MARIJUANA USE IN AN AGING POPULATION: GLOBAL BRAIN STRUCTURE AND COGNITIVE FUNCTION

by

RACHEL ERIN THAYER

B.S., University of California, San Diego, 2009

M.A., University of Colorado Boulder, 2013

A thesis submitted to the

Faculty of the Graduate School of the

University of Colorado in partial fulfillment

of the requirement for the degree of

Doctor of Philosophy

Department of Psychology and Neuroscience

2017

This thesis entitled:
Marijuana Use in an Aging Population: Global Brain Structure and Cognitive Function
written by Rachel Erin Thayer
has been approved for the Department of Psychology and Neuroscience

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Kent E. Hutchison, Ph.D.

Committee Chair

Angela D. Bryan, Ph.D.

Committee Member

Date_____

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.

IRB protocol #15-0459

Thayer, Rachel Erin (Ph.D., Psychology and Neuroscience)

Marijuana Use in an Aging Population: Global Brain Structure and Cognitive Function

Thesis directed by Professor Kent E. Hutchison

Scientific literature delineating potential negative impacts or neuroprotective benefits of marijuana consumption has not kept pace with societal changes in acceptance of its recreational use. Further exploration of marijuana use among older adults may help clarify global risks or neuroprotective benefits of using marijuana. This study collected structural MRI and cognitive assessments within a sample of recreational marijuana users age 60 years and older and healthy control non-users in order to report basic associations between marijuana use and brain structure, and, importantly, associations between brain structure and cognitive function.

Marijuana users (n=28) and controls (n=28) were not different in terms of global brain structural measures, but groups showed diffuse areas of difference throughout the brain. Users (n=28) showed slightly poorer working memory than controls (n=10). Lifetime users (n=15) performed poorly compared to both short-term users (n=13) and controls (n=10) in executive function, and poorly compared to controls in general cognition. Estimated total THC consumption in the last 90 days showed negative association with total gray matter volume and diffuse clusters in whole-brain models, and years of regular marijuana use showed consistent negative associations with cognitive performance in executive function, processing speed, and total cognition.

The current study is an important contribution to the field in terms of addressing several of the common limitations of existing research and providing innovation in exploring marijuana use in a novel and growing population. Study results suggest that lifetime marijuana use at a recreational level does not have a strong and consistent effect on brain structure in comparison to

substances like alcohol, but it does appear to have a negative association with aspects of cognitive functioning. From a harm reduction perspective, it is valuable to note that any cognitive harms associated with long-term marijuana use may be reduced by consuming strains with lower THC concentrations with less frequency.

ACKNOWLEDGMENTS

This research was primarily supported by the National Institute on Drug Abuse under Award Number R36DA040020, PI: Thayer. Additionally, it was supported in part by the National Institute on Aging under Award Number R01AG043452, PI: Bryan. The content is solely the responsibility of the author and does not necessarily represent the official views of the National Institutes of Health or the University of Colorado Boulder.

I would first like to thank all study participants for their time, enthusiasm, and best wishes in completing this project. I would also like to acknowledge and express my sincerest gratitude for the assistance and support of several faculty, staff, research assistants, and volunteers whose help was instrumental in completion of data collection: Nitin Beri, Audrey Davis, Greg Giordano, Tim Helmuth, Raeghan Mueller, Suzanne Taborsky, Teryn Wilkes, and Dr. Emily Richardson.

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INTRODUCTION

Enormous changes concerning the acceptance of marijuana use have occurred in the United States over the last decade, leading to legalized recreational use in several states and decriminalized marijuana use or legalized medical use in many others. Marijuana consumption is increasing, and perceived risks of using marijuana are decreasing, particularly among young people (Johnston et al., 2015). One body of literature suggests negative impacts of marijuana consumption on the brain, particularly during adolescence (e.g., Lisdahl, Gilbart, Wright, & Scollenbarger, 2013), including risk for future cognitive and mental health problems such as increased risk for psychosis (Chadwick, Miller, & Hurd, 2013; Rubino & Parolaro, 2014). However, a number of preclinical studies suggest that cannabinoids may have neuroprotective effects (Chiarlone et al., 2014; Hermann & Schneider, 2012; Scotter, Abood, & Glass, 2010), and there is corresponding interest in developing new pharmaceutical treatment of neurodegenerative conditions [e.g., epilepsy (Devinsky et al., 2014); multiple sclerosis (Pryce & Baker, 2012)] based on cannabinoids. Scientific literature clearly delineating potential negative impacts or neuroprotective benefits of marijuana consumption has not kept pace with social, political, and legal changes. Increasing acceptance of marijuana among adolescents and adults suggests that prevalence of recreational use, and by extension long-term use among the aging population, will increase over time. In addition, epidemiological studies have suggested that

substance use among older adults will be an increasing problem and represents additional health burdens. An epidemiological study estimated that the number of older adult marijuana users will triple by 2020 to include more than 3 million individuals (Colliver et al., 2006). Further, data from the 2002-2007 National Survey on Drug Use and Health suggested that 90% of persons aged 50-59 years who reported drug use within the last year had initiated substance use before age 30 (Wang & Andrade, 2013). These studies suggest increasing rates of chronic marijuana use among older adults.

Although there have been dozens of studies on the effects of marijuana in adolescent and young adult populations, there have been very few studies of the effects of marijuana use in the aging population. Older adults are a largely ignored portion of the developmental spectrum in terms of substance use research, but likely represent as much "neural risk" as adolescents or younger adults given onset of most neurocognitive disorders in late adulthood and ongoing cognitive decline. On one hand, it is possible that chronic use of marijuana into late adulthood may have increased negative cognitive effects. On the other hand, it is possible that the neuroprotective effects of certain cannabinoids may have beneficial effects on the aging brain (e.g., to slow typical cognitive and structural decline). Further exploration of chronic marijuana use among older adults may help clarify global risks or neuroprotective benefits of using marijuana long-term, as well as provide clues to mechanisms underlying marijuana's harm or benefit to brain structure and cognition.

The following review will briefly highlight preclinical studies and current challenges in translating animal to human models; heterogeneous results for associations between marijuana use and brain structure; evidence for acute cognitive deficits as a result of marijuana use, but limited cognitive effects of chronic use, and brief considerations for the relationship between

brain structure and cognitive function in aging adults; and, finally, specific aims of the current study.

Preclinical Studies

Using marijuana influences the endocannabinoid system of the brain, which broadly modulates neural function through various forms of synaptic plasticity (e.g., Goodman & Packard, 2015). The main molecular targets of endogenous cannabinoids in the brain as well as the main psychoactive compound in marijuana, delta-9-tetrahydrocannabinol (THC), are the cannabinoid receptors, which are mostly highly expressed in the hippocampus, amygdala, and dorsolateral striatum (Goodman & Packard, 2015). Importantly, different genetic strains of marijuana may greatly differ on potency of THC and 80 or more additional cannabinoids [e.g., cannabidiol (CBD), cannabinol, cannabigerol, tetraydrocannabivarin; Russo, 2007], which may act as agonists, partial agonists, or antagonists (e.g., CBD; Niesink & van Laar, 2013) at cannabinoid receptors.

In preclinical models of acute brain damage as well as chronic neurodegeneration, activation of cannabinoid receptors has been shown to have neuroprotective effects (e.g., Gowran, Noonan, & Campbell, 2011), perhaps via inhibition of excessive synaptic activity leading to excitotoxicity (Chiarlone et al., 2014). Among otherwise healthy subjects, effects of marijuana administration are less clear. An early meta-analysis of THC administration among healthy animals suggested that chronic exposure (e.g., equivalent to approximately 10 years of the human lifespan) is associated with blunted dendrites, smaller neurons, and increased extracellular space in the hippocampus of rats (Scallet, 1991). It was also noted that there may be developmental or age of initiation effects (i.e., decreased effects among older rats; Scallet, 1991). However, translational correspondence to human models may be limited since animal models

most often administer pure THC or synthetic compounds, which does not directly compare to the complexities of chemical compounds found within marijuana used medicinally and recreationally. In particular, the ratio of THC to CBD in different genetic strains of marijuana may be associated with relative harm versus benefit (e.g., Fagherazzi et al., 2012; Wright et al., 2013). Gaining increased understanding of how marijuana use is associated with global outcomes (e.g., increased versus decreased structural volume/density or cortical thickness and associations with cognitive function) may provide additional context for further exploration of particular mechanisms among aging individuals.

Marijuana Use and Brain Structure

It is still unclear what impact heavy marijuana use may have on human brain structure, including cortical thickness and gray and white matter volume. Neuroimaging studies using functional or diffusion modalities typically suggest small negative effects of marijuana use. In general, marijuana use impacts brain response patterns during acute administration, with regular marijuana users often demonstrating decreases in cerebral blood flow following acute marijuana exposure (Cousijn et al., 2012; Nestor, Hester, & Garavan, 2010; O'Leary et al., 2002). Decreases in blood flow were found in brain areas that have been previously associated with attentional modulation and sensory processing, including temporal lobe auditory regions and the visual cortex (O'Leary et al., 2002). These acute alterations in blood flow, if repeated during many administrations, would suggest likely changes to brain gray matter over time. Poorer white matter microstructure has also been found in heavy marijuana users (Filbey et al., 2014; Shollenbarger, Price, Weiser, & Lisdahl, 2015), particularly in fronto-temporal areas that develop through late adolescence and young adulthood (Ashtari et al., 2009). In addition, heavy marijuana use during adolescence has been linked to decreased attention and processing speed

related to differences in white matter microstructure (Jacobus et al., 2009). These differences were often found in small clusters, but given even small changes over relatively short use history, some evidence suggests likely changes to brain white matter over time.

However, the literature has yet to document consistent brain morphological changes as might be suggested by studies using other neuroimaging modalities. One systematic review suggested converging evidence for alterations of the frontal and medial temporal cortices and cerebellum, but also noted that results of the review highlighted variability in study methodologies and results (Batalla et al., 2013). In contrast, a meta-analysis of fourteen structural imaging studies among marijuana users with no potential confounds from psychosis symptoms found no consistent group differences in total gray or white matter, but reduced hippocampal volumes (Rocchetti et al., 2013). Several studies have found that marijuana use is associated with increased volume of specific subcortical structures (Cousijn et al., 2012), but others have found decreased volumes (Demirakca et al., 2011; Lorenzetti et al., 2015; Solowij et al., 2011; Yücel et al., 2008;).

A study that received wide coverage in the popular press reported morphological differences between young adult recreational users and non-users (Gilman et al., 2014). Among density, volume, and shape (surface morphometry) comparisons, recreational marijuana users showed greater gray matter density in the left nucleus accumbens and left amygdala; marginally greater volume in left nucleus accumbens, which did not meet criteria for correction of multiple comparisons; and shape differences in the left nucleus accumbens and right amygdala. The authors suggested that observed changes in the left nucleus accumbens were exposure-dependent. Recreational users (n=20) were a mean age of approximately 21 years, and reported using marijuana between 3 and 4 days each week over the last 90 days, with age of onset of use

between age 16 and 17 years. Importantly, their alcohol use over the prior 90 days significantly differed from non-users (n=20), and was covaried in follow-up analyses on extracted peak values but not in group difference models within the imaging data (Gilman et al., 2014). This practice could inflate cluster-based outputs of imaging models. Beyond methodological concerns, group differences were reported without any linkage to behavioral data beyond substance use (e.g., the reported dose-dependent effect; density and shape differences that were identified based on user group status were significantly associated with aspects of use such as joints smoked per occasion), and it remains unclear what functional implications any of the reported morphological changes might have for other behavior.

A subsequent study by Weiland, Thayer et al. (2015) attempted to replicate methods reported in Gilman et al. (2014) as closely as possible. Brain morphology was compared in marijuana users who had daily consumption over the last 60 days versus non-users. Groups were matched on a critical confounding variable, alcohol use, to a greater degree than in previously published studies, and daily users would likely demonstrate any dose-dependent effects suggested in the previous report. Adult users were 27 years old on average, and showed no differences in structural measures based on recent daily use that survived standard statistical corrections compared to controls. Neither were any structural differences observed between adolescent (mean age approximately 17 years) daily users versus non-users. This project also examined results from published studies that appeared in a recent review, which tend to report equivocal effects (Lorenzetti et al., 2014). Effect size between marijuana users and control groups varied considerably across studies, structures, and hemispheres (see Figure 1). This is likely due to confounds from alcohol use, relatively short-term or limited use compared to other substances, focus on adolescent or young adult samples, or other variability in methodology.

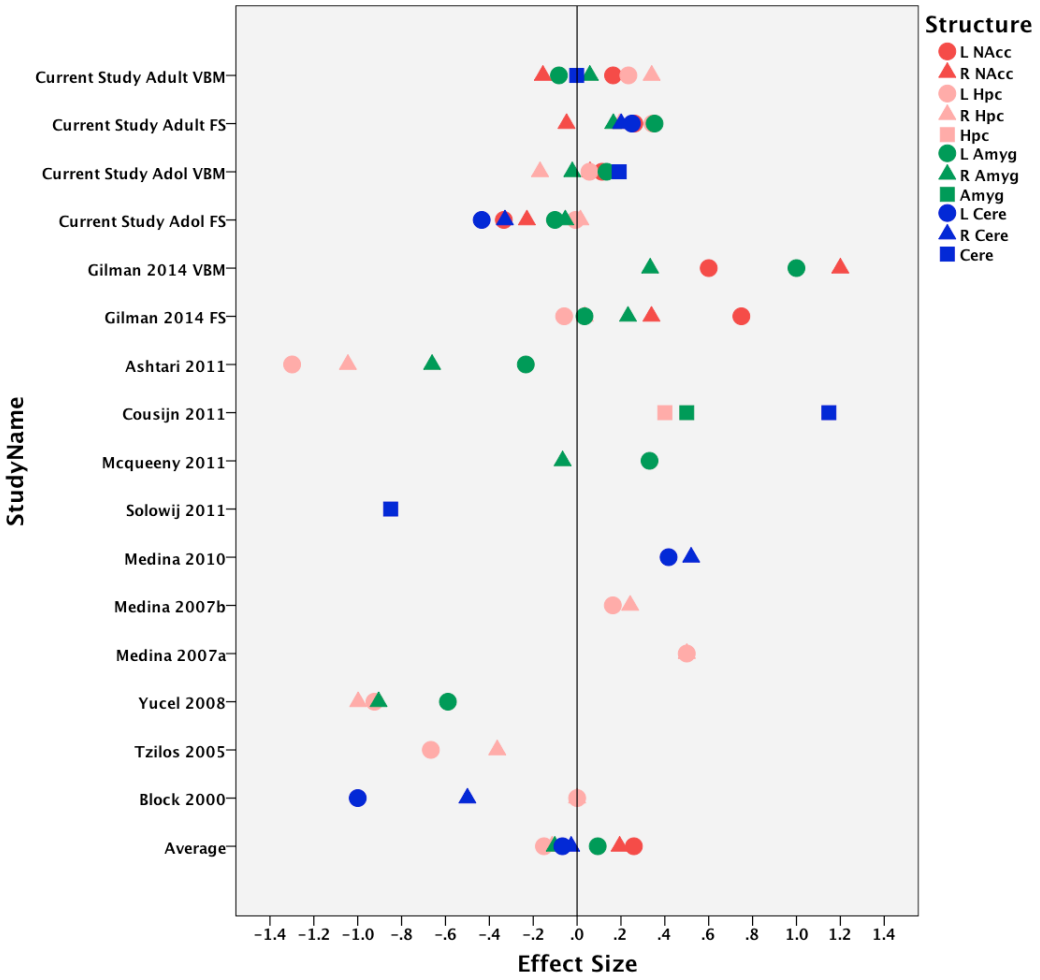

Figure 1. Effect sizes of differences in structure volume between marijuana users and non-users in published studies [Weiland, Thayer et al., 2015; L=Left, R=Right; NAcc=nucleus accumbens, Hpc=hippocampus, Amyg=amygdala, Cere=cerebellum].

A study on long-term use compared occasional users to regular users (age range for both groups 18 to 30 years, with reported median 7 years of marijuana use), and found regions of decreased as well as increased volume (Battistella et al., 2014). Although participants had no history of neurologic or psychiatric conditions, including problems with alcohol or other substances (Battistella et al., 2014), construct validity for long-term chronic use could be improved. The age range for the study remains young, especially in comparison to studies on structural associations of chronic alcohol use (i.e., exposure over many decades; Pfefferbaum et

al., 1992). In a similar study, Yücel et al. (2008) demonstrated that long-term, heavy marijuana users showed bilaterally reduced hippocampal and amygdala volumes compared with non-using controls, and marijuana users performed significantly worse on a verbal learning task compared with controls. This study used defined regions of interest for bilateral hippocampus and amygdala, and did not report on possible global effects. The chronic marijuana users had at least 10 years of heavy use, with mean duration of regular use of 20 years and mean age of 40 years (Yücel et al., 2008). Alternatively, Tzilos and colleagues (2005) reported no difference in total gray matter volume, total white matter volume, or bilateral hippocampal volume among long-term (i.e., mean regular use for 23 years, with mean 19 years of daily use but range of 1 to 33 years of daily use) marijuana users with a mean age of 38 years (range 30 to 55 years), although results were based on number of episodes of marijuana use rather than years of use. These two studies (Tzilos et al., 2005; Yücel et al., 2008) had similarly aged participants with similar mean years of regular use, but defined heavy/chronic use differently, and one study utilized a whole-brain approach in addition to a region of interest, whereas the other used specific regions of interest. These differences in methodology are certainly not uncommon across other studies, and it is therefore difficult to directly compare results.

