Brain Morphological Changes and Early Marijuana Use: A Magnetic Resonance and Positron Emission Tomography Study

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ABSTRACT. Background: The focus of this report is on the possible role that the age of first use of marijuana may play on brain morphology and function.

Methods: Magnetic resonance imaging (MRI) and positron emission tomography (PET) were utilized to study 57 subjects. Brain volume measures (whole brain, gray matter, white matter and lateral ventricle volumes), global cerebral blood flow (CBF) and body size were evaluated.

Results: There are three primary findings related to age of first use of marijuana. Subjects who started using marijuana before age 17, compared to those who started later, had smaller whole brain and percent cortical gray matter and larger percent white matter volumes. Functionally, males who started using marijuana before 17 had signifi-

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cantly higher CBF than other males. Both males and females who started younger were physically smaller in height and weight, with the effects being greater in males.

Conclusions: These findings suggest that the age at which exposure to marijuana begins is important. Early adolescence may be a critical period for effects that are not present when exposure begins later. These results are discussed in light of reported effects of marijuana on gonadal and pituitary hormones. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: getinfo@haworthpressinc.com <Website: http://www.haworthpressinc.com>]*

KEYWORDS. Brain morphology, marijuana (THC), Positron Emission Tomography (PET), Mangetic Resonance Imaging (MRI), adolescence, cerebral blood flow (CBF)

INTRODUCTION

The issue of whether there are long-term CNS effects from the use of marijuana remains a point of controversy. While there are substantial data that marijuana has acute effects,[1] there are few data to show chronic use of cannabis to be harmful.[2] Amidst this controversy, marijuana continues its status as the most commonly used illegal drug in the USA. Of particular concern is the marked increase of its popularity among young people.[3] Early use may present particular concern because of reported effects of marijuana on hormones that have increased activity during early adolescence.

Drug induced phenomena may be subsumed under acute effects, withdrawal, chronic functional (possibly reversible) and gross morphological effects. In the case of marijuana, although an impressive number of studies are available on its possible chronic effects,[1,4,5] little is known about possible irreversible functional or gross morphological changes. Initial findings of cerebral atrophy following exposure to marijuana were identified with pneumoencephalography.[6] However, those findings were not confirmed with computed tomography.[7-9] While MRI and automated techniques for differentiation of gray and white matter have been available,[10] they have not been applied to marijuana research. While numerous studies have attempted to deal with the difficult problem of sorting out the potential effects of chronic exposure, no study has examined the possible role that age of first exposure may play. The study reported here utilized MRI and PET to study effects of marijuana smoking on the brain. Data related to marijuana intoxication are presented elsewhere.[11]

METHODS

Subjects

This project was reviewed and approved by the Institutional Review Board at Duke University Medical Center. Volunteers were recruited through local advertising, and all volunteers signed a written informed consent prior to their participation in the study. Study participants were screened by a psychiatrist (RJM) who excluded significant physical or psychiatric disorders, abuse or addiction to any drug other than marijuana during the previous six months, current use of any prescribed or unprescribed medication and current vascular disorders including migraine. Males with alcohol use of more than two drinks per day and females with more than one drink per day were also excluded. Substance abuse was evaluated according to the Structured Clinical Interview for DSM-III-R.[12] Volunteers were required to have used marijuana either in the past or at present. None met criteria for abuse or dependence for other substances. All subjects completed the Drug Abuse Screening Test (DAST),[13] Brief Michigan Alcoholism Screening Test (MAST),[14] the short version of the Beck Depression Inventory[15] and the Trait Anxiety scores of the State-Trait anxiety scale.[16] Participants were predominantly right handed. Medical history, physical examination, electrocardiogram and clinical laboratory tests were obtained on all. None gave history or findings suggestive of neurological disorder, brain damage, head trauma, epilepsy, recurrent vascular headache or cognitive deficit. Pregnancy was excluded with plasma HCG tests. Subjects completed an interview including questions about age at first use of marijuana, current use and a checklist of the number of different types of drugs tried including alcohol and nicotine. Of 59 subjects recruited for study two were excluded; one because of age over 50 and one because an adequate determination of total brain volume could not be made. The remaining 57 ranged in age from 19 to 48 (mean 31.3 $\pm$ 7) years. There were 32 males (mean age of 33.5 $\pm$ 8.3) and 25 females (mean age of 29.8 $\pm$ 6.6). A total of 17 subjects either currently smoked cigarettes (n = 14) or had, but now did not (n = 3). All reported use of less than a pack per day. There was no sex difference in nicotine use. Most used alcohol (48/57) or had, but stopped (2/57); but there was no age-of-onset or sex difference for alcohol use. Most began using marijuana in their teens (16.8 $\pm$ 3.6 years with a median of 16 yr). ANOVA indicated no difference in age of first marijuana use by sex, or in duration of use (defined as current age minus age at first use). All completed high school with 54.4% completing college. Most were employed (77.8%) or in school (16.1%). There were no differences (chi-square) for education or employment by sex. The mean Beck Depression score was 1.4 $\pm$ 2.0, and the Trait Anxiety scores of the State-Trait anxiety scale was 30.1 $\pm$ 6.1. The mean MAST was 3.0 $\pm$ 4.8 and the

