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Cannabis Use Is Quantitatively Associated with Nucleus Accumbens and Amygdala Abnormalities in Young Adult Recreational Users

Jodi M. Gilman,[1,4,5] John K. Kuster,[1,2]* Sang Lee,[1,6]* Myung Joo Lee,[1,6]* Byoung Woo Kim,[1,6] Nikos Makris,[3,5] Andre van der Kouwe,[4,5] Anne J. Blood,[1,2,4,5]† and Hans C. Breiter[1,2,4,6]†

[1]Laboratory of Neuroimaging and Genetics, Department of Psychiatry, [2]Mood and Motor Control Laboratory, [3]Center for Morphometric Analysis, Department of Psychiatry, and [4]Athinoula A. Martinos Center in Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Charlestown, Massachusetts 02129, [5]Harvard Medical School, Boston, Massachusetts 02115, and [6]Warren Wright Adolescent Center, Department of Psychiatry and Behavioral Sciences, Northwestern University Feinberg School of Medicine, Chicago, Illinois 06011

Marijuana is the most commonly used illicit drug in the United States, but little is known about its effects on the human brain, particularly on reward/aversion regions implicated in addiction, such as the nucleus accumbens and amygdala. Animal studies show structural changes in brain regions such as the nucleus accumbens after exposure to Δ9-tetrahydrocannabinol, but less is known about cannabis use and brain morphometry in these regions in humans. We collected high-resolution MRI scans on young adult recreational marijuana users and nonusing controls and conducted three independent analyses of morphometry in these structures: (1) gray matter density using voxel-based morphometry, (2) volume (total brain and regional volumes), and (3) shape (surface morphometry). Gray matter density analyses revealed greater gray matter density in marijuana users than in control participants in the left nucleus accumbens extending to subcallosal cortex, hypothalamus, sublenticular extended amygdala, and left amygdala, even after controlling for age, sex, alcohol use, and cigarette smoking. Trend-level effects were observed for a volume increase in the left nucleus accumbens only. Significant shape differences were detected in the left nucleus accumbens and right amygdala. The left nucleus accumbens showed salient exposure-dependent alterations across all three measures and an altered multimodal relationship across measures in the marijuana group. These data suggest that marijuana exposure, even in young recreational users, is associated with exposure-dependent alterations of the neural matrix of core reward structures and is consistent with animal studies of changes in dendritic arborization.

Key words: cannabis; gray matter density; marijuana; multimodal imaging; reward; topology/shape

Introduction

Marijuana (cannabis) is the most commonly used illicit drug in the United States (15.2 million past-month users; US Department of Health and Human Services, 2008). It is also the most widely used illicit drug on college campuses (Mohler-Kuo et al., 2003). Moreover, its use is increasing among adolescents and young adults (Henry et al., 2003), partially due to society's changing beliefs about cannabis use and its legal status.

Cannabis use is associated with impairments of cognitive functions, including learning and memory, attention, and decision-making. Animal studies show structural changes in brain regions underlying these functions after exposure to Δ9-tetrahydrocannabinol (THC), the main psychoactive component of cannabis (Lawston et al., 2000; Downer et al., 2001). In the nucleus accumbens, the length of the dendrites and number of dendritic spines increases with THC exposure in rats (Kolb et al., 2006). Less is known about the relationship between cannabis use and brain structure in humans. Although some studies have shown volume reductions in the hippocampus, amygdala, and cerebellum, others have not shown such effects (see Lorenzetti et al., 2010 for review). Differences in methodology may have contributed to these mixed results, suggesting that using a variety of structural methods together to quantify brain morphology may be important.

In the present study, we collected high-resolution T1 MRI scans on young adult (age 18–25 years) cannabis/marijuana users and matched nonusing controls. We conducted three blinded, automated, and independent analyses of brain structure and their

interaction: (1) gray matter density using voxel-based morphometry, (2) volume (regional volumes of deep gray matter structures) to investigate gross volumetric differences, and (3) shape deformation (surface topology) to investigate localized shape differences of subcortical structures. Although most previous studies have investigated the effect of cannabis use in heavy, dependent users, the present study posed the question of whether even recreational use may be associated with brain abnormalities.

Although THC acts centrally across a number of brain regions, the amygdala and the nucleus accumbens are especially likely to be affected. THC binds to endogenous cannabinoid (CB1) receptors located in brain regions including the nucleus accumbens and amygdala (Burns et al., 2007). Converging findings in the addiction literature implicate these regions as being important to the impact that marijuana may have on the brain (for review, see Koob and Volkow, 2010). First, the nucleus accumbens and amygdala have been implicated in non-drug reward processing (Blood and Zatorre, 2001; Breiter et al., 2001), as well as with the euphoria associated with the rewarding effects of drugs (Breiter et al., 1997; Gálvez-Buccollini et al., 2008; Gilman et al., 2008). Second, animal studies have demonstrated that exogenous and endogenous cannabinoids can significantly alter synaptic transmission in the nucleus accumbens (Lupica et al., 2004) and in the amygdala (Azad et al., 2004). Third, animal studies have shown that structural abnormalities can occur in the nucleus accumbens of THC-treated animals (Kolb et al., 2006). Accordingly, our hypothesis was that cannabis use would be associated with gray matter density alterations in the nucleus accumbens and the amygdala, potentially affecting volume and shape measures of these regions or relationships among these measures.

Materials and Methods

Participants. Participants in this study were 20 young adult (age 18–25 years) current marijuana users and 20 controls. Marijuana and control participants were matched on age, sex (9 males and 11 females in each group), handedness, race, and years of education. All participants were right-handed. Marijuana participants used marijuana at least once a week, but were not dependent, according a Structured Clinical Interview for the DSM-IV (SCID; First et al., 2002). Marijuana participants were not excluded if they had used other illegal drugs in the past; however, they were excluded if they met abuse criteria for any drug other than marijuana. Control participants had not used marijuana in the past year and had used marijuana on less than five occasions in their lifetime. Marijuana and control participants were medically healthy and did not meet DSM-IV criteria for any current or lifetime Axis I disorder. All participants completed the Alcohol Use Disorder Identification Test (AUDIT; Saunders et al., 1993) to check for problem drinking; any potential participant scoring above an 8 was excluded from inclusion in the final cohort of matched participants.