In an effort to explore shared genetic and environmental effects versus those of marijuana, one study examined associations between marijuana use and brain structure between siblings who had differing levels of use (Pagliaccio et al., 2015). Minor differences, which the authors describe as within the range of normal variability, in the volume of the left amygdala and right ventral striatum were observed when comparing lifetime non-users to those who had ever used marijuana, and volumetric differences in left amygdala were strongly associated with shared genetic factors. Among sex-matched siblings who were discordant for marijuana use, no

differences were observed in whole brain volumes, bilateral amygdala, hippocampus, and ventral striatum, or orbitofrontal cortex. Overall, discordant siblings showed reduced amygdala volumes compared to siblings with similar marijuana use. These results suggest that marijuana use was unlikely to have causal effects in reduced structure volumes (Pagliaccio et al., 2015).

In sum, the literature on marijuana use and brain structure is widely heterogeneous, and has methodological limitations that need to be carefully considered in future research. A general limitation of existing structural neuroimaging studies is how variations in morphology of cortical or subcortical structures should be interpreted, because functional or behavioral measures are frequently not reported in conjunction with brain data. Studies vary widely in their analytical approach (i.e., whole-brain versus region of interest), which often prevents direct comparison of results. Similarly, many studies focus on how marijuana use may interact with other substances (e.g., comorbid alcohol and marijuana use; Lisdahl et al., 2013) or psychological disorders (e.g., marijuana use and the onset of psychosis; Rapp et al., 2013). Importantly, associations of marijuana use have most often been reported in conjunction with conditions with known, strong effects on brain structure (i.e., alcohol and schizophrenia). It is also possible that any observed effects represent premorbid differences in brain structure rather than consequences of marijuana use (e.g., Pagliaccio et al., 2015). Finally, studies have largely been unable to report differences in genetic strains that are most frequently used by study participants, which may be a driving factor in studies that observe likely harms versus potential benefits.

Overall, the literature lacks a clear foundation of basic associations between chronic marijuana use and global brain structure. Without a better understanding of global marijuana effects on the brain, focused region of interest analyses (see Figure 2) may be missing important information. Similarly, studying combined or interactive effects of comorbid alcohol and

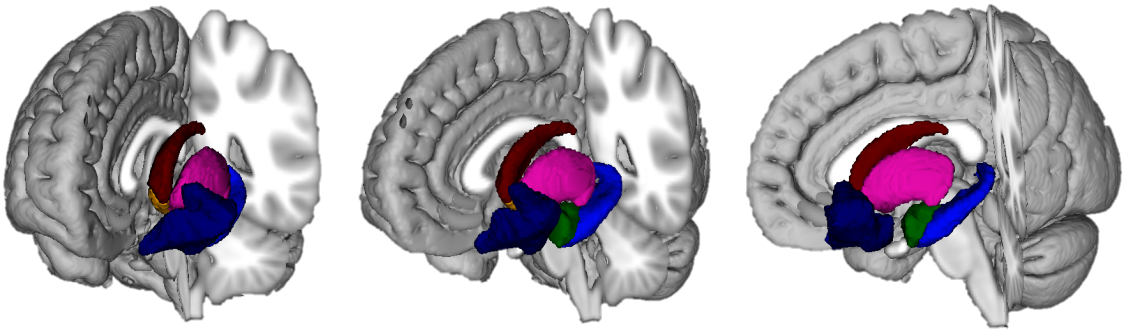

Figure 2. Brain structures commonly used in region of interest analyses of marijuana use [left hemisphere: nucleus accumbens, orange; dorsal striatum (caudate, red; putamen, pink); orbitofrontal cortex, navy; amygdala, green; and hippocampus, blue].

marijuana use is difficult without clear expectations of typical marijuana effects alone. A focused study of chronic marijuana use and brain structure would address an important gap in the literature in order to inform future research as well as provide useful information about potential health risks or benefits for dissemination to the public at large. Chronic marijuana users are the most likely to show a cumulative effect on brain structure, especially in comparison to substances with known long-term effects (i.e., alcohol; Pfefferbaum et al., 1992). Structural brain measures of cortical thickness and volume are arguably among the most accessible and quantifiable brain characteristics (i.e., compared to functional measures like connectivity), and it is well understood how brain structure changes over the course of lifespan development (e.g., cortical atrophy; Sowell, Thompson, & Toga, 2004). Structural brain imaging studies have consistently shown an inverse relationship between aging and total cerebral brain volume (Pfefferbaum et al. 1994) at an estimated rate of 1.9% per decade (Seshadri et al. 2004). Expected declines among older adults will also better allow the possibility of distinguishable neuroprotective effects of marijuana use (e.g., preserved volume).

Cognitive Effects of Marijuana

The literature on associations between brain structure and marijuana use is heterogeneous

at best, but acute cognitive effects are clearly documented. Studies that were published as early as the 1970s suggest that marijuana disrupts immediate and delayed free recall of information (Abel et al., 1971; Darley et al., 1974). More recently, acute cognitive impairment has been observed during marijuana intoxication in working memory, processing speed, and attention (Lundqvist, 2005; Ranganathan & D'Souza, 2006; Wilson et al., 1994). In contrast to existing studies on acute marijuana effects, evidence in regard to long-term cognitive deficits is less clear overall and often introduces methodological confounds. Dose-dependent deficits in learning and memory processes have been observed among adults with chronic exposure to marijuana (Solowij & Battisti, 2008). It has also been suggested that regular marijuana use has long-term implications including decreased IQ (Meier et al., 2012). Similarly, one study reported that marijuana and other substance use in adolescence was associated with poorer cognitive function 10 years later (Hanson et al., 2011). Some commentaries have highlighted that studies on chronic exposure did not account for several likely confounds (e.g., socioeconomic status; Rogeberg, 2013), but more importantly, others have documented recovery of observed deficits after abstinence (e.g., Schulte et al., 2014). Other studies have substantiated specific long-term impacts on learning and memory. In particular, although other areas of cognitive function likely recover even when use earlier in life was chronic, hippocampal-dependent short-term memory may remain impaired after last marijuana use (Abush & Akirav, 2012).

Taken together, converging evidence from structural neuroimaging and cognitive data suggests that if any effects of chronic marijuana use are observed, those are most likely to be changes to the hippocampus (e.g., reduced volume; Demirakca et al., 2011) and poorer memory (e.g., Solowij & Battisti, 2008). Global cortical thinning and gray matter volume loss, as well as other indicators of atrophy such as ventricle size, are associated with poorer neuropsychological

test scores and cognitive decline (Benedict et al., 2006; Draganski, Lutti, & Kherif, 2013; Zivadinov et al., 2001). More specifically, atrophy of the hippocampus is often implicated in memory decline during aging, and in greater degrees underlies more serious memory impairments of neurodegenerative diseases (e.g., dementias; Bilello et al., 2015; Fotuhi, Do, & Jack, 2012). Therefore, exploring associations among chronic marijuana use, brain structure, and cognitive function among older adults offers an interesting opportunity to observe potential harm or benefit both in terms of brain structure (i.e., positive or negative associations with global structural measures) and in terms of cognitive function (e.g., worse or better memory).

Study Aims and Hypotheses

The current proposal sought to more definitively answer the question of whether long-term marijuana use is associated with brain structure and cognition. This study collected structural MRI and cognitive assessments within a mixed sample of older adult marijuana users and age-matched non-users in order to report basic associations between marijuana use and brain structure, and, importantly, associations between brain structure and cognitive function. Cognitive assessments offer high functional value and clear comparisons to healthy aging individuals. However, it is also important to note that this research remains cross-sectional, and it is expected to be very difficult to accurately assess past substance use histories through interviews (e.g., estimating substance use over decades) as well as potential confounds related to general health. In particular, exclusionary criteria were selected to reduce potential contributions of heavy alcohol use history and medical conditions with known cognitive effects.

Aim 1. The first aim of the current study was to assess structural MRI in older adults who are current marijuana users. Measures of gray and white matter volume and cortical thickness in marijuana users were compared to a group of similarly aged healthy non-users to provide

preliminary information about how marijuana use might be associated with brain structure among adults age 60 years and older. It was hypothesized that marijuana users would show no differences in gray and white matter volumes compared to non-users, and that marijuana users would show no differences in cortical thickness compared to non-users. Alternatively, if marijuana use has long-term negative effects or neuroprotective effects on brain structure, group differences in volumetric and cortical thickness measures could be observed (e.g., users showing relative decreases or increases, respectively).

Aim 2. The second aim of the study was to collect brief assessments representing major areas of cognitive function (e.g., executive function, attention, memory, language, processing speed) in order to investigate functional correlates of marijuana use and brain structure. It was hypothesized that marijuana users would show poorer memory than non-users, but no other group differences. Deficits were not expected to be of large magnitude (i.e., less than one standard deviation). It was further hypothesized that brain structural measures would be positively associated with cognitive function.

Aim 3. The third aim of the study was to characterize associations between measures of marijuana use and brain structure and cognitive function within the group of marijuana users. First, the marijuana group was further divided into individuals who reported lifetime marijuana use and those who reported short-term regular use. It was hypothesized that short-term users would show better memory performance than lifetime users, but no brain structural differences. Further exploratory analyses investigated associations of marijuana use measures of estimated years of regular use and past 90-day estimated THC consumption and days of marijuana use with brain structure and cognitive function. It was hypothesized that greater THC consumption would be associated with poorer memory performance.

CHAPTER II

METHOD

Overview

Adults age 60 years and older were recruited from the Boulder-Denver metro area to complete a single experimental session involving questionnaires, cognitive testing, and magnetic resonance imaging (MRI). Adults with weekly or greater marijuana use were recruited from the community through online advertisements and direct mail flyers. A supplementary group of non-marijuana-using adults from an existing large study on age, exercise, and cognitive function was included in initial neuroimaging analyses. Analyses investigated group differences in global brain structure and cognitive function, associations between cognitive function and brain structure, and associations between measures of marijuana use and brain structure within the user group. All study procedures were approved by the Institutional Review Board of the University of Colorado Boulder.

Power Analysis

Estimates of effect size follow Cohen (1988) and were conducted in G*Power 3.1 (Faul et al., 2009). Power analysis suggested a minimum total sample size of 55 was needed in order to detect a moderate effect size ($f^2 = 0.15$) at a two-tailed alpha of .05 and power level of at least .80 for a single regression coefficient in linear multiple regression. Recruitment thus aimed to complete data collection for 28 marijuana users and 28 non-using controls.

Participant Eligibility

Participants were drawn from a study on brain structure and cognitive function targeting current marijuana users over the age of 60 as well as non-using controls (R36 study; PI: Thayer), and from an existing large study on exercise, aging, and cognitive function (FORCE study; PI: Bryan). Exclusionary criteria for the R36 study were matched to existing eligibility criteria for the FORCE study in order to increase the sample size of the non-using control group.

Healthy Control Adults

In order to be eligible for inclusion in the FORCE study on exercise, aging, and cognitive function, participants had to be age 60 or older; be able to pass a brief mental status screen (i.e., to rule out existing cognitive problems; Pfeiffer, 1975); and be physically healthy as assessed by a study physician (i.e., no injuries, physical impairments, or pre-existing contraindications to exercise).

Individuals are ineligible to participate in the FORCE study if they:

(1) Are a heavy tobacco smoker (> 20 pack years)

(2) Have diabetes that is (a) not controlled (i.e. hemoglobin A1C above 7%) or (b) treated by insulin or sulfonylureas

(3) Have a body size exceeding the capacity of the MRI machine (approximately 23" in diameter)

(4) Are on antipsychotic medications

(5) Have any of the following conditions: bipolar disorder, schizophrenia, dementia, Alzheimer's disease

(6) Have MRI contraindications (i.e., non-removable metallic implants, claustrophobia, traumatic brain injury, etc.)

(7) Have uncontrolled hypertension (systolic BP ≥160 and/or diastolic BP ≥100 mmHg)

Marijuana-Using Adults

Active recruitment targeted marijuana users age 60 years old and older who reported consuming marijuana at least once per week for at least the last year. In addition to the exclusionary criteria listed above, participants with any history of an alcohol or other substance use disorder (other than cannabis use disorder) were excluded. This exclusion limited generalizability of findings considering high comorbidity of marijuana use with alcohol use in particular, but the current study aimed to reduce possible confounds related to other substances.

Participant Enrollment

Recruitment utilized online advertisement through Craigslist, posting flyers in public places including senior centers, and finally directly mailing flyers (*N*=5,000) to adults over the age of 60 living in Boulder County. Overall, a low rate of individuals contacted the study for more information (see Figure 3; approximately 2.6% return rate out of 4,875 flyers likely delivered). Recruitment of marijuana users appeared to be largely limited by reported medicinal use for exclusionary health conditions. Of *n*=55 individuals who completed screening but were deemed ineligible to participate, *n*=7 were not yet 60 years old; *n*=11 did not qualify as regular users (i.e., occasional use falling between the user and control groups); *n*=7 reported MRI contraindications (e.g., claustrophobia, pacemakers); *n*=5 had poorly controlled diabetes or were managing their diabetes with insulin; *n*=6 endorsed history of alcohol or other substance use disorder; *n*=3 endorsed history of serious mental illness; *n*=5 reported history of traumatic brain injury; and *n*=11 reported history of other contraindicated neurological conditions (e.g., narcolepsy, essential tremor, stroke). Among potential non-using controls, *n*=7 individuals

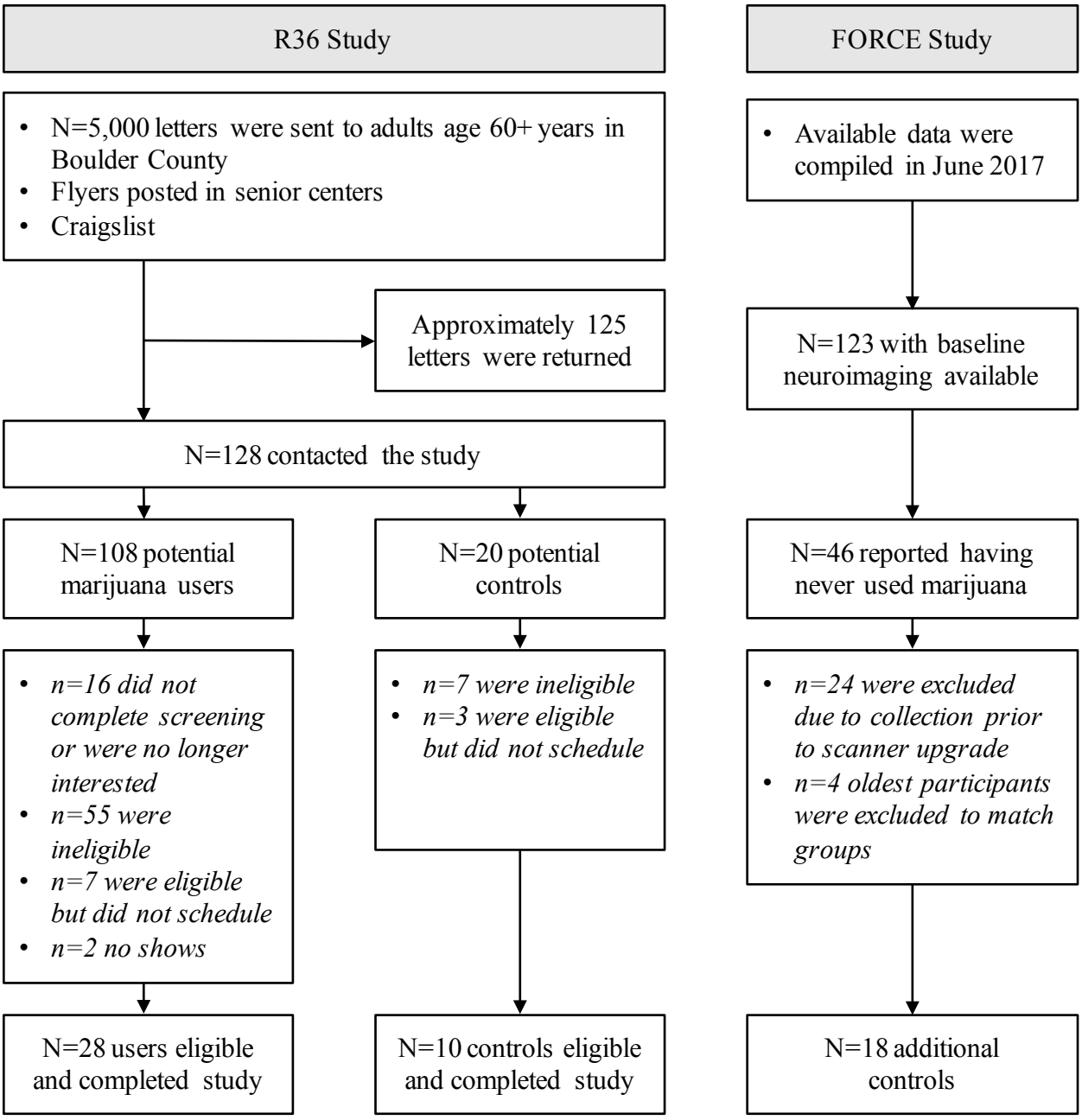

Figure 3. Participant recruitment and enrollment.

completed screening but were ineligible to participate due to reported claustrophobia (*n*=1), history of serious mental illness (*n*=1), history of traumatic brain injury (*n*=3), and history of contraindicated neurological conditions (i.e., seizures, neuropathy under medical evaluation; *n*=2). In total, the R36 study successfully recruited and completed data collection for *N*=28 marijuana users and *N*=10 controls.