mean DAST was 2.9 ± 2.5. The most common other drugs to have been tried were LSD (39/57), cocaine (34/57), amphetamine (21/57) and psilocybin (23/57).

Procedures

Subjects came to the laboratory once for PET scans and a second day for MRI. Before PET scans they were required to abstain from marijuana for two weeks, alcohol for 24 hours, and nicotine and caffeine for four hours. Urine drug screens conducted before PET scans were negative for all participants. Blood was drawn for determination of plasma THC before PET scan. MRI utilized both T2-weighted and T1-weighted images. Transaxial MRI scans were performed with a General Electric 1.5 Tesla Sigma System. For all but four subjects, 3 mm contiguous, axial images were obtained parallel to the canthomeatal line. Two subjects had 5 mm, one had 4 mm and one had a 2 mm slice thickness images. The MR (proton density) image was utilized to establish segmentation criteria to determine whole brain (cerebrum) volume (WBV) (which excluded CSF and cerebellum volumes) and gray matter. Determination of the lateral ventricle volumes utilized T2. Use of segmentation criteria to identify tissue types has been shown to be a reliable method.[10,17] White matter volume was determined by subtracting gray plus ventricle from whole brain volume. Segmentation criteria were established on an individual basis. This process consisted of identifying signal levels on the MRI for predominantly gray matter and predominantly white, then using the midpoint of these activity levels as the cut criteria. A pixel was included as gray matter if its signal strength was at or above that cut point. ROI volumes were determined for each MRI slice (taking into consideration the slice thickness) and summed across all slices. Lateral ventricular volumes were determined by outlining the ventricles on each appropriate T2-weighted MRI slice, and using segmentation criteria to compute the volume. A set of rules for identifying ROI bilaterally (including frontal, temporal, parietal and occipital lobes, the insula, cingulate gyrus, basal ganglia, thalamus, amygdala and hippocampus) were developed with consultation from a neuroanatomist (JMP) and standard anatomical texts.[18,19] Gray matter regions were first delineated by hand on each MRI slice and then further distinguished from the surrounding tissue by the threshold technique mentioned above. ROI analysis was used to determine gray matter volume only. One person (TCH), who was blind to group assignment, did all region identification on all subjects. TCH has a BS in human physiology and 12 years of supervised experience at UCLA and Duke, including supervision by JMP who is Chief of Neuroradiology at Duke University Medical Center.

We examined the reliability of our ROI identification process on the images of ten subjects. Four ROI (which included cortical and subcortical structures)

were identified in the right hemisphere for all slices and procedure repeated one week later. Reliability was determined by computing an intraclass correlation (ICR), taking into the model brain, region and slice, achieving an ICR = 0.97, $p \leq .001$.

