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CANNABIS USE AND AFFECTIVE PROCESSING: A BRAIN STRUCTURE ANALYSIS

by

Kristin E. Maple

A Thesis Submitted in

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ABSTRACT

CANNABIS USE AND AFFECTIVE PROCESSING: A BRAIN STRUCTURE ANALYSIS

by

Kristin E. Maple

The University of Wisconsin—Milwaukee, 2016
Under the Supervision of Professor Krista Lisdahl

Cannabis is the most commonly used illicit drug amongst adolescents and young adults in the United States. Previously, cannabis and its components have been associated with differences in affective processing and neural functioning. Participants (ages 16-25) were cannabis users and non-users excluded for psychiatric disorders, major medical conditions, and excessive other drug use. A series of multiple regressions examined whether past year cannabis use and cannabis x gender predicted measures of emotional face processing (using the PennCNP affective battery) as well as volumes in bilateral prefrontal, temporal, limbic, and cerebellar regions, as well as frontolimbic white matter tracts. Subsequently, Pearson correlations were conducted within the cannabis group to assess whether brain regions significantly associated with cannabis use predicted mood and affective processing. Increased cannabis use was associated with higher Acuity Neutral scores, smaller left rostral anterior cingulate (rACC) volumes, larger superior temporal volumes, and reduced right uncinate fasciculus mean diffusivity. Significant cannabis x gender interactions were observed for left rACC and forceps minor fractional anisotropy (FA). Greater cannabis use was associated with smaller left rACC volumes only within females, as well as greater forceps minor FA in females and reduced FA in males. Within the cannabis-using group, smaller rACC

volumes were correlated with lower Emotion Discrimination Correct scores. These findings suggest that cannabis use exerts dose dependent effects on frontolimbic circuitry, which are in turn associated with deficits in affective processing. This may be one potential mechanism underlying high comorbidity rates between chronic cannabis use and psychiatric disorders.

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2-AG	2-Arachidonoylglycerol
ACC	Anterior Cingulate Cortex
AFNI	Analysis of Functional Neuroimages
ANOVA	Analysis of Variance
ATR	Anterior Thalamic Radiations
BDI-II	Beck Depression Inventory—Second Edition
cACC	Caudal Anterior Cingulate Cortex
CB1	Cannabinoid receptor-1
CBD	Cannabidiol
CC	Corpus Callosum
CDDR	Customary Drinking and Drug Use Record
CNR1	Cannabinoid receptor-1 gene
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders—Fourth Edition
DTI	Diffusion Tensor Imaging
eCB	Endocannabinoid
FA	Fractional Anisotropy
FAAH	Fatty Acid Amide Hydrolase
FDR	False Discovery Rate
fMRI	Functional Magnetic Resonance Imaging
FOV	Field of View
GE	General Electric
HPA	Hypothalamic-Pituitary-Adrenal

lOFC	Lateral Orbital Frontal
MCW	Medical College of Wisconsin
MD	Mean Diffusivity
MDD	Major Depressive Disorder
MDEFT	Modified Driven Equilibrium Fourier Transform
MINI	Mini International Psychiatric Interview
MNI	Montreal Neurological Institute template
mOFC	Medial Orbital Frontal Cortex
mPFC	Medial Prefrontal Cortex
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
ms	milliseconds
OFC	Orbital Frontal Cortex
PCA	Principal Components Analysis
PCP	Phencyclidine
PFC	Prefrontal Cortex
rACC	Rostral Anterior Cingulate Cortex
sMRI	Structural Magnetic Resonance Imaging
SPSS	Statistical Package for the Social Sciences
ROI	Region of Interest
TE	Echo Time
THC	Δ-9-tetrahydrocannabinol
TLFB	Timeline Follow Back

TMD   MDEFT preparation time

TR   Repetition Time

TRACULA Tracts Constrained by Underlying Anatomy

WRAT-4 Wide Range Achievement Test—Fourth Edition

ACKNOWLEDGEMENTS

Cannabis Use and Affective Processing: A Brain Structure Analysis

Cannabis is the most commonly used illicit drug in the United States, with 35.1% of 12th graders and 31.6% of 19-28 year-olds using in the past year (Johnston et al., 2015a; Johnston et al., 2015b). Furthermore, the perceived risk of using cannabis has dropped substantially amongst this population since 2005 (Johnston et al., 2015a; Johnston et al., 2015b). This decrease in perceived risk has occurred in parallel with many states proposing, and some passing, legislation to legalize medicinal and recreational cannabis use. Currently, recreational use is legal in Colorado, Washington, Oregon, Alaska, and Washington, D.C. Experts have predicted that with legalization, the price of cannabis is likely to drop, and consumption is likely to increase (Caulkins et al., 2012). Given that cannabis use is widespread and may increase further, research on its potential neurocognitive effects in young people is critical.

Adolescents and emerging adults may be especially vulnerable to the negative effects of cannabis use due to the substantial neurodevelopment occurring during this period (Lisdahl et al., 2013; Lisdahl et al., 2014; Jacobus & Tapert, 2014). Although at age six the brain is approximately 90% of its adult weight, neurodevelopment continues into the late 20's (Casey, Jones, & Hare, 2008). White matter volume and integrity increase, enhancing neural efficiency (Giedd et al., 1999; Lebel et al., 2008; Lebel & Beaulieu, 2011). The adolescent brain also undergoes significant synaptic pruning in regions associated with higher-order and executive function, such as the prefrontal cortex (PFC) (Casey et al., 2008). The PFC is one of the last areas to mature, lagging behind subcortical limbic regions involved in affect, such as the amygdala (Casey et al., 2008). According to Casey and colleagues' (2008) model, when adolescents are

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presented with an emotionally-charged situation, limbic regions will override the prefrontal executive system, leading to increased risk taking, impulsivity, and emotional reactivity. Thus, adolescents may be more likely to engage in risky behavior such as substance use, and are at greater risk of developing a substance use disorder compared to those initiating use as adults (Chambers et al., 2003; Casey et al., 2008; Bava & Tapert, 2010). Given the increased likelihood to abuse substances, including cannabis, during a time when the brain is particularly vulnerable to their neurotoxic effects, adolescence and emerging adulthood is the ideal period to research and target substance use interventions (Squeglia, et al., 2009; Lisdahl et al., 2013).

The Endogenous Cannabinoid System: Neurodevelopment & Affective Processing

The main psychoactive component of cannabis, Δ-9-tetrahydrocannabinol (THC), exerts its effects by binding brain cannabinoid receptor-1 (CB1) in the endocannabinoid (eCB) system (Howlett, 1995). Cannabidiol (CBD) is another cannabinoid found in herbal cannabis, but typically in low doses. In cannabis, THC content is typically much higher compared to CBD, with published mean THC levels at 8.8% and CBD at 0.4% in the U.S. in 2008 (Mehmedic et al., 2010). A more recent report of cannabis seized in Australia noted 14.88% THC (Swift et al., 2013). Recent research has suggested that CBD may antagonize some negative effects of THC, such as anxiety (Niesink & van Laar, 2013).

CB1 receptors are located on pre-synaptic terminals of both excitatory and inhibitory neurons, as well as on glial cells, including astrocytes, microglia, and oligodendrocytes (e.g., Domenici et al., 2006; Pazos et al., 2005). The eCB system includes the primary

ligands anandamide (AEA) and 2-AG, which also bind CB1 (Mechoulam & Parker, 2013). After AEA interacts with CB1 at the pre-synaptic terminal, it is degraded by the enzyme fatty acid amide hydrolase (FAAH) (Ho & Hillard, 2005). Thus, higher levels of FAAH are typically associated with lower levels of eCBs (Giang & Cravatt, 1997).

From prenatal stages through adulthood, the eCB system influences neurodevelopment (e.g., Aguado et al., 2006; Berghuis et al., 2007; Mulder et al., 2008; Schneider et al., 2008; Chadwick et al., 2013). Activation of CB1 receptors can initiate a signal transduction pathway that affects developmental processes, such as synaptic plasticity, cell migration, axon guidance, and neuronal growth (Pazos et al., 2005; Mechoulam & Parker, 2013; Melis et al., 2014; Zhou et al., 2014). The eCB system is dispersed throughout the brain and undergoes dynamic changes throughout neurodevelopment (Chadwick et al., 2013). CB1 receptors are highly expressed in the PFC, cingulate cortex, hippocampus, amygdala, basal ganglia, and cerebellum, with relatively low levels in the brainstem (Herkenham et al., 1990; Glass et al., 1997; Mackie et al., 2005; Svizenska et al., 2008). While distribution of CB1 receptors stays relatively constant across development, density is altered over time (Glass et al., 1997); expression of CB1 mRNA and CB1 density and binding are highest during puberty onset and adolescence, decreasing by adulthood (Ellgren et al., 2008; Schneider, 2008; Heng et al., 2011; Chadwick et al., 2013; Higuera-Matas et al., 2015). This pattern is particularly evident in the medial PFC, cingulate cortex, and insula, which demonstrate the most dramatic and progressive decrease in CB1 expression across adolescence (Heng et al., 2011). This decline in CB1 levels occurs in tandem with an increase in AEA and decrease in 2-AG within the PFC (Ellgren et al., 2008). These differences in

eCB activity across adolescence, particularly in maturing prefrontal regions, indicate a role for this system in the neurodevelopmental processes occurring during this period. Therefore, age-related changes in eCB functioning indicate that adolescents and emerging adults may be at greatest risk to incur the negative consequences of cannabis exposure (Higuera-Matas et al., 2015).

The eCB system, among other functions (e.g., sleep, pain, memory, feeding, and inflammation), modulates mood and stress response. Indeed, genetic variants in the eCB system, including multiple *FAAH* and *CNR1* (gene coding for CB1) single nucleotide polymorphisms, are associated with both subclinical depressive symptoms and mood disorders in humans (Maple et al., in press; Barrero et al., 2005; Monteleone et al., 2010; Mitjans et al., 2013). Moreover, several rodent studies have revealed that increases in eCB activity elicit antidepressant and anxiolytic responses, while decreases in activity lead to depression (Martin et al., 2002; Hill et al., 2005; Hill & Gorzalka, 2005; Gobbi et al., 2005; Bortolato et al., 2007; Naidu et al., 2007; Serra & Fratta, 2007; Adamczyk et al., 2008; McLaughlin & Gobbi, 2012). Steady eCB tone in the mPFC prevents unnecessary limbic and HPA activation, promoting positive emotionality and healthy coping responses to stress (McLaughlin, Hill, & Gorzalka, 2014). However, under chronic, unpredictable stress, eCB tone in the mPFC can decrease, leading to HPA hyperactivation. Indeed, this heightened HPA activity increases vulnerability to anxiety and mood disorders (McLaughlin et al., 2014). This suggests a crucial role for the eCB system in modulating frontolimbic activity in response to stress.

More specifically, evidence suggests that the eCB system is associated with facial emotion processing. Studies have demonstrated that both *FAAH* (Hariri et al., 2008)

and *CNR1* (Chakrabarti et al., 2006) genotypes modulate brain activation to emotional faces. Recognizing and interpreting facial expressions is a critical aspect of functioning in human social networks. Facial expressions provide information about an individual's emotional state, and interpretation of these expressions by others can elicit an emotional state in response (Phillips et al., 2003; Bourke et al., 2010). Therefore, it is possible that any inaccuracy in identifying facial emotions may negatively influence one's affective state and emotional well-being (Phillips et al., 2003). In addition, mood may bias one's appraisals regarding facial emotions, therefore perpetuating a cycle of appraisals impacting emotional responses, which in turn influence further appraisals (e.g., Mogg et al., 2000; Joormann & D'Avanzato, 2010; Duque & Vazquez, 2015). In fact, abnormal facial emotion processing is common in those with psychiatric disorders, including major depressive disorder (MDD), bipolar disorder, social anxiety disorder, and schizophrenia (e.g., Marwick & Hall, 2008; Morris et al., 2009; Bourke et al., 2010; Stuhrmann et al., 2011; Staugaard, 2010; Samame, 2013).

