

Altered brain tissue composition in heavy marijuana users

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Abstract

Marijuana is the most widely used illicit substance in the United States; however, previous imaging studies have not detected altered brain structure in marijuana users compared to non-users. Voxel-based morphometry was used to investigate possible differences in brain tissue composition in a group of 11 heavy marijuana users and a group of 8 non-users. All participants were male. Statistical comparisons were made at the voxel level on T1-weighted magnetic resonance images to determine differences in gray matter and white matter tissue density. Compared to non-users, marijuana users had lower gray matter density in a cluster of voxels in the right parahippocampal gyrus ($P = 0.0001$), and greater density bilaterally near the precentral gyrus and the right thalamus ($P < 0.04$). Marijuana users also had lower white matter density in the left parietal lobe ($P = 0.03$), and higher density around the parahippocampal and fusiform gyri on the left side compared to non-users ($P < 0.002$). Longer duration of marijuana use (in years) was significantly correlated with higher white matter tissue density in the left precentral gyrus ($P = 0.045$). Our preliminary results suggest evidence of possible structural differences in the brain of heavy marijuana users, and localize regions for further investigation of the effects of marijuana in the brain.

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1. Introduction

Marijuana (*Cannabis sativa*) is the most widely used illicit substance in the United States, and its prevalence of use in other countries is also high. According to estimates for the year 2000, almost 1 in 20 persons (nearly 11 million) used marijuana within the past 30 days (Substance Abuse and Mental Health Services Administration, 2001). Estimates also suggest that approximately 2.4 million persons in the United States used marijuana in 2000 for the first time. There have been several debates on the possible behavioral and physiological consequences of marijuana use and abuse. Negative effects of heavy marijuana use include

impairments in short-term memory, attention, and coordination of movement (Bolla et al., 2002; Onaivi, 2002; Solowij, 1998). Even though marijuana has been classified as an illegal substance in the United States, efforts have been made to promote possible medical applications (Nahas et al., 1999). For example, acute administration of medicinal marijuana and its cannabinoid constituents has been recommended as a treatment for multiple sclerosis-related symptoms (e.g., muscle spasm and tremor) and glaucoma, as an appetite stimulant, or as an anti-emetic agent for medication-induced emesis in cancer patients (Williamson and Evans, 2000). Therefore, better description and elucidation of the behavioral and physiological effects of marijuana is of paramount importance for us in order to make informed decisions about its use.

A number of imaging studies conducted in the 1970s and 1980s failed to demonstrate any significant structural differences in the brain of marijuana users when compared to

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non-users. The original finding in 1971 of marijuana-associated cerebral atrophy (Campbell et al., 1971), evidenced by increased ventricular size using air encephalography, was not subsequently replicated using the then newly developed technique of computed axial tomography (Co et al., 1977; Hannerz and Hindmarsh, 1983; Kuehnle et al., 1977). The development of magnetic resonance imaging (MRI) to visualize brain structure and the contrast between gray matter and white matter tissue and cerebrospinal fluid has led to the application of image processing techniques to quantify various parameters of brain regions of interest (e.g., region volume). In 2000, Block and colleagues (Block et al., 2000), using volumetric MRI, did an analysis of global (whole brain) and regional volumes of the cortical lobes, and found no structural differences in marijuana users compared to a non-using comparison group. The only other study (Wilson et al., 2000) that used MRI to evaluate morphological changes in marijuana users found a lower volume of whole brain gray matter and higher white matter volume, but no differences in whole brain or ventricular volumes in users who started using marijuana before age 17 compared to individuals who started using at age 17 or later. This study did not include a non-using comparison group.

With more recent advances in the analysis of structural MR images, we thought it opportune to again address the question of possible structural alterations in the brain of marijuana users. Voxel-based morphometry is an automated and non-biased method to evaluate brain tissue composition (gray matter or white matter) differences on a voxel-by-voxel basis using a measure of tissue density or concentration (Ashburner and Friston, 2000). Volumetric measurements and tissue density measures are not equivalent, since a change in tissue density within a region could occur without a corresponding volumetric change. Voxel-based morphometry has the advantage of being able to detect differences in tissue composition at the voxel level that may not be apparent with analysis of regional volumes or from visual examination of MR images.