Procedure. Participants completed screening and scanning during one study visit. All marijuana users were asked to refrain from using substances on the day of the study. We performed a urine drug screen that tested for cannabis, amphetamines, cocaine, barbiturates, methamphetamines, benzodiazepines, codeine, morphine, and ethanol. Because THCCOOH, the main secondary metabolite of THC, can be detected in urine several weeks after last use (Fraser et al., 2002), a urine test alone could not determine with certainty whether participants were acutely intoxicated. Therefore, we ensured that no participant exhibited overt signs of intoxication based on a four-item marijuana intoxication scale developed in our laboratory that was designed to assess four signs of acute intoxication (Karschner et al., 2011): increased resting heart rate (>100 beats/minute), congestion of the conjunctival blood vessels (red eyes), slowed speech response, and giddiness. No marijuana participants were excluded based on these criteria. All study procedures were approved by the Massachusetts General Hospital (MGH) Institutional Re-

view Board and informed consent was obtained after the nature and possible consequences of the studies were explained.

Acquisition and processing of neuroimaging data. Participants were scanned using a 3T Siemens Trio scanner with a 32-channel head coil at the Martinos Center for Biomedical Imaging. Whole-brain T1-weighted 1 mm isotropic structural scans were collected using a 3D multiecho MPRAGE sequence (176 sagittal slices, FOV 256 mm, TR 2530 ms, TI 1200 ms, $2\times$ GRAPPA acceleration, TE 1.64/3.5/5.36/7.22 ms, BW 651 Hz/px, T_{acq} 6:03 min; van der Kouwe et al., 2008). Functional scans were collected using a 2D gradient echo EPI sequence (31 slices, 3 mm thick, 0.6 mm gap, FOV 216 mm, 3 mm^2 in-plane resolution, TR 2 s, TE 30 ms, BW 2240 Hz/px). All acquisitions were automatically positioned using AutoAlign (van der Kouwe et al., 2005). All scans were checked for movement in case any participant moved >3 mm (Breiter et al., 1997; Gasic et al., 2009). No participants were excluded based on these criteria.

Voxel-based morphometry analysis. Structural data were analyzed in a blinded fashion with the standard automated processing stream of FSL-VBM (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM), an optimized voxel-based morphometry protocol performed with FSL tools. First, structural images were brain extracted and gray matter segmented before being registered to the 2 mm MNI 152 standard space using nonlinear registration (Andersson and Smith, 2007). The resulting images were averaged and flipped along the x-axis to create a left-right symmetric, study-specific gray matter template. Second, all native gray matter images were nonlinearly registered to this study-specific template and "modulated" to correct for local expansion (or contraction) due to the nonlinear component of the spatial transformation. The modulated gray matter images were then smoothed with an isotropic Gaussian kernel with a σ of 3 mm (FWHM = 6.9 mm). Finally, a voxelwise general linear model was applied using permutation-based nonparametric testing, correcting for multiple comparisons across space.

Clusters were identified using two concurrent criteria: (1) a significance threshold for peak *t* values corrected for multiple comparisons using a Bonferroni correction and (2) a cluster threshold for voxels adjacent to the peak voxel, along with a minimum cluster size. The maximum voxel (max vox) threshold for each cluster was set based on the total search volume of 4920 mm^3 for the bilateral nucleus accumbens and amygdalae, which, divided by a minimum target cluster of five voxels (40 mm^3), implied a correction of 0.05/123 = 0.0004 (z = 3.5) for the max vox of putative clusters. The second threshold for cluster voxels outside of the max vox was selected on the basis of what correction would suffice for a search volume of just one of the four a priori structures. The largest of the nucleus accumbens and amygdala volumes was the left amygdala (1848 mm^3), which, divided by a minimum cluster size of five voxels (40 mm^3), suggested a correction of 0.05/46.2 = 0.001 (z = 3.3) as a significance level for all non-max voxels in a target cluster. These methods have been used previously (Breiter et al., 1997; Makris et al., 2008; Blood et al., 2010; Blood et al., 2012). To supply information for follow-up hypotheses by other investigators, all clusters outside of the nucleus accumbens and amygdala that met these two criteria were also tabulated in Table 3.

In addition to a cluster-based analysis, we also investigated focal voxel-based morphometry differences in morphometric segmentations of a priori regions (left and right nucleus accumbens and amygdala; Gasic et al., 2009). To investigate these segmented regions in a blinded manner, we averaged voxel-based morphometry values over a 3 × 3 × 3 voxel volume (27 total voxels) around the peak within each MNI segmentation and extracted these data from each participant, randomly represented by a number from 001 to 040. The coordinates (in millimeters) of these ROIs were based on the max vox of the difference cluster constrained by the anatomical segmentations of the nucleus accumbens and amygdala (Gasic et al., 2009) observed in the left hemisphere structures and the mirror location of these maxima in the right hemisphere. These locations were as follows: left nucleus accumbens = −12, 8, −12; right nucleus accumbens = 12, 8, −12; left amygdala = −24, −10, −10; and right amygdala = 24, −10, −10. These values were entered into a multivariate general linear model (SPSS Version 19), which allowed us to determine whether group differences were significant after covarying for factors including age, sex, alcohol use, and cigarette smoking. In addition, to

Table 1. Participant demographics

	CON (n = 20)	MJ (n = 20)	p-value
Sex (M/F)	9 M/11 F	9 M/11 F	N/A
Age	20.7 (1.9)	21.3 (1.9)	0.30
Years of education	14.3 (3.4)	12.6 (4.8)	0.20
STAI[a]			
State	28.9 (7.94)	27.7 (7.38)	0.65
Trait	29.8 (7.32)	29.5 (5.56)	0.89
HAM-D[b]	0.80 (1.40) [range: 0–5]	1.10 (1.37) [range: 0–5]	0.50
TIPI[c]			
Extroversion	10.9 (2.36)	10.7 (2.13)	0.78
Agreeableness	10.8 (2.47)	10.7 (1.81)	0.94
Conscientiousness	11.9 (2.08)	11.7 (2.13)	0.76
Emotional stability	10.5 (2.52)	11.4 (2.64)	0.27
Openness	12.1 (1.90)	12.4 (1.61)	0.57
Substance use			
Alcohol			
No. alcoholic drinks/week	2.64 (2.38)	5.09 (4.69)	0.10
AUDIT score	3.30 (1.78)	5.50 (2.21)	0.05
Cigarettes			
No. of occasional smokers[d]	0	7	N/A
No. of daily smokers	0	1	N/A
Marijuana			
No. days/week	0	3.83 (2.36)	N/A
No. joints/week	0	11.2 (9.61)	N/A
No. joints/occasion	0	1.80 (0.77)	N/A
No. smoking occasions/day	0	1.80 (0.70)	N/A
Age of onset (years)	—	16.6 (2.13)	N/A
Duration of use (years)	—	6.21 (3.43)	N/A