Supplementary control group neuroimaging data were accessed through the FORCE study according to existing data available by June 30, 2017. The FORCE study collects baseline neuroimaging data for adults interested in beginning an exercise intervention. Of 123 participants with baseline neuroimaging data available, *n*=46 reported having never tried marijuana. Participants were excluded if data collection occurred prior to scanner upgrade in June 2016 (*n*=24), leaving *n*=22 available supplementary controls. Finally, *n*=4 of the oldest participants were excluded to more closely match groups according to age, leaving a final sample of *N*=18 controls from the FORCE study.

Procedures

Participants in the R36 study completed a single appointment consisting of questionnaires, cognitive testing, and MRI. Participants were instructed to not drink alcohol within 48 hours, consume any marijuana within 6 hours, or consume caffeine or smoke cigarettes within 3 hours of their scheduled participation. All study procedures were approved by the Institutional Review Board of the University of Colorado Boulder.

Measures

Merged Sample. All participants (i.e., across both participation sources) completed a demographics questionnaire which collected information on age, sex, marital status, SES, occupation, income, education, and race/ethnicity as well as basic questions related to marijuana use, including whether they had ever used marijuana, first age of use, and whether they currently use marijuana.

The Alcohol Use Disorder Identification Test (AUDIT; Babor et al., 2001) was used to measure alcohol use and problems related to alcohol use. The AUDIT includes general consumption items (e.g., How many drinks do you have on a typical day when you are

drinking?) as well as symptoms of alcohol use disorder within the last three months (e.g., How often during the three months have you found that you were not able to stop drinking once you had started?). Scores range from 0 to 40 with a clinical cutoff of 8 signifying likely problematic drinking.

The Beck Depression Inventory-II (BDI-II) consists of 21 scaled statements designed to assess symptoms of depression (e.g., pessimism, loss of pleasure), with a coefficient alpha of .91 (Beck, Steer, Ball, & Ranieri, 1996). Participants rate each item from 0 to 3 for severity over the last two weeks. The BDI-II was administered to account for comorbid depression as a possible factor related to group differences.

The Beck Anxiety Inventory (BAI; Beck, 1990) consists of 21 items, each describing a common symptom of anxiety (e.g., heart pounding or racing, unable to relax). Participants rate each item according to how much they were bothered (not at all, mildly, moderately, or severely) by the symptom during the past week. The items are summed to obtain a total score that ranges from 0 to 63. The BAI was administered to account for comorbid anxiety as a possible factor related to group differences.

R36 Sample. In addition to the above questionnaires, participants in the R36 sample completed the following additional measures related to substance use.

The Fagerstrom Test of Nicotine Dependence (FTND; Heatherton, Kozlowski, Frecker, & Fagerstrom, 1991) was used to collect information on nicotine dependence.

The Timeline Follow-Back (TLFB; Sobell & Sobell, 1992) assessed substance use for the 90 days prior to the scanning session. The TLFB is a calendar-assisted structured interview that provides temporal cues to increase the accuracy of recall. This interviewer-administered instrument has demonstrated test-retest reliability and validity (Sobell, Sobell, & VanderSpek,

1979). Primary measures derived from the TLFB were days of marijuana use and amount used per day. Preferred marijuana potency and method of administration (e.g., smoked, vaporized, consumed in edible form) were also collected in order to estimate milligrams (mg) of THC consumption according to cannabis potency equivalencies developed for the State of Colorado (i.e., one mg of THC in edible form is equivalent to 5.71 mg of THC in smoked marijuana; Orens et al., 2015). If a participant was unable to report their preferred potency of smoked marijuana, average potency of 15% was assumed for current strains in the Denver metro area (Vergara et al., 2017) and average potency of 4% THC was assumed for participants who maintained use of the same strain since the 1960s or 1970s (ElSohly et al., 2016).

The Marijuana Dependence Scale (MDS) is based on DSM-V criteria for cannabis use disorder. Individuals respond 'yes' or 'no' to each dependence item (e.g., "When I smoked marijuana, I often smoked more or for longer periods of time than I intended"). The items are then summed to form the scale. The internal consistency of the MDS (based on the DSM-IV) is high in previously published reports (α=.85; see Stephens, Roffman, & Curtin, 2000).

The extended Marijuana Motives Measure (MMM; Benschop et al., 2015) assesses reasons for using marijuana through 24 items rated on a 5-point Likert scale (1=Almost never/never, 2=Some of the time, 3=Half of the time, 4=Most of the time, 5=Almost always/always). Items of the MMM form subscales for coping (e.g., “to forget about my problems”), enhancement (e.g., “because I like the feeling”), social (e.g., “because it makes social gatherings more fun”), conformity (e.g., “to fit in with the group I like”), expansion (e.g., “because it helps me to be more creative and original”), and routine (e.g., “out of habit”) reasons. Two additional text entry items were included for participants to report their own reasons not already included.

Cognitive Testing

Participants completed the NIH Toolbox Cognition Battery of tests (Gershon et al., 2013). This brief computerized battery includes seven tasks assessing various cognitive functions. The Cognition Battery for ages 12 years and older includes the NIH Toolbox Flanker Inhibitory Control and Attention Task (attention), Picture Sequence Memory Test (episodic memory), List Sorting Working Memory Test (working memory), Picture Vocabulary Test (vocabulary knowledge), Oral Reading Recognition Test (oral reading skill), Dimensional Change Card Sort Test (executive function), and Pattern Comparison Processing Speed Test (processing speed). Primary measures of interest were standard scores normed for age for each test, and the age-normed Total Composite standard score as a measure of current general cognition. Standard scores have a mean of 100 and standard deviation of 15 points, such that scores falling between 90 and 85 are considered low average and scores below 85 are impaired.

Structural MRI

The Intermountain Neuroimaging Consortium MRI Suite houses a 3T research-only Siemens MAGNETOM Prisma system with a 32-channel head coil. Scanner instability and quality control on a phantom are monitored weekly. For optimal contrast between gray matter, white matter and cerebrospinal fluid, a multi-echo MPRAGE (MEMPR) sequence was collected with the following parameters: TR/TE/TI=2400/2.07/1000 ms, flip angle=8°, FoV=256x256 mm, Slice thickness=0.8 mm, Slices per slab=224, 3D voxel resolution=0.8 x 0.8 x 0.8 mm, Pixel bandwidth=240 Hz. A fieldmap for distortion correction was also acquired: TR/TE=7220 ms/73 ms, FoV=248x248 mm, in-plane voxel resolution=3.0 x 3.0 x 3.0 mm, 56 slices.

Data Processing

Self-Report and Cognition Measures

Questionnaire and cognition data were imported into the SPSS Statistics software package for all analyses. Continuously scaled variables were examined for normality of distributions according to the Shapiro-Wilk test. Variables showing non-normal distributions were log transformed prior to further analysis.

Structural MRI Processing

Two separate but similar techniques for structural data processing and analysis were used in order to explore consistency of results across two common analysis techniques (e.g., Thayer et al., 2016). Global brain structure was examined using both voxel-based morphometry and surface-based morphometry.

Voxel-Based Morphometry. Voxel-based morphometry provides a measure of volume/density. Tools from FMRIB Software Library (FSL v5.0.1) were used for automated segmentations of subcortical structures and whole-brain probability maps. Automated segmentations were obtained through the FIRST model-based segmentation and registration tool (Patenaude et al., 2011). Whole-brain maps were prepared through the FSL-VBM analysis pipeline (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM) following standard automated processing (Ashburner & Friston, 2000; Good et al., 2001). Images were brain-extracted and segmented before non-linear registration to Montreal Neurological Institute (MNI) standard space (Andersson, Jenkinson, & Smith, 2007). Resulting images were averaged to create a study-specific template, to which native images were non-linearly registered and modulated. The modulated segmented images were smoothed with an isotropic Gaussian kernel with a sigma of 3, yielding full-width half-maximum (FWHM) of 6.9 mm. Resulting subject-specific probability

maps were input into general linear models via FSL's Randomise program. Models were corrected for multiple comparisons through Monte Carlo simulations with 5000 permutations and threshold free cluster enhancement for whole-brain corrected $p<.05$. If results did not survive multiple comparison correction, clusters were then viewed under voxelwise $p<.001$ and minimum cluster size of 100 voxels.

Surface-Based Morphometry. Surface-based morphometry provides measures of volume for gray and white matter and cortical thickness for gray matter, which may be defined across total brain, hemisphere, or individual anatomical regions. Analyses used FreeSurfer v5.3 (https://surfer.nmr.mgh.harvard.edu/) standard processing to perform cortical reconstruction and volumetric segmentation. These methods included skull stripping, Talairach transformation, and segmentation and parcellation of cortical and subcortical structures (Dale et al., 1999; Fischl et al., 2004). Resulting subject-specific volume maps were input into general linear models in FreeSurfer's Qdec with initial multiple comparison correction through Monte Carlo simulations for $p<.05$. If results did not survive multiple comparison correction, clusters were then viewed under voxelwise $p<.001$ and minimum cluster size of 100 voxels.

Statistical Analyses

Aim 1

In order to test hypotheses under Aim 1, marijuana user and control groups were first examined for group differences in potential confounding variables (age, gender, depression and anxiety symptoms, intracranial volume) to be included as covariates in statistical analyses. Automated segmentation values for global volumes (cerebrospinal fluid, total gray matter, total white matter) and subcortical structures were examined through independent samples *t*-tests in SPSS and subjected to false discovery rate (FDR) correction. Whole-brain general linear models

categorically coded group status to explore difference in volume (FreeSurfer for surface-based morphometry, and FSL for voxel-based morphometry) between marijuana users and controls above and beyond covariates. Further models explored differences in whole-brain cortical thickness (FreeSurfer).

Aim 2

Given the smaller sample of non-using controls with available cognitive data, groups were again examined for differences in demographic characteristics to be included as covariates in further analyses. In order to test the first hypothesis under Aim 2 (i.e., that marijuana users would show poorer memory than non-users but no other group differences), independent samples *t*-tests examined group differences in age-normed standard scores across individual cognitive tests and total composite score. In order to test the second hypothesis under Aim 2 (i.e., that brain structural measures would be positively associated with cognitive function), analyses planned to use any cognitive performances showing group differences as predictors in structural models.

Alternatively, in the event of no group differences in cognitive performance, analyses were planned such that partial correlations would examine associations between cognitive performance and automated segmentations above and beyond intracranial volume. Cognitive tests showing associations with volume were selected as predictors in whole-brain models, and clusters showing associations with cognitive performance were extracted for inclusion in *t*-tests examining group differences in structural measures.

Aim 3

In order to test the first hypothesis under Aim 3, the marijuana user group was further divided into individuals who reported lifetime marijuana use and those who reported short-term

regular use. Prior group analyses were replicated including independent samples *t*-tests of cognition age-normed standard scores and structural analyses in automated segmentations and whole-brain general linear models.

For exploratory analyses using marijuana use characteristics (estimated years of regular marijuana use, past 90-day estimated THC consumption and days of use), partial correlations were examined with automated brain segmentations while controlling for intracranial volume. Marijuana use characteristics were then entered into whole-brain general linear models with intracranial volume as a covariate. Finally, bivariate correlations were examined between use measures and cognition standard scores.

CHAPTER III

RESULTS

Aim 1: Differences in Brain Structure between Marijuana Users and Controls

Sample Characteristics

Sample characteristics are presented in Table 1. Across the entire sample, participants were 68 years old (*SD*=5.66), 48% female, and college educated on average. The majority of participants identified their race and ethnicity as White Not Hispanic, with very few participants of Latino (*n*=2) and Asian (*n*=4) ancestry. None of the participants were current tobacco cigarette

Table 1

Aim 1 sample characteristics.

	Whole Sample *Mean (SD; Range)*	Controls *Mean (SD; Range)*	Marijuana Users *Mean (SD; Range)*
N	56	28	28
*Race/Ethnicity**			
White	50	23	27
Latino	2	1	1
Asian	4	4	0
Females:Males	27:29	17:11	10:18
Age*	68.29 (5.66; 60-83)	69.79 (5.71; 61-83)	66.79 (5.28; 60-80)
Years Education	16.38 (2.45; 12-22)	16.29 (2.59; 12-22)	16.46 (2.33; 13-22)
AUDIT Total Score	3.77 (3.02; 0-18)	3.39 (2.22; 0-9)	4.14 (3.66; 0-18)
BDI-II Total Score*	4.38 (4.10; 0-18)	5.00 (3.36; 0-11)	3.75 (4.71; 0-18)
BAI Total Score	3.04 (3.69; 0-21)	3.68 (4.35; 0-21)	2.39 (2.83; 0-12)

AUDIT: Alcohol Use Disorders Identification Test; BDI-II: Beck Depression Inventory-Second Edition; BAI: Beck Anxiety Inventory

*$p \leq .05$

smokers. On average, users reported 23.55 years of regular use (*SD*=19.89, range 1.5 to 50 years). Marijuana users (*n*=28) and controls (*n*=28) did not differ in terms of gender (*p*=.06) or years of education, problematic alcohol use, or anxiety symptoms (all *p*>.27), but controls endorsed slightly greater symptoms of depression [*t*(54)=1.99, *p*=.05]. Marijuana users were slightly younger than non-using controls [*t*(54)=2.06, *p*=.04]. Both depression symptoms and age were therefore included as covariates in group neuroimaging analyses.

Automated Segmentations

Voxel-Based Morphometry. Marijuana users and controls were not statistically different in terms of total intracranial volume or total volume of cerebrospinal fluid, gray matter, or white matter (all *p*>.13, accounting for age and depression symptoms). Intracranial volume was therefore not included as a covariate in whole-brain analyses.

In terms of automated segmentations of subcortical structures, and accounting for age and depression symptoms, marijuana users showed greater volume than controls in left putamen [$F(1,53)=11.49$, FDR corrected $p=.02$, $\eta_p^2=.18$; see Figure 4] but no other subcortical structures.

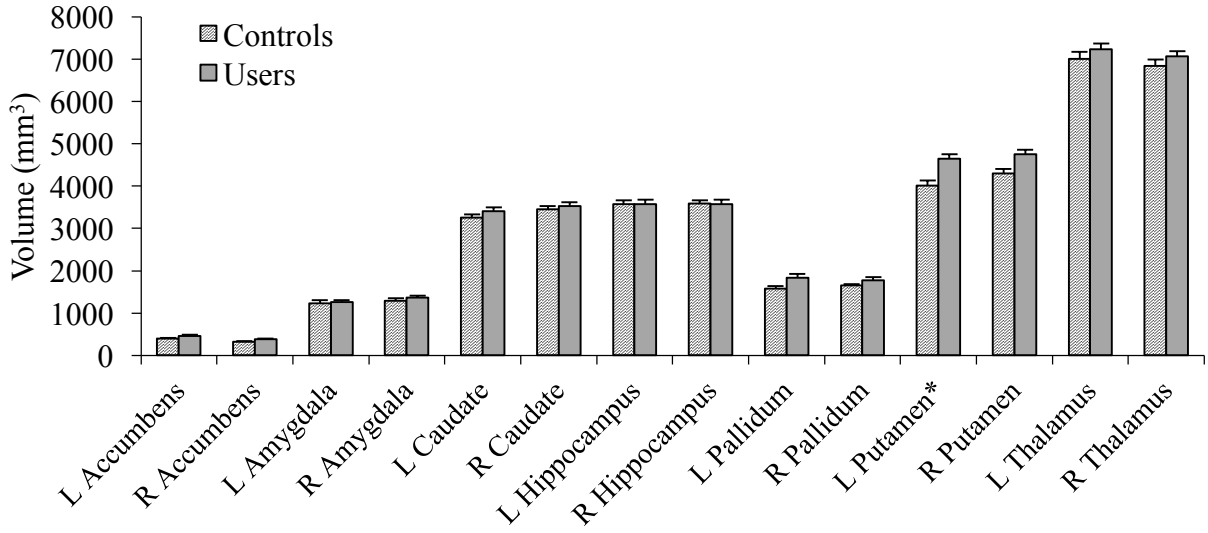

Figure 4. Voxel-Based Morphometry subcortical structure volumes for marijuana users and controls [*p<.05; L=Left, R=Right].