In the present study, we investigated tissue composition differences in both gray matter and white matter in a group of heavy marijuana users, abstinent for 20 days, and a non-using comparison group. Based on our previous work showing alterations in neurocognitive performance (Bolla et al., 2002) and cerebral blood flow (Bolla et al., submitted), we hypothesized that heavy marijuana users would show alterations in brain tissue composition, especially in the hippocampus, an area rich in cannabinoid receptors (Glass et al., 1997).

2. Methods

2.1. Participants

The Institutional Review Boards of the National Institute on Drug Abuse and the Johns Hopkins Medical Institutions approved the research protocol. All participants, recruited through community advertisements, gave written informed

Table 1
Demographic characteristics of participants

A	Comparison group (<i>n</i> = 8)	Marijuana group (<i>n</i> = 11)
Age (years)	29.7 ± 4.7	25.4 ± 5.0
Range	22–34	21–35
Education (years)	13.9 ± 2.7	12.8 ± 1.9
Shipley IQ	103.7 ± 8.6	101.6 ± 7.8
SES	4.4 ± 1.4	4.2 ± 1.5
Marijuana use/week (joints)	–	34.7 ± 17.6
Range	–	8–63
Marijuana use duration (years)	–	7.5 ± 5.5
Range	–	2–22
Marijuana start age (years)	–	15.7 ± 2.5
Range	–	12–21
Alcohol use/week (drinks)	1.5 ± 2.0	1.4 ± 2.2
Ethnicity		
African American	6	7
Asian	–	–
Caucasian	2	2
Hispanic	–	2

Values are expressed as mean ± S.D. or as frequency counts. All participants were male. Shipley IQ is the estimated intelligence quotient from the Shipley Institute of Living Scale (Zachary et al., 1985). Socioeconomic status (SES) is from the Hollingshead Two-Factor Index of Social Position, based on occupation and education (Hollingshead, 1965).

consent prior to participating in the study, and were remunerated for their participation. All participants in the present study were men. The participants in the marijuana and comparison groups were matched for age, level of education, and socioeconomic status (see Table 1). Drug use history was obtained from each participant's self-report using the Drug Survey Questionnaire (Smith, 1991), Addiction Severity Index (McLellan et al., 1992), and the Diagnostic Interview Schedule (Robins et al., 1981). Due to the documented effects of alcohol on brain structure (Pfefferbaum et al., 1998), current consumption of less than seven alcoholic drinks per week was required for both groups. All participants had a complete medical evaluation and urine toxicology screens. Participants were excluded for a past or current Axis I psychiatric diagnosis by the DSM-IV criteria (American Psychiatric Association, 1994), a past or current diagnosis of dependence or abuse of any substance except for marijuana including alcohol, a past or current history of neurological problems (e.g., loss of consciousness due to head trauma, seizure disorder, stroke), abnormal findings on the neurological examination, or left-handedness.

2.1.1. Marijuana group

This group consisted of 11 men who claimed marijuana as their drug of choice, smoked marijuana for a minimum of 2 years, currently used four or more times per week, and had a positive urine toxicology screen for marijuana and its metabolites when admitted to the study. Study participants were housed at the NIDA Clinical Inpatient Research Unit for approximately 25 days (to monitor abstinence from drug

use for a uniform period of time). The MR scans were acquired on Day 20 after admission. The apparent half-life for plasma THC (delta 9-tetrahydrocannabinol, the main psychoactive component in marijuana) mean \pm S.D. is 4.1 ± 1.1 days (Johansson et al., 1988), which may not reflect actual clearance time from brain.

2.1.2. Comparison group

The comparison group consisted of eight men who had not used marijuana according to drug report scales. Potential participants were excluded if they reported using any illicit substance. The results of the urine toxicology screens confirmed this. Participants in the comparison group were housed at the Johns Hopkins Bayview General Clinical Research Center on the night before their MR scan. We have no reason to believe that the differential housing of the two groups contributed to tissue density differences reported below.

2.2. Structural magnetic resonance image acquisition

The MR images were obtained on a General Electric 1.5 T Signa scanner at the MR Research Center of Johns Hopkins School of Medicine. Volumetric T1-weighted MR axial images were acquired with a three-dimensional spoiled gradient protocol. The acquisition parameters were: TE/TR = 6/35 ms; flip angle = 35° ; number of excitations = 1; field of view = 24 cm; matrix size = 256^2 ; with 124 slices of 1.5 mm thickness (with no inter-slice gap) through the entire brain.