All values are expressed in means and SDs. CON, controls; MJ, marijuana users.
[a]State Trait Anxiety Inventory Form (Spielberger et al., 1983).
[b]Hamilton Depression Rating Scale (Hamilton, 1960).
[c]Ten-Item Personality Inventory (Gosling et al., 2003).
[d]Occasional smokers reported from 1 cigarette/week to 1 cigarette every 3 months.

determine whether gray matter density was associated with drug use severity, we performed linear regressions between the average values of each ROI and measures of drug use behavior including: number of joints smoked per week, smoking occasions per day, smoking days per week, and number of joints smoked per smoking occasion (see Behavioral Measures below). Because we conducted these tests in four regions (left and right nucleus accumbens and amygdala), we performed a Bonferroni correction on the resulting p-values ($p < 0.05/4 = 0.0125$) for the voxel-based morphometry analysis. We did not correct for the number of drug use measures because these measures tend not be independent of each other (i.e., smoking occasions per day, joints per occasion, joints per week, and smoking days per week were all highly correlated, with $p < 0.01$).

Volume analysis. Intracranial volume (ICV), total brain, gray matter, white matter, and subcortical volumes were estimated using the standard automated cortical and subcortical segmentations created by Freesurfer (http://surfer.nmr.mgh.harvard.edu), again performed in a blinded manner. Summary images for the segmentation outputs were generated to check the quality of segmentations and these segmentations were also spot checked using a blinded approach by an expert neuroanatomist (N.M.), who directs the MGH Center for Morphometric Analysis (CMA). We extracted volumes of our a priori regions (left and right nucleus accumbens and amygdala) in each of the 40 participants. As with the gray matter density measures from described in Voxel-based morphometry analysis, above, these volume measurements were entered into a multivariate general linear model, which allowed us to determine whether group differences were significant after covarying factors such as age, sex, alcohol use, and cigarette smoking. In addition, we performed linear regressions between the volume of each region and measures of drug use behavior (see Behavioral Measures below). Because we conducted these tests in four regions (left and right nucleus accumbens and amygdala), we performed a Bonferroni correction on the resulting p-values ($p < 0.05/4 = 0.0125$).

We also extracted values of other striatal structures (i.e., caudate and putamen), the medial temporal regions (i.e., hippocampus) and thala-

mus, to assess whether there were differences between groups in these measures that met the threshold set for a priori regions. Values for these brain volumes, along with a priori regions, are listed in Table 5. In these analyses, ICV and sex were used as covariates.

Shape analysis. Shape of subcortical structures was computed using FMRIB's Integrated Registration and Segmentation Tool (FIRST), a model-based segmentation/registration tool that segments all of the subcortical structures, producing mesh and volumetric outputs (applying boundary correction) of subcortical structures. The shape/appearance models used in FIRST were constructed from manually segmented images provided by the CMA, which were segmented in a blinded fashion. Further analyses by FIRST were also performed in a blinded fashion. The manual labels were parameterized as surface meshes and modeled as a point distribution model. Deformable surfaces were used to automatically parameterize the volumetric labels in terms of meshes; the deformable surfaces were constrained to preserve vertex correspondence across the training data. Furthermore, normalized intensities along the surface normals were sampled and modeled. The shape and appearance model was based on multivariate Gaussian assumptions. Shape was then expressed as a mean with modes of variation (principal components). More information about these analyses can be found at http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIRST/UserGuide#Vertex_Analysis_.28with_NEW_features_in_v5.0.0.29.

After vertices were calculated for each ROI, each a priori region (left and right nucleus accumbens and amygdala) was compared between control and marijuana participants using FSL's "Randomise" a permutation test enabling modeling and inference using standard general linear model design setup (Nichols and Holmes, 2002). Of these, two met a cluster correction threshold for familywise error of $p < 0.05$ (left nucleus accumbens and right amygdala). The right nucleus accumbens and left amygdala did not meet the cluster correction threshold for significant differences between groups. From the resulting statistical maps generated by "Randomise" we identified the peak voxel showing the maximum

deformation between the groups for each ROI. We then extracted individual scalar values (representing shape change from the average normal surface) for all 40 participants. The data extracted from these ROIs were then entered into a general linear model, allowing us to investigate differences between groups while controlling for other factors such as age, sex, alcohol use, and cigarette smoking. In addition, we performed linear regressions between individual scalar values of maximum shape deformation and measures of drug use behavior (see Behavioral Measures below). Because we conducted these tests in four regions (left and right accumbens and amygdala), we performed a Bonferroni correction on the resulting p-values ($p < 0.05/4 = 0.0125$).

Multimodal data integration. For multimodal analysis of gray matter density, volume, and shape (topology) measures, we focused on assessments between marijuana users and control groups for each of the four a priori structures alone (a) and the relationship between hemispheres for the nucleus accumbens and amygdala (b). The relationship among gray matter density, volume, and shape measures was evaluated for (a) and (b) using covariance matrices with SPSS Version 19 software and tabulated as bivariate correlations in Tables 6 and 7. The data used as input to these matrices included the following: (1) extracted ROI-based gray matter density data per participant, (2) volumes per participant, and (3) scalar values (representing shape change from the average normal surface) for participants from the peak voxel showing the maximum deformation between the groups. For analyses of (a) and (b), differences of $r > 0.4$ between matrix elements from each group were further evaluated graphically (see Fig. 2C) and assessed to determine whether significant effects found in one group were absent in the other. These techniques were developed by the concerted effort of M.J.L., A.J.B., and H.C.B. and were then used in this study as a first application.