Surface-Based Morphometry. Marijuana users and controls were not statistically different in terms of total intracranial volume; total volume of cerebrospinal fluid, gray matter, or white matter; or cerebellum gray or white matter volume (all $p>.13$, accounting for age and depression symptoms). Intracranial volume was therefore not included as a covariate in whole-brain analyses.

Accounting for age and depression symptoms, marijuana users showed slightly greater volume of right putamen [$F(1,52)=4.37$, uncorrected $p=.04$, $\eta_p^2=.08$] and trend-level difference in left putamen [$F(1,52)=3.96$, uncorrected $p=.05$, $\eta_p^2=.07$], although these differences did not survive FDR correction. No group differences were observed in bilateral accumbens, amygdala, caudate, hippocampus, pallidum, or thalamus (all $p>.17$).

Whole-Brain General Linear Models

Voxel-Based Morphometry. No group differences survived multiple comparison correction according to Monte Carlo simulations at thresholding of $p<.05$. At uncorrected $p<.001$ and while accounting for age and depression symptoms, marijuana users showed greater volume in several large clusters in posterior and temporal regions as well as one smaller cluster in orbitofrontal cortex (see Table 2 and Figure 5). The greatest effect was observed in a large cluster spanning bilateral precuneus. Controls showed greater volume than users in large clusters in superior lateral occipital cortex and cerebellum, as well as several smaller clusters in similar regions and frontal gray and white matter (see Table 2 and Figure 5).

Table 2

Voxel-Based Morphometry clusters (≥100 voxels) of group difference (uncorrected p<.001) between marijuana users (n=28) and controls (n=28) while accounting for age and depression symptoms.

Annotation[a]	Peak *t*	Cluster Size (mm^3)	MNI Coordinates[a] X	Y	Z	Partial Eta Squared[b]

Users > Controls						
L lingual gyrus	4.83	2120	-6	-88	-14	.29
L/R precuneus	4.44	1592	0	-64	12	.32
L middle temporal gyrus	4.42	536	-60	-26	10	.27
L temporal fusiform cortex; inferior temporal gyrus	4.79	512	-42	-36	-20	.30
L frontal orbital cortex	4.44	216	-18	6	-16	.25
Controls > Users						
L lateral occipital cortex	5.76	3136	-20	-62	56	.47
R cerebellum I-IV	4.79	1208	16	-38	-20	.30
L cerebellum I-IV	4.27	344	-10	-38	-16	.24
L genu corpus callosum	4.62	344	-16	32	-2	.27
L cerebellum crus II	4.02	264	-48	-48	-54	.23
R postcentral gyrus	4.44	256	20	-36	58	.27
L lateral occipital cortex	3.80	144	-32	-80	22	.24
L middle frontal gyrus; frontal pole	3.77	112	-46	32	30	.24
L precentral gyrus	3.96	104	-8	-16	64	.24

[a]Peak association
[b]Calculated from cluster values in SPSS
L=Left; R=Right

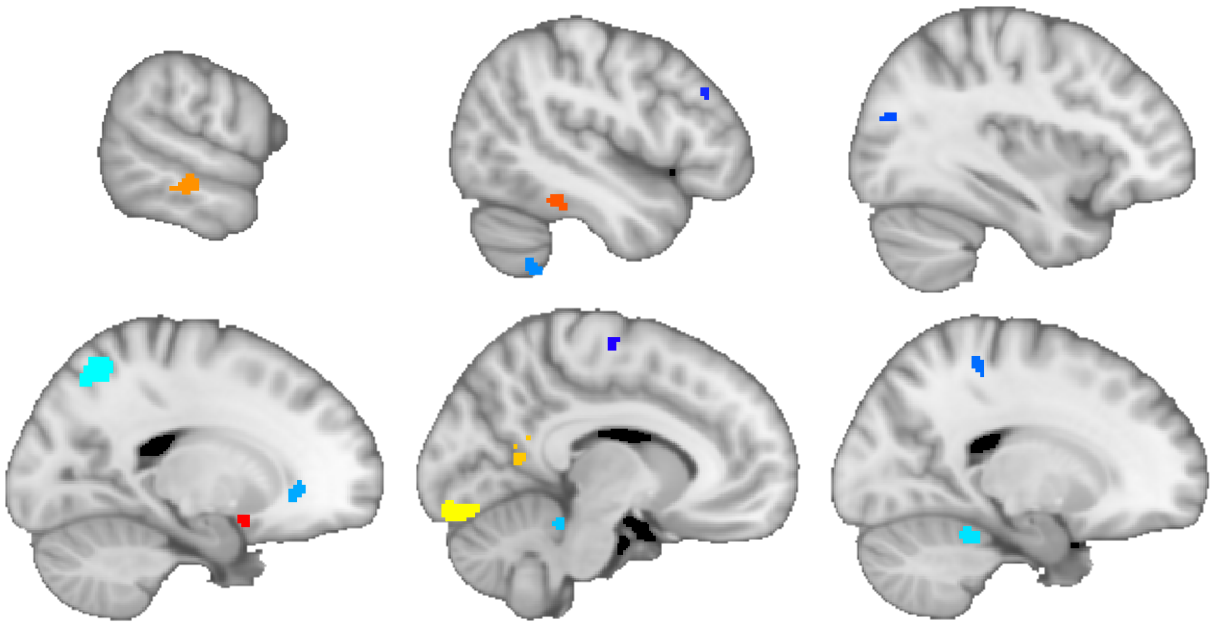

Figure 5. Voxel-Based Morphometry clusters showing group differences between marijuana users and controls [Yellow-red, users>controls; Light blue-dark blue, controls>users; From upper left, X=-60, -46, -35, -18, -9, 19].

Surface-Based Morphometry. Marijuana users showed greater cortical volume than controls in left lingual cortex and rostral middle frontal cortex (accounting for age and depression symptoms, corrected $p<.05$; see Table 3 and Figure 6). No group differences were observed in right hemisphere. In terms of cortical thickness, no group differences survived multiple comparison correction according to Monte Carlo simulations at thresholding of $p<.05$, and no clusters exceeded size of 100 voxels at uncorrected $p<.001$.

Table 3

Surface-Based Morphometry clusters (≥100 voxels) of greater volume (corrected p<.05) in marijuana users (n=28) than controls (n=28) while accounting for age and depression symptoms.

Annotation[a]	Peak *t*	Cluster Size (mm^2)	Talairach Coordinates[a] X	Y	Z	Partial Eta Squared[b]
Users > Controls						
L lingual	3.62	1617.8	-23.0	-53.3	3.8	.21
L rostral middle frontal	3.89	1433.0	-33.7	48.1	11.9	.21

[a]Peak association
[b]Calculated from cluster values in SPSS
L=Left

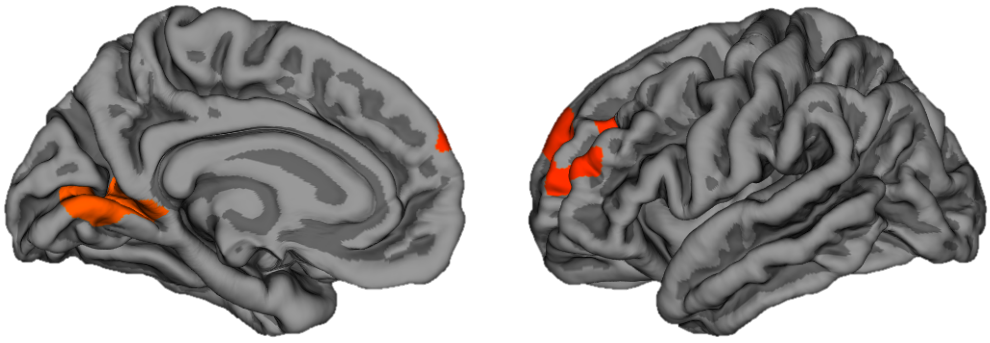

Figure 6. Surface-Based Morphometry clusters showing greater cortical volume in marijuana users than controls.

Summary of Aim 1 Results

Marijuana users and non-using controls were not different in terms of global structural measures, and showed possible differences in subcortical volumes such that volume of putamen was greater in marijuana users than controls. In whole-brain models, users showed clusters of greater cortical volume than controls in bilateral precuneus, left lingual gyrus, rostral middle frontal cortex, and several smaller clusters in left middle and inferior temporal gyrus and left orbitofrontal cortex. Controls showed greater volume than marijuana users in lateral occipital cortex and cerebellum; several smaller clusters in precentral and postcentral gyrus and middle frontal gyrus; and volume of white matter in the genu of the corpus callosum. Group status on average accounted for approximately 25% of the variance remaining in brain structure when already accounting for age and depression symptoms.

Aim 2: Cognitive Function Associations with Brain Structure Between and Across Groups

Sample Characteristics

Cognitive data were available for R36 study participants comprising n=28 marijuana users and n=10 controls. Groups differed in terms of age [controls greater than users, $t(36)=2.51$, $p=.02$], but did not differ in terms of depression symptoms ($p=.09$), or gender, race/ethnicity, years of education, problematic alcohol use, or anxiety symptoms (all $p>.15$; see Table 4).

Table 4

Aim 2 sample characteristics.

	Whole Sample *Mean (SD; Range)*	Controls *Mean (SD; Range)*	Marijuana Users *Mean (SD; Range)*
N	38	10	28
Race/Ethnicity			
White	35	9	26
Latino	2	1	1
Females:Males	16:22	6:4	10:18

Age*	68.18 (6.11; 60-83)	72.10 (6.84; 62-83)	66.79 (5.28; 60-80)
Years Education	16.13 (2.37; 12-22)	15.20 (2.35; 12-18)	16.46 (2.33; 13-22)
AUDIT Total Score	3.82 (3.31; 0-18)	2.09 (1.91; 0-5)	4.14 (3.66; 0-18)
BDI-II Total Score	4.18 (4.43; 0-18)	5.40 (3.44; 0-11)	3.75 (4.71; 0-18)
BAI Total Score	2.76 (4.02; 0-21)	3.80 (6.39; 0-21)	2.39 (2.83; 0-12)

AUDIT: Alcohol Use Disorders Identification Test; BDI-II: Beck Depression Inventory-Second Edition; BAI: Beck Anxiety Inventory
*$p<.05$

Group Differences in Cognitive Measures

Overall, marijuana users and controls did not show significant differences in cognition age standard scores (see Table 5 and Figure 7). Controls performed slightly better than marijuana users on the List Sorting Working Memory test [$t(36)=2.07$, $p<.05$], although this difference did not survive FDR correction across cognition scores.

Table 5

Cognition age standard scores for controls (n=10) and marijuana users (n=28).

	Controls	Users
	Mean (SD)	*Mean (SD)*
Picture Vocabulary	113.60 (9.71)	118.39 (8.54)
Flanker Inhibitory Control	97.90 (8.41)	93.25 (11.85)
List Sorting Working Memory*	112.00 (10.65)	103.21(11.81)
Dimensional Change Card Sort	119.30 (12.97)	112.46 (17.34)
Pattern Comparison Processing Speed	94.40 (22.54)	94.75 (20.69)
Picture Sequence Memory	94.10 (19.40)	94.86 (12.77)
Oral Reading	107.80 (12.97)	111.04 (8.16)
Total Composite	109.50 (13.67)	108.87 (10.96)

*Uncorrected $p<.05$

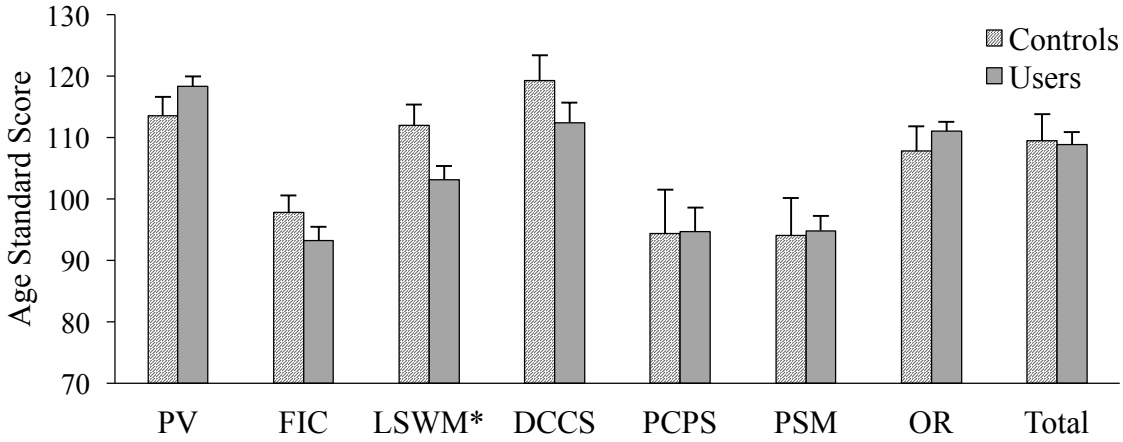

Figure 7. Cognitive performance of controls and marijuana users [*Uncorrected $p<.05$; PV=Picture Vocabulary, FIC=Flanker Inhibitory Control, LSWM=List Sorting Working Memory, DCCS=Dimensional Change Card Sort, PCPS=Pattern Comparison Processing Speed, PSM=Picture Sequence Memory, OR=Oral Reading, Total=Total Composite].

Automated Segmentations

Voxel-Based Morphometry. Across groups, global structural volumes were not significantly associated with cognition age standard scores above and beyond intracranial volume. A negative correlation was observed between Dimensional Change Card Sort performance and left accumbens volume [$r(35)=-.36$, uncorrected $p<.05$; see Table 6]. In contrast, positive correlations between Picture Sequence Memory standard score and volume were observed for left and right thalamus [$r(35)=.37$ and .43 respectively, uncorrected $p<.05$]. Picture Sequence Memory standard score was the only cognition measure to show a consistent pattern of associations with structural volumes.

Surface-Based Morphometry. A similar pattern was observed such that volumes showed a pattern of positive correlation with Picture Sequence Memory score, with significant associations observed for left and right cerebellum white matter [$r(35)=.36$, uncorrected $p<.05$] and right thalamus [$r(35)=.34$, uncorrected $p<.05$; see Table 7].

Table 6

Partial correlations between Voxel-Based Morphometry global and subcortical volumes and cognition age standard scores while controlling for intracranial volume.

	PV	FIC	LSWM	DCCS	PCPS	PSM	OR	Total
CSF	.02	.06	.03	.16	.17	-.29	.05	.06
GM	.06	-.06	.00	-.10	-.10	.24	.08	.03
WM	-.11	-.04	-.04	-.16	-.17	.22	-.18	-.13
L Acc	.06	-.04	-.23	-.36*	-.24	.11	-.02	-.17
R Acc	.03	.07	-.04	-.13	-.10	.15	-.02	-.03
L Amyg	.09	.06	.01	-.02	-.05	.19	-.03	.05
R Amyg	-.11	.00	.00	-.02	-.09	.07	-.07	-.06
L Caud	-.08	-.01	-.20	-.06	-.02	-.03	.00	-.09
R Caud	-.10	.13	-.17	.10	.17	-.05	-.04	.02
L Hipp	-.18	.20	-.04	.04	-.09	.01	-.17	-.09
R Hipp	-.07	-.07	.11	-.01	-.20	.18	-.10	-.07
L Pall	.14	-.05	-.13	-.11	.16	.02	.04	.04
R Pall	-.13	-.16	-.17	-.18	.08	-.10	-.21	-.19
L Put	.17	.09	-.13	.05	.04	.24	.12	.14
R Put	.03	-.03	-.02	-.06	-.15	.18	.07	-.01
L Thal	-.08	.03	.11	-.04	-.18	.37*	-.01	.01
R Thal	-.14	.07	.09	.03	-.08	.43**	-.06	.05
Average	-.03	.01	-.05	-.06	-.06	.14	-.04	-.03

**p<.01 uncorrected, *p<.05 uncorrected; L=Left, R=Right; CSF=cerebrospinal fluid, GM=gray matter, WM=white matter, Acc=accumbens, Amyg=amygdala, Caud=caudate, Hipp=hippocampus, Pall=pallidum, Put=putamen, Thal=thalamus; Average=average correlation not including CSF; PV=Picture Vocabulary, FIC=Flanker Inhibitory Control, LSWM=List Sorting Working Memory, DCCS=Dimensional Change Card Sort, PCPS=Pattern Comparison Processing Speed, PSM=Picture Sequence Memory, OR=Oral Reading, Total=Total Composite

Table 7

Partial correlations between Surface-Based Morphometry global and subcortical volumes and cognition age standard scores while controlling for intracranial volume.