2.3. Voxel-based morphometry

The MR images were received in DICOM format and reformatted to the ANALYZE 7.5 image volume format. Voxel-based morphometry was performed using statistical parametric mapping software (SPM99; <http://www.fil.ion.ucl.ac.uk/spm/>). Several pre-processing steps are required to analyze structural MR images using voxel-based morphometry in SPM99 (Ashburner and Friston, 2000). The MR images were spatially normalized by linear and non-linear transformations into the standard stereotaxic coordinate space developed at the Montreal Neurological Institute (i.e., the MNI coordinate space). The normalized MR images were resampled to an isotropic voxel size of 2 mm. The images were segmented, using a modified mixture cluster algorithm with bias correction for magnetic field inhomogeneity, to produce separate images of gray matter and white matter for each subject. The gray matter and white matter images were then spatially normalized to the MNI gray matter or white matter template images to reduce the probability of voxel misclassification. The segmented gray matter and white matter images were finally smoothed with a 12 mm^3 isotropic Gaussian kernel to conform the images to the assumptions of random fields theory for statistical analysis (Salmond et al., 2002).

2.4. Statistical analysis of MR images

Using the general linear model approach implemented in SPM99, relative differences in voxel tissue density between the two groups was determined. All images were proportionally scaled to account for global differences. One-sided contrasts were performed to evaluate greater or lesser tissue density between groups.

Because the hippocampal region has a very high density of CB₁ cannabinoid receptors (Glass et al., 1997) and marijuana has effects on hippocampal function (Hampson and Deadwyler, 1998), we hypothesized a priori that this region would show alterations in tissue composition in marijuana users when compared to non-users. We used a small volume correction (see Matochik et al., 2003) to test our hypothesis of tissue density differences between the two groups in the hippocampal region. Briefly, a volume of interest was created for the hippocampal region bilaterally with voxels outside this region set to an intensity value of zero. By limiting the analysis to the hippocampal region (search volume of 562 voxels for the left hippocampal region and 541 for the right), rather than the entire gray matter volume, the statistical power to detect possible differences was increased.

Covariate analysis of the relationship of duration of marijuana use (in years), use per week (number of joints), and age of starting marijuana use (in years) with gray matter and white matter density in the marijuana group was also performed.

For all statistical analysis, the voxel-wise threshold was set at $P < 0.001$, and clusters of contiguous voxels that passed this threshold were considered significant at $P < 0.05$ corrected for multiple comparisons within the entire gray matter or white matter search volume. Cluster significance is the probability of obtaining a cluster (i.e., a group of contiguous voxels) as large as or larger than the observed cluster in the search volume analyzed.

3. Results

3.1. Characteristics of participants

The demographic characteristics of the two groups are presented in Table 1. Only for measures of marijuana use did the groups differ. Individuals in the marijuana group reported marijuana as their drug of choice; and did not report current or past use of other drugs, including cocaine, heroin, opiates, amphetamines, and barbiturates. Participants in both groups reported smoking on average between zero and seven cigarettes per week. The present study, as with many drug abuse studies, is limited by reliance on self-reports of past drug use. All the MR images were read as within normal limits by a neuroradiologist.

3.2. Gray matter tissue density

The results of voxel-based morphometry for brain gray matter tissue density are presented in Table 2 and Fig. 1. The

Table 2
Results of voxel-based morphometry

	BA	Cluster	Spatial	MNI coordinates			Z-score (max)
		P-value	extent	x	y	z	
Brain gray matter							
Marijuana users < comparison group							
R parahippocampus gyrus	28	0.0001	598	22	−18	−10	4.32
Marijuana users > comparison group							
L precentral gyrus	4	0.0001	733	−32	−30	58	5.00
R precentral gyrus	4	0.042	135	28	−30	56	4.03
R thalamus	–	0.001	313	2	−12	0	4.47
Brain white matter							
Marijuana users < comparison group							
L parietal lobule WM		0.029	108	−20	−48	64	4.44
Marijuana users > comparison group							
L parahippocampal gyrus WM		0.002	201	−22	−24	−8	4.26
R lentiform nucleus WM		0.001	224	18	−8	−8	4.15
R brainstem WM		0.0001	256	10	−20	−24	3.50
L fusiform gyrus WM		0.001	223	−46	−6	−30	4.90

BA = Brodmann area number for cortical gray matter region; L = left; MNI = Montreal Neurological Institute; R = right; WM = white matter around named region. Marijuana users: $n = 11$, Comparison group: $n = 8$. All P -values in table are corrected for multiple comparisons.

non-using comparison group had greater tissue density in a cluster of voxels in the right parahippocampal gyrus.