Behavioral measures. Marijuana users completed a timeline follow-back (Sobell et al., 1986) asking them to indicate, for the past 90 d, the days that they smoked marijuana. They were given a calendar and asked to check off to the best of their ability the days that they smoked marijuana, focusing on patterns of use. They were also asked to designate, on the days that they used marijuana, how many separate times in a day they used and how many joints (or joint equivalents) they consumed per smoking occasion. All participants (marijuana users and controls) also completed a timeline follow-back for alcohol use, (Sobell et al., 1986) asking them to detail their drinking behavior in the past 90 d to determine whether alcohol consumption differed and to use as a covariate in contrasts between the two groups. All participants also completed the State Trait Anxiety Inventory Form (STAI; Spielberger et al., 1983) to assess anxiety, the Hamilton Depression Rating Scale (HAM-D; Hamilton, 1960) to assess depression, and the Ten-Item Personality Inventory (TIPI; Gosling et al., 2003) to assess personality characteristics.

Results
Participants
Control and marijuana participants were not significantly different in age, sex, years of education, handedness, or race; they also did not differ significantly on any of the behavioral questionnaires (STAI,

A Gray Matter Density: Marijuana > Control Participants
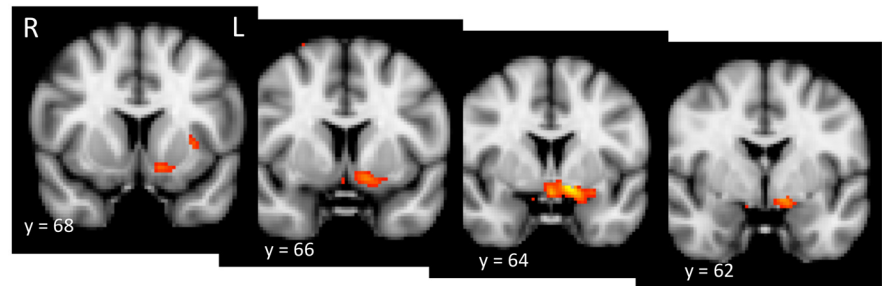
B Associations Drug Use Behavior and Gray Matter Density in Left Nucleus Accumbens
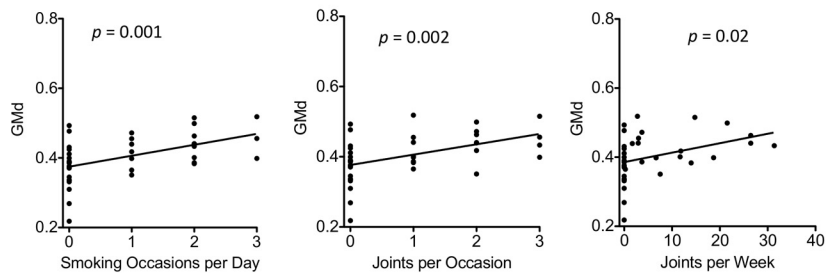
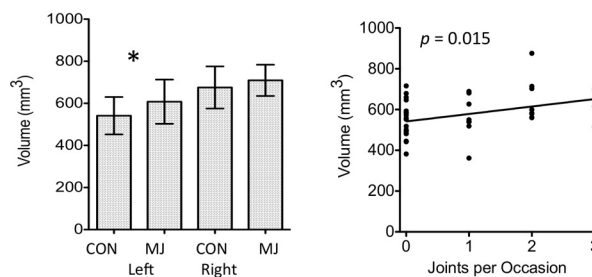**C** Volume and Associations with Drug Use in Left Nucleus Accumbens

Figure 1. ***A***, Whole-brain voxel-based morphometry between marijuana users and control participants. Images are thresholded at $z = 2.5$. The most significant increases in gray matter density were in the left nucleus accumbens extending to the hypothalamus, sublenticular extended amygdala, and amygdala (Tables 2 and 3). ***B***, Associations between gray matter density and drug use measures; $p < 0.0125$ was considered to be significant after correcting for 4 comparisons (Table 4). GMd, Gray matter density. ***C***, Nucleus accumbens volume was increased in marijuana users and was associated with drug use measures. Error bars represent SE. An asterisk above the bar chart on left indicates that significance met $p < 0.05$ uncorrected ($p = 0.037$; Table 2), which was a trend effect after correcting multiple comparisons. The association with drug use, after correcting for 4 comparisons ($p = 0.05/4$, or 0.0125), was determined to be a trend toward significance (Table 4). CON, Controls; MJ, marijuana participants.

HAM-D, and TIPI; all $p > 0.05$; Table 1). Marijuana users reported drinking a greater number of alcoholic drinks per week than control participants and showed a trend toward a significant difference on AUDIT (Table 1), so alcohol use was used as a covariate in statistical analyses (see Table 2 footnotes).

Gray matter density measures
The whole-brain gray matter density analysis revealed greater density values in marijuana users than in control participants in the left nucleus accumbens, extending to subcallosal cortex, hypothalamus, sublenticular extended amygdala, and left amygdala (Fig. 1A, Tables 2, 3). When we extracted data from each participant using peak ROI values, these differences remained significant after controlling for age, sex, alcohol use, and cigarette smoking (Table 2). Gray matter density in the left nucleus accumbens and the left amygdala was further associated significantly with marijuana drug use behaviors (Fig. 1B, Table 4), but not with age of onset of marijuana use, potentially due to the tight range of onset in this cohort.