Cannabis & Affective Processing

Considering the critical role the eCB system plays in modulating mood, and its apparent role in processing facial emotions, it is unsurprising that both acute and chronic exposure to exogenous cannabinoids, including THC and CBD, can alter affective functioning. Multiple studies have found that acute THC administration significantly impairs participants' emotion identification abilities (Ballard et al., 2012; Bossong et al., 2013; Hindocha et al., 2015), while CBD enhances accuracy (Hindocha et al., 2015). Acute THC administration affects neural activation to emotional faces in limbic (Phan et al., 2008; Bhattacharyya et al., 2010; Bossong et al., 2013), temporal

(Fusar-Poli et al., 2009), frontal, parietal, and occipital (Fusar-Poli et al., 2009; Bossong et al., 2013) areas. In response to emotional faces, CBD impacts activity in the left amygdala, anterior and posterior cingulate cortices, anterior parahippocampal gyrus, left middle occipital gyrus, and the cerebellum (Fusar-Poli et al., 2009; Bhattacharyya et al., 2010). While some have found acute THC (Gorka et al., 2015) and CBD (Fusar-Poli et al., 2010) to impact frontolimbic connectivity, others have found no effect (Fusar-Poli et al., 2010). Cannabis smoking, which typically has substantially higher levels of THC compared to CBD (Mehmedic et al., 2010), leads to poorer acute emotion recognition (Clopton et al., 1979). Therefore, the current literature suggests that acute THC and CBD impact many frontolimbic and associated areas with high eCB activity during facial emotion processing.

Chronic cannabis exposure is associated with long-term affective and mood problems. Cannabis users score higher on measures of anxiety and depression compared to non-using controls (Troisi et al., 1998; Dorard et al., 2008; Medina & Shear, 2007; Medina et al., 2007; Wright et al., under review). In fact, cannabis use has a high rate of comorbidity with anxiety and mood disorders in both adolescents (e.g., McGee et al., 2000; Wittchen et al., 2007; Dorard et al., 2008) and adults (e.g., Troisi et al., 1998; McGee et al., 2000; Agrawal et al., 2011; Lev-Ran et al., 2013). A substantial number of studies suggest that cannabis use increases one's risk for subsequently developing affective disorders, especially if use begins in early adolescence (e.g., Bovasso, 2001; Patton et al., 2002; Rey & Tennant, 2002; Hayatbakhsh et al., 2007; van Laar et al., 2007; de Graaf et al., 2010; Fairman & Anthony, 2012; Rasic et al., 2013). Yet, other studies report anxiety and/or depression predicting later cannabis use

(e.g., Wittchen et al., 2007). Thus, the relationship between cannabis and affect is complicated and likely nuanced, especially given that many people reportedly use cannabis to ameliorate internalizing symptoms, such as depression and anxiety (Boys et al., 2001; Walsh et al., 2013). One mechanism underlying mood symptoms in cannabis users may be damage to the affective neural network, resulting in deficits in basic affective processing such as facial emotion processing.

A limited number of studies have investigated the relationship between chronic cannabis use and facial emotion processing. The available evidence suggests that cannabis users are significantly slower (Platt et al., 2010) and less accurate (Hindocha et al., 2014; Bayrakci et al., 2015) in identifying emotions compared to controls. Further, the ability of cannabis users to discriminate whether two faces show the same emotion is reduced (Bayrakci et al., 2015). Findings from another group suggest that lower levels of cannabis use might not be related to facial emotion processing, while more frequent and recent cannabis use is associated with affective processing deficits (Huijbregts et al., 2014). Chronic cannabis use has been associated with blunted ACC (Gruber et al., 2009) and amygdala (Gruber et al., 2009; Cornelius et al., 2010) activation to negative facial emotions, while in younger (14 years of age) cannabis users, amygdala activation is increased (Spechler et al., 2015).

Cannabis Use and Brain Structure Associated with Affective Processing

Morphometry Studies. Facial emotion processing is a complex process, recruiting many visual, limbic, temporoparietal, and prefrontal areas, as well as the cerebellum and putamen (Ishai et al., 2005; Fusar-Poli et al., 2009; Said et al., 2011). Further, many regions involved in facial emotion processing are high in CB1 receptor density,

including the PFC, cingulate cortex, amygdala, hippocampus, and cerebellum. Chronic cannabinoid exposure is associated with CB1 receptor downregulation in areas associated with affect, including cortical regions (McKinney et al., 2008; Hirvonen et al., 2012), the hippocampus, and cerebellum (Villares, 2007; McKinney et al., 2008). This downregulation of CB1 receptors is likely one mechanism by which long-term cannabis use can impact affective processing differentially from acute exposure.

Although some studies have examined functional brain relationships underlying facial emotion processing in cannabis users, no known studies have investigated structural relationships associated with these differences. Yet, several researchers have examined basic brain structural differences related to cannabis use. Indeed, many of these structural differences have been found in regions classically associated with emotional face processing. Cannabis users have exhibited volumetric differences in the mOFC (Churchwell et al., 2010; Cheetham et al., 2012; Batistella et al., 2014; Price et al., 2015), total PFC (Medina et al., 2009), and fusiform gyrus (Jarvis et al., 2008). Only one known study has investigated superior temporal gyrus volume in cannabis users compared to non-users, yielding no difference between groups in either the right or left superior temporal gyrus (DeLisi et al., 2006). Cannabis use has also been associated with volume differences in subcortical affective regions such as the amygdala (Yucel et al., 2008; McQueeny et al., 2011; Schacht et al., 2012; Cousijn et al., 2012; Gilman et al., 2014; Pagliaccio et al., 2015), hippocampus (Matochik et al., 2005; Medina et al., 2007a; Yucel et al., 2008; Ashtari et al., 2011; Demirakca et al., 2011; Schacht et al., 2012; Cousijn et al., 2012; Solowij et al., 2013), and cerebellum (Jarvis et al., 2008; Medina et al., 2010; Solowij et al., 2011; Cousijn et al., 2012; Batistella et al., 2014;

Nurmedov et al., 2015). Most studies in emerging adult samples have demonstrated decreased volumes across brain regions in association with cannabis use (eg, Matochik et al., 2005; Yucel et al., 2008; Churchwell et al., 2010; Ashtari et al., 2011; Demirakca et al., 2011; Schacht et al., 2012; Cousijn et al., 2012; Solowij et al., 2013; Batistella et al., 2014; Price et al., 2015; Pagliaccio et al., 2015).

Some studies have reported increased volumes in association with cannabis use (e.g., Medina et al., 2009; Medina et al., 2010; McQueeny et al., 2011). However, these samples were comprised exclusively of younger adolescents (eg, 16-18 years). In these studies, increased volumes were associated with functional deficits such as poorer executive functioning and increased internalizing symptoms. The authors of these studies hypothesize that cannabis may interfere with significant synaptic pruning occurring during adolescence (Medina et al., 2009; Medina et al., 2010; McQueeny et al., 2011; Lisdahl et al., 2014). Further, gender may moderate these findings; adolescent females appear to be particularly vulnerable to the impact of cannabis on PFC and amygdala volumes (Medina et al., 2009; McQueeny et al., 2011). Despite this substantial literature, some studies have reported null findings for each of these brain regions (eg, Tzilos et al., 2005; Medina et al., 2007b; Ashtari et al., 2011; Cousijn et al., 2012; Mashhoon et al., 2015; Pagliaccio et al., 2015; Smith et al., 2015; Lorenzetti et al., 2015; Price et al., 2015; Weiland et al., 2015). Thus, more research is needed to clarify how gender might moderate the relationship between cannabis use and PFC, limbic, and cerebellar volumes.

White Matter Integrity. As CB1 receptors are present on axons and oligodendrocytes (Domenici et al., 2006; Pazos et al., 2005), which comprise white

matter, exogenous cannabinoids may alter white matter functioning. Several diffusion

tensor imaging (DTI) studies have found associations with cannabis use and white

matter integrity in regions throughout the brain (e.g., Gruber & Yurgelun-Todd, 2005;

DeLisi et al., 2006; Arnone et al., 2008; Ashtari et al., 2009; Bava et al., 2009; Allin et

al., 2009; Bava et al., 2010; Gruber et al., 2011; Zalesky et al., 2012; Bava et al., 2013;

Jacobus et al., 2013a; Jacobus et al., 2013b). Of particular note, Shollenbarger and

colleagues (2015) found an association between cannabis use and decreased white

matter integrity in the corpus callosum forceps minor, uncinate fasciculus, and anterior

thalamic radiations (ATR). As the CC forceps minor, uncinate fasciculus, and ATR

connect frontolimbic regions (Catani & Thiebaut de Schotten, 2012), reduced integrity in

these tracts may be of particular relevance to affective processing. Reduced uncinate

integrity has been associated with major depression (Bracht et al., 2015; Taylor et al.,

2007; Cullen et al., 2010; Dalby et al., 2010; Carballedo et al., 2012; Zhang et al., 2012)

and poorer facial emotion recognition (Fuije et al., 2008). Similarly, several studies have

associated integrity in the ATR (Henderson et al., 2013; Bessette et al., 2014; Lai & Wu,

2014, Bracht et al., 2015) and CC forceps minor (e.g., Alves et al., 2014; Serafini et al.,

2014) with mood disorders. Thus, considering the association that the uncinate, ATR,

and forceps minor have with affective disorders, it is possible they are involved in

affective processing in cannabis users. Consistent with this, Shollenbarger and

colleagues (2015) found that increased subclinical depressive symptoms in cannabis

users were associated with decreased integrity in bilateral ATR and right uncinate

fasciculus. However, more research is needed to determine if these tracts are

associated with specific aspects of affective processing, such as identifying facial emotions.

Gender Differences

Evidence indicates that male and female brains may process affective information differently. Women have twice the rate of affective disorders as men (e.g., Gater et al., 1998), and research findings suggest that healthy females are generally more accurate at identifying facial emotions compared to males (Kret & De Gelder, 2012). Further, males and females appear to recruit different networks while processing emotional faces (Killgore & Yurgelun-Todd, 2001; Killgore et al., 2001; Killgore & Yurgelun-Todd, 2004; Whittle et al., 2011). Although the literature is not wholly consistent, females tend to exhibit increased temporal, ACC, and limbic activation while males demonstrate increased frontal and parietal activation to emotional faces (Hall et al., 2004; Kempton et al., 2009; Whittle et al., 2011). In addition, female affective circuitry may be more susceptible to the negative effects of cannabis use. For instance, compared to males, adolescent female rats exposed to THC are more vulnerable to its anxiety and depression producing properties (Rubino et al., 2008). In humans, female cannabis users have exhibited disadvantages in PFC and amygdala volumes compared to their male counterparts (Medina et al., 2009; McQueeny et al., 2011). Additionally within females compared to males, increased CB1 desensitization to THC is observed within frontolimbic regions, including the PFC, amygdala, and hippocampus (Crane et al., 2013). Thus, cannabis-using females may process facial emotions differently from male users, and this may be related to underlying brain structure in frontolimbic regions.

Aims and Hypotheses

Despite evidence that chronic cannabis use may impact facial emotion processing in adolescents and emerging adults, few studies have examined affective processing in regular cannabis users and whether these relate to structural abnormalities in the affective processing network. Thus, the current study seeks to elucidate these relationships.

Primary Aims. *(1) Cannabis Use and Affective Processing.* We will investigate whether increased cannabis use or cannabis x gender predict affective processing and mood symptoms in adolescents and emerging adults. Specifically, we will examine whether these variables predict (a) facial emotion processing and (b) depressive symptoms. Based on the aforementioned literature, we hypothesize that cannabis users will have impaired emotional processing and increased depressive symptoms compared to non-users. It is predicted that females will be more susceptible to the potential effects of cannabis on (a) affective processing and (b) depressive symptoms compared to males.

(2) Cannabis Use and Brain Structure. We will assess whether cannabis use and cannabis x gender are associated with abnormal brain structure in regions underlying affective processing. (a) We will investigate whether cannabis use and cannabis x gender predict volumes of regions implicated in affective processing, including the bilateral medial orbital frontal cortex (mOFC), lateral orbital frontal cortex (lOFC), rostral ACC (rACC), caudal ACC (cACC), Pars triangularis, superior temporal gyrus, fusiform gyrus, amygdala, hippocampus, and cerebellum. Based on other studies with a similar mean age, we hypothesize that increased cannabis use will be associated with decreased gray matter volumes in prefrontal regions, superior temporal gyrus, fusiform

gyrus, amygdala, and hippocampus, as well as increased cerebellar volumes. Additionally, it is predicted that greater cannabis use in females will be associated with the most marked volumetric relationships. (b) We will also explore whether cannabis use and cannabis x gender predict white matter integrity in major frontolimbic tracts involved in affective processing: R/L uncinate fasciculus, R/L anterior thalamic radiations, and corpus callosum forceps minor. We hypothesize that higher levels of cannabis use will be associated with decreased white matter integrity in these regions. It is additionally predicted that females with more cannabis use will have the greatest reduction in white matter integrity.