The marijuana group had greater gray matter tissue density than the comparison group in three significant clusters of voxels: left precentral gyrus, including portions of the postcentral gyrus; the right precentral gyrus, also including portions of the postcentral gyrus; and in the thalamus, including the dorsomedial nucleus, on the right side.

3.3. White matter tissue density

The results of voxel-based morphometry for white matter are presented in Table 2 and in Fig. 2. The comparison group had greater tissue density than the marijuana group in white matter in the left parietal lobule.

The reverse contrast of marijuana users more than non-users in white matter density found four regions with significant clusters. These regions included two areas in the temporal lobe of the left hemisphere: white matter near the fusiform gyrus and in the parahippocampal gyrus. The other regions with higher white matter density in the marijuana group included white matter near the lentiform nucleus and in the pons of the brainstem.

3.4. Hippocampal small volume correction

Using the a priori small volume correction analysis for the hippocampal region, we found that the comparison group had higher gray matter density in a cluster in the right hippocampal region (cluster: $P = 0.0001$, extent = 33 voxels; MNI coordinates at peak voxel: $x = 24$, $y = -18$, $z = -14$, Z-score (max) = 3.89), and two significant clusters in the left hippocampal region (cluster: $P = 0.001$, extent = 27; peak voxel: $x = -20$, $y = -16$, $z = -14$, Z-score (max) = 3.63, and cluster: $P = 0.009$, extent = 8; peak voxel: $x = -32$, $y = -6$,

$z = -20$, Z-score (max) = 3.30). Small volume correction for the hippocampal region found no voxels where the marijuana group had a greater density of gray matter.

3.5. Covariate analysis with tissue density in marijuana users

Longer duration of marijuana use (in years) was associated with an area of higher white matter density around the left precentral gyrus (cluster: $P = 0.045$, extent = 92; peak voxel: $x = -48$, $y = -8$, $z = 26$, Z-score (max) = 3.82). There were no significant correlations between gray matter density and duration of use. There was no relationship of marijuana use/week (joints) or starting age of marijuana use with gray matter or white matter tissue density.

4. Discussion

Using voxel-based morphometry, we detected differences in both gray matter and white matter tissue density in a sample of heavy marijuana users compared to a non-using comparison group. Our results provide evidence, using non-invasive imaging, of structural alterations in the brain of heavy marijuana users. While the present study cannot provide conclusive evidence that marijuana use causes alterations in brain structure, our preliminary results suggest that this may be a possibility that deserves further investigation.

Two considerations are important in understanding the results of voxel-based morphometry. One is the definition of the difference in tissue density and the second issue relates to why the difference in tissue density is detected (i.e., the mechanism of action). In voxel-based morphometry, the density or concentration of a particular class of tissue (e.g., gray matter) is evaluated on a voxel-by-voxel basis. Within each voxel,