PET Scans: CBF measurements were performed in a semi-dark room with eyes and ears unoccluded. Subjects were instructed not to move. The left radial artery was catheterized under local anesthesia for arterial samples. A venous line was established in the opposite arm for infusion of ^{15}O-water and THC or placebo. Beginning simultaneously with each bolus injection of ^{15}O-water, 20 arterial blood samples (1-2 ml each) were drawn manually during each scan for determination of the ^{15}O-water input function. PET scans, utilizing the autoradiographic method,[20-22] were obtained with a General Electric 4096-Plus scanner. Data were acquired simultaneously from 15 planes (8 direct and 7 cross planes) with an approximate intrinsic axial and transaxial resolution of 6 mm FWHM with an axial field of view of 10 cm. A five-minute transmission scan was obtained for attenuation correction. Accurate CBF values measured with PET are dependent on appropriate corrections being made to the data. Attenuation, which is specific to each subject because of head size and shape and skull thickness, was measured and corrected individually via the transmission scan.

A bolus injection of 50 mCi ^{15}O-water was administered intravenously, and emission counts were accumulated over ten-second intervals for two minutes. Images were reconstructed with attenuation correction. They were then examined and the first image to show cerebral counts was taken as time zero. The next six subsequent scans were summed to obtain a 60-second autoradiographic image. Parametric images of CBF were computed according to the method of Meyer,[23] quantitative CBF being determined for each pixel and the mean determined over all pixels in an ROI volume.

We registered the PET image with each subject's own MRI, following methods developed by Chen et al.[24] and Pelizzari et al.[25] As implemented in our laboratory, the accuracy of this technique is on the order of 1-2 mm when performed with head phantom and human studies.[26,27] The registration results in a one-to-one anatomical correspondence between pixels in the PET data and the re-sliced MRI data; therefore, application of MRI-determined regions to the PET images is straightforward. The application of the ROIs to the PET data is automated, so that once the registration is done and regions are determined, application to the PET scan for determination of area weighted mean CBF require no operator intervention, other than routine inspection.

ANALYSIS

The median age of first use of marijuana (FIRST) was used to categorize subjects for analysis. The primary analysis method was ANCOVA using a

sex by FIRST model to examine brain volume measures, CBF, and other variables. Analysis of multiple ROI in the cortex was by multi-variate ANOVA with regions and hemisphere treated as repeated measures. Post hoc analyses used Tukey's comparisons between means.[28] Chi-square analysis was used for frequency data.

RESULTS

Subjects were stratified as starting marijuana use early (before age 17) or late (age 17 or later). Because the median of the total group (age 16) was used to set this cut point, the distribution of males and females is not equal, but a chi-square of sex by FIRST did not reach statistical significance. Age at first use of marijuana ranged from 11 to 26 years. The group sample sizes and mean ages were as follows: 13 early males (EM) = 14.5 $\pm$ 1.3, 19 late males (LM) = 19.3 $\pm$ 2.7, 16 early females (EF) = 14.5 $\pm$ 1.3, and 9 late females (LF) = 19.1 $\pm$ 2.0. ANOVA indicated no difference in the age at first use between males and females, and there was no significant interaction of sex by age at first use.

Demographic and Rating Scale Variables

Presented in Table 1 are the means and sd for current age, height, weight, estimated yearly marijuana use and duration of use. This table separates subjects by sex and age at first use of marijuana. ANOVA of current age indicated no group difference related to FIRST. Analysis of height indicated significant main effects for sex ($F = 56.50$, $p \leq .001$) and FIRST ($F = 5.07$, $p \leq .028$), and a significant interaction ($F = 3.98$, $p \leq .050$). Post hoc analyses indicated the EM group was shorter than LM, but there was no difference between EF and LF. There were also sex ($F = 38.82$, $p \leq .001$) and FIRST ($F = 5.44$, $p \leq .023$) main effects for weight, but no significant interaction. The frequency distributions for weight and height are presented in Figure 1. Analyses of duration of first use (defined as current age minus age of first use), baseline plasma THC, MAST and DAST indicated no significant main effects or interactions.

Males reported having tried more different drugs besides marijuana than females (4.2 $\pm$ 2.3 vs. 2.9 $\pm$ 1.8; $F = 10.37$, $p \leq .002$) and early users tried more than late (4.3 $\pm$ 1.9 vs. 2.9 $\pm$ 2.2; $F = 11.57$, $p \leq .001$), but the interaction was not significant. There was no significant difference by sex or FIRST for the number reporting alcohol use.