	PV	FIC	LSWM	DCCS	PCPS	PSM	OR	Total
CSF	.04	.23	.14	.30	.20	.03	.05	.22
GM	.01	-.08	-.02	-.10	-.03	.25	.03	.03
WM	-.15	-.09	-.11	-.30	-.24	.19	-.14	-.21
L Cere GM	.02	.02	-.06	-.14	-.09	.17	.10	.01
R Cere GM	.01	-.01	-.07	-.16	-.08	.11	.09	-.03
L Cere WM	.09	.11	.03	.09	.15	.36*	.02	.20
R Cere WM	.08	.03	.02	.08	.12	.36*	.07	.18
L Acc	.01	-.01	-.14	-.20	.02	.26	-.07	-.03
R Acc	-.05	.11	-.15	-.19	-.21	.09	-.10	-.14
L Amyg	-.05	.11	.06	.23	.17	.15	.00	.17
R Amyg	.02	-.15	.06	.11	-.08	.06	-.05	.00
L Caud	-.19	-.04	-.22	-.12	.05	-.06	-.10	-.15
R Caud	-.10	.00	-.21	-.10	.16	-.05	.03	-.04
L Hipp	-.18	-.10	-.01	-.04	-.09	.23	-.17	-.09
R Hipp	-.15	-.13	-.01	-.02	-.21	.19	-.11	-.12
L Pall	.29	-.15	-.01	-.03	.15	-.11	.19	.11
R Pall	-.07	.03	.07	.07	.00	.08	.00	.03
L Put	.22	-.06	.00	.10	.10	.15	.20	.18
R Put	.02	-.16	-.15	-.03	-.09	.03	.05	-.07
L Thal	-.26	.05	-.13	-.12	-.17	.26	-.12	-.14
R Thal	-.01	-.08	-.04	-.18	-.19	.34*	.00	-.05
Average	-.02	-.03	-.05	-.05	-.03	.15	.00	-.01

*p<.05 uncorrected; L=Left, R=Right; CSF=cerebrospinal fluid, GM=gray matter, WM=white matter, Cere=cerebellum, Acc=accumbens, Amyg=amygdala, Caud=caudate, Hipp=hippocampus, Pall=pallidum, Put=putamen, Thal=thalamus; Average=average correlation not including CSF; PV=Picture Vocabulary, FIC=Flanker Inhibitory Control, LSWM=List Sorting Working Memory, DCCS=Dimensional Change Card Sort, PCPS=Pattern Comparison Processing Speed, PSM=Picture Sequence Memory, OR=Oral Reading, Total=Total Composite

Whole-Brain General Linear Models

Given the observed correlations between volumes and cognition scores, Picture Sequence Memory and Dimensional Change Card Sort performance were selected as predictors of interest for inclusion in whole-brain general linear models of brain structure along with intracranial volume as a covariate.

Voxel-Based Morphometry. No associations survived multiple comparison correction according to Monte Carlo simulations with thresholding of $p<.05$. For Picture Sequence Memory score (uncorrected $p<.001$), three clusters showed positive association in left frontal and parietal regions while controlling for intracranial volume (see Table 8 and Figure 8). Similarly, three frontal and parietal clusters were positively associated (uncorrected $p<.001$) with performance on the Dimensional Change Card Sort test (see Table 8 and Figure 8), although one cluster in right anterior cingulate gyrus showed negative association.

Table 8

Voxel-Based Morphometry clusters (≥100 voxels) associated (uncorrected p<.001) with cognition age standard scores.

Annotation[a]	Peak *t*	Cluster Size (mm^3)	MNI Coordinates[a] X	Y	Z	Partial Eta Squared[b]
Picture Sequence Memory						
L precentral gyrus	4.39	448	-30	-26	54	.37
L frontal pole	4.66	104	-30	58	16	.38
L postcentral gyrus	4.19	104	-36	-32	66	.35
Dimensional Change Card Sort						
R postcentral gyrus	4.08	200	62	-14	36	.34
R anterior cingulate gyrus	-3.91	160	2	38	20	.32
L frontal operculum cortex; inferior frontal gyrus	4.11	144	-48	14	-2	.33
R precuneus	4.21	136	6	-52	64	.33

[a]Peak association
[b]Calculated from cluster values in SPSS
L=Left; R=Right

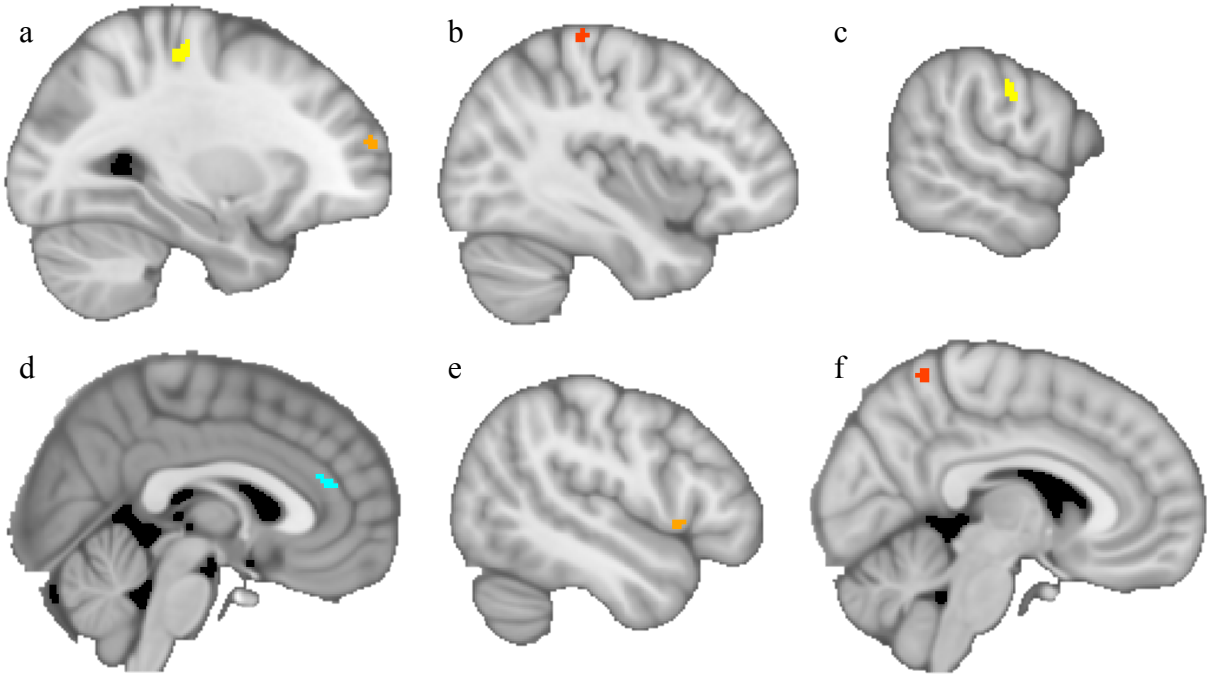

Figure 8. Voxel-Based Morphometry clusters associated with Picture Sequence Memory (a, b) and Dimensional Change Card Sort (c-f) standard scores [red-yellow=positive, blue=negative; from upper left, X=-28, -38, 60, 2, -48, 6].

Surface-Based Morphometry. One large cluster in left postcentral cortex was positively associated with Picture Sequence Memory performance for both cortical thickness and volume, while controlling for intracranial volume and surviving multiple comparison correction according to Monte Carlo simulations with thresholding of $p<.05$ (see Table 9 and Figure 9). A left subregion of dorsolateral prefrontal cortex also showed positive association with Picture Sequence Memory score. Similarly, positive association was observed between Dimensional Change Card Sort performance and cortical thickness in overlapping left postcentral cortex as well as right precuneus (see Table 9 and Figure 9). No clusters of negative association were observed across cognition measures and both cortical thickness and volume.

Table 9

Surface-Based Morphometry clusters (≥100 voxels) associated (corrected p<.05) with cognition age standard scores.

Annotation[a]	Peak *t*	Cluster Size (mm^2)	Talairach Coordinates[a] X	Y	Z	Partial Eta Squared[b]
Picture Sequence Memory						
Cortical Thickness						
L postcentral	4.54	1384.0	-52.5	-21.0	44.8	.36
Cortical Volume						
L postcentral	3.66	1382.5	-42.4	-28.7	48.0	.38
L pars triangularis	4.15	1081.8	-48.2	34.1	-3.6	.30
Dimensional Change Card Sort						
Cortical Thickness						
L postcentral	3.98	1350.8	-54.6	-16.2	44.8	.38
R precuneus	3.56	839.0	7.1	-45.6	58.5	.32

[a]Peak association
[b]Calculated from cluster values in SPSS
L=Left; R=Right

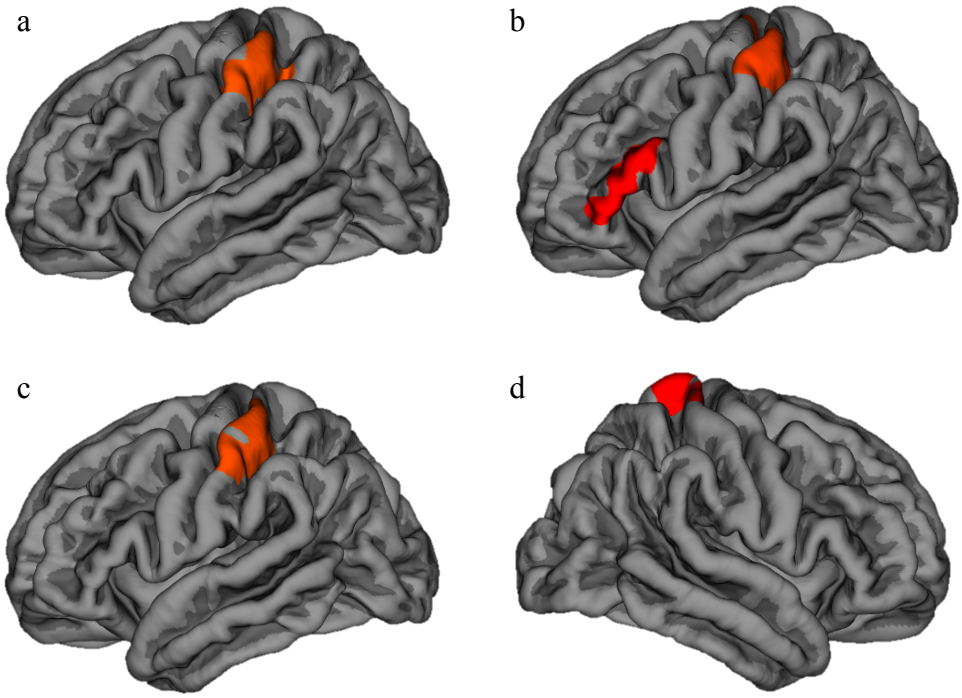

Figure 9. Surface-Based Morphometry clusters associated with Picture Sequence Memory (a, cortical thickness, and b, cortical volume) and Dimensional Change Card Sort (c, d, cortical thickness) standard scores.

Post Hoc Group Differences. Values were extracted from all clusters showing significant associations with cognition standard scores for group comparison. Marijuana users and controls did not show any difference in cortical volume or thickness across extracted clusters (all $p>.11$).

Summary of Aim 2 Results

Marijuana users and controls were not different in terms of their cognitive performance overall, although controls performed slightly better than marijuana users on a working memory test. Correlations between cognition standard scores and structural measures from automated segmentations showed a pattern of positive associations between Picture Sequence Memory performance and volumes, with significant association observed for thalamus volume. One negative correlation was observed between volume of left accumbens and Dimensional Change Card Sort score. Entering Picture Sequence Memory score and Dimensional Change Card Sort score into general linear models resulted in mainly positive associations between cognitive performance and volume in frontoparietal regions, especially precentral and postcentral cortex and precuneus. Marijuana users and controls did not show group differences in extracted regions associated with cognitive performance.

Aim 3: Marijuana Use Associations with Brain Structure and Cognitive Function

Marijuana Use Characteristics

Sample characteristics are reported in Table 10. Participants generally reported following one of two patterns of marijuana use: lifetime users who had used regularly since initiating use in late adolescence or early adulthood (n=15), and short-term regular users who first tried marijuana at a similar age but used inconsistently, discontinued use during middle adulthood (e.g., while raising a family), and then initiated regular use later in life (e.g., particularly following retirement or with legalization of recreational use; n=13). Lifetime and short-term marijuana users did not

Table 10

Aim 3 sample characteristics.

	Marijuana Users *Mean (SD; Range)*	Short-term Users *Mean (SD; Range)*	Lifetime Users *Mean (SD; Range)*
N	28	13	15
Race/Ethnicity			
Caucasian	26	13	14
Latino	1	0	1
Females:Males	10:18	6:7	4:11
Age	66.79 (5.28; 60-80)	67.38 (4.65; 61-80)	66.27 (5.89; 60-80)
Years Education	16.46 (2.33; 13-22)	16.69 (2.50; 13-20)	16.27 (2.25; 14-22)
AUDIT Total Score	4.14 (3.66; 0-18)	3.23 (2.28; 0-7)	4.93 (4.46; 0-18)
BDI-II Total Score	3.75 (4.71; 0-18)	3.69 (4.99; 0-18)	3.80 (4.63; 0-18)
BAI Total Score	2.39 (2.83; 0-12)	2.23 (3.35; 0-12)	2.53 (2.42; 0-7)
Age at First Use	20.04 (8.11; 14-58)	22.31 (11.41; 14-58)	18.07 (2.49; 14-22)
Years of Regular Use*	23.55 (19.89; 1.5-50.0)	4.19 (1.55; 1.5-7.0)	40.33 (10.56; 19.0-50.0)
MDS	0.79 (1.17; 0-4)	0.85 (1.21; 0-4)	0.73 (1.16; 0-3)
TLFB Alcohol Use Days	33.89 (29.59; 0-88)	34.92 (29.56; 0-88)	33.00 (30.62; 0-88)
TLFB Total Drinks	68.08 (72.68; 0.00-264.00)	56.28 (54.57; 0.00-163.68)	78.31 (85.99; 0.00-264.00)
TLFB Drinks/Drinking Day	1.50 (1.02; 0-4)	1.21 (0.86; 0-3)	1.74 (1.11; 0-4)
TLFB Marijuana Use Days	63.46 (24.87; 12-90)	53.92 (27.52; 12-90)	71.71 (19.63; 32-90)
TLFB Total mg THC	1033.27 (923.20; 99.82-3502.63)	840.74 (806.81; 99.82-3150.00)	1200.13 (1010.69; 168.13-3502.63)

AUDIT: Alcohol Use Disorders Identification Test; BDI-II: Beck Depression Inventory-Second Edition; BAI: Beck Anxiety Inventory; MDS: Marijuana Dependence Scale; TLFB: Timeline Follow-back

*Group difference $p<.001$

differ in age, race/ethnicity, gender, years of education, or anxiety, depression, or problematic alcohol use.

Participants reported a low number of symptoms on the Marijuana Dependence Scale. Consistent with this reporting, participants most frequently reported using marijuana for enhancement reasons, followed by expansion and social reasons on the Marijuana Motives Measure. Participants generally reported a low level of using marijuana for coping, social conformity, or routine reasons. Lifetime users reported greater routine use [$t(26)=-2.82$, $p=.01$], but groups did not otherwise differ in their reasons for using marijuana. A total of 10 participants (36%) additionally reported using marijuana for sleep or pain concerns.

On the Timeline Follow-back, $n=16$ participants reported smoking marijuana, $n=9$ participants reported consuming edibles, and $n=3$ reported both smoking and consuming edibles in the past 90 days. A total of $n=7$ participants were able to report a preference for strength of marijuana strain in terms of THC content ($n=3$ preferred THC of 20% and $n=4$ preferred THC of 25% or greater), compared to $n=11$ participants who were unsure of strain characteristics and were assumed to consume marijuana with average THC content (15% in Colorado; Vergara et al., 2017) and $n=1$ participant who reported having grown the same strain on her property for the last 40 to 50 years (assumed 4% THC content; ElSohly et al., 2016). On average, short-term regular users reported using on approximately 54 days ($SD=27.52$) out of the past 90 days, and lifetime users reported using on approximately 72 days [$SD=19.63$; $t(26)=-1.99$, $p=.06$]. Estimated total THC consumption in the past 90 days was 841.74 mg ($SD=806.81$) for short-term users and 1200.13 mg ($SD=1010.69$) for lifetime users, which did not significantly differ between groups ($p=.31$). Finally, participants reported consuming alcohol on approximately 34 days ($SD=29.59$) out of the past 90 days across groups, with an average of 1.5 ($SD=1.02$) drinks

per drinking day. Reported alcohol consumption did not differ between short-term and lifetime user groups (all $p>.18$).

Group Differences in Brain Structure

Automated Segmentations. User groups were not different in Voxel-Based Morphometry global structural or subcortical volumes (all $p>.13$), or in Surface-Based Morphometry global structural or subcortical volumes (all $p>.13$).

Whole-Brain General Linear Models. No group differences survived multiple comparison correction in whole-brain models. At uncorrected $p<.001$ and minimum cluster size of 100 voxels, short-term regular users showed greater volume in Voxel-Based Morphometry models than lifetime users in four small clusters in parietal and occipital regions (see Table 11 and Figure 10). No group differences were observed in Surface-Based Morphometry models.