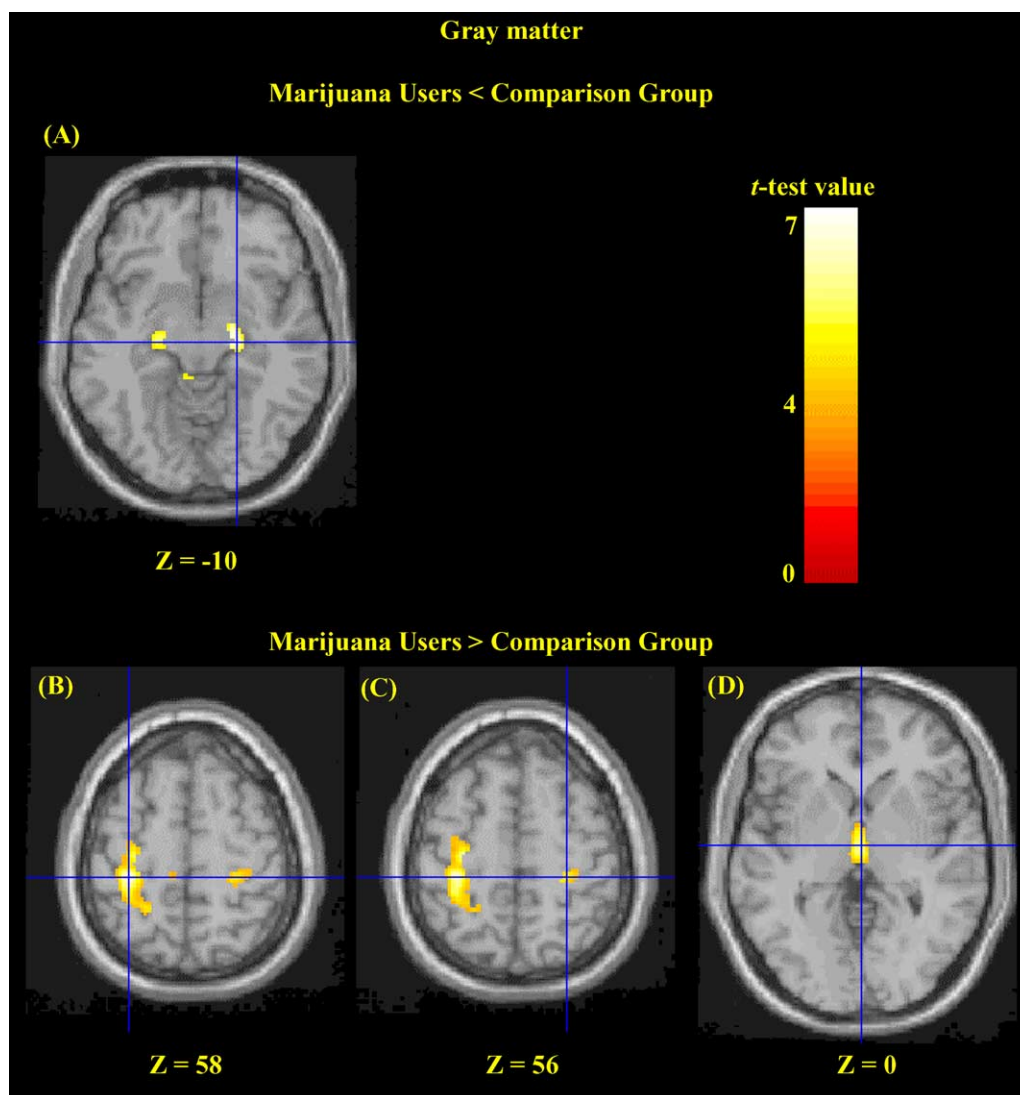


Fig. 1. Overlay of significant gray matter clusters on a T1-weighted MR image in MNI coordinate space (refer to data in Table 2). The location of slices is given by the transverse (z) direction, with the cursor at the peak voxel within the cluster. Clusters (A): parahippocampal gyrus; (B) and (C): precentral gyrus; and (D): thalamus. The images are in neurological orientation (i.e., right = right side). The voxel-wise threshold is set at $P < 0.001$. Color scale bar indicates t -test value.

tissue density (of a particular tissue class) is inferred from a weighted average of the intensity values of the neighboring voxels contained within the applied smoothing kernel. Since different tissue compositions have, on a T1-weighted MR image, a range of different signal intensities, the weighted average reflects the tissue density or concentration of a voxel in the segmented gray matter or white matter image.

The mechanisms that underlie the differences in brain tissue composition in heavy marijuana users compared with non-users found in the present study are unclear. Voxel-based morphometry (and also measurements of regional volumes made from structural MR images) cannot provide information about the microstructure of a brain region or cytoarchitectonic details. Changes in neuronal or glial cell numbers (e.g., atrophy), alterations within neuronal or glial cells (e.g., inflammation), or even structural changes in the neuropil

(e.g., changes in synaptic density) may possibly affect the signal intensity within the “smoothed” voxel. While voxel-based morphometry cannot provide direct evidence for a particular mechanism underlying the alterations in tissue density, the method is valuable for identifying focal brain regions for further investigation, and for detecting subtle structural alterations that may not be apparent on visual examination of MR images or with analysis of regional volumes.