Secondary Aim. Once we determine regions that are associated with either cannabis or cannabis x gender, we will determine if those areas predict (a) emotional processing and (b) depressive symptoms within the cannabis user group. We hypothesize that brain structure will predict affective processing and depressive symptoms within cannabis users.

Method

Participants

Participants included 77 (two of these did not complete the MRI scan due claustrophobia) cannabis using and non-using adolescents and emerging adults ages 16-25 years, recruited from a larger parent study (R01 DA030354).

Recruitment. Individuals were recruited from the community. Flyers were posted around universities, cafes, bars, headshops, recreation centers and festivals. Best attempts were made to recruit an ethnically representative sample balanced for gender.

Inclusion/Exclusion Criteria. *Inclusion criteria.* *Inclusion criteria for cannabis use.* Used cannabis at least 10 times in past three months or at least 50 times in life. *Inclusion criteria for non-using controls.* Used cannabis fewer than 50 times in life and fewer than five times in the past year. ***Exclusion criteria (both groups).*** Non-English speaker, left-handed, has non-removable metal (or any other MRI contraindication), younger than 16 years of age, older than 25 years of age, prenatal alcohol (>6 drinks per week or >4 per day) or nicotine exposure, birth complications, premature birth (<33 weeks gestation), no neurological disorders (ie, seizures, migraines, tumors, chemotherapy, multiple sclerosis, movement disorders), head trauma with >2 minute loss of consciousness, learning disability, intellectual disability, vision or hearing impairments, major health problems, independent DSM-IV psychological disorder diagnosis (aside from substance use disorder), current use of psychoactive medication, using more than 10 cigarettes per day, heavy other drug use (>25 lifetime uses of non-cannabis drugs), possible pregnancy, failure to remain abstinent from substances throughout the study (as indicated by positive urine toxicology and/or continuous sweat patch testing).

In order to assess inclusion and exclusion criteria, screening included both an initial and a detailed phone screen, each with both the youth participant and a parent/guardian (required for ages 16-17; highly preferable for ages 18-25).

Screening. *Initial Screening.* When a potential participant called in response to an advertisement, study staff obtained verbal consent/assent to conduct a 5-10 minute phone screen (if youth was younger than 18, study staff gained permission from a parent/guardian) with each the parent and youth separately to determine basic eligibility

with non-sensitive questions (eg, age, ethnicity, MRI contraindications, vision and hearing problems, yes/no questions about psychiatric history and yes/no questions about substance use).

Detailed Screening. Before conducting the 45-minute detailed phone screen, study staff obtained written consent/assent from participants and parents. Comprehensive substance use history (lifetime) was obtained from youth with the Customary Drinking and Drug Use Record (CDDR) (Brown et al., 1998; Stewart and Brown, 1995). Information regarding youth psychiatric history was gathered by administering the Mini International Psychiatric Interview (MINI) (Sheehan et al., 1998) to youth and parents. Youth and parents were informed if ineligible and each paid $20 upon completion of the detailed screen. To maintain study integrity, specific reasons for ineligibility were not disclosed to participants.

Procedure

Eligible participants attended five study sessions over the course of four weeks. Sessions 1-3 were weekly sessions consisting of drug testing and brief neuropsychological testing. One week after Session 3, Sessions 4 and 5 were conducted within 24-48 hours of each other. Session 4 included a 3-hour neuropsychological battery, while Session 5 consisted of MRI scanning.

Verifying abstinence. *Biological measures.* Participants were expected to remain abstinent from alcohol and drugs throughout the course of the study in order to prevent acute intoxication. Thus, all participants were monitored for substance abstinence during each of five study sessions. At each session, participant abstinence was evaluated using urine toxicology, specifically the ACCUTEST SplitCup 10 Panel drug

test, which measured whether someone was positive or negative for amphetamines, barbiturates, benzodiazepines, cocaine, ecstasy, methadone, methamphetamines, opiates, PCP, and THC. Urine samples were also used to measure cotinine (metabolite of nicotine) levels with NicAlert. In addition to the urine toxicology, continuous sweat toxicology was conducted with the PharmChek Drugs of Abuse Patch, which tested for cocaine, benzoylecgonine, heroin, 6MAM, morphine, codeine, amphetamines, methamphetamine, THC, and phencyclidine. Furthermore, at each session, participants underwent breathalyzer testing for recent alcohol use. Participants who tested positive for any drugs, or who had a blood alcohol concentration of >.000 directly prior to Session 4 (extended neuropsychological battery) and Session 5 (MRI scanning) were removed from the study.

Measures

Demographic Information. Participants completed a background questionnaire outlining demographic variables including age, gender, ethnicity, self and biological parents' educations, incomes, and employments, marital status, history of medical or neurological illness, psychological disorders or use of psychiatric medication, and learning disability.

Substance use. During each session, participants were asked to report the last time they used alcohol, marijuana, cigarettes, or other drugs. In addition, at Session 3, participants were asked to complete the Timeline Follow Back (TLFB; Sobell & Sobell, 1992). This is a highly reliable and valid measure of past year substance use, using holidays and other memory cues. As part of completing the TLFB, participants were asked to report the quantity of specific substances (e.g., alcohol, cannabis, nicotine,

etc.) used on each day during the past year. Substance use was measured in standard units (e.g., joints for cannabis). Lifetime and past 3-month substance use was measured using the Customary Drinking and Drug Use Record (CDDR), which also assesses withdrawal symptoms, DSM-IV abuse and dependence criteria, and substance-related difficulties (Brown et al., 1998; Stewart and Brown, 1995).

Self-reported Mood. Participants completed the Beck Depression Inventory-II (BDI-II; Beck et al., 1996) at Session 4. This measure contains 21 items measuring past two-week depressive symptoms on a 0-3 point scale. Total scores indicate either "minimal" depression (0-13), "mild" depression (14-19), "moderate" depression (20-28), and "severe" depression (29-63). Independent depressive disorders were excluded as part of the larger imaging study.

Affective Processing. During Session 4, participants completed an affective battery (PennCNP) containing three facial emotion processing tasks of interest to the current analysis: Emotion Recognition, Emotion Discrimination, and Emotional Acuity tasks. In Emotion Recognition, participants were presented with a series of faces (shown individually) and instructed to identify which emotion each face was expressing. Possible answer choices included happy, sad, anger, fear, and no emotion. The facial stimuli are described in Gur et al. (2002) and Pinkham et al. (2008). In the Emotion Discrimination task, participants were presented with pairs of faces, one pair for each trial. Each pair of faces contained two pictures of the same individual, with or without a subtle (computer-generated) change in facial expression, which may or may not have indicated a difference in intensity between the two facial emotions. During each trial, the participant had to decide which picture expressed the specified emotion (happy or sad)

more intensely, or whether they were equal. In the Emotional Acuity task, participants were presented with one face per trial. Faces were randomly presented and participants were instructed to rate the facial expression's emotional valence on a 7-point scale: very sad, moderately sad, somewhat sad, neutral, somewhat happy, moderately happy, or very happy. Facial stimuli for the Emotion Discrimination and Emotional Acuity tasks are described in Erwin et al. (1992). As normative scores are not available for this battery, all analyses were conducted with raw scores.

MRI Data Acquisition. Participants were scanned on a 3T GE scanner at Medical College of Wisconsin (MCW) during Session 5, which was completed within 24-48 hours of Session 4 (when affective measures were obtained). ***Structural Image Acquisition.*** This took approximately 15 minutes. A T1-weighted, 3-D anatomical brain scan was obtained with a modified driven equilibrium Fourier transform (MDEFT) sequence (TMD=1.1 s, TR=13 ms, FOV=25.6 x 19.2 x 19.2 cm., matrix 256 x 192 x 96 pixels, flip angle=20 degrees). ***Diffusion Tensor (DTI) Image Acquisition.*** DTI was obtained using 48 diffusion directions with b ≈ 700s/mm2 (FOV= 25.6 cm, 128 x 128 matrix, resolution=4x4x4 mm, TR = 9,300 ms, TE = 81.4 ms (minimum), flip angle 90˚.

MRI Processing. ***Image Preprocessing.*** Structural images were initially preprocessed using the Analysis of Functional Neuroimages (AFNI) software package. This sequence included converting 2D slice data into a 3D dataset (BRIK and HEAD files). This file was then converted into a file readable by FreeSurfer (.mgz). Using FreeSurfer software, all T1-weighted 3D anatomical datasets underwent motion correction, non-parametric non-uniform intensity normalization, MNI transformation, removal of non-brain materials, and skull-stripping. This was followed by whole-brain

segmentation of white and gray matter and registration of anatomical brain regions. Automatic subcortical segmentation took place in six steps, including registration to a template, canonical normalization, canonical registration, neck removal, registration w/skull, and subcortical labeling. Bilateral cortical ROIs (mOFC, rACC, cACC, Pars triangularis, Pars orbitalis, OFC, superior temporal gyrus, fusiform gyrus) were parcellated using the Desikan-Killiany atlas (Desikan et al., 2006) in FreeSurfer. This is a gyral-based atlas; regions include a gyrus plus adjacent sulci. Using these ROIs, regional cortex volumes (in cubic millimeters) were obtained. In the current analysis, Pars orbitalis and OFC were combined to create the lateral OFC (lOFC) region. All automated FreeSurfer steps were inspected for processing errors, and manual edits were made as needed. For each case, automatic segmentation and parcellation masks were manually edited for accurate segmentation, using multiple views for visual inspection.

   DTI Processing. Following whole-brain segmentation, the software program, Tracts Constrained by Underlying Anatomy (TRACULA) (within FreeSurfer) was used to analyze tractography data. TRACULA is a global probabilistic tractography program allowing reconstruction of white matter pathways from diffusion tensor images. This yields measures of white matter integrity, including fractional anisotropy (FA) and mean diffusivity (MD) (Yendiki et al., 2011). Each image underwent the following preprocessing steps: (1) Image Corrections (e.g., for B0 inhomogeneities, eddy currents, and simple head motion), (2) Further head motion correction, (3) Intra-subject and Inter-subject registration (4) Mask creation (White matter is extracted from FreeSurfer's segmentation and parcellation and combined into a mask), (5) Tensor fit,

(6) Estimation of pathway priors (the atlas data was combined with the individual's own masks). Following this preprocessing, a ball-and-stick model of diffusion was fitted to the images. After diffusion measures in each voxel were determined by Markov Chain Monte Carlo sampling, likelihood of the locations of uncinate fasciculus, ATR, and corpus callosum forceps minor pathways within each individual were established. From these estimated pathways, statistics on diffusion measures (average weighted FA and MD) within each individual were extracted. We performed group analyses along the entire corpus callosum forceps minor, bilateral uncinate fasciculus, and bilateral anterior thalamic radiations tracts. For nine of these participants, forceps minor could not be adequately reconstructed in TRACULA. Thus, they were excluded from the regressions investigating forceps minor FA and MD.

Data Analysis

Preliminary Analysis. All analyses were conducted in the statistical program SPSS. For each primary aim, demographic and other drug use was examined using ANOVA (or Mann-Whitney for any variables not normally distributed) and Chi-Square analyses to detect differences between cannabis users and non-users. Variables that either differed between groups or have known relationships with affective processing or brain structure were entered as covariates. For Aim 1, these included gender, past year alcohol use, and past year nicotine use. Aim 2 included these covariates as well as intracranial volume for volumetric analyses.

Principal Components Analysis (PCA) for Emotional Processing Tasks. We conducted three principal components analyses (PCA) to extract components from each of the Emotion Recognition, Emotion Acuity, and Emotion Discrimination tasks.

Accuracy and response time variables were selected for each task and entered into PCA (see Tables 2, 3, 4).

