavier MJ users tended to report: a) greater lifetime exposure to different substances (i.e., more likely to initiate different uses); b) regular use of alcohol and nicotine along with MJ use; and c) infrequent but recent use of other illicit drugs. These characteristics occurred despite significant effort to recruit appropriate groups for comparison, but speaks to the culture of MJ use among emerging adults in this region (i.e., chronic, recent comorbid nicotine, alcohol, and MJ use with increased recreational use of hallucinogens) as well as nationally (Ramos et al., 2012). Future studies should seek to address the comorbidity of other drug use, particularly nicotine, through appropriate recruitment and study design in order to compare patterns as well as frequency of nicotine and MJ use associated with neurocognition (Fried et al., 2006; Jacobsen et al., 2005; Musso et al., 2006). Despite these concerns, however, results demonstrate independent prediction according to MJ use as analyses controlled for past year nicotine, alcohol, and hallucinogen use. Finally, because this project examined anatomical structure in a sample of emerging adults, results may not generalize to individuals outside of this cohort and should be interpreted in the context of neurodevelopment.
Future studies should aim to a) assess individual differences, b) utilize multi-modal imaging techniques, and c) examine MJ-users longitudinally. Goals of such large-scale research would be to provide clearer information regarding the timing of neurodevelopmental processes and impacts of initiation of MJ use. Multi-modal imaging techniques could characterize the relationship between brain structure and function locally at the cellular level (spectroscopy) or within regions of interest (structural MRI or DTI), as well as globally across several regions (PET, fMRI, functional connectivity). For example, Behan et al. (2013) found no difference in brain activation patterns among adolescent MJ users vs. controls on the Go/No-Go task; however, correlational analyses yielded evidence of aberrant connectivity patterns associated with chronic marijuana consumption, between inferior parietal lobes and the cerebellum. Additionally, identification of biomarkers associated with particular endophenotypes is also an important consideration in behavioral imaging. Variations of the cannabinoid receptor gene (CNR1) have been shown to modulate several neurotransmitter systems associated with mood, (Viveros et al., 2005; Lazary et al., 2009), including anxiety. Variations have also been linked to reduced fronto-temporal white matter volumes, poorer cognition, and increased risk for substance use disorders (Ho et al., 2010; Ruiz-Contreras et al., 2011; 2013; Zuo et al., 2007; Benyamina et al., 2011). Improved characterization of at-risk populations could help identify individual differences related to phenotypes such as mood and cognition that may predate adverse neurocognitive consequences of addiction. In turn, this information could be used to develop individualized, scientifically informed treatments. For example, this may be MI and contingency management supplemented with coping strategies for adolescents with a particular risk allele and self-reported subclinical symptoms of anxiety; or MI, CBT, and adjunctive
cognitive training for emerging adults meeting criteria for CUD and positive family history of SUD.

In conclusion, this project found that for an emerging adult sample, MJ user group status significantly predicted smaller mOFC and inferior parietal volumes and marginally predicted lateral orbitofrontal volumes. There was a dose-dependent relationship between past year MJ use and smaller mOFC volumes. In addition, smaller volumes were associated with poorer complex attention and inhibitory processing among MJ users. There was also a significant group difference in brain-behavior relationships, with MJ users demonstrating smaller mOFC volumes and poorer LNS performance whereas the opposite was true for controls. Results were consistent with previous research suggesting disruption of neurodevelopmental processes associated with chronic MJ use in adolescents and emerging adults; in particular, these findings represented a degree of systematic replication of findings from Medina et al. (2009) and Churchwell et al. (2010). On average, MJ users in the present study smoked just over one joint per day, ranging from twice monthly usage all the way up to over four joints per day. Further, one-quarter of participants met criteria for CUD. MJ users were abstinent for approximately 13 days per self-report; unfortunately, more detailed THC toxicology results were not available for all participants. These results suggest neuroanatomical and cognitive alterations associated with past year marijuana use among otherwise healthy emerging adults. Future directions should expand upon these findings towards examination of other tissue classes underlying volumetric abnormalities, including white matter structure and quality and indices of cortical architecture such as surface area, gyrification, and cortical thickness. In addition, FreeSurfer has developed semi-automated methods for applying masks measuring the entire PFC, which would allow for a closer comparison to Medina and colleagues’ (2009) adolescent study. In closing, several
current societal issues are noteworthy, including legalization of medical MJ, increased potency of medical MJ (Sevigny et al., 2012), and diversion of medical MJ among youth (Salomonsen-Sautel et al., 2012). Thus, it is both timely and critical that researchers work towards improved understanding of neurocognitive deficits associated with use as well as towards development of effective treatments aimed at delaying onset of MJ use among youth.
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