Table 11

Voxel-Based Morphometry clusters (≥100 voxels) showing greater volume (uncorrected p<.001) in short-term (n=13) than lifetime (n=15) marijuana users.

Annotation[a]	Peak *t*	Cluster Size (mm^3)	MNI Coordinates[a] X	Y	Z	Cohen's *d*[b]
L occipital pole	4.56	344	-12	-92	-12	1.79
L postcentral gyrus	4.77	280	-14	-32	76	1.87
R superior parietal lobule; postcentral gyrus	4.38	144	22	-46	56	1.72
R occipital pole	4.51	128	16	-102	-12	1.77

[a]Peak association
[b]Calculated from peak *t* value
L=Left; R=Right

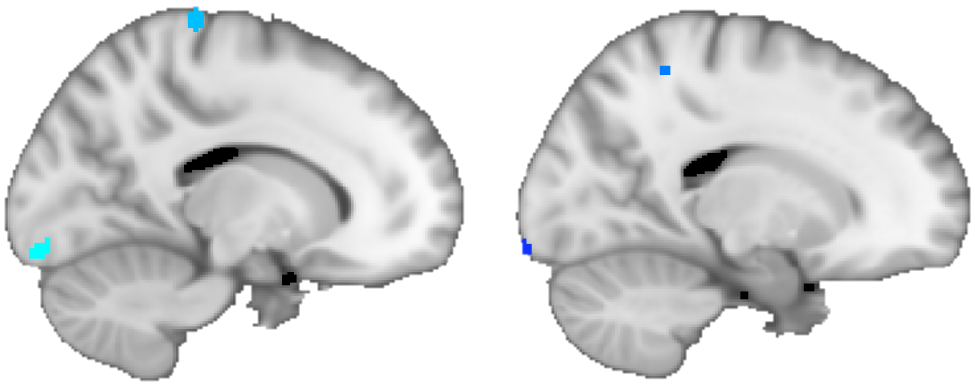

Figure 10. Voxel-Based Morphometry clusters showing lower volume in lifetime marijuana users compared to short-term users [From left, X=-14, 18].

Group Differences in Cognitive Function

Short-term users performed significantly better than lifetime users on the Dimensional Change Card Sort test [$t(26)=3.15$, FDR corrected $p=.02$], Pattern Comparison Processing Speed test [$t(26)=2.69$, FDR corrected $p=.03$], and overall Total Composite score [$t(26)=3.83$, FDR corrected $p<.01$; see Table 12 and Figure 11]. Lifetime users performed in the low average range on the Flanker Inhibitory Control and Pattern Comparison Processing speed tests, but otherwise all standard scores fell in the average to above average ranges.

Table 12

Cognition age standard scores for short-term (n=13) and lifetime (n=15) marijuana users.

	Short-term Users *Mean (SD)*	Lifetime Users *Mean (SD)*
Picture Vocabulary	120.85 (7.59)	116.27 (8.98)
Flanker Inhibitory Control	97.08 (11.62)	89.93 (11.39)
List Sorting Working Memory	106.54 (13.31)	100.33 (9.89)
Dimensional Change Card Sort*	122.08 (12.38)	104.13 (16.97)
Pattern Comparison Processing Speed*	104.92 (18.59)	85.93 (18.70)
Picture Sequence Memory	97.31 (13.71)	92.73 (11.94)
Oral Reading	114.54 (5.59)	108.00 (8.96)
Total Composite*	115.81 (9.05)	102.85 (8.82)

*FDR corrected $p<.05$

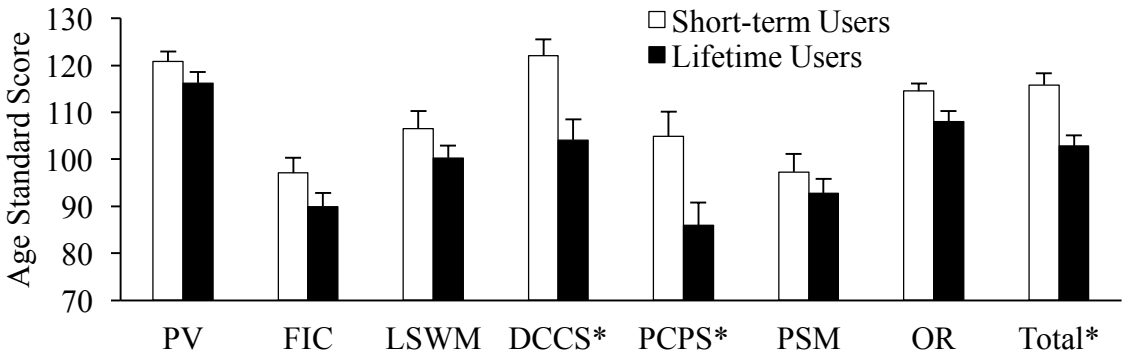

Figure 11. Cognitive performance of short-term and lifetime marijuana users [*FDR corrected $p<.05$; PV=Picture Vocabulary, FIC=Flanker Inhibitory Control, LSWM=List Sorting Working Memory, DCCS=Dimensional Change Card Sort, PCPS=Pattern Comparison Processing Speed, PSM=Picture Sequence Memory, OR=Oral Reading, Total=Total Composite].

Post Hoc Group Comparisons with Controls. Given observed differences between short-term and lifetime marijuana users, a post hoc analysis then included non-using controls (n=10) in an ANOVA. Significant differences in cognitive performance across groups were observed in Dimensional Change Card Sort score [$F(2,35)=6.13$, FDR corrected $p<.04$] and Total Composite score [$F(2,35)=5.46$, FDR corrected $p<.04$; see Figure 12]. Post hoc Tukey tests showed that

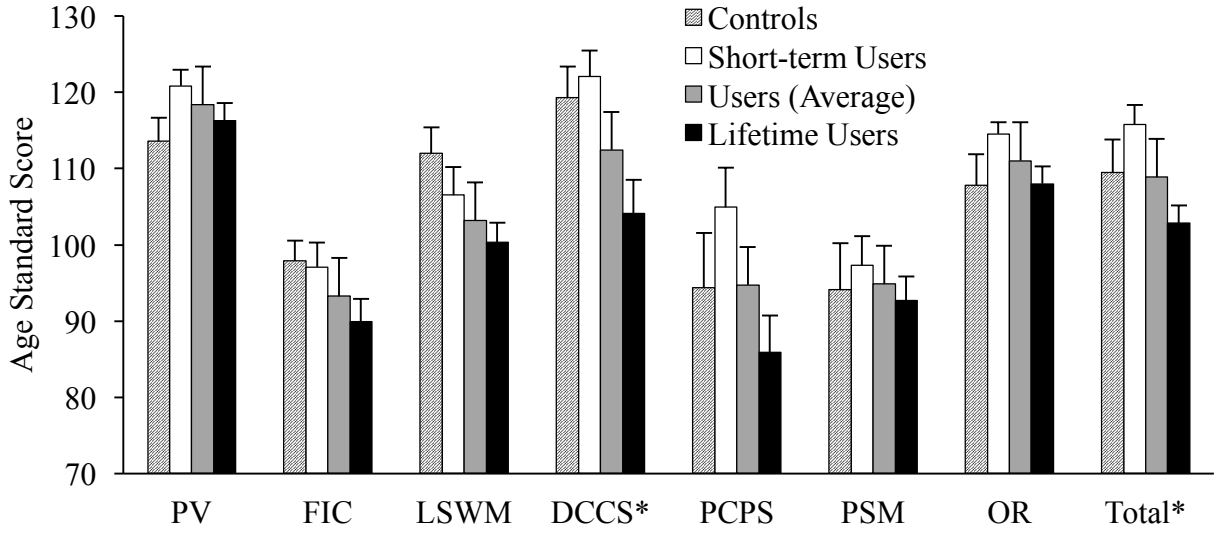

Figure 12. Cognitive performance across controls and marijuana user groups [*FDR corrected $p<.05$; PV=Picture Vocabulary, FIC=Flanker Inhibitory Control, LSWM=List Sorting Working Memory, DCCS=Dimensional Change Card Sort, PCPS=Pattern Comparison Processing Speed, PSM=Picture Sequence Memory, OR=Oral Reading, Total=Total Composite].

lifetime users performed poorly both compared to controls [p<.04, 95% CI (-29.68, -0.65)] and short-term users [p<.01, 95% CI (-31.42, -4.47)] on the Dimensional Change Card Sort test, and poorly relative to short-term users [p<.01, 95% CI (-22.56, -3.35)] but not controls in terms of Total Composite score. No significant differences were observed between controls and short-term users (all p>.13).

Marijuana Use Measures and Brain Structure

Automated Segmentations. No significant associations were observed between marijuana use metrics of estimated years of regular use or TLFB days of marijuana use and global structural or subcortical volumes controlling for intracranial volume. TLFB estimated THC consumption showed a general pattern of negative correlations with volume, with significant negative association between THC consumption and total gray matter in both Voxel-Based Morphometry [r(25)=-.44, uncorrected p<.05] and Surface-Based Morphometry [r(25)=-.42, uncorrected p<.05; see Table 13]. THC consumption was also negatively correlated with volume of right accumbens in Voxel-Based Morphometry [r(25)=-.41, uncorrected p<.05].

Table 13

Partial correlations between marijuana use measures and structural automated segmentations controlling for intracranial volume.

	Voxel-Based Morphometry			*Surface-Based Morphometry*		
	Years	Days	THC	Years	Days	THC
CSF	-.05	.13	.33	.09	.21	.27
GM	-.23	-.29	-.44*	-.03	-.29	-.42*
WM	.32	.09	-.07	.22	-.12	-.37
L Cere GM				.19	-.19	-.07
R Cere GM				.19	-.28	-.19
L Cere WM				-.08	-.07	-.06
R Cere WM				.04	.07	.07

L Acc	.15	-.04	-.32	-.04	-.08	-.22
R Acc	-.16	-.15	-.41*	.01	-.22	-.25
L Amyg	.21	-.18	-.03	-.18	-.14	-.22
R Amyg	-.01	-.14	-.12	.06	.03	-.16
L Caud	-.09	.20	.05	-.04	.17	.03
R Caud	-.22	.12	.19	-.04	.23	.07
L Hipp	.12	.02	-.06	.01	-.16	-.10
R Hipp	.26	.10	-.10	.15	-.06	-.05
L Pall	.15	.16	-.05	.15	.23	.19
R Pall	.07	.06	-.20	-.12	.26	.23
L Put	-.06	-.03	.01	-.16	.04	.03
R Put	-.17	-.18	-.18	-.24	.10	-.03
L Thal	.06	-.10	.02	-.04	-.14	-.20
R Thal	.06	-.22	-.03	.12	-.25	-.22
Average	.03	-.04	-.11	.01	-.04	-.10

*p<.05 uncorrected; L=Left, R=Right; CSF=cerebrospinal fluid, GM=gray matter, WM=white matter, Cere=cerebellum, Acc=accumbens, Amyg=amygdala, Caud=caudate, Hipp=hippocampus, Pall=pallidum, Put=putamen, Thal=thalamus; Average=average correlation not including CSF; Years=estimated years of regular marijuana use, Days=days of marijuana use on 90-day Timeline Follow-back, THC=estimated total THC consumption on 90-day Timeline Follow-back

Whole-Brain General Linear Models. Overall, negative associations were observed between marijuana use measures and brain structure. In Voxel-Based Morphometry models, two large clusters showed negative association (corrected p<.05) between TLFB days of use and volume in right occipital cortex and right cerebellum (see Table 14). Smaller diffuse clusters of negative association, typically in frontal and occipital regions, were observed for years of regular use and estimated total THC (uncorrected p<.001), with one small cluster of positive association between total THC and volume spanning bilateral thalamus (see Table 14 and Figure 13).

Table 14

Voxel-Based Morphometry clusters (≥100 voxels) associated with marijuana use measures.

Annotation[a]	Peak *t*	Cluster Size (mm^3)	MNI Coordinates[a] X	Y	Z	Partial Eta Squared[b]
Estimated years of regular use (uncorrected p<.001)						
R lateral occipital cortex	-4.96	152	58	-64	4	.47
L frontal orbital cortex	-5.02	112	-12	16	-28	.49
TLFB days of use (corrected p<.05)						
R occipital fusiform gyrus	-5.81	944	28	-88	-14	.55
R cerebellum crus I	-5.63	840	48	-76	-26	.50
TLFB estimated total THC (uncorrected p<.001)						
R inferior temporal gyrus	-4.40	312	46	-22	-34	.44
L occipital fusiform gyrus	-4.91	296	-36	-72	-16	.49
R thalamus	4.31	232	4	-24	8	.46
L superior frontal gyrus	-4.68	208	-20	10	46	.46
L supramarginal gyrus	-4.74	144	-58	-30	34	.49
R precentral gyrus	-4.51	120	40	-6	38	.49

[a]Peak association
[b]Calculated from cluster values in SPSS
L=Left, R=Right; TLFB=Timeline Follow-back

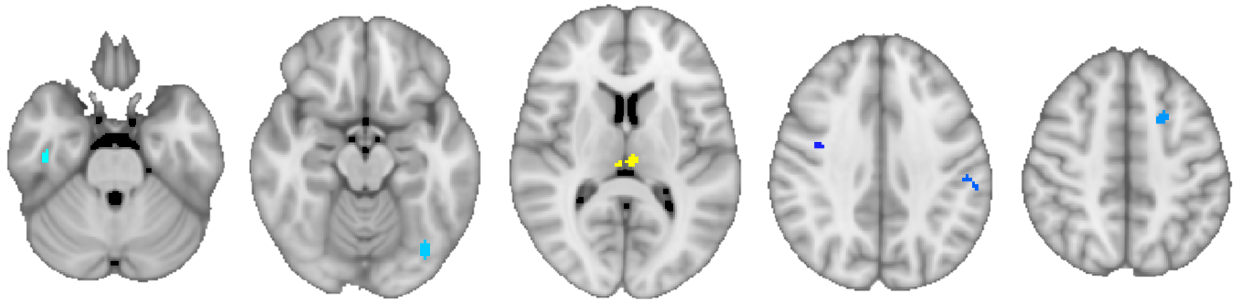

Figure 13. Voxel-Based Morphometry clusters associated with 90-day Timeline Follow-back estimated total THC consumption [From left, Z=-28, -16, 10, 36, 48].

In Surface-Based Morphometry models, two large clusters showed negative association (corrected *p*<.05) between TLFB estimated total THC and cortical volume in frontal and temporal regions (see Table 15 and Figure 14). Two other small clusters of negative association

(uncorrected $p<.001$) were observed between TLFB days of use and left frontal cortical volume and between total THC and right superior temporal cortical thickness.

Table 15

Surface-Based Morphometry clusters (≥100 voxels) associated with marijuana use measures.

Annotation[a]	Peak *t*	Cluster Size (mm^2)	Talairach Coordinates[a] X	Y	Z	Partial Eta Squared[b]
TLFB days of use						
Cortical Volume (uncorrected p <.001)						
L caudal middle frontal	-4.46	269.3	-34.1	26.1	41.4	.55
TLFB estimated total THC						
Cortical Thickness (uncorrected p <.001)						
L lateral occipital	-4.34	122.1	-20.5	-96.1	4.6	.46
Cortical Volume (corrected p<.05)						
L caudal middle frontal	-4.53	1437.0	-35.3	24.3	42.3	.44
R superior temporal	-3.46	958.3	48.8	-15.0	-2.8	.42

[a]Peak association
[b]Calculated from cluster values in SPSS
L=Left, R=Right; TLFB=Timeline Follow-back

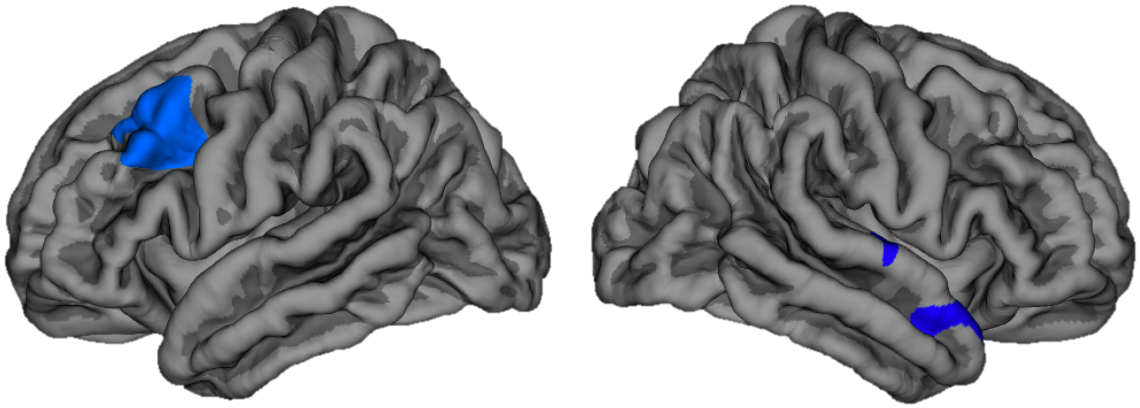

Figure 14. Surface-Based Morphometry clusters of cortical volume negatively associated with 90-day Timeline Follow-back estimated total THC consumption.