    Primary Analysis. *Aim 1.* Multiple regressions (including appropriate covariates) were used to examine whether past year cannabis use and cannabis x gender predicted (a) emotional processing (as defined by components extracted from PCA) and (b) BDI-II scores. *Aim 2.* To determine the relationship between past year cannabis use, cannabis x gender, and brain structure: (a) Multiple regressions (including appropriate covariates) were used to predict bilateral mOFC, lOFC, Pars triangularis, superior temporal gyrus, fusiform gyrus, rACC, cACC, amygdala, hippocampus, and cerebellum volumes from past year cannabis use and cannabis x gender. (b) Multiple regressions (including appropriate covariates) were used to predict fractional anisotropy (FA) and mean diffusivity (MD) of the corpus callosum forceps minor, bilateral uncinate fasciculus, and bilateral anterior thalamic radiations from past year cannabis use and cannabis x gender. Main effects and covariates were entered into the first block; interaction variables were included in the second block. In order to assess if any participants disproportionately influenced the regression models, DFBETA analyses for past year cannabis use and cannabis x gender were conducted. One outlier for past year cannabis use was observed in predicting depressive symptoms; thus it was removed from the model. Results are reported with outliers removed. False Discovery Rate (FDR) correction (Benjamini & Hochberg, 1995 method) was conducted in Aims 1, 2a, and 2b. In aim 2a, FDR corrections were run separately for subcortical volume and cortical volume variables within each brain hemisphere (R/L) and white matter integrity variables. Additionally, f^2 was used to assess effect sizes for multiple regression

analyses (small= 0.02-0.14, medium= 0.15-0.34, and large= >0.35) (Cohen et al., 2003).

Secondary Analysis: Brain-Behavior Relationships. We conducted brain-behavior analyses on brain regions that were significantly associated with past year cannabis use or cannabis x gender. For those regions that differed, we ran Pearson correlations within the cannabis group to determine whether brain abnormalities were related to (a) emotional processing and (b) BDI-II scores. For all analyses, significance was determined if p <0.05.

Results

Preliminary Results

Aim 1 Subgroup: Demographic Information (see Table 1). ANOVAs and chi-square tests revealed no significant difference between cannabis users and non-users in age ($F(1,75)$= 1.17, p= .28), race ($\chi^2(5)$= 5.30, p=.38), ethnicity ($\chi^2(2)$= .92, p=.63), gender ($\chi^2(1)$= 2.50, p=0.11), and reading ability ($F(1,75)$= 1.81, p= .18).

Aim 1 Subgroup: Drug Use Information. Cannabis users and non-users significantly differed on measures of past year cannabis use ($F(1,75)$= 24.54, p<.01), past year alcohol use ($F(1,75)$= 15.18, p<.01) and past year nicotine use ($F(1,75)$= 7.61, p<.01). Past year alcohol and nicotine use were included as covariates in the regressions.

Aim 2 Subgroup: Demographic Information (see Table 1). ANOVAs and chi-square tests revealed no significant difference between cannabis users and non-users

in age ($F(1,73)= 0.98$, $p= .32$), race ($\chi^2(5)= 5.68$, $p=.34$), ethnicity ($\chi^2(2)= .92$, $p=.63$), gender ($\chi^2(1)= 1.88$, $p=0.24$), and reading ability ($F(1,73)= 1.54$, $p= .22$).

Aim 2 Subgroup: Drug Use Information. Cannabis users and non-users significantly differed on measures of past year cannabis use ($F(1,73)= 22.89$, $p<.01$), past year alcohol use ($F(1,73)= 14.58$, $p<.01$), and past year nicotine use ($F(1,73)= 7.69$, $p<.01$). Past year alcohol and nicotine use were included as covariates in the regressions.

Principal Components Analysis. Three principal components analyses (PCA) were conducted to reduce variables from the each of the tasks (Emotion Recognition, Acuity, and Discrimination) in the PennCNP affective battery. Variables with loadings ≥ 0.6 were considered to define a component. *Emotion Recognition.* For the Emotion Recognition task, nine variables were subjected to PCA with varimax rotation. This yielded four components meeting Kaiser's criterion (eigenvalues >1). The following components were obtained (see Table 2): Recognition Time (29.54% of variance), Anger/Fear Correct (14.15% of variance), Sad Correct (13.91% of variance), and Happy Correct (12.57% of variance). These components accounted for 70.17% of the total variance observed in the Emotion Recognition variables. *Emotional Acuity.* Twelve variables were subjected to PCA for the Emotional Acuity task, yielding three components meeting Kaiser's criterion (eigenvalues >1). Components included Acuity Time (34.32% of variance), Acuity Neutral (28.15% of variance), and Acuity Intensity (13.08% of variance) (see Table 3). Together, these components explained 75.55% of the variance observed. *Emotion Discrimination.* For the Emotion Discrimination task, eight variables underwent PCA with varimax rotation. Two components meeting

Kaiser's criterion (eigenvalues >1) were obtained (see Table 4): Discrimination Time (46.72% of variance) and Discrimination Correct (33.86% of variance). These components accounted for 80.58% of the total variance.

Primary Results (see Table 5 for significant findings)

All regions of interest demonstrating a significant relationship with either cannabis use or cannabis x gender are represented in Figure 1. Covariates included gender, past year alcohol use, and past year nicotine use. Intracranial volume (ICV) was included as an additional covariate in sMRI regressions.

Behavioral Outcomes. *Cannabis.* Past year cannabis use did not significantly predict Acuity Time [t (67) = .07, *beta*=.01, *p*=.95], Acuity Intensity [t (67) = 1.12, *beta*= .14, *p*=.30], Recognition Time [t (71) = -.34, *beta*= -.04, *p*=.73], Anger/Fear Correct [t (72) = 1.17, *beta*= .14, *p*=.25], Happy Correct [t (72) = .40, *beta*= .05, *p*=.69], Sad Correct [t (72) = .13, *beta*=.02, *p*=.90], Discrimination Time [t (72) = -.55, *beta*=-.07, *p*=.58], Discrimination Correct [t (72) = -.64, *beta*=-.08, *p*=.53], or depressive symptoms [t (71) = .84, *beta*=.11, *p*=.40]. Increased cannabis use was significantly associated with increased Acuity Neutral scores [t (67) = 2.02, *beta*= .25, *p*<.05, f^2=.06, FDR-corrected: *p*=.47] (see Figure 2). ***Cannabis*Gender.** Cannabis use and gender did not interact to predict Acuity Time [t (66) = .51, *beta*=.06, *p*=.62], Acuity Intensity [t (66) = -.54, *beta*= -.07, *p*=.59], Acuity Neutral [t (66) = -.26, *beta*= -.03, *p*=.80], Recognition Time [t (71) = -.17, *beta*=-.02, *p*=.87], Anger/Fear Correct [t (71) = .70, *beta*= .09, *p*=.49], Happy Correct [t (71) = -1.50, *beta*= -.18, *p*=.14], Sad Correct [t (71) = .62, *beta*=.08, *p*=.54], Discrimination Time [t (71) = 1.15, *beta*=.14, *p*=.25], and depressive symptoms [t (70) =

-.19, *beta*=-.03, *p*=.85]. Cannabis and gender marginally interacted to predict

Discrimination Correct such that in females, greater cannabis use was associated with

lower Discrimination Correct scores; males exhibited the opposite pattern [*t* (71) = -1.93,

beta=-.23, *p*<.06]. **Covariates.** Gender significantly predicted Happy Correct, such that

females had higher scores on this component compared to males [*t* (72) = 2.17, *beta*=

.26, *p*= .03].

    sMRI Outcomes. *Cannabis.* Past year cannabis use did not significantly predict left

hippocampus [*t* (69) = 1.08, *beta*=.10, *p*=.29], right hippocampus [*t* (69) = -.31, *beta*=-

.03, *p*=.76], left amygdala [*t* (69) = 1.28, *beta*=.13, *p*=.21], right amygdala [*t* (69) = -.34,

beta=-.04, *p*=.76], left cerebellum [*t* (69) = 1.24, *beta*=.11, *p*=.22], right cerebellum [*t*

(69) = 1.57, *beta*=.14, *p*=.12], right cACC [*t* (69) = -.84, *beta*=-.09, *p*=.40], left cACC [*t*

(69) = -1.19, *beta*=-.14, *p*=.24], right rACC [*t* (69) = .41, *beta*=.05, *p*=.68], right mOFC [*t*

(69) = -.08, *beta*=-.01, *p*=.94], left mOFC [*t* (69) = -.51, *beta*=-.05, *p*=.61], left lOFC [*t*

(69) = .29, *beta*=.03, *p*= .77], right pars triangularis [*t* (69) = -.85, *beta*=-.10, *p*=.40], left

pars triangularis [*t* (69) = -1.00, *beta*=-.12, *p*=.32], right fusiform gyrus [*t* (69) = .45,

beta=.04, *p*=.65], left fusiform gyrus [*t* (69) = -.28, *beta*=-.03, *p*=.78], and left superior

temporal [*t* (69) = 1.35, *beta*=.12, *p*= .18] volumes. Increased past year cannabis use

marginally predicted smaller right lOFC volumes [*t* (69) = -1.93, *beta*=-.18, *p*<.06].

Greater past year cannabis use significantly predicted smaller left rACC volumes [*t* (68)

= -3.26, *beta*=-.29, *p*<.01, f^2= .11; FDR corrected: *p*= 0.01] and larger right superior

temporal volumes [*t* (69) = 2.27, *beta*=.19, *p*= .03, f^2= .06; FDR corrected: *p*= 0.19] (see

Figure 3). **Cannabis*Gender.** Cannabis use and gender did not interact to predict left

hippocampus [*t* (68) = -1.04, *beta*=-.10, *p*=.30], right hippocampus [*t* (68) = .11,

beta=.01, p=.91], left amygdala [t (68) = -.22, beta=-.02, p=.83], right amygdala [t (68) = -.24, beta=-.03, p=.81], left cerebellum [t (68) = 1.06, beta=.10, p=.29], [t (68) = .17, beta=.02, p=.86], right cerebellum [t (68) = .17, beta=.02, p=.86], right cACC [t (68) = .30, beta=.03, p=.76], left cACC [t (68) = .69, beta=.08, p=.50], right rACC [t (68) = -1.54, beta=-.17, p=.13], right mOFC [t (68) = .53, beta=.05, p=.60], left mOFC [t (68) = .84, beta=.08, p=.40], right lOFC [t (68) = .64, beta=.06, p=.53], left lOFC [t (68) = 1.12, beta=.67, p=.27], right pars triangularis [t (68) = .23, beta=.03, p=.82], left pars triangularis [t (68) = .18, beta=.02, p=.86], right fusiform gyrus [t (68) = -.24, beta=-.02, p=.81], left fusiform gyrus [t (68) = .47, beta=.04, p=.64], right superior temporal [t (68) = .98, beta=.08, p=.33], or left superior temporal [t (68) = -2.70, beta=-.26, p<.01] volume. Cannabis use and gender significantly interacted to predict left rACC volumes, such that only females demonstrated reduced volumes with increased cannabis use [t (68) = -2.14, beta=-.19, p=.04, f^2= .07; FDR corrected: p=.25] (See Figure 4). **Covariates.** Gender was significantly associated with left amygdala [t (69) = -2.13, beta=-.28, p=.04], left cerebellum [t (69) = -2.78, beta=-.34, p<.01], and right cerebellum [t (69) = -3.12, beta=-.37, p<.01] volumes, such that females exhibited smaller volumes compared to males.

   DTI Outcomes. *Cannabis.* Past year cannabis use did not significantly predict right uncinate fasciculus FA [t (70) = .10, beta=.01, p= .92], right ATR FA [t (70) = -.08, beta= -.01, p= .94], right ATR MD [t (70) = -.95, beta= -.11, p= .35], left uncinate fasciculus FA [t (70) = -.01, beta=-.00, p= .99], left ATR FA [t (70) = .67, beta= .08, p= .50], left ATR MD [t (70) = -1.06, beta= -.13, p= .29], forceps minor FA [t (61) = -.05, beta=-.01, p= .96], or forceps minor MD [t (61) = -.84, beta=-.11, p= .40]. Cannabis use marginally

predicted left uncinate MD [t (70) = -1.85, *beta*=-.22, *p*= .07]. Greater cannabis use was significantly associated with reduced MD in the right uncinate fasciculus [t (70) = -2.00, *beta*=-.24, *p*= .05, f^2= .06; FDR corrected: *p*= .34] (see Figure 5). ***Cannabis*Gender.*** Cannabis*Gender did not significantly predict right uncinate fasciculus FA [t (69) = 1.19, *beta*=.15, *p*=.24], right uncinate fasciculus MD [t (69) = -.70, *beta*= -.08, *p*=.49], right ATR FA [t (69) = .54, *beta*=.06, *p*=.59], right ATR MD [t (69) = -.57, *beta*=-.07, *p*=.57], left uncinate fasciculus FA [t (69) = 1.22, *beta*=.15, *p*=.23], left uncinate fasciculus MD [t (69) = -.45, *beta*=-.05, *p*=.65], left ATR FA [t (69) = .21, *beta*=.02, *p*=.83], left ATR MD [t (69) = -.84, *beta*=-.10, *p*=.40], or forceps minor MD [t (60) = -.69, *beta*=-.09, *p*=.50]. Cannabis*Gender significantly predicted forceps minor FA, such that females demonstrated increased FA with more use while the opposite pattern was observed in males [t (60) = 2.06, *beta*=.26, *p*=.04, f^2= .07; FDR corrected: *p*=.44] (see Figure 6). ***Covariates.*** Increased past year nicotine use was significantly associated with reduced right ATR FA [t (70) = -2.07, *beta*= -.24, *p*= .04]. Gender significantly predicted right [t (70) = 2.27, *beta*= .27, *p*= .03] and left [t (70) = 2.42, *beta*=.28, *p*=.02] ATR MD, such that females had higher values compared to males. Gender also significantly predicted left uncinate MD [t (70) = 2.14, *beta*=.25, *p*= .04] and left ATR FA [t (70) = -2.70, *beta*= -.31, *p*<.01], such that males had higher values relative to females.