At present, there is debate about whether or not marijuana is toxic to the brain or could cause permanent structural damage (review by Iversen, 2003). There is a large literature demonstrating impairment of hippocampal-related memory functions associated with marijuana use (Solowij, 1998), and it is this region that has received the most attention in trying to understand the effects of marijuana in the brain. The hippocampal region also has one of the highest densities of

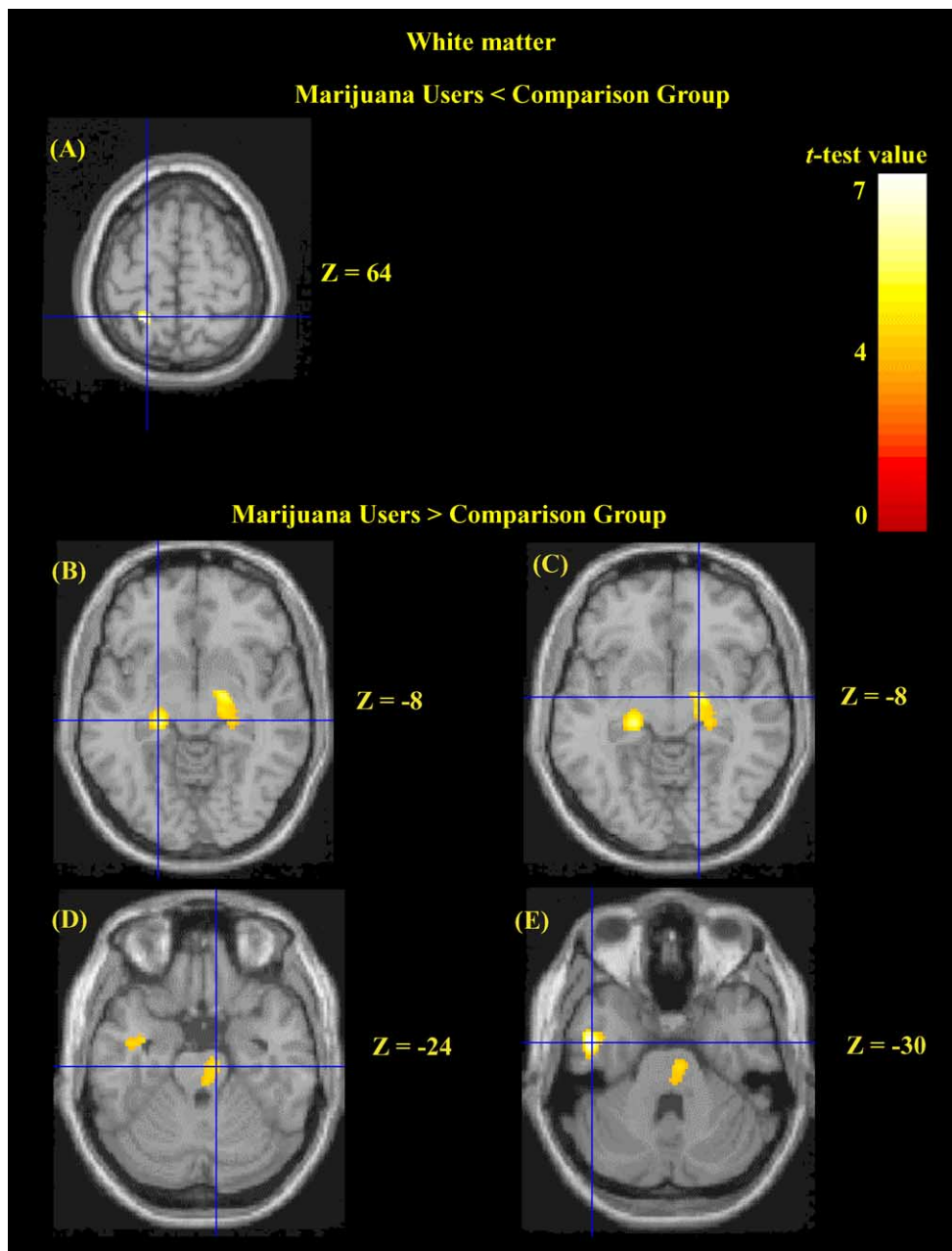


Fig. 2. Overlay of significant white matter clusters on a T1-weighted MR image in MNI coordinate space (refer to data in Table 2). The location of slices is given by the transverse (z) direction, with the cursor at the peak voxel within the cluster. Clusters (A): parietal lobule; (B): parahippocampal gyrus; (C): lenticular nucleus; (D): brainstem; and (E): fusiform gyrus. The images are in neurological orientation (i.e., right = right side). The voxel-wise threshold is set at $P < 0.001$. Color scale bar indicates t -test value.