The number of marijuana joints per year had a very high variance, and the distribution was skewed, requiring log transformation before analysis. This analysis indicated a FIRST main effect that approached significance ($F =$

TABLE 1. Demographic and Baseline Assessments: Subjects Grouped by Age at First Use of Marijuana

		MALES		FEMALES	
		< 17	≥ 17	< 17	≥ 17
Age (*)	M	31.5	33.2	27.9	33.1
	sd	6.5	8.5	6.3	6.7
Height (*)	M	69.0	72.3	64.8	64.9
	sd	2.7	3.0	2.8	2.8
Weight (*)	M	171.1	191.7	138.6	145.2
	sd	19.1	26.6	22.1	28.0
Marijuana (*)	M	240.8	205.6	146.5	128.2
	sd	198.1	587.0	138.7	186.8
Duration (*)	M	16.9	13.9	13.4	14.0
	sd	6.4	6.9	6.0	6.6

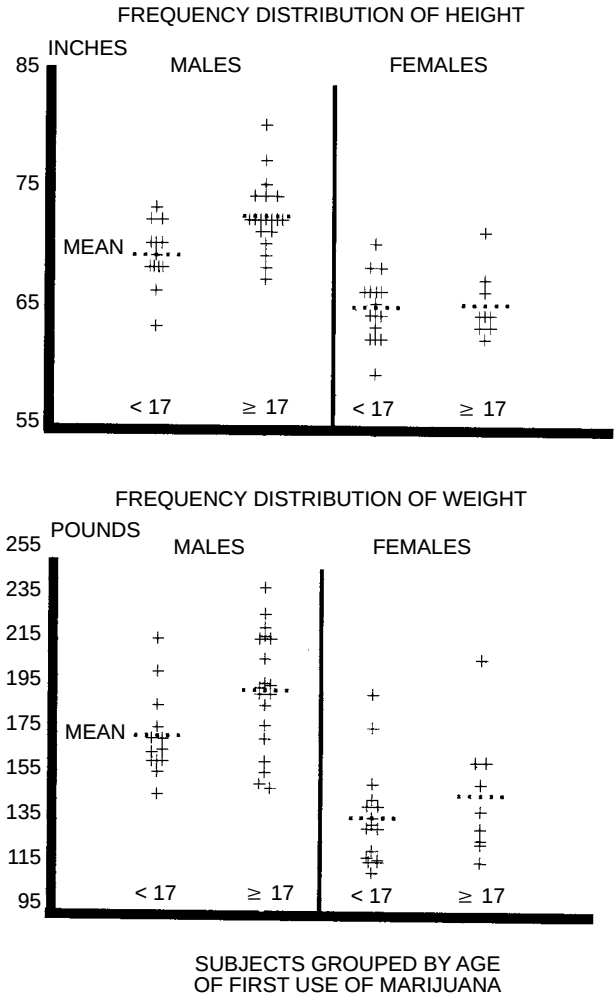
(*) Definitions of variables: Age at testing, Height in inches, Weight in pounds, Marijuana use in number of joints per year, Duration of use in years since first use of marijuana.

3.91, $p \leq .06$) with Early greater than Late, but there was no sex main effect or interaction. Subjects had been requested not to smoke marijuana for two weeks before coming for the PET scan, and ANOVA of plasma THC levels indicated no significant group differences. Because the ANOVA for estimated number of marijuana cigarettes per year (log transformed) approached significance, we included this transformed score as well as age as covariates in all ANOVAs of MRI and CBF data.