Secondary Results (Brain-Behavior Relationships in Cannabis users)

Within cannabis users, smaller left rACC volumes were significantly associated with lower Discrimination Correct scores [r= .37, *p*= .04] (see Figure 7). Since cannabis*gender predicted left rACC volumes, the relationship between gender and left

rACC was explored. Within female cannabis users, smaller left rACC volumes significantly predicted lower Discrimination Correct scores [r= .68, *p*= .01]. Within male cannabis users, no significant relationship was observed between left rACC volume and Discrimination Correct scores [r= .31, *p*= .18]. No other affective measures were significantly associated with left rACC, right superior temporal volumes, or right uncinate fasciculus MD.

Discussion

To our knowledge, this is the first study to assess the dose-dependent relationships between cannabis use, affective processing, and brain structure. Our first primary aim was to examine the association between cannabis use, depressive symptoms, and performance on an affective processing battery. Contrary to our predictions, we found that greater past year cannabis use predicted higher Acuity Neutral component scores. In the second primary aim, we investigated relationships between cannabis use and brain structures previously shown to be associated with affective processing. Most notably, increased past year cannabis use significantly predicted smaller left rACC volumes. Gender moderated this relationship such that only females exhibited significantly smaller left rACC volumes with increased cannabis use. Secondary analyses revealed that within the cannabis-using group, decreased left rACC volumes were significantly correlated with lower Discrimination Correct scores. This suggests that cannabis-related differences in the left rACC are associated with functional deficits. Past year cannabis use predicted larger right superior temporal volumes and lower MD in the right uncinate fasciculus. Finally, a significant cannabis x gender interaction was observed in the forceps minor, with females exhibiting increased FA with more use,

while the opposite pattern occurred in males. The only relationship that survived

correction for multiple comparisons was the main effect of increased cannabis use

predicting smaller left rACC volumes.

Our finding that greater cannabis use predicted *higher* scores on one component,

Acuity Neutral, is, to our knowledge, the first report of chronic cannabis use being

related to improved performance on an emotional faces task. This was in contrast to our

hypothesis that chronic cannabis use would be associated with poorer affective

processing due to downregulation of CB1 receptors in brain regions regulating affect

(Hirvonen et al., 2012). In previous studies, cannabis users have exhibited deficits in

facial emotion processing, although literature in this area is sparse (Platt et al., 2010;

Hindocha et al., 2014; Huijbregts et al., 2014; Bayrakci et al., 2015). Hindocha et al.

(2014) found that cannabis users were more impaired than controls in recognizing static

facial emotions of moderate and higher intensity; this difference was not observed in

processing more subtle emotions (20% emotional). Further, using a dynamic facial

morphing task, Platt and colleagues (2010) found that cannabis users required a greater

intensity of emotion for correct identification. Our findings deviate from both of these

studies, in that cannabis use was not associated with more or less accurate intense

emotion identification (Acuity Intensity component), but was associated with improved

accuracy for subtle emotions (Acuity Neutral component). This may be due to

differences in task construction (e.g., static versus dynamic stimuli, providing wider

range of response options) across studies.

Another possible explanation is differences in cannabis exposure; mean use

amongst cannabis users was higher (25-27 uses per month) in both the studies by

Hindocha et al. (2014) and Platt et al. (2010) compared to the current study (20 uses per month). Thus, it is possible that emotional face processing deficits are only observable in heavier (closer to daily) cannabis users. Some research suggests that adolescents who engage in a low to moderate level of cannabis use (no more than monthly) have improved interpersonal relations compared to those who abstain and heavier users (Shedler & Block, 1990). This may provide insight into the higher Acuity Neutral scores in the current study; 39.4% of the cannabis users in the current study used monthly or less frequently in the past year. Further, cannabis samples can vary widely in levels of THC and CBD (Swift et al., 2013), which may account for differences across studies. As acute CBD has been shown to improve facial affect recognition at moderate intensity (Hindocha et al., 2015), perhaps more participants in the current study used cannabis higher in CBD compared with participants in studies by Platt et al. (2010) and Hindocha et al. (2014). Future work will be needed in order to address this possibility. However, as the relationship between cannabis use and Acuity Neutral in the current study did not survive correction for multiple comparisons, it should be interpreted with caution.

Our most robust finding was that greater cannabis use was associated with reduced left rACC volumes, driven primarily by females. Null findings reported by other groups (Cousijn et al., 2012; Lorenzetti et al., 2015) may be due to the gender distribution. This finding is consistent with a recent report in young adult cannabis users with comorbid ADHD (Lisdahl et al., in press). Further, earlier age of cannabis use onset has been associated with reduced right rACC thickness within a sample of alcohol and cannabis using adolescents (Jacobus et al., 2014). Additionally, research amongst psychosis

patients has revealed cannabis-dependent relationships with decreased bilateral ACC volumes (Szeszko et al., 2007), left ACC volumes (Rapp et al., 2013), and increased cortical thinning in the left ACC during the first five years of schizophrenia (Rais et al., 2010). Thus, the evidence suggests that chronic cannabis use is related to reductions in ACC gray matter, particularly in the left hemisphere. This may be because the left hemisphere contains a higher CB1 receptor density (Glass et al., 1997). The ACC itself is rich in CB1 receptors (Herkenham et al., 1990; Glass et al., 1997) and chronic cannabis use leads to downregulation of CB1 receptors in the cingulate cortex (Hirvonen et al., 2012). The eCB system modulates synaptic plasticity and, via CB1 receptors on glial cells, neuronal support (Pazos et al., 2005; Melis et al., 2014). Thus, CB1 downregulation may lead to reduced dendritic branching or neuronal atrophy, resulting in gray matter reductions (Lisdahl et al., 2014). Importantly, females may have increased CB1 desensitization to THC in frontolimbic regions compared to males (Crane et al., 2013). This may explain why females, but not males, in the current study, demonstrated smaller left rACC volumes with increased cannabis exposure. Further, reduced left rACC volumes were related to lower Discrimination Correct scores within cannabis using females, but not males. This is consistent with other studies showing volumetric differences in the PFC and amygdala associated with functional deficits in female, but not male, cannabis users (Medina et al., 2009; McQueeny et al., 2011).

The link between exogenous cannabinoids, the ACC, and emotion processing has been previously documented. Chronic cannabis users, compared to healthy controls, have demonstrated altered ACC activation to masked emotional faces (Gruber et al., 2009) and ACC hypoactivity in response to emotional scenes (Wesley et al., 2016).

Acute THC administration enhanced functional connectivity between the rACC/mPFC and amygdala during an emotion discrimination task in which participants were instructed to decide which of two emotional (angry, fearful, or happy) faces matched a third face (Gorka et al., 2015). On the other hand, acute CBD has been shown to attenuate ACC activation and decrease connectivity between the ACC and amygdala in response to fearful faces (Fusar-Poli et al., 2009; Fusar-Poli et al., 2010). The present study supplements the fMRI literature by providing evidence for left rACC structural abnormalities with chronic cannabis use in association with abnormal affective processing. Notably, cannabis use predicted rostral, but not caudal ACC volumes. Substantial past work suggests the rostral portion of the ACC supports emotional processing, while the caudal region contributes more to cognitively demanding tasks by influencing response selection, error detection, working memory, and monitoring competition (Devinsky et al., 1995; Bush et al., 2000). Given the specialization of the rACC, it is unsurprising that reduced left rACC volumes were associated with lower Emotion Discrimination Correct scores in cannabis using females.

It is worth considering why Discrimination Correct was the only affective measure for which a relationship was observed with left rACC. Perhaps the rACC serves a crucial function in an aspect of the Emotion Discrimination task that is not present in the other tasks. For example, it is possible that the rACC is critical in comparing two emotional stimuli and making a decision regarding them. Previous research has identified the ACC as important in emotion discrimination when one is presented with multiple faces (Munro et al., 2007). Additionally, research suggests a role for the rACC in shifting attention toward or away from emotional faces (Klumpp et al., 2012), which may be

important if attending to multiple faces simultaneously. Another possibility is that the

Emotion Discrimination task was more difficult compared to the Emotion Recognition

and Emotional Acuity tasks, and thus the Discrimination task was more sensitive to

rACC abnormalities. In functional neuroimaging studies of cannabis users, aberrant

activation patterns often occur without differences in task performance (e.g., Cousijn et

al., 2014; Wesley et al., 2016), suggesting that cannabis users may be able to

compensate by adjusting which regions are recruited for a particular task. Thus,

perhaps female cannabis users in our sample were able to rely more heavily on other

brain areas besides the rACC while completing the Emotion Recognition and Emotional

Acuity tasks, but not the Emotion Discrimination task. However, a functional

neuroimaging study would be necessary in order to address this possibility.

Greater past year cannabis use also predicted larger right superior temporal

volumes, although this was not related to any affective processing measure in the

current sample. One past investigation revealed no superior temporal volume

alterations in cannabis users (DeLisi et al., 2006). However, others have reported

increased cortical thickness in the superior temporal gyrus in cannabis users compared

to non-users (Lopez-Larson et al., 2011; Epstein & Kumra, 2015). Moreover, adolescent

cannabis users have reduced cerebral blood flow in the left superior temporal gyrus

prior to abstinence (Jacobus et al., 2012), which may impact synaptic pruning

(Takahashi et al., 1999). Thus, structural aberrations in the superior temporal gyrus

could reflect insufficient synaptic pruning related to cannabis use. Alternatively,

increased right superior temporal volumes may indicate abnormal connectivity patterns

(Lisdahl et al., 2014), in which this region compensates for reduced efficiency in other

regions more susceptible to exogenous cannabinoids, such as the PFC (Mackie et al., 2005). Yet, it is not possible to conclude that this association between cannabis use and increased superior temporal volumes reflects cortical damage or compensation without evidence for behavioral deficits in our sample. Importantly, the association between cannabis use and the superior temporal region did not survive correction for multiple comparisons, indicating it may be a spurious finding. The superior temporal region is involved in facial perception, auditory processing, and multisensory audiovisual language processing (Ishai et al., 2005; Gazzaniga et al., 2014). Thus, future studies should continue to investigate these functions in cannabis users in relation to superior temporal areas.

The uncinate fasciculus is a tract connecting the anterior temporal lobe with frontal regions, passing through the amygdala (Catani & Thiebaut de Schotten, 2012; Von Der Heide et al., 2013). It supports episodic memory, language, and socio-emotional processing (Von Der Heide et al., 2013). In contrast to our hypothesis, cannabis use was significantly related to increased integrity, indicated by decreased mean diffusivity, in the right uncinate fasciculus. Most studies have shown cannabis use to be associated with decreased white matter integrity in various tracts, including in the uncinate fasciculus (e.g., Jacobus et al., 2013a). Our own group found increased MD and decreased FA in the uncinate fasciculus in a different sample of young adult cannabis users (Shollenbarger et al., 2015). Importantly, mean past year use cannabis use amongst users in that sample (548 joints) was *more than double* the amount in the current study (228 joints). Therefore, it is possible that at lower doses, cannabis use may actually improve white matter integrity. In fact, others have also noted increased

white matter integrity in association with cannabis use, although in a different tract (forceps minor) (Filbey et al., 2014). Cannabis has anti-inflammatory properties and has been shown to specifically decrease inflammation in myelin, a major component of white matter (for review, Burstein, 2015; Kozela et al., 2015). Reduced inflammation leads to decreased tissue water and diffusion, which results in lower MD values (Alexander et al., 2007). Additionally, cannabinoids possess neuroprotective and antioxidant properties that may benefit white matter (Karl et al., 2012). Another possibility is that the right uncinate fasciculus has abnormally strong connectivity in order to compensate for observed structural abnormalities in the right temporal region, which it connects with the PFC. Perhaps this initial compensation disappears with heavier cannabis use. Age of cannabis use onset may also influence white matter integrity and lead to differences in findings. Mean age of onset amongst cannabis users in the present study as well as in the study by Filbey and colleagues (2014) was relatively high at 17 and 18 years, respectively. Further, eCB genetics (i.e., *FAAH* genotype) has been shown to moderate white matter integrity in cannabis users (Shollenbarger et al., 2015). However, the relationship between greater cannabis use and reduced right uncinate fasciculus MD did not survive correction for multiple comparisons and as such, is of limited interpretability.