Table 2. Structural measures of marijuana users (MJ) and controls (CON) in the amygdala and nucleus accumbens

Modality	Region	Hemisphere	x	y	z	Max z/F	Max p-value	Cluster volume[a]
Voxelwise significant comparisons in GMd and shape								
GMd	Accumbens	L	−12	2	−10	4.19	**<0.0001***	376
	Amygdala	L	−24	−12	−8	3.56	**0.0004***	40
Shape	Accumbens	R	6	11	−5	13.9	**0.0006***	50
	Accumbens	L	−9	15	−9	8.06	**0.0072***	78
	Amygdala	R	29	−8	−16	19.4	**<0.0001***	309

		CON		MJ		t test results		GLM results	
Modality	Region	Mean	SD	Mean	SD	t	p-value	F	Adjusted p-value
ROI-based comparisons in GMd and volume									
GMd	R accumbens	0.44	0.08	0.47	0.05	1.31	0.20	0.95	0.34
	R amygdala	0.33	0.03	0.34	0.03	2.05	**0.047**	4.47	**0.042**
	L accumbens	0.37	0.06	0.43	0.05	3.53	**0.001***	10.38	**0.003***
	L amygdala	0.32	0.03	0.35	0.04	2.77	**0.009***	8.75	**0.006***
Volume	R accumbens	675.40	100.22	709.40	103.80	1.22	0.23	0.85	0.36
	R amygdala	1713.20	222.24	1764.75	222.81	0.75	0.46	0.70	0.41
	L accumbens	541.35	88.87	607.95	86.24	2.16	**0.037**	5.37	**0.027**
	L amygdala	1698.45	323.56	1711.35	323.03	0.15	0.88	0.01	0.93

		Group difference (mm)		t test results		GLM results	
Modality	Region	Mean	SD	t	p	F	Adjusted p
ROI-based comparisons in shape							
Shape	R accumbens	0.27	0.40	3.26	**0.002***	6.73	**0.014**
	R amygdala	0.46	0.66	4.39	**<0.0001***	11.24	**0.002***
	L accumbens	0.17	0.27	2.84	**0.007***	8.27	**0.007***
	L amygdala	−0.06	0.32	1.16	0.26	2.31	0.14

Coordinates are in MNI space and are listed in millimeters. Reported p values are uncorrected; please see Materials and Methods for whole-brain correction. Gray matter density (GMd) measures reflect ROI average density. Volume is indicated in cubic millimeters. Shape values were obtained by identifying the voxel showing the peak differences between groups within each ROI and extracting the value of that peak voxel in each individual subject. The mean difference between relative group averages is shown in millimeters. For measures, group differences between the 20 controls and 20 MJs are reported as t statistics. Individual subject values were covaried in a general linear model (GLM) to account for age, sex, alcohol use, and cigarette smoking, yielding F and adjusted p values. Values at p < 0.05 uncorrected are shown in bold; an asterisk indicates that these values met correction for multiple comparisons (e.g., p < 0.0125).
[a]GMd difference cluster volume is shown in cubic millimeters and shape difference cluster volume in square millimeters.

Table 3. Significant differences in gray matter density between marijuana users (MJ) and healthy controls (CON): whole-brain analysis

Area	Region	Hemisphere	x	y	z	Peak z	Volume, mm³
MJ > CON							
Frontal	Middle frontal gyrus	R	30	−10	44	3.71	48
Parietal	Precuneus	R	24	−60	26	4.16	472
	Postcentral gyrus	L	−24	−36	58	3.75	184
Temporal	Insula	L	−30	14	2	3.63	80
Subcortical	Nucleus accumbens/putamen	L	−12	2	−10	4.19	296
	Amygdala	L	−24	−12	−8	3.56	40
Occipital	Lateral occipital cortex	R	36	−76	30	3.69	48
	Occipital cortex	L	−20	−78	26	3.73	56
CON > MJ							
Frontal	Frontal pole	R	32	54	6	3.94	760
	Dorsolateral prefrontal cortex	R	36	−16	48	3.94	448
	Middle frontal gyrus	R	30	20	58	3.67	80
	Frontal pole	L	−20	68	−6	4.62	40
Temporal	Temporal pole	L	−32	8	−48	4.42	312
	Superior temporal gyrus	L	−48	−6	−12	3.60	88
Occipital	Lateral occipital cortex	R	8	−66	66	3.77	72

All clusters were derived from a VBM analysis. Significant clusters consisted of at 40 mm³ (5 voxels) thresholded at z = 3.3 (p < 0.001), with a peak voxel of z > 3.5 (p < 0.0005). Coordinates are in MNI space and are listed in millimeters.

Volumes

Of the four a priori volumes, the left nucleus accumbens volume was larger in marijuana users (Figure 1C, Table 2), mirroring the increase in gray matter density, although this increase did not meet significance after controlling for multiple comparisons. Alterations in left nucleus accumbens volume were associated, at trend level, with both the number of joints per occasion and smoking occasions per day (Fig. 1C, Table 4), but not with age of onset of marijuana use. To verify that volumetric alterations did not merely reflect group differences in global brain volume, we determined that there were no significant differences in ICV, total brain volume, or gray or white matter volume; there were also no differences in any other deep gray matter regions after controlling for ICV and sex (Table 5).

Topology/shape measures

Significant shape differences were detected between marijuana users and control participants using both voxelwise and ROI approaches in the right amygdala and left nucleus accumbens (Fig. 2A,B, Table 2); these differences remained significant after con-

Table 4. Linear regression between smoking behavior and structural measures

	Smoking occasions/day		Joints/occasion		Joints/week		Smoking days/week	
	r^2	p	r^2	p	r^2	p	r^2	p
GMd								
R accumbens	0.050	0.171	0.059	0.137	0.055	0.156	0.049	0.174
R amygdala	0.071	0.100	0.035	0.251	0.007	0.610	0.027	0.321
L accumbens	0.255	**0.001***	0.235	**0.002***	0.141	**0.020**	0.115	**0.035**
L amygdala	0.191	**0.005***	0.061	0.130	0.021	0.380	0.042	0.212
Volume								
R accumbens	0.015	0.450	0.097	0.051	0.027	0.307	0.003	0.714
R amygdala	0.009	0.558	0.069	0.100	0.072	0.094	0.008	0.565
L accumbens	0.082	0.073	0.145	**0.015**	0.066	0.110	0.048	0.230
L amygdala	0.000	0.867	0.000	0.885	0.001	0.831	0.001	0.835
Shape								
R accumbens	0.231	**0.002***	0.265	**0.001***	0.232	**0.018**	0.232	**0.002***
R amygdala	0.296	**< 0.001***	0.414	**< 0.0001***	0.186	**0.001***	0.186	**0.007***
L accumbens	0.158	**0.012***	0.170	**0.009***	0.120	0.536	0.120	**0.031**
L amygdala	0.004	0.685	0.006	0.643	0.016	0.439	0.002	0.785

Values of gray matter density (GMd), volume, and shape were entered into linear regressions with the four measures of marijuana use, yielding the reported r^2 and p-values. $p < 0.05$ uncorrected are in bold; an asterisk indicates that these values met correction for multiple comparisons across four regions (e.g., $p < 0.0125$).