CBF

ANCOVA of whole brain gray matter CBF (ml/100 gm/min) indicated that males had lower flow ($F = 5.940$; $df = 1,51$; $p \leq .018$), and there was a significant interaction of FIRST by sex ($F = 4.35$; $df = 1,51$; $p \leq .042$). Post hoc analyses showed EM blood flows (48.8 ± 9.6) were significantly higher than LM (40.0 ± 6.3), but there was no difference between the two groups of females (EF = 49.2 ± 8.4; LF = 49.4 ± 6.8), nor was there a difference between EM and EF. It has been suggested that sex differences in cerebral metabolism may be accounted for by total brain volume, and if one corrects for this the sex differences go away.[29] With this in mind, we repeated the ANCOVA on global CBF adding WBV as a covariate, and this analysis indicated a stronger statistical interaction between First and sex ($F = 5.03$, $df = 1,50$, $p \leq .029$), and the sex main effect remained essentially unchanged

FIGURE 1. Height and Weight of Marijuana Smokers



(F = 5.56, df = 1,50, $p \leq .013$). There was no significant relationship between CBF and duration of use.

Brain Volumes

Whole brain (cerebrum) volume (WBV) showed a sex difference (F = 35.42, df = 1,51, $p \leq .001$) (Table 2). Therefore, to adjust for this sex

TABLE 2. MRI Volume Measurements: Subjects Grouped by Age at First Use of Marijuana

		Variables Expressed as Cubic Centimeters			
		MALES		FEMALES	
		< 17	≥ 17	< 17	≥ 17
Cerebrum (A) (*)	M	1295.8	1316.1	1149.9	1097.9
	sd	130.0	90.6	110.9	86.5
Gray (*)	M	688.0	729.7	631.4	631.5
	sd	70.3	68.9	58.0	82.3
White (*)	M	583.0	554.5	495.5	440.3
	sd	76.6	68.0	82.9	53.3
Ventricles (*)	M	24.8	31.8	23.0	26.1
	sd	6.5	15.2	9.9	11.2

(*) Definitions of variables: Cerebrum volume, Gray, White and Ventricle volumes in cubic centimeters. Cerebrum is whole brain volume.

difference, we expressed gray matter, white matter, and ventricular volumes as a percent of WBV, and all analyses were based on the percent volumes. The whole brain, gray matter, white matter and ventricular volumes are presented in Table 2, and are expressed as percents of WBV in Table 3. None of the volume measures showed a relationship to duration of use. ANCOVAs indicated significant FIRST main effects, but no sex differences or interactions for percent whole brain gray matter ($F = 5.940$, $df = 1,51$, $p \leq .018$). Both EM and EF had a lower volume. Percent whole brain white matter volume also showed a significant FIRST effect ($F = 7.334$, $df = 1,51$, $p \leq .009$) with those starting early having greater volume. The percent ventricular volumes did not reach significance.

We determined cortical gray matter volumes for six ROI (frontal, temporal, parietal and occipital lobes, cingulate gyrus and insula) in each hemisphere (Table 4), and expressed these volumes as a percent of WBV (Table 5). These data were analyzed in a sex by First repeated measures MANCOVA model with ROI and hemisphere treated as repeated measures, and age and current marijuana use as covariates. This analysis indicated a significant FIRST main effect ($F = 4.53$, $df = 1,51$, $p \leq .038$), a FIRST by Hemisphere interaction ($F = 6.08$, $df = 1,53$, $p \leq .017$) and a FIRST by Hemisphere by ROI interaction (Pillai $F = 2.48$, $df = 5,49$, $p \leq .044$), and there was a significant sex by Region interaction (Pillai $F = 3.23$, $df = 5,49$, $p \leq .013$). Females tended to have slightly greater percents for regional gray matter volumes (Table 5).

TABLE 3. MRI Volume Measurements: Subjects Grouped by Age at First Use of Marijuana

		Variables Expressed as Percents of Whole Brain Volume			
		MALES		FEMALES	
		< 17	≥ 17	< 17	≥ 17
Gray (*)	M	53.2	55.5	55.1	57.4
	sd	2.6	4.0	4.2	4.6
White (*)	M	44.9	42.1	42.9	40.2
	sd	2.7	4.3	4.4	4.7
Ventricle (*)	M	1.9	2.4	2.0	2.4
	sd	0.6	1.0	0.8	1.0
Left C-Gray (*)	M	25.2	26.2	26.1	26.6
	sd	1.4	2.0	1.9	2.3
Right C-Gray (*)	M	25.3	26.6	26.1	27.8
	sd	1.4	2.0	2.1	2.1

(*) Definitions of variables: Gray, White and Ventricle Volumes as percents of whole brain volume. Gray and white are total cerebrum volumes, C-gray is sum of cortical gray for each hemisphere including frontal, temporal, parietal and occipital lobes, cingulate gyrus and insula ROI.