Cannabis use and gender interacted to predict forceps minor FA, such that females tended to have increased FA with more use, while the opposite pattern was observed in males. Previous studies demonstrated reduced white matter integrity in the forceps minor (Jacobus et al., 2013; Shollenbarger et al., 2015) and anterior portions of the corpus callosum (Arnone et al., 2008; Gruber et al., 2011; Gruber et al., 2014) in

cannabis users compared to controls. Yet, increased forceps minor FA aligns with Filbey and colleagues' (2014) findings, although this was observed as a main effect in their entire sample, which was predominantly male. The forceps minor is a fiber bundle connecting the frontal lobes, including the rACC (Catani & Thiebaut de Schotten, 2012). It is possible that females with greater cannabis use exhibited increased FA in the forceps minor in order to compensate for reductions in rACC volume and function, while this was not necessary for males, who did not exhibit rACC effects. As the rACC is high in CB1 receptor density, perhaps the forceps minor is able to uniquely adjust to exogenous cannabinoid influence (Filbey et al., 2014). Another possibility is that males were more susceptible than females to the negative effects of cannabis on the forceps minor, as frontal white matter takes longer to develop in males compared to females (Simmonds et al., 2014). Further, factors such as THC: CBD ratios and genetics may have differed between males and females in our sample, driving these differences in FA. Additionally, this finding may be spurious as it did not survive correction for multiple comparisons.

The present study has some important limitations. Firstly, in order to address whether through use of PCA, more subtle behavioral relationships were missed, supplementary regressions were run for cannabis use predicting individual PennCNP variables. The only significant relationships were with Emotional Acuity Total Correct and Emotional Acuity Happy Neutral Correct, which both loaded onto the Acuity Neutral component, indicating that all variables demonstrating a significant relationship with cannabis use were represented in components yielding significance. Second, due to the cross-sectional, correlational nature of the study, it is not possible to determine the

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causal nature of the relationship between cannabis use and outcome variables. Further, the cannabis exposure was lower than what is typically reported in previous publications (average exposure 228 past year joints, with 39.4% using monthly or less frequently and 24.2% using daily or more); therefore, these results may not generalize to young adults with heavier use patterns. Similarly, length of cannabis abstinence was much longer in our sample compared with most previous research (mean length of abstinence 57 days, minimum of 19 days). In most studies, users are abstinent for an average of 2-3 days, and research suggests there may be significant recovery with abstinence (Lisdahl et al., 2014; D'Souza et al., 2016). Additionally, only one relationship (cannabis predicting left rACC volumes) survived correction for multiple comparisons. As such, the other significant associations found may be spurious and must be interpreted with caution. We did not meet the sample size recommended (*N*=133) for 80% power at the smallest effect size observed (f^2=0.06); therefore, we only had adequate power (recommended *N*= 74) for the effect size observed in the rACC (f^2=0.11). Two brain region measures, right lOFC volume and left uncinate fasciculus MD, exhibited trend level relationships (*p*= 0.058 and *p*= 0.06, respectively) with past year cannabis use. It is possible that with a larger sample size, significant effects may have been revealed. Therefore, these findings should be replicated in a larger sample for increased power. Finally, considering the study's naturalistic design, there may other variables, including age, genetics, THC:CBD ratios (for reviews, see Niesink & van Laar, 2013; Lorenzetti et al., 2016), sex hormones, and physical fitness levels (Herting et al., 2012), moderating the results. Future research should use longitudinal designs with larger sample sizes and also measure these potential moderators.

The current study provides evidence for a relationship between past year cannabis use and reduced left rACC volumes in females, larger superior temporal volumes, and decreased right uncinate fasciculus MD. Gender moderated the relationship between cannabis use and forceps minor FA, with females demonstrating increased FA with more use and males demonstrating the opposite relationship. Our most clinically relevant and robust finding was greater past year cannabis use predicting smaller left rACC volumes and within cannabis users, smaller left rACC volumes being associated with lower scores on the Discrimination Correct component. Thus, chronic cannabis use during adolescence and emerging adulthood, a time of continued neurodevelopment (Casey et al., 2008), may negatively impact brain regions important for affective processing. These neural abnormalities, in turn, are associated with difficulty discriminating emotions, which may negatively influence mood and emotional well-being (Phillips et al., 2003). Given the potential negative consequences of cannabis use as evidenced by current and prior findings, more research on interventions for cannabis use in youth is needed.

Tables

Table 1
Participant Demographics

	Aim 1: Affective/Behavioral (*N*=77)		Aim 2: Brain Structure (*N*=75)	
	Cannabis Users (n=33)	Non-Users (n=44)	Cannabis Users (n=32)	Non-Users (n=43)
	% or *M* ± *SD* (range)	% or *M* ± *SD* (range)	% or *M* ± *SD* (range)	% or *M* ± *SD* (range)
Gender (% female)	36.4%	54.5%	37.5%	53.5%
Race (% Caucasian)	65.9%	69.7%	68.8%	67.4%
Ethnicity (% Non-Hispanic)	87.9%	88.6%	87.5%	88.4%
Age (years)	21.6±2.2 (18-26)	21.0±2.7 (16-25)	21.6 ± 2.2 (18-26)	21.0±2.7 (16-25)
WRAT-4 (raw score)	60.7±5.2 (41-69)	62.0 ±3.7 (53-69)	60.7 ± 5.3 (41-69)	62.0 ±3.8 (53-69)
Beck Depression Inventory-II (BDI-II)	5.7 ± 4.6 (0-18)*	2.6 ± 3.1 (0-10)*	5.8 ± 4.7 (0-18)*	2.6 ± 3.1 (0-10)*
Age of weekly cannabis use onset (years)	17.5 ± 1.8 (13-21)	-	17.5 ± 1.8 (13-21)	-
Lifetime cannabis use (uses)	796.45 ± 1095.9 (25-6000)*	3.4 ± 6.8 (0-25)*	790.1 ± 1112.8 (25-6000)*	3.4 ± 6.8 (0-25)*
Past year cannabis use (joints)	228.6 ± 306.2 (0-1394)*	0.4 ± 1.1 (0-5)*	226.8 ± 310.9 (0-1394)*	0.4 ± 1.1 (0-5)*
Length of cannabis abstinence at Session 4 (days)	57.2 ± 85.9 (19-422)	-	58.1 ± 87.1 (19-422)	-
Past year alcohol use (standard drinks)	313.2 ±272.9 (0-897)*	112.4 ± 178.8 (0-698.5)*	316.4 ± 276.7 (0-897)*	114.7 ± 180.2 (0-698.5)*
Past year nicotine use (cigarettes)	143.4 ±344.4 (0-1165)*	0.5 ±1.9 (0-12)*	147.8 ± 348.9 (0-1165)*	0.5 ±1.9 (0-12)*
Session 4 cotinine levels (0-6)	2.06 ± 1.7 (0-6)*	1.09 ± .6 (0-3)*	2.10 ± 1.7 (0-6)*	1.09 ± .6 (0-3)*
Session 5 cotinine levels (0-6)	2.03 ± 1.6 (0-6)*	1.09 ± .7 (0-3)*	2.03 ± 1.6 (0-6)*	1.09 ± .7 (0-3)*
% Positive THC Urine Toxicology Session 1	54.5%*	0%*	53.1%*	0%*
% Positive THC Urine	3%	0%	3.1%	0%

Toxicology Session 4				
% Positive THC Urine	-	-	3.1%	0%
Toxicology Session 5				

Note. *Group differences: *p*< .05.

Table 2. *Emotion Recognition Principal Components Analysis Factor Loadings*

Variable	Component Labels			
	Recognition Time	Anger/Fear Correct	Sad Correct	Happy Correct
Anger Correct	.121	***.849***	-.152	-.156
Fear Correct	-.262	***.627***	.328	.323
Sad Correct	-.001	-.021	***.935***	.003
Happy Correct	.031	-.018	-.001	***.950***
Anger Correct Median Response Time (ms)	***.660***	.107	-.296	-.033
Fear Correct Median Response Time (ms)	***.725***	-.362	.014	-.261
Sad Correct Median Response Time (ms)	***.611***	-.007	-.099	-.026
Happy Correct Median Response Time (ms)	***.765***	.107	.380	-.009
Neutral Correct Median Response Time (ms)	***.809***	-.071	.069	.173

Table 3. *Emotional Acuity Principal Components Analysis Factor Loadings*

Variable	Component Labels		
	Acuity Time	Acuity Neutral	Acuity Intensity
Total Correct	-.096	**.915**	.380
Very Happy Correct	-.074	-.093	**.758**
Happy Neutral Correct	-.003	**.879**	-.129
Neutral Correct	-.154	**.940**	-.135
Sad Neutral Correct	-.252	**.746**	-.025
Very Sad Correct	.163	.076	**.747**
Correct Trials Median Response Time (ms)	**.947**	-.199	.148
Correct Very Happy Trials Median Response Time (ms)	**.806**	.112	-.245
Correct Happy Neutral Trials Median Response Time (ms)	**.744**	-.388	.173
Correct Neutral Trials Median Response Time (ms)	**.887**	-.293	.182
Correct Sad Neutral Trials Median Response Time (ms)	**.860**	-.155	.265
Correct Very Sad Trials Median Response Time (ms)	**.603**	.015	-.206

Table 4.

Emotion Discrimination Principal Components Analysis Factor Loadings

	Component Labels	
Variable	Discrimination Time	Discrimination Correct
Total Correct	.094	***.989***
Happy Trials Correct	.114	***.896***
Sad Trials Correct	.041	***.844***
Correct Happy Trials Median Response Time (ms)	***.921***	-.013
Incorrect Happy Trials Median Response Time (ms)	***.726***	.375
Correct Sad Trials Median Response Time (ms)	***.886***	-.019
Incorrect Sad Trials Median Response Time (ms)	***.778***	.271
Total Correct Trials Median Response Time (ms)	***.974***	-.017

Table 5.