Table 5. Volumes of total brain and subcortical ROIs in marijuana users (MJ) and healthy controls (CON)

	Controls		MJ		Variance accounted for by ICV		Variance accounted for by sex		Variance accounted for by group	
	Mean	SD	Mean	SD	F	p	F	p	F	p
Total ICV	1460962.45	186411.89	1429117.14	191802.18	—	—	12.89	**	0.37	NS
Total brain	1228316.23	107399.90	1207892.96	107013.81	37.75	***	5.61	*	0.12	NS
Total gray	652429.33	58089.89	643401.98	58569.35	28.87	***	5.39	*	1.03	NS
Total white	575886.9	56070.39	564490.98	53863.34	25.21	***	2.91	NS	0.10	NS
R accumbens	675.4	100.22	709.4	103.8	0.05	NS	0.12	NS	1.35	NS
R amygdala	1713.2	222.24	1764.75	222.81	9.49	**	1.75	NS	1.47	NS
R caudate	3980.15	432.73	3936.7	393.26	3.04	NS	2.22	NS	0.03	NS
R putamen	5790.8	610.46	5811.65	605.85	1.14	NS	3.39	NS	0.05	NS
R thalamus	7737.45	1053.86	7496.6	1065.25	12.06	**	0.49	NS	0.45	NS
R hippocampus	4325.05	443.61	4340.5	440.4	13.47	**	1.58	NS	0.27	NS
L accumbens	541.35	88.87	607.95	86.24	0.28	NS	0.01	NS	4.66	*
L amygdala	1698.45	323.56	1711.35	323.03	1.19	NS	10.76	**	0.09	NS
L caudate	3989.55	372.12	3863.45	358.7	4.07	*	3.10	NS	0.85	NS
L putamen	6204.25	620.05	6171.9	622.58	2.36	NS	3.58	NS	0.00	NS
L thalamus	7698.95	950.39	7471.8	950.78	4.77	*	0.43	NS	0.51	NS
L hippocampus	4231.7	379.52	4209.2	380.75	7.36	*	0.96	NS	0.00	NS

Total brain is composed of gray matter + white matter + intraventricular CSF. All volumes are reported in cubic millimeters. All structures were entered into a univariate general linear model to examine how ICV, sex, and group (MJ or CON) affected variance of volumes of structures.

$*p < 0.05$; $**p < 0.01$; $***p < 0.001$, uncorrected for multiple comparisons.

trolling for age, sex, alcohol use, and cigarette smoking (Table 2). These regions showed significant associations between shape measures and marijuana drug use behaviors (Table 4), but not between shape measures and age of onset of marijuana use.

Multimodal relationships across structural measures
When we investigated the relationship among gray matter density, volume, and vertices, we found that multimodal relationships in control participants were altered in marijuana users, particularly in the left nucleus accumbens. In control participants, there was a positive relationship (i.e., $r > 0.4$) between gray matter density and both volume and vertices in the left nucleus accumbens (Fig. 2C, left colorized covariance matrix, Table 6); in the marijuana users, those relationships were largely insignificant (Fig. 2C, right colorized covariance matrix, Table 6). In contrast, for both groups, across all measures, left and right regions were highly correlated (Table 7). Therefore, abnormalities were observed across hemispheres, although they were strongest in the left nucleus accumbens.

Discussion
The present study demonstrates that, even in young, nondependent marijuana users, morphometric abnormalities relative to nonusers are observable, many of which are exposure dependent. These observations also demonstrate that fundamental relationships observed in covariance analyses among structural measures of controls are absent in marijuana users, suggesting that marijuana use may be associated with a disruption of neural organization in the a priori regions of the nucleus accumbens and amygdala. These findings emphasize the importance of multimodal imaging for two primary reasons. First, convergent evidence across modalities makes a convincing case for a robust finding. In this study, all three structural measures in the left nucleus accumbens were parametrically associated with drug use measures, raising a hypothesis that the etiology of these changes may have been due to drug exposure. Second, multimodal imaging can demonstrate that certain abnormalities may be more detectable using one modality than another due to the different

A Surface Morphology (Shape): Right Amygdala
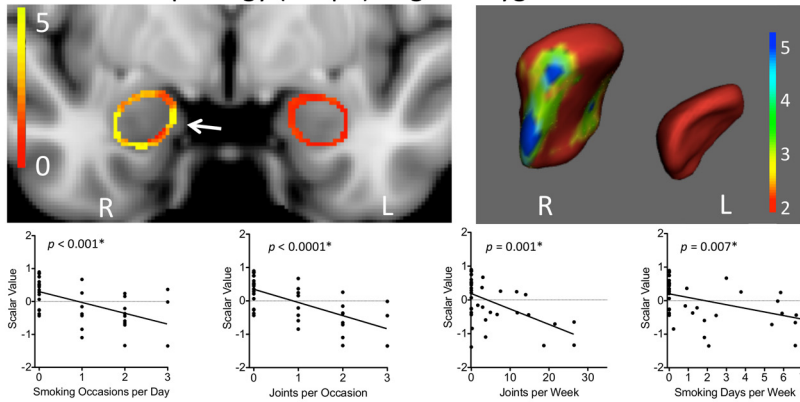
B Surface Morphology (Shape): Nucleus Accumbens
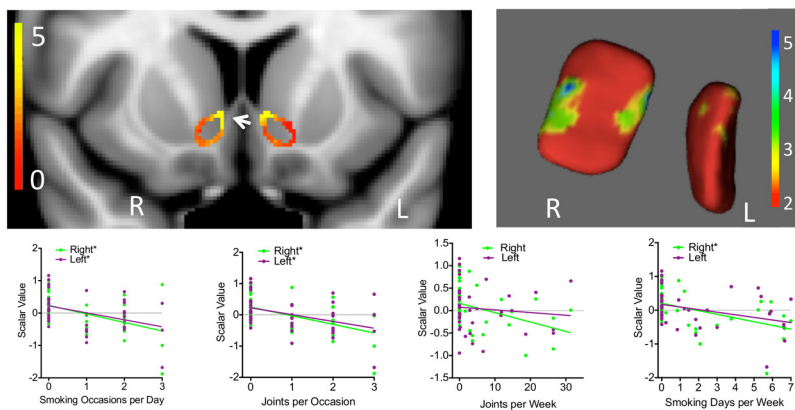
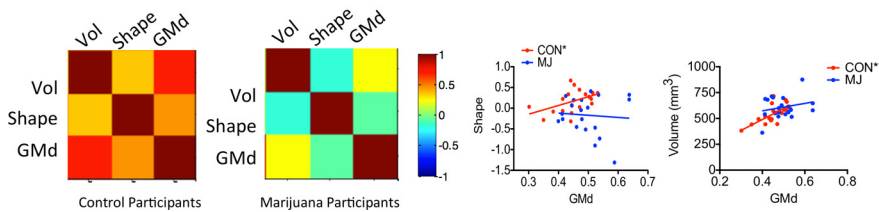## C Correlations Among Structural Measures of the Left Nucleus Accumbens