To analyze the above three-way interaction, we computed separate repeated measures ANCOVAs for each ROI using a sex by FIRST by Hemisphere model. These analyses indicated that the frontal gray matter ROI showed a significant FIRST by Hemisphere interaction ($F = 11.99$, $df = 1,53$, $p \leq .001$), and for the parietal gray matter region there was a significant main effects of FIRST ($F = 4.89$, $df = 1,51$, $p \leq .032$). Separate sex by First ANCOVAs of cortical gray for each hemisphere (summed over all 6 ROI) indicated no significant differences for the left, but the right hemisphere showed a significant main effect for FIRST ($F = 10.15$, $df = 1,51$, $p \leq .002$). Presented in Figure 2 are the means plus the upper 95% confidence interval for males and females for the left and right hemisphere cortical gray matter (percent of WBV). Both males and females showed a larger right hemisphere, and those who started early (< 17) had smaller volumes.

Analysis of subcortical gray matter (percent of WBV), determined by subtracting cortical from whole brain gray matter, failed to show any significant sex or FIRST differences. Because it has been reported that marijuana may affect the hippocampal region,[30] we computed the gray matter volume for hippocampus and amygdala and analyzed these in an ANCOVA model,

TABLE 4. Regional Volumes of Gray Matter: Subjects Grouped by Sex and Age of First Use of Marijuana

	Males				Females			
	< 17		≥ 17		< 17		≥ 17	
LEFT	Mean (**)	sd	Mean	sd	Mean	sd	Mean	sd
FRNT (*)	112.2	18.8	119.3	20.9	111.2	15.0	103.2	17.5
TEMP (*)	83.3	11.9	86.8	13.1	69.8	9.7	69.1	19.0
PARI (*)	66.9	15.6	74.4	16.9	71.5	11.7	74.3	9.4
OCCI (*)	45.9	11.8	47.6	17.0	32.6	11.4	31.2	12.2
INSU (*)	10.3	1.7	10.0	1.8	9.2	1.1	9.4	1.2
CING (*)	6.7	1.8	6.1	1.1	5.2	1.2	5.5	1.3
RIGHT								
FRNT (*)	120.7	19.3	131.4	22.8	116.0	18.5	117.4	17.1
TEMP (*)	91.4	16.6	96.5	9.7	75.5	11.1	75.9	16.5
PARI (*)	60.3	15.3	68.0	17.8	65.4	10.3	68.9	10.1
OCCI (*)	38.0	7.1	36.8	15.1	27.1	10.5	28.3	12.7
INSU (*)	10.4	1.9	10.2	1.6	9.7	1.3	9.7	0.8
CING (*)	6.5	1.8	6.3	1.2	5.6	1.4	5.6	1.2

(*) ROI–frontal, temporal, parietal and occipital lobes, insula and cingulate gyrus.
(**) ROI volumes are expressed as cubic centimeters.

but there were no significant effects. Neither did this region show effects related to duration of use.

It has been reported that ratio of gray to white matter is 1.3 in adults ages 25-39, as determined by *in vivo* MRI measurements.[31] Our data found a ratio of 1.31 ± 0.24 in total sample. However, ANCOVA indicated a significant differences on the basis of FIRST ($F = 5.23$, $df = 1,51$, $p \leq .026$) with Early having a significantly lower ratio (E: 1.25 ± 0.18 vs. L: 1.38 ± 0.26), and this difference was still significant when WBV was also covaried.