Significant Relationships Observed With p-values Before and After Correcting for Multiple Comparisons

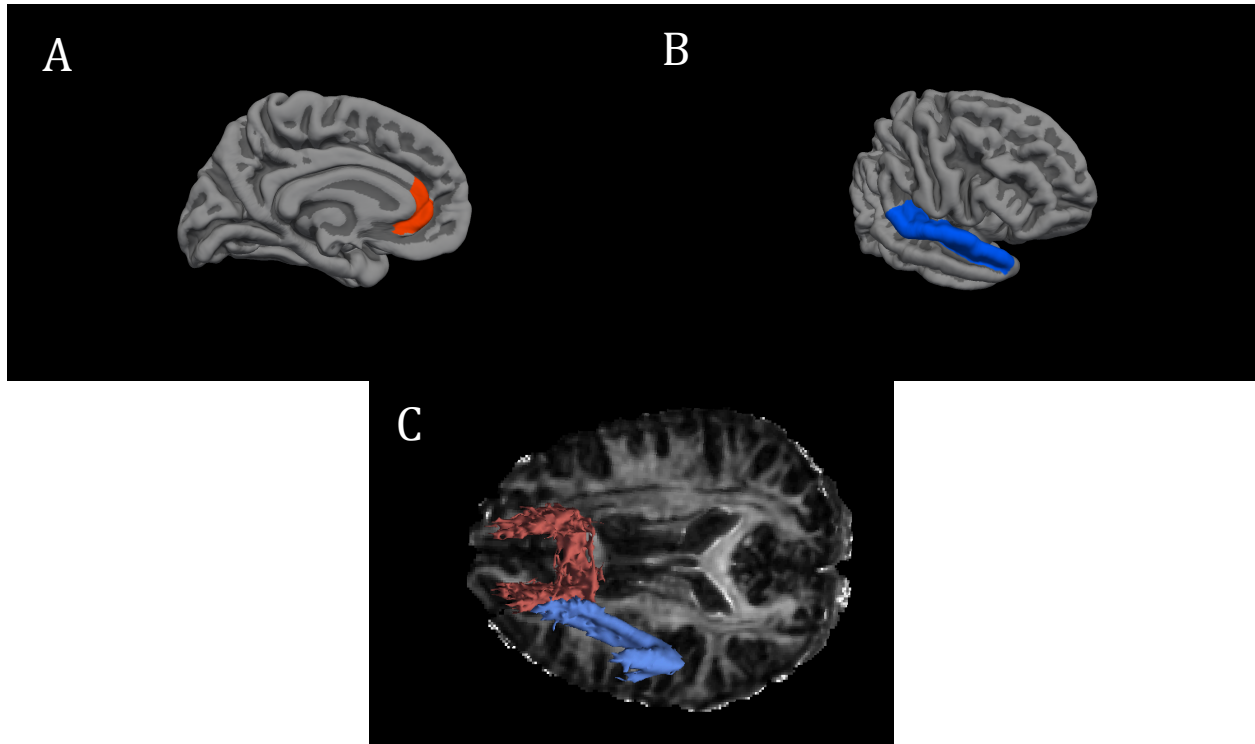| Relationship	p-value	Effect Size (f^2)	FDR-corrected p-value
Cannabis →Acuity Neutral Component	0.047*	.06	0.470
Cannabis→Left rACC volume	0.002*	.11	***0.014****
Cannabis*Gender→Left rACC volume	0.036*	.07	0.252
Cannabis→ Right superior temporal volume	0.027*	.06	0.189
Cannabis→ Right uncinate MD	0.050*	.06	0.340
Cannabis*Gender→forceps minor FA	0.044*	.07	0.440

Note. *$p \leq$ .05.