Figure 2. Shape analysis. 2D results of the shape analysis of the amygdala (**A**) and the nucleus accumbens (**B**) show areas that are significantly different between marijuana and control participants (left); 3D results are shown on right. Below each colorized statistical map, differences in shape were significantly associated with several drug use measures. An asterisk after the *p*-value in **A** and after the left/right color legend in **B** indicates a significant correlation (corrected). Please see Table 4 for significance after correcting for multiple comparisons. **C**, Color maps indicate bivariate correlations between modalities in the left nucleus accumbens (left). The relationship between gray matter density and shape and between gray matter density and volume differed between marijuana usersr and control participants; one asterisk indicates a trend correlation; two asterisks indicate a significant correlation (corrected). Please see Table 6 for significance after correcting for multiple comparisons. GMd, Gray matter density; Vol, volume.

etiological sensitivities of different neuroimaging modalities. For example, the amygdala showed salient shape differences in the absence of volumetric differences. Assessing each of these measures alone, along with their relationship together (i.e., covariance), can provide evidence of more robust differences between groups and, potentially, of etiology that can be tested as a hypothesis in subsequent work.

Examining these measures together is important because no one measure is a gold standard; each has caveats associated with it. For example, MRI-based volumetry is approximate for brain structures that have no clear borders between them due to similarity in image intensity values on T1-MRI. Furthermore, global measures of volume lack information on detailed topography

within a given brain structure (Filipek et al., 1989; Filipek et al., 1994; Makris et al., 1999; Fischl et al., 2002). In these cases, information provided by other techniques, such as voxel-based morphometry and shape analyses, may be useful in generating a multifactorial perspective of abnormalities. In voxel-based morphometry analyses, gray matter density is compared across the entire brain at every voxel, allowing the detection of differences in small or large regions, which can be topographically specified with high accuracy (Ashburner and Friston, 2000) even though all brains have been normalized. Finally, shape analyses allow for a comparison of surface geometrical properties of structures between groups (Heimann and Meinzer, 2009) that may not have had an overall volume change or alteration in gray matter density. These multiple aspects of structural information enrich our knowledge related to a structure in terms of size, density, and topography and give us more confidence of the veracity and accuracy of the MRI techniques used and the derived anatomical results.

This study demonstrates that different aspects of brain morphology may be affected by cannabis. In this study, shape- and voxel-based morphometry measures of subcortical regions were consistently affected in an exposure-dependent manner by cannabis, whereas volume was less affected. Since their initial application in addiction and psychiatric research (e.g., Makris et al., 2004), shape analyses are becoming more common in the psychiatric literature, providing useful information about locations and patterns of structural changes. For example, subcortical structural alterations have been reported in thalami of patients with schizophrenia (Harms et al., 2007; Kang et al., 2008; Coscia et al., 2009; Harms et al., 2010), obsessive-compulsive disorders (Kang et al., 2008), Parkinson's disease (McKeown et al., 2008), and Tourette's syndrome (Wang et al., 2007). Subcortical surface alterations may be more sensitive to the effects of cannabis than total volumes. Volume alterations may also be more pronounced with greater duration of use. In our study, the nucleus accumbens and amygdala showed altered surface topology compared with controls, which could potentially lead to volume alterations with continued use.

Both gray matter density and average volume were increased in marijuana participants in the left nucleus accumbens. The density increase was localized to a large area, which clearly represents the extended amygdala as defined by Heimer and Alheid (1991), a neuroanatomical entity implicated in addiction (Koob, 2003). The structures comprising the extended amygdala may be key substrates for the reinforcing actions of drugs and in the negative reinforcing aspect of dependence (Koob, 2003).

Table 6. Bivariate correlations between modalities in marijuana users (MJ) and healthy controls (CON)

	CON			MJ		
	Volume	Shape	GMd	Volume	Shape	GMd
R accumbens						
Volume						
r	1	**0.486**	0.429	1	0.116	**0.532**
p	0	**0.030***	0.059	0	0.628	**0.017***
Shape						
r	**0.486**	1	0.353	0.116	1	−0.011
p	**0.030***	0	0.126	0.628	0	−0.33
GMd						
r	0.429	0.353	1	**0.532**	−0.011	1
p	0.059	0.126	0	**0.017***	0.964	0
R amygdala						
Volume						
r	1	0.169	−0.244	1	0.253	0.115
p	0	0.476	0.299	0	0.282	0.630
Shape						
r	0.169	1	−0.037	0.253	1	**0.445**
p	0.476	0	0.876	0.282	0	**0.049***
GMd						
r	−0.244	−0.037	1	0.115	**0.445**	1
p	0.299	0.876	0	0.630	**0.049***	0
L accumbens						
Volume						
r	1	0.358	**0.688**	1	−0.173	0.235
p	0	0.121	**0.001****	0	0.466	0.320
Shape						
r	0.358	1	**0.459**	−0.173	1	−0.077
p	0.121	0	**0.042***	0.466	0	0.748
GMd						
r	**0.688**	**0.459**	1	0.235	−0.077	1
p	**0.001****	**0.042***	0	0.320	0.748	0
L amygdala						
Volume						
r	1	**0.731**	0.085	1	0.134	0.021
p	0	**<0.001****	0.722	0	0.573	0.929
Shape						
r	**0.731**	1	0.06	0.134	1	−0.179
p	**<0.001****	0	0.801	0.573	0	0.449
GMd						
r	0.085	0.06	1	0.021	−0.179	1
p	0.722	0.801	0	0.929	0.449	0

$p < 0.05$ uncorrected are bolded. Please note, after correcting for multiple comparisons ($p < 0.002$), that only the left accumbens correlation between volume and gray matter density (GMd) and the left amygdala correlations between volume and shape were significant in controls.