CB₁ receptors in the human brain (Glass et al., 1997). While earlier animal studies (monkeys: Harper et al., 1977; Heath et al., 1980; rats: Landfield et al., 1988; Scallet et al., 1987) have provided some evidence of neuronal damage, primarily in the hippocampal region, related to chronic THC administration, other animal studies (rats: Chan et al., 1996; rats and monkeys: Westlake et al., 1991; review: Scallet, 1991) have not found a consistent effect after chronic THC administration. However, more recent studies (with rats) have suggested that THC is toxic in cultured preparations to hippocampal

(Chan et al., 1998; Lawston et al., 2000) and cortical (Downer et al., 2001) neurons. Based on our a priori hypothesis using small volume correction analysis, we found lower gray matter tissue density bilaterally in the hippocampal region in marijuana users compared to non-users (results presented in the text). However, we cannot determine whether differences in density are related to neuronal death, changes in synaptic density, or another mechanism.

The detection of tissue density differences in the hippocampal region is not unexpected given a large body of

literature on which our a priori hypothesis was based. The functions of altered tissue density in other regions, such as the parietal lobe and the nearby precentral gyrus and in the fusiform gyrus, are less clear. These regions have been implicated in working memory and in attention, and may be related to functional deficits observed in marijuana users (Bolla et al., 2002; Solowij, 1998).

Similar to our observations, other studies using voxel-based morphometry have reported both increases and decreases in tissue density in different regions in the same subject group (see Wilke et al., 2001). Changes in one component of the brain (e.g., gray matter) may be compensated for by changes in a neighboring component, for example, gray matter displacement caused by the reduction of nearby white matter. This idea is supported by our finding that the bilateral clusters with higher gray matter density around the precentral gyrus in marijuana users are located very close to clusters with higher white matter density in the comparison group (see MNI coordinates in Table 2). Similarly, the higher parahippocampal gray matter density cluster in non-users is near a cluster of higher white matter density in the marijuana users. It is possible that neuronal shrinkage or atrophy may have contributed to the differences in gray matter tissue density. Oligodendrocytes, the myelin-forming cells within the brain, are also sites for the effects of marijuana (Molina-Holgado et al., 2002), which might relate to differences in white matter density. Voxel-based morphometry can detect a difference or a change in voxel intensity (presumably reflecting tissue density), but the mechanism for that effect cannot be determined with current methods. It should also be noted that artificial compensations could affect tissue density (e.g., template warping), although we have tried to minimize these effects by normalizing each participant's image to the MNI gray matter or white matter templates and by visual inspection.

The results of the present study cannot determine if the differences between groups may have existed prior to initiation of marijuana use, or if other variables, either not controlled for or not recognized, may have contributed to the differences we detected in tissue density. Our results could be interpreted as a chance difference between groups and not as a deficit in marijuana users (Solowij, 1998). However, we do not believe that our results are spurious. First, we have shown that this sample of heavy marijuana users is impaired on a decision-making task (Iowa gambling task; Bechara et al., 1994) compared to our non-user group (Bolla et al., submitted), and that this impairment in performance is related to the amount of marijuana used. Second, there was a significant correlation between longer duration of use and higher white matter density in the left precentral gyrus, a gray matter region where we also found differences between users and non-users (see Table 2). Third, the results are robust considering that our sample of marijuana users was relatively small and yet highly significant differences in tissue density were detected. Also, the mean duration of marijuana use in our sample was longer than for participants in an earlier MR study (Block

et al., 2000). It should be emphasized that the level of marijuana use was greater in our sample than for the occasional or recreational user and for medicinal administration.

5. Conclusion

Using voxel-based morphometry, we found brain tissue composition alterations in heavy marijuana users that were not detected by previous methods of image analysis. We have identified areas in both gray matter and white matter of the brain that are target regions for further investigation. Correlative neuropathological and histological studies are needed to understand what the structural results mean at the cellular level. Given the limitations of a cross-sectional design as employed here, replication of these preliminary results in another and larger sample of marijuana users is needed. Within-subject imaging would be useful to track possible structural changes with heavy marijuana use, especially before initiation and after long-term use. This would allow us to determine if the differences detected in the present study existed prior to the initiation of marijuana use. The question of whether marijuana has harmful effects on brain structure remains open. Although alterations in brain tissue composition associated with heavy marijuana use may be subtle, understanding the effects of these changes may be important for informed decision-making about its use and abuse.

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