Figure 1. All regions of interest that demonstrated a significant relationship with either cannabis use or cannabis x gender prior to correction for multiple comparisons are presented here on representative participants. A) Increased cannabis use and gender significantly interacted to predict smaller left rACC volumes, such that only females demonstrated this relationship. The left rACC is shown in red on this sagittal slice. B) Greater cannabis use was associated with larger right superior temporal volumes, highlighted in blue. C) This axial slice presents a ventral view of the brain. Increased cannabis use was related to decreased right uncinate fasciculus (shown in blue) MD; gender moderated the relationship between cannabis use and forceps minor (red) FA, such that males had decreased FA with more cannabis use and females exhibited the opposite pattern.
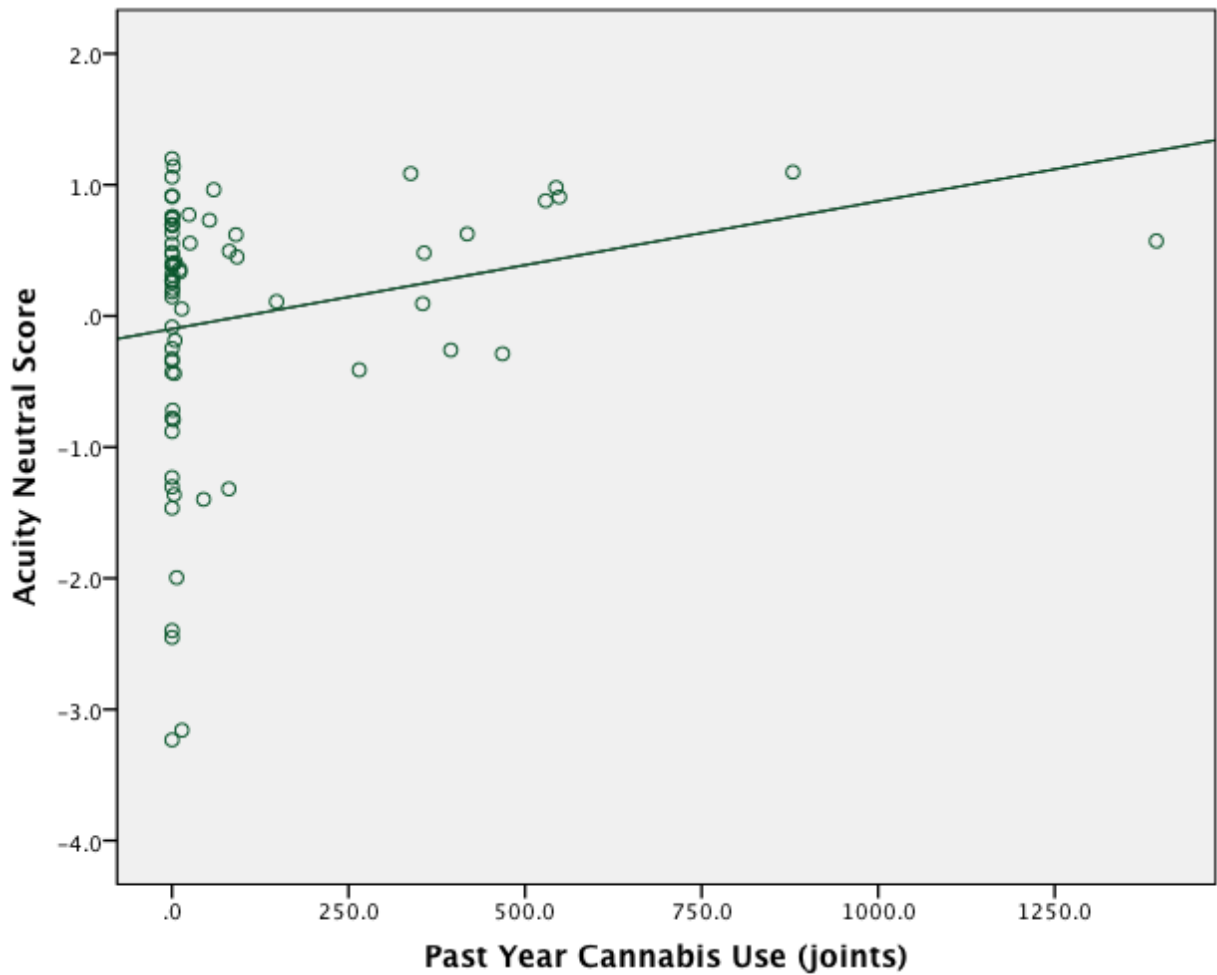
Figure 2. After controlling for gender, past year alcohol use, and past year nicotine use, a dose-dependent relationship was observed between greater past year cannabis use and higher scores on the Acuity Neutral component.
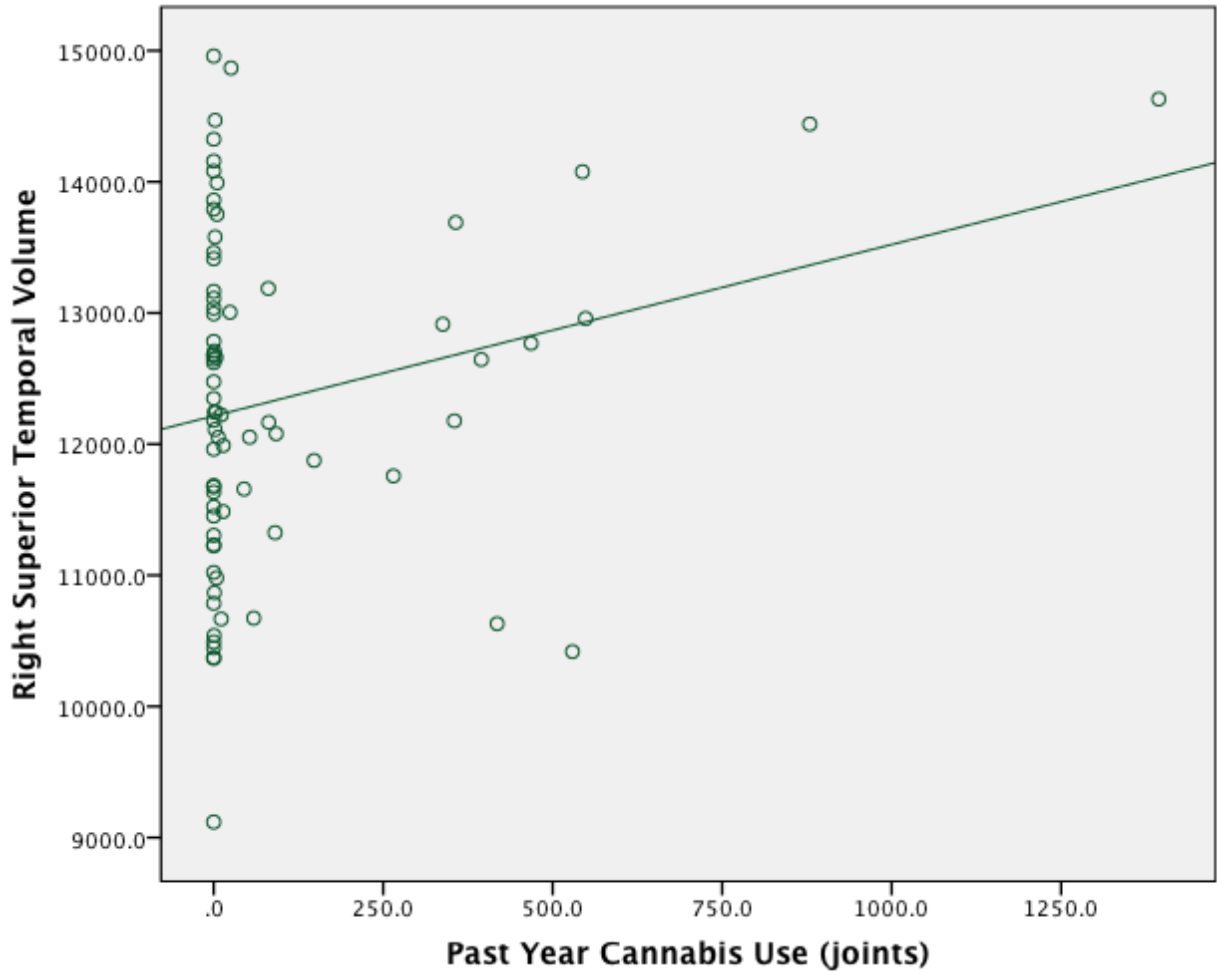
Figure 3. After controlling for gender, past year alcohol use, and past year nicotine use, increased past year cannabis use was associated with larger right superior temporal volumes. However, this relationship did not survive correction for multiple comparisons.
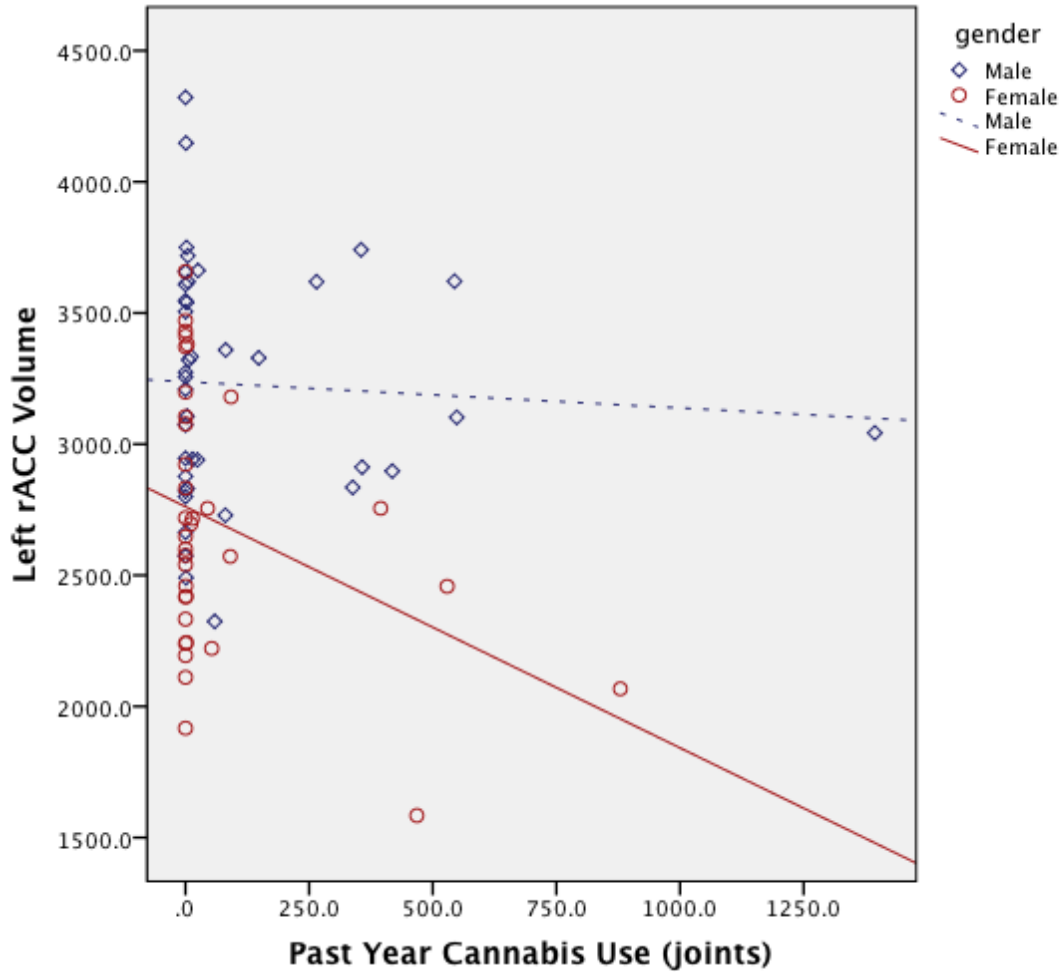
Figure 4. Greater past year cannabis use significantly predicted smaller left rostral ACC volumes. Gender significantly moderated this relationship, such that in females, cannabis use was associated with reduced volumes, while in males, no significant association between cannabis use and left rACC emerged. Covariates included gender, past year alcohol use, and past year nicotine use. The main effect (cannabis use predicting left rACC volumes) survived FDR corrections; however, the cannabis*gender interaction was no longer significant after correcting for multiple comparisons.
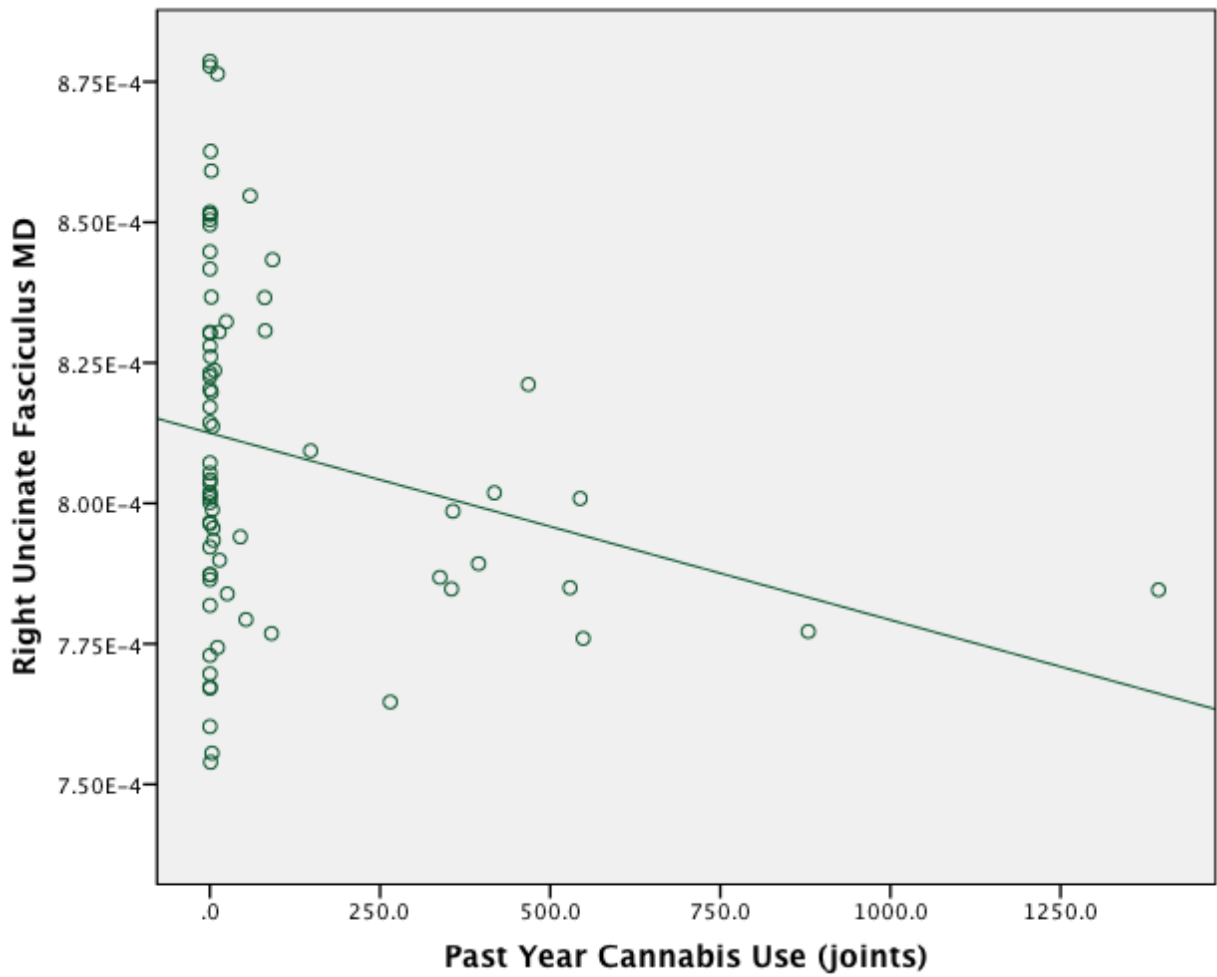
Figure 5. Greater past year cannabis use was significantly associated with reduced mean diffusivity in the right uncinate fasciculus, indicating increased integrity.
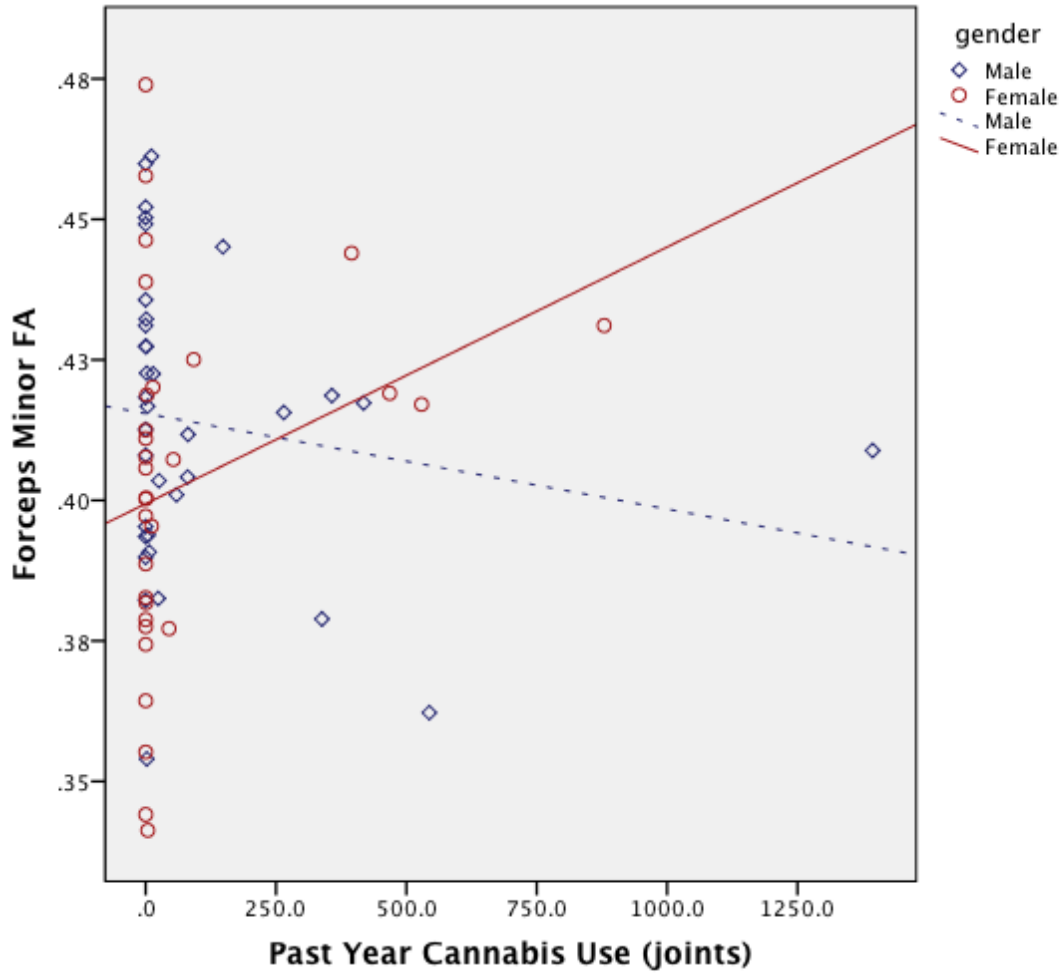
Figure 6. Cannabis*Gender significantly predicted fractional anisotropy (FA) in the corpus callosum forceps minor, such that males exhibited reduced FA with increased use; females had higher FA with increased cannabis use.
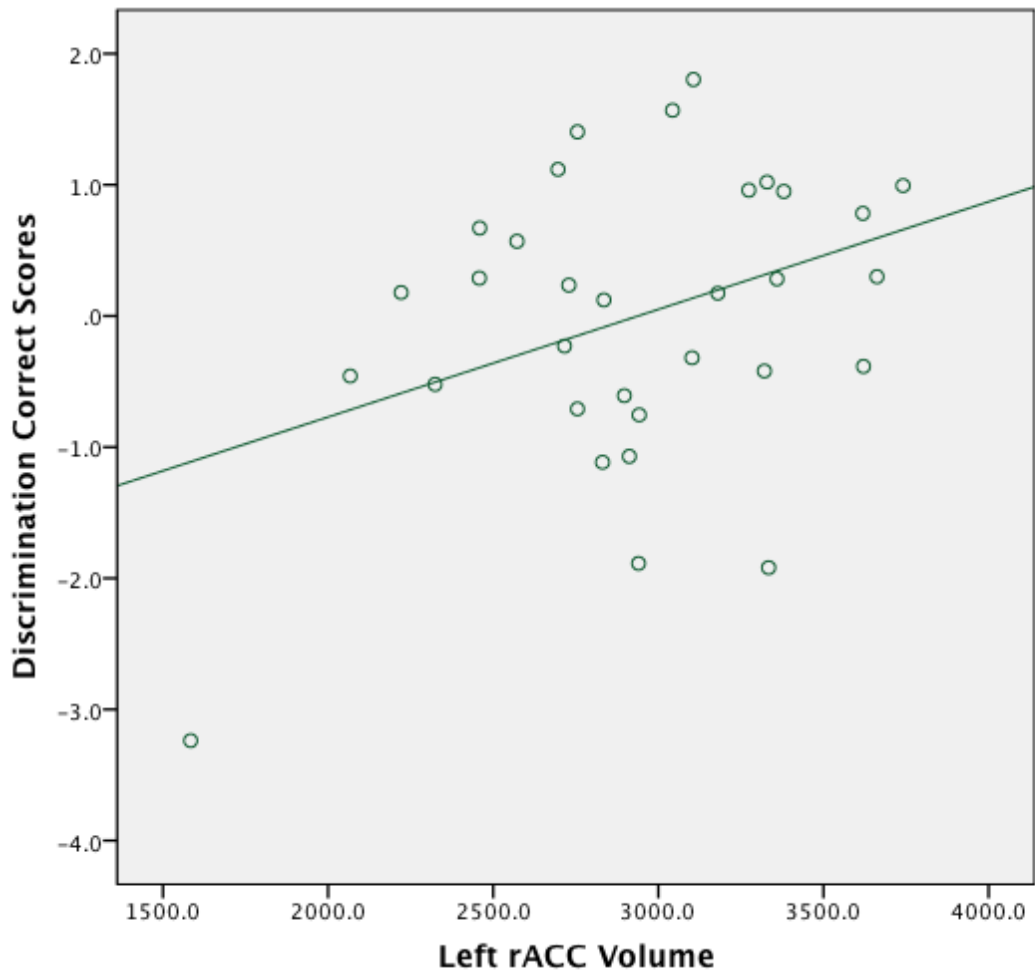
Figure 7. Within the cannabis-using group, Pearson correlations revealed that smaller left rACC volumes were associated with lower scores on the Discrimination Correct component.

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