Marijuana Use Measures and Cognitive Function

Estimated years of regular marijuana use was consistently negatively correlated with cognitive performance (average r=-.32; see Table 16), and significantly associated with

Dimensional Change Card Sort score [$r(26)=-.53$, uncorrected $p<.01$], Pattern Comparison Processing Speed score [$r(26)=-.46$, uncorrected $p<.05$], and Total Composite score [$r(26)=-.51$, uncorrected $p<.01$], although these associations did not survive FDR correction across all correlations (all $p\geq.06$). Consistent negative correlations were also observed for past 90-day TLFB days of marijuana use (average $r=.21$), although only the correlation between days of use and List Sorting Working Memory score reached significance [$r(26)=-.43$, uncorrected $p<.05$]. No significant associations were observed between TLFB estimated total THC consumption and cognitive performances.

Table 16

Pearson's r correlations between marijuana use measures and cognition age standard scores.

	Years	Days	THC	Average
PV	-.17	-.08	.01	-.08
FIC	-.29	-.07	.16	-.07
LSWM	-.13	-.43*	-.30	-.29
DCCS	-.53**	-.02	.25	-.10
PCPS	-.46*	-.21	-.02	-.23
PSM	-.20	-.35	-.02	-.19
OR	-.24	-.22	-.19	-.22
Total	-.51**	-.31	-.01	-.28
Average	-.32	-.21	-.02	-.18

**$p<.01$ uncorrected, *$p<.05$ uncorrected; PV=Picture Vocabulary, FIC=Flanker Inhibitory Control, LSWM=List Sorting Working Memory, DCCS=Dimensional Change Card Sort, PCPS=Pattern Comparison Processing Speed, PSM=Picture Sequence Memory, OR=Oral Reading, Total=Total Composite; Years=estimated years of regular marijuana use, Days=days of marijuana use on 90-day Timeline Follow-back, THC=estimated total THC consumption on 90-day Timeline Follow-back

Summary of Aim 3 Results

Participants within the marijuana user group reported either being lifetime regular users or short-term users who had used regularly post-retirement or since legalization. Reported use

patterns did not appear to be consistent with problematic use (i.e., cannabis use disorder). Lifetime users reported marginally greater number of marijuana use days out of the past 90 days, but did not report consuming a significantly higher amount of estimated THC than short-term users during the past 90 days.

Groups were then examined for differences in brain structure and cognitive function. Short-term users showed small clusters of greater volume than lifetime users in bilateral postcentral gyrus and bilateral occipital pole, but groups were otherwise not different in terms of brain structure. In terms of cognitive function, lifetime users performed significantly worse than short-term users on an executive function task, a processing speed task, and cognition total composite score. In post hoc analyses including both user groups and non-using controls, lifetime users performed poorly compared to both short-term users and controls in executive function and poorly compared to controls in total composite score, but short-term users showed no significant differences compared to controls.

Specific measures of marijuana use were then examined for associations with brain structure and cognitive function. Of estimated years of regular use, days of use out of the past 90 days, and past 90-day estimated total THC consumption, only estimated total THC consumption showed negative association with total gray matter volume. Past 90-day THC consumption and days of use showed consistent negative associations with brain structure in clusters frequently localized in frontal and occipital regions. Finally, estimated years of regular marijuana use showed consistent negative associations with cognitive performance, again in executive function, processing speed, and total composite score. Past 90-day measures appeared to be more related to poorer working memory performance.

CHAPTER IV

DISCUSSION

Overview

The current study sought to clarify the extent to which long-term marijuana use is associated with brain structure and cognition. The study aimed to compare adults age 60 years and older who were current marijuana users to healthy non-using controls in terms of global brain structural measures and cortical and subcortical gray matter. Groups were also compared in terms of their current cognitive functioning on a brief measure of attention, executive function, working and episodic memory, vocabulary, oral reading, processing speed, and general cognition. Associations between cognition measures and brain structure were also explored in order to provide a functional context for interpretation of group differences in brain structure. Finally, marijuana use was further examined by comparing groups of participants who reported regular marijuana use over their lifetime or over the last several years, and by exploring structural and cognitive associations of long-term (years of use) and short-term (past 90-day THC consumption and days of use) measures of marijuana use.

A summary of study results is presented in Table 17. Results were partially consistent with the hypothesis that marijuana users would show no differences in gray and white matter volumes or cortical thickness compared to non-using controls. Marijuana users and controls were not different in terms of global brain structural measures, but groups did show areas of diffuse

Table 17

Summary of results across study aims according to brain region.

	Aim 1	Aim 2	Aim 3
Total Gray Matter			- THC
Cerebellum	Controls > Users	+ PSM	- Days
Subcortical Structures			
Thalamus		+ PSM	+ THC
Putamen	Users > Controls		
Accumbens		- DCCS	- THC
Occipital Regions			
Lateral occipital cortex, occipital pole	Controls > Users		Short > Lifetime; - Years, - Days, - THC
Lingual gyrus	Users > Controls		
Temporal Regions			
Middle temporal gyrus, temporal fusiform cortex	Users > Controls		
Inferior temporal gyrus	Users > Controls (left)		- THC (right)
Parietal Regions			
Precuneus	Users > Controls (inferior)	+ DCCS (superior)	
Postcentral gyrus	Controls > Users	+ PSM, + DCCS	Short > Lifetime
Supramarginal gyrus			- THC
Frontal Regions			
Anterior cingulate gyrus		- DCCS	
Precentral gyrus	Controls > Users	+ PSM	- THC
Superior frontal gyrus			- THC
Inferior frontal gyrus		+ DCCS	
Middle frontal cortex	Users > Controls (rostral)		- Days, - THC (caudal)
Frontal pole	Controls > Users	+ PSM	
Orbitofrontal cortex	Users > Controls		- Years

+ = positive association, - = negative association; PSM = Picture Sequence Memory, DCCS = Dimensional Change Card Sort; THC = Timeline Follow-back estimated total THC consumption, Days = Timeline Follow-back days of marijuana use, Years = estimated years of regular marijuana use

difference throughout the brain. However, results appeared in both directions and did not support one group consistently showing greater volume or cortical thickness than the other. It was also hypothesized that marijuana users would show poorer memory than non-users, but no other group differences. Results were supportive of this hypothesis in that marijuana users showed slightly poorer (i.e., less than one standard deviation difference in standard score between groups) working memory than controls. It was further hypothesized that brain structural measures would be positively associated with cognitive function. Positive associations between structural measures and cognitive performance were observed for measures of episodic memory and executive function, but this was not an entirely consistent pattern in that two additional regions showed negative association between volume and executive function. Finally, despite some overlap in terms of larger anatomical regions between clusters showing group difference and clusters associated with cognitive performance, user groups did not show structural differences in extracted clusters associated with episodic memory or executive function.

The final aim of the study was to characterize associations between measures of marijuana use and brain structure and cognitive function. Participants within the user group reported either being long-term, lifetime users or using regularly for only the last several years, but across groups participants did not generally report symptoms of cannabis use disorder. In terms of recent use, short-term and lifetime users did not differ in estimated THC consumption over the last 90 days, and lifetime users reported consuming marijuana on slightly more days out of the past 90 days than short-term users. It was hypothesized that short-term users would show better memory performance than lifetime users, but no brain structural differences. Short-term and lifetime users did show localized brain differences such that short-term users had greater volume in bilateral postcentral gyrus and bilateral occipital pole, regions which were previously

shown to have greater volume in controls compared to users. The hypothesis that lifetime users would show poorer memory was not supported; rather, lifetime users performed significantly worse than short-term users in executive functioning and processing speed, which also impacted general cognition score. Interestingly, when also compared to non-using controls, lifetime users performed poorly compared to both short-term users and controls in executive function and poorly compared to controls in total composite score, but short-term users showed no significant differences compared to controls. Estimated total THC consumption did show negative association with total gray matter volume, as well as negative association with diffuse clusters in whole-brain models (see Table 17). In comparison, estimated years of regular marijuana use showed association with brain structure in only two small clusters. In terms of cognitive performance, years of regular marijuana use showed consistent negative associations with cognitive performance in executive function, processing speed, and total composite score. The hypothesis that greater THC consumption would be associated with poorer memory performance was not clearly supported, as days of use out of the last 90 days was negatively associated with working memory but THC consumption associations were nonsignificant.

Overall, regions that emerged as significant across study aims were precentral and postcentral gyri, and cerebellum (crus I and I-IV; see Figure 15). These regions showed volume

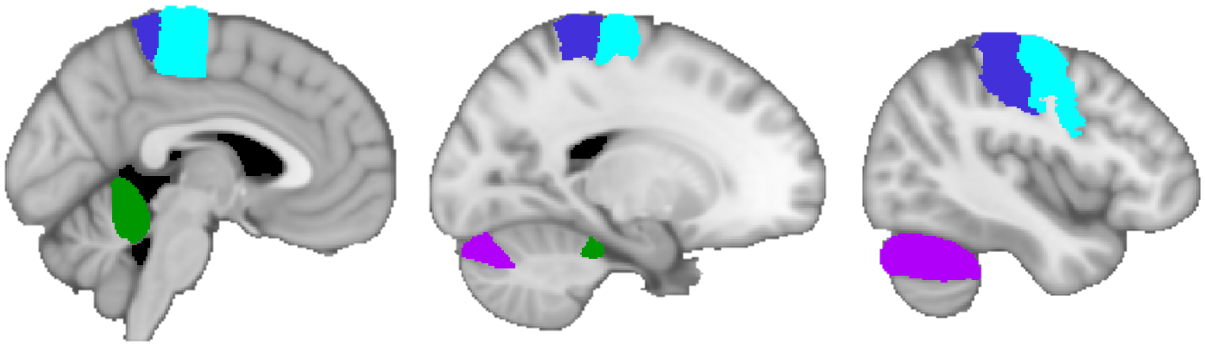

Figure 15. Regions that emerged across study aims showing associations with measures of marijuana use and cognitive function [precentral gyrus, cyan; postcentral gyrus, blue; cerebellum crus I, purple, and I-IV, green; X=-3, -18, -42].

greater in controls than users; positive association between volume and cognitive performance in episodic memory or executive function; and volume greater in short-term users compared to lifetime users or negative association with past 90-day use measures.

Marijuana Users versus Controls

Brain Structure

In the context of marijuana use that was regular for approximately 24 years on average, marijuana users did not differ from controls in terms of global brain structural measures. This is generally consistent with studies suggesting that marijuana use has no effect on cortical volume in young adults (Orr, Paschall, & Banich, 2016), but the current study is the first to document this finding among older adults. Cortical atrophy occurs over the course of aging and results in increasing cerebrospinal fluid and decreasing cerebral volume (Pfefferbaum et al. 1994; Sowell, Thompson, & Toga, 2004), and the results of the current study suggest that marijuana use does likely not have a strong neurodegenerative impact on overall cortical volume beyond aging. This is in contrast to the well-documented effects of alcohol on cortical volumes beyond age (e.g., Thayer et al., 2016), and it is important to note that both groups in the current study reported relatively low alcohol consumption on average and no history of alcohol use disorder.

However, it remains likely that long-term marijuana use has a more subtle impact on brain structure. A recent systematic review of 31 structural neuroimaging studies (Lorenzetti, Solowij, & Yücel, 2016) reported consistent effects of altered structure (i.e., most frequently decreased volume or decreased cortical thickness) in users compared to non-using controls across hippocampus, prefrontal cortex, and amygdala. These regions are all relatively dense in CB1 receptors, and preclinical models consistently suggest THC is neurotoxic to hippocampus, amygdala, striatum, and lateral prefrontal cortex and anterior cingulate. Regions that were

reported with less frequency included insula, parietal (e.g., paracentral gyri, inferior parietal cortex; Lopez-Larson et al., 2011), temporal (e.g., temporal pole, superior temporal gyrus; Gilman et al., 2014), and occipital (e.g., fusiform gyrus; Matochik et al., 2005) regions. Cerebellum showed mixed results [volume decreased compared to controls in Medina et al. (2010) but greater than controls in Solowij et al. (2011) and Cousijn et al. (2012)]. Of the studies included in the review, the highest mean age was 40 years, but four studies included participants up to age 60, and the greatest reported years of regular use was 23 years.

In the current study, controls did show diffuse small clusters of greater volume than users throughout the brain consistent with regions reported above, including cerebellum, postcentral and precentral gyri (e.g., Lopez-Larson et al., 2011), and frontal pole (e.g., Gilman et al., 2014) in addition to superior lateral occipital cortex. These regions are implicated in the sensorimotor and executive control networks (Shirer et al., 2012). In comparison, users showed greater volume than controls in putamen, lingual gyrus, middle and inferior temporal gyrus, precuneus, middle frontal cortex, and orbitofrontal cortex, which are implicated in default mode and salience networks (Shirer et al., 2012) as well as models of substance use (e.g., precuneus and orbitofrontal cortex; e.g., Durazzo et al., 2011). Interestingly, serious symptoms of chronic alcoholism (e.g., withdrawals and relapses) have been shown to be negatively correlated with gray matter volume in regions including the orbitofrontal cortex (Durazzo et al. 2011; Le Berre et al. 2014), which contrasts with current study results. Given that the user group did not report symptoms of cannabis use disorder on average and generally reported using marijuana for benign reasons (e.g., enjoying the feeling, being more creative or feeling greater connection), it is worth further investigating whether these regions of greater volume represent a beneficial impact among older adults in future studies.

Groups were not different in terms of hippocampal or amygdala volume, which was somewhat surprising given the relative consistency of these effects in the literature. This may reflect that the level of use reported in this sample (i.e., more than 20 years of regular and recreational use on average but not representative of cannabis use disorder) was lower than the heavy use reported in other studies. For example, in one of the few studies with an average age above the 20s (mean age of 40 years; Yücel et al., 2008), marijuana users reporting at least 10 years of heavy use (here defined as smoking more than 5 joints daily; mean duration of regular use of 20 years on average) showed bilaterally reduced hippocampal and amygdala volumes compared with non-using controls. However, several more recent studies provide important considerations for reported hippocampal changes. The same research group (Yücel et al., 2016) reported that marijuana users showed 11% smaller hippocampal volume than controls, but further investigation suggested users who were also exposed to CBD or were abstinent from use did not differ from controls. Another study among young adults (mean age approximately 21 years) compared heavy users (i.e., smoked marijuana approximately 5 days per week) and controls at baseline and approximately 3 years later, and found no differences in hippocampal volumes as increases over time were observed in both groups (Koenders et al., 2017). Finally, the authors of a systematic review of adult marijuana use concluded that many studies provided evidence for hippocampal changes, but evidence was insufficient to clarify whether those findings represented a consequence of marijuana use or premorbid group differences (Nader & Sanchez, 2017). Given that the marijuana user group in the current study included participants reporting a considerable duration of use (i.e., up to 50 years) and reasonably controlled for depression symptoms and age via covariates and other factors via exclusionary criteria (e.g.,

general health factors, history of alcohol use disorder), it seems plausible that factors other than marijuana use may largely contribute to existing hippocampus and amygdala findings.

Cognitive Function

In comparing marijuana users and controls, the only area of group difference in cognitive function was working memory, such that users showed slightly poorer (i.e., less than one standard deviation difference in standard score between groups) performance than controls. These results align with the existing literature that finds poorer performance for users in verbal learning and memory across group comparison studies, but these differences tend to be of relatively small magnitude and thus less likely to have a strong impact on daily functioning. In particular, THC has been shown to negatively affect encoding of verbal information as opposed to retrieval, such that acute THC did not impact memory for information learned prior to acute administration (Ranganathan et al., 2017). However, chronic THC exposure may impact both encoding and retrieval of verbal information. Broyd and colleagues (2016) conducted a systematic review of neuropsychological findings in acute and long-term cannabis use, and found that attention and verbal learning and memory were most impacted by both acute and chronic exposure. It is important to note that in the current study, the group difference in working memory occurs in the context of no differences in episodic memory, attention, executive function, processing speed, or language (oral reading, vocabulary), and all age standard scores across both groups fell within the average range overall.

Despite findings suggesting some differences in cognitive function based on duration of marijuana use, a consistent pattern did not clearly emerge linking associations between cognitive function and brain structure and those observed with marijuana use. Global cortical thinning and gray matter volume loss, as well as other indicators of atrophy such as ventricle size, are

associated with poorer neuropsychological test scores and cognitive decline with aging (Benedict et al. 2006; Draganski et al. 2013; Zivadinov et al. 2001). Positive associations between structural measures and cognitive performance were observed for episodic memory and executive function in postcentral gyrus clusters in particular, but user groups did not show a significant difference in extracted cluster values. In terms of larger anatomical structures, however, regions where controls showed greater volume than users were positively associated with performance in episodic memory and executive function, whereas regions of greater volume in users did not appear to overlap with cognition clusters (see Table 17). Direct statistical models of these relationships could be explored in larger sample sizes with adequate power.