*$p < 0.05$; **$p < 0.01$.

Table 7. Bivariate correlations within modalities in the right and left hemispheres in marijuana users (MJ) and healthy controls (CON)

	CON		MJ	
	r	p	r	p
Accumbens				
Volume	0.659	**0.002***	0.532	**0.016***
Shape	0.581	**0.007***	0.674	**0.001***
GMd	0.623	**0.003***	0.878	**<0.0001***
Amygdala				
Volume	0.791	**<0.0001***	0.700	**0.001***
Shape	0.531	**0.016***	0.466	**0.039***
GMd	0.690	**0.001***	0.509	**0.022***

$p < 0.05$ uncorrected are bolded. GMd, Gray matter density.

*$p < 0.05$; **$p < 0.01$.

Increased gray matter density in this region is consistent with findings in animal studies of drug use. Researchers have reported a 6.9% increase in the number of dendritic branches and increased density of dendritic spines in the shell of the nucleus

accumbens in amphetamine- and cocaine-treated rats (Robinson and Kolb, 1999) and this increase appears to persist long after acute administration. These results have been replicated using nicotine (Gonzalez et al., 2005) and cannabis (Kolb et al., 2006). In a study in which rats were given doses of THC that were within the range that might be experienced by human marijuana users, prior exposure to THC increased the length of the dendrites and the number of dendritic branches in the shell of the nucleus accumbens (in contrast to an absence of effects in the hippocampus, striatum, orbital frontal cortex, parietal cortex, or occipital cortex; Kolb et al., 2006). Dendritic arborization changes may relate to an increase in dopamine, because acute administration of drugs of abuse (e.g., THC; Chen et al., 1990) produces increases in extracellular levels of dopamine (DA) in the nucleus accumbens (Pontieri et al., 1995).

Interestingly, the left nucleus accumbens was consistently affected by cannabis use across all three modalities. This structure also showed differences between marijuana and control participants in the multimodal relationship between gray matter density and both shape and volume. Few other associations of measures across modalities differed between marijuana and control participants. It will be important for follow-up work to assess the emergence of further abnormalities with prolonged marijuana use.

The present study did not find any differences in amygdala volumes between marijuana users and control participants, but did find that surfaces of the right amygdala were deformed inwards and this diminishment was highly correlated with drug use behavior. These exposure-dependent deformations may precede more pronounced volumetric changes, because others have reported a reduction in amygdala volumes in long-term, heavy users (Yücel et al., 2008; Schacht et al., 2012). Such an observation is also consistent with data showing a negative correlation between level of cannabis dependence and amygdala gray matter density (Cousijn et al., 2012). Our observations indicate that such changes may occur before dependence, which is intriguing in the context that the amygdala appears to play an important role in drug craving (Breiter et al., 1997; Childress et al., 1999; Bonson et al., 2002).

In addition to the a priori regions of the nucleus accumbens and amygdala, the whole-brain gray matter density analysis revealed other brain regions that showed reduced density in marijuana users compared with control participants, including several regions in the prefrontal cortex: right/left frontal pole, right dorsolateral prefrontal cortex, and right middle frontal gyrus (although another small region in the right middle frontal gyrus showed higher gray matter density in marijuana users). Countless studies have shown that prefrontal cortex dysfunction is involved with decision-making abnormalities in addiction (e.g., Bechara, 2001), and functional MRI and magnetic resonance spectroscopy studies have shown that cannabis use may affect the function of this region (Quickfall and Crockford, 2006; Hermann et al., 2007). Furthermore, diffusion tensor imaging studies have shown that cannabis use may affect the integrity of white matter fiber tracts in prefrontal regions (Gruber and Yurgelun-Todd, 2005). A study examining the structural effects of cannabis on the prefrontal cortex reported greater gray matter density in the precentral gyrus of marijuana users (Matochik et al., 2005). More studies are needed with larger cohorts to fully examine the effect of cannabis use on prefrontal structures.

This preliminary study has several caveats. First, the sample size does not provide power to examine complex interactions such as sex differences. Because this is a cross-sectional study, causation cannot be determined, although marijuana exposure

parametrically correlated with structural differences, which suggests the possibility of causation. Longitudinal studies are needed to determine whether marijuana exposure explicitly leads to the differences observed in this study. Furthermore, this study did not include quantifiable marijuana metabolite levels, which would have provided further information about the amount of marijuana exposure. This measure could be incorporated into future studies as a complementary measure to detailed timeline follow-back measures of drug use. Finally, age of onset was collected for marijuana use only. Early exposure to alcohol may have also affected brain structure (although no participant met criteria for past alcohol abuse or dependence).

The results of this study indicate that in young, recreational marijuana users, structural abnormalities in gray matter density, volume, and shape of the nucleus accumbens and amygdala can be observed. Pending confirmation in other cohorts of marijuana users, the present findings suggest that further study of marijuana effects are needed to help inform discussion about the legalization of marijuana. These results extend prior studies showing that drugs of abuse that are known to elevate DA release are associated with structural abnormalities in the brain and related disruptions in behavior (Makris et al., 2004; Makris et al., 2008). The multimodal convergence of these findings also points to the salience of structural differences in the brain related to drug exposure and strongly argues that human addiction research, if not all psychiatric study, must move past a predominant focus on neurotransmission.

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