Marijuana Use Metrics

Years of Regular Marijuana Use

First, estimated years of regular marijuana use was used as a basis for further group analyses comparing short-term and lifetime marijuana users. In terms of brain structure, short-term users showed localized greater volume compared to lifetime users in bilateral postcentral gyrus and bilateral occipital pole, regions which were previously shown to have greater volume in controls compared to users. In terms of cognitive function, short-term users performed significantly better than lifetime users in executive function and processing speed as well as total cognition. Interestingly, further investigation including the initial control group suggested that short-term marijuana use did not appear to have an impact on cognitive function overall. Not surprisingly, differences across controls, short-term, and lifetime users were observed in executive function and processing speed, and driven by those scores, total cognition; but these differences were generally driven by lifetime users, as short-term users and controls were not statistically different in term of age standard scores. Lifetime users showed scores below

expectation in processing speed and executive function in the context of educational attainment at the college level and above average vocabulary, which is a common and validated estimate of premorbid cognitive function and tends to be resistant to change over time (Lezak et al., 2012). Most strikingly, processing speed for lifetime users was borderline impaired, and executive function was low average. Lifetime users also showed episodic memory at the low end of the average range. These findings are consistent with studies suggesting psychomotor function and executive functions are impaired in acute intoxication but may persist in chronic users (Broyd et al., 2016). Group difference in total cognition score also may suggest a more generalized influence or mild changes in daily functioning for lifetime users.

When treated as a continuous measure within the marijuana user group, estimated years of regular use was consistently negatively associated with cognitive function but did not show compelling associations with brain structure. Small clusters in occipital cortex and orbitofrontal cortex were negatively associated with years of regular use. Decreased volume in orbitofrontal cortex is consistent with a study showing smaller medial and lateral orbitofrontal cortex among dependent marijuana users, although overall marijuana users were not different from non-using controls in orbitofrontal cortex volume (Chye et al., 2017). This finding is also somewhat consistent with a systematic review supporting associations between duration of regular use and prefrontal cortex and hippocampus but not amygdala, parahippocampal gyrus, cerebellum, or striatum, although the review also notes that most studies did not examine specific measures of marijuana use (Lorenzetti, Solowij, & Yücel, 2016). In terms of cognitive performance, years of regular marijuana use paralleled group analyses in showing negative associations with executive function, processing speed, and total cognition score.

Past 90-Day THC Consumption and Days of Use

Measures of marijuana use in the last 90 days were also examined as continuous measures. Days of use was negatively associated with volume of fusiform gyrus, cerebellum, and caudal middle frontal cortex. This result parallels a recent study finding that higher marijuana use (here defined as average amount smoked in grams per day multiplied by days per week used over the past month) was associated with lower volume of entorhinal cortex and fusiform gyrus (Thames et al., 2017). In comparison, estimated total THC consumption in the past 90 days showed negative association with total gray matter volume, as well as negative association with diffuse clusters in whole-brain models (see Table 17).

For cognitive function, days of use in the last 90 days was negatively associated with working memory but THC consumption associations were nonsignificant. This finding is somewhat inconsistent with existing studies that have suggested influence of THC in particular on cognitive function, but it also underlines the importance of improved quantification of marijuana consumption in future studies. In human research, greater acute cognitive impairments were associated with use of higher THC/lower CBD strains (Colizzi & Bhattacharyya, 2017), which may extend to longer-term exposure, and higher THC strain content was associated with greater symptoms of addiction (Curran et al., 2016). Further, total grams consumed over the last month was associated with lower global cognition score (average score across attention/working memory, processing speed, verbal fluency, learning and memory, and executive function; Thames et al., 2017). One preclinical study found that THC administration in rats was associated with choosing to exert less cognitive effort on a task, and co-administration of CBD resulted in modest improvement over THC effects (Silveira et al., 2016). Sustained CB1 activation leading to downregulation is likely a strong contributing factor for cognitive deficits in chronic users, but

it is also the case that cognitive impairments do not generally last beyond 4 to 6 weeks of abstinence, which directly maps onto the timeline of recovery of downregulation (Ceccarini et al., 2015). This mechanism is especially linked with modest cognitive impairments of long-term marijuana use in aspects of verbal learning and memory (Curran et al., 2016; Mizrahi, Watts, & Tseng, 2017).

The findings of the current study are particularly interesting given that measures of recent use appeared to show more consistent association with brain structure than cognitive function, and the measure of long-term exposure to marijuana showed more consistent associations with cognitive function than structural measures. This pattern may suggest long-term alterations at the synaptic level (e.g., Ceccarini et al., 2015) more so than in overall neural structure with more moderate levels of use on average. Further studies could examine models including measures of both short-term and long-term use to further clarify effects, as the variability among findings in the current literature may be partially explained by studies capturing short-term versus long-term associations.

Strengths and Limitations

The current study attempted to address many of the pitfalls that have limited prior research examining associations between marijuana use and brain structure (e.g., use of other drugs like alcohol, mental health problems; Curran et al., 2016). In particular, exclusionary criteria were selected to reduce potential contributions of heavy alcohol use history (e.g., Weiland et al., 2015) and medical conditions or history of serious mental illness with known cognitive effects. Control and marijuana user groups were carefully examined for differences in reported recent alcohol use and contributions of mental health concerns of depression and anxiety, as well as the major contributing factor of age to brain structure (e.g., Thayer et al.,

2016). The study also included collection of cognitive data that offer high functional value and clear comparisons to healthy aging individuals, so that in using age standard scores, age is already controlled in analyses of possible group difference. Another common limitation of existing research is lack of baseline data for cognitive functioning (Volkow et al., 2016), which is certainly still true for this study, but estimates of premorbid functioning are common in clinical usage and rely on expectations of prior cognitive functioning based on educational attainment and vocabulary (Lezak et al., 2012). Educational attainment and vocabulary age standard scores were similar across groups and commensurate with above average premorbid functioning, which provides a more meaningful context for consideration of the few lower cognitive performances among lifetime users. In terms of neuroimaging analyses, including both common approaches to analyzing structural data (i.e., FreeSurfer's Surface-Based Morphometry measures and FSL's Voxel-Based Morphometry and FIRST output) provides greater opportunities to compare results to the existing literature. Finally, in the context of careful consideration of these factors, the current study offers an innovative opportunity to examine potential harms or benefits of recreational marijuana use given its focus on an aging population.

On the other hand, the current study is highly exploratory in nature, and it is therefore especially important to consider general limitations and factors that could influence interpretability of the current results. Marijuana-using adults did not complete the physical health assessment that was required for control participants in the FORCE study (i.e., to rule out contraindications to exercise such as cardiac abnormalities), and it is therefore possible that the user group contained a disproportionate number of participants with undetected health concerns that could influence neuroimaging or cognitive data. In particular, about a third of marijuana users reported sleep concerns as one reason for their marijuana use, which may reflect health

problems that could confound results (e.g., obstructive sleep apnea; Andreou, Vlachos, & Makanikas, 2014). The R36 study requested that all participants refrain from consuming marijuana for at least 6 hours prior to study participation, but toxicology data were not collected and it is impossible to rule out the potential influence of acute exposure on study results. User and control groups in the initial group analyses were also marginally different in terms of proportion of males and females in each group, and previous research has suggested a differential impact of THC according to sex due to possible interactions with the endocrine system (Ketcherside, Baine, & Filbey, 2016). Sex effects were not examined, but could be explored in future studies.

It also remains unclear whether brain structural effects of marijuana use are in any way causal versus premorbid given the cross-sectional nature of most studies. This issue has been examined more thoroughly among young adult populations. For example, one longitudinal study (Koenders et al., 2016) among young adults found no changes with continued marijuana use between neuroimaging sessions 3 years apart in volume of orbitofrontal cortex, anterior cingulate cortex, insula, striatum, thalamus, amygdala, hippocampus, or cerebellum, but cross-sectional analyses at both time points suggested negative associations between recent consumption in grams of marijuana and hippocampus, amygdala, and superior temporal gyrus volumes. The authors suggested those findings could represent premorbid risk factors for substance use (Koenders et al., 2016). Large consortium projects are currently collecting longitudinal neuroimaging and substance use data among adolescent populations, but older adults remain under-studied. A longitudinal study among older adult users would be extremely useful in further examining harms versus benefits of marijuana use on cognitive function in particular.

One of the most significant general limitations was lack of power. Only the first aim was fully powered to detect an effect of moderate size, and due to limitations in collecting a sufficient number of controls with cognitive data, subsequent aims were decidedly underpowered. Estimated power was only .50 to detect moderate effect sizes within the user group (calculated in G*Power 3.1; Faul et al., 2009). As such, it is not surprising that few neuroimaging results exceeded thresholds including whole-brain corrections for multiple comparisons. However, these preliminary neuroimaging results are still useful for examining potential patterns in the data for further study, and it is important to note that several whole-brain results did meet accepted cutoffs for significance thresholding (Aim 1: users showed greater volume than controls in lingual and rostral middle frontal cortex in FreeSurfer; Aim 2: positive associations between cognitive performance and FreeSurfer cortical thickness and volume in postcentral gyrus, pars triangularis, and precuneus; Aim 3: days of use negatively associated with occipital cortex and cerebellum in FSL, and THC negatively associated with caudal middle frontal and superior temporal volume in FreeSurfer).

Similarly, cognition analyses were likely impacted both by limited sample size and by somewhat limited construct validity. The NIH Toolbox Cognition Battery (Gershon et al., 2013) is well-validated in terms of norms but likely best conceptualized as a cognitive screen, and power to detect cognitive associations was likely impacted by having only a single estimate of performance in each of the tested cognitive domains. Further, it is difficult to determine whether poorer cognitive performance reflects a true deficit versus lower motivation during testing (e.g., Silveira et al., 2016). Thorough examination of the associations between marijuana use and cognitive function would require more comprehensive neuropsychological testing.

Finally, the most significant limitations of the current study relate to challenges in quantifying marijuana use. This is a well-known limitation across the literature both in terms of estimating long-term use based on subjective memory and in terms of measuring acute use in the absence of simple measures similar to breath alcohol content (Volkow et al., 2016), but in particular the current study was impacted by the number of users reporting consuming marijuana primarily through edibles versus smoking. Although formulas exist for equating exposure considering factors such as how much THC is lost burning a joint (e.g., suggested by the State of Colorado and used in the current study; Orens et al., 2015), consuming THC via edibles versus smoking results in different rates of metabolism (e.g., Newmeyer et al., 2017) and therefore likely impacts the concentration of THC available to reach the brain. All analyses using past 90-day estimated THC consumption should be cautiously interpreted. Given the popularity of using edibles in the current study (i.e., 43% of users reported primarily consuming edibles or using both edibles and smoking), further study of this issue is clearly warranted and future analyses could examine differences between smokers and edible users.

Future Directions

Findings in Related Research

Functional MRI. Briefly, potentially due to the inconsistent findings in the literature across structural neuroimaging studies, there has been great interest in whether marijuana use shows the same pattern of alterations in functional response to tasks and cerebral blood flow as other substance use. In the current study, symptoms of cannabis use disorder were low even among the lifetime users, which supports that use was primarily at a recreational level, although it is also possible that symptoms that might otherwise be endorsed (e.g., interfering with responsibilities) may be less relevant in a population that was largely not employed full-time

(i.e., 75% of the user group was retired or working part-time). A recreational level of use might not be expected to show the same functional patterns as in individuals reporting lack of control of their substance use, but examining patterns of brain function among recreational users could further clarify the potential harms versus benefits of use and relationships with cognitive function. Given that group differences in structure appeared to occur in regions associated with identified functional networks (e.g., users showing greater volumes in regions of default mode and salience networks and controls showing greater volume in regions of sensorimotor and executive control networks), a future line of research could examine this pattern in task-based or functional connectivity data. Marijuana use has been associated with alterations in anticipatory reward (Martz et al., 2016) including sensitization to cannabis cues (Filbey et al., 2016), but again these findings are among young adult populations. In a recent study utilizing SPECT scanning to examine cerebral blood flow, marijuana users showed lower perfusion on average compared to non-using controls, and lower perfusion in right hippocampus was most predictive in distinguishing users from controls (Amen et al., 2017). Given lower perfusion of blood flow overall, this finding could suggest a more vascular than cortical profile for cognitive and structural changes and point to greater involvement of white matter changes than cortical change with marijuana use.

Diffusion Tensor Imaging. Alterations in white matter microstructure have also been associated with marijuana use, and in particular diffusion tensor imaging may best capture anti-inflammatory properties of CBD and inflammatory properties of THC in terms of potential changes to myelin. Briefly, gray matter structural alterations in areas rich in CB1 receptors could account for downstream changes in areas low in CB1 receptor density (e.g., parietal cortex) via impaired functional and structural connectivity (Lorenzetti, Solowij, & Yücel, 2016), or it could

be the case that white matter is more vulnerable to changes in cerebral blood flow (Amen et al., 2017). Users of high potency cannabis demonstrated poorer white matter microstructure in the corpus callosum compared to low-potency users and non-using controls, and daily users also demonstrated poorer microstructure than occasional or non-users (Rigucci et al., 2016). An examination of continuing heavy use over time among young adults also suggested heavy marijuana use was associated with both alterations in white matter integrity and impairments in verbal learning (Becker et al., 2015). The finding in the current study that lifetime users performed especially poorly in processing speed suggests an impact on white matter, and occasional associations with white matter volume were observed (i.e., controls greater than users in volume of the genu of the corpus callosum; positive association between cerebellum white matter volume and episodic memory). Processing speed deficits are often observed in cognitive decline associated with white matter lesions (Lezak et al., 2012), which is particularly interesting given that exclusionary criteria addressed medical conditions with major vascular impacts (e.g., diabetes). Future studies measuring both white matter microstructure and cognitive performance could potentially best clarify associations between cognitive function and marijuana use, especially in a developmental context where marijuana use in adolescence and young adulthood is linked to poor verbal learning (e.g., and provides a possible link to marijuana use being associated with poorer educational attainment overall; Volkow et al., 2016), but marijuana use in older adults may have greater impact on other cognitive functions as suggested by the current study. In speculation, if white matter associations are found among older users, the potential vascular cognitive pattern of marijuana use could be further elucidated by examining participants who primarily consume marijuana by smoking versus edibles, in which case smokers may show poorer white matter integrity than users of edibles.

Neurodegenerative Conditions. Briefly, there is increasing interest in cannabis as a source of new pharmacologic interventions for neurodegenerative conditions, as marijuana use has shown potential in reducing neuroinflammation and behavioral disturbances in models of Alzheimer's disease (e.g., Ahmed et al., 2015 for review). An equal ratio of THC to CBD administration was shown to be beneficial in a preclinical model of Alzheimer's pathology (Aso et al., 2016), and chronic low doses of THC were associated with improvements in cognitive function and increased hippocampal neural spine density in older mice (Bilkei-Gorzo et al., 2017). These studies underlie the importance of continuing to examine potential benefits of chemical compounds in cannabis, particularly for older adults facing accelerated cognitive and functional decline.

Conclusions

In sum, current study results suggest that marijuana use among older adults is not likely associated with global differences in brain structure, but may be associated with subtle diffuse differences that should be further investigated for possible detriment versus benefit. In group comparisons of cognitive performance, working memory performance was lower in users than controls, but when users were grouped according to duration of use, processing speed and executive function along with total cognition emerged as areas of lower function for lifetime users. In terms of brain structure, regions where controls showed greater volume than users appeared to overlap with anatomical regions associated with executive function and episodic memory performance. Finally, group differences in cognitive function were driven by lower performance of lifetime users compared to short-term users or controls, but whether in group analyses or treated as continuous, long-term marijuana use did not show large effects on brain structure. In comparison, measures of recent marijuana use were associated with brain structure

such that days of use and estimated THC consumption in the last 90 days showed an overall pattern of negative association with gray matter volumes.

The current study is an important contribution to the field in terms of addressing several of the common limitations of existing research and providing innovation in exploring marijuana use in a novel and growing population. In a recent study examining survey data from 47,140 adults age 50 years or older in the United States (Han et al., 2017), there was a significant increase in marijuana use from 2006 to 2013, with a 250% relative increase among adults age 65 years or older over that time. Further, 6.9% of older adult marijuana users met criteria for cannabis use disorder (Han et al., 2017). Given greater prevalence in use among older adults, it is important to clarify interactions among marijuana use and brain structure and function to inform health recommendations. The current study suggests that lifetime marijuana use at a recreational level does not have a strong and consistent effect on brain structure in comparison to substances like alcohol, but it does appear to have a negative association with aspects of cognitive functioning. Future studies should examine harm versus benefit of edible versus smoking consumption as well as collect detailed assessment of strain THC versus CBD composition, especially considering evidence that cognitive impairments related to THC may be offset by CBD (Broyd et al., 2016). Similarly, differences in brain structure observed in other studies have suggested that users exposed to CBD in addition to THC did not differ from controls, and observed effects may recover with abstinence (Yücel et al., 2016). From a harm reduction perspective, it is valuable to note that any cognitive harms associated with long-term marijuana use may be reduced by consuming strains with lower THC concentrations with less frequency.

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