SUMMARY

The relationships between marijuana use, brain function and morphology were studied in 57 marijuana smokers (32 males and 25 females). Analyses indicated that five variables showed a significant relationship to age of first use of marijuana. These analyses indicate that both males and females who started using marijuana before age 17 have a lower percent of cortical gray

TABLE 5. Regional Gray Matter Volumes as a Percent of Whole Brain: Subjects Grouped by Sex and Age of First Use of Marijuana

	Males				Females			
	< 17		≥ 17		< 17		≥ 17	
LEFT	Mean (**)	sd	Mean	sd	Mean	sd	Mean	sd
FRNT (*)	8.6	1.0	9.1	1.5	9.7	1.3	9.4	1.2
TEMP (*)	6.5	1.4	6.6	1.0	6.1	1.0	6.3	1.5
PARI (*)	5.1	1.0	5.7	1.2	6.2	0.9	6.8	0.9
OCCI (*)	3.5	0.8	3.6	1.2	2.8	0.9	2.8	1.0
INSU (*)	0.8	0.2	0.8	0.1	0.8	0.1	0.9	0.1
CING (*)	0.5	0.2	0.5	0.1	0.5	0.1	0.5	0.1
RIGHT								
FRNT (*)	9.3	1.0	10.0	1.7	10.1	1.4	10.6	0.9
TEMP (*)	7.1	1.0	7.4	0.8	6.6	1.0	6.9	1.3
PARI (*)	4.7	1.1	5.2	1.3	5.7	0.9	6.3	0.9
OCCI (*)	2.9	0.5	2.8	1.1	2.4	0.9	2.6	1.0
INSU (*)	0.8	0.1	0.8	0.1	0.8	0.1	0.9	0.1
CING (*)	0.5	0.2	0.5	0.1	0.5	0.1	0.5	0.1

(*) ROI–frontal, temporal, parietal and occipital lobes, insula and cingulate gyrus.
(**) ROI volumes are expressed as percent of total whole brain volume.

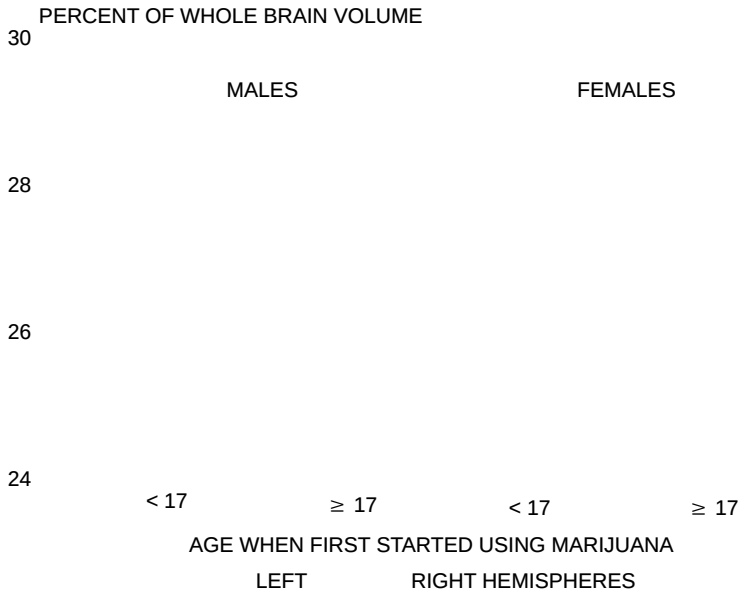
matter compared to those who started later (after adjusting for whole brain volume), increased percent brain white matter, although whole brain and ventricular volumes were not different. The mean CBF for males who started smoking before age 17 was significantly higher than males who started after 17, and their mean CBF was not different from females. Males who started later had significantly lower mean CBF than females. In addition subjects who had started smoking earlier had lower body weight and reduced height.

It is important to note that none of these measures showed a significant relationship to duration of use. Thus, the findings are not simply that those who started younger have smoked longer.

DISCUSSION

The results of this study demonstrate possible effects of early marijuana use in three areas. First, both males and females who start early have a smaller percent of cortical gray matter and increased white (adjusted for

FIGURE 2. Percent Hemispheric Cortical Gray Volume: Mean + 95% Confidence Interval



whole brain volume) than those who start later. Second, males who start early have a global CBF that is not different from females, and is significantly higher than males who start smoking later. Third, both males and females who start using marijuana before the age of 17 are physically smaller in terms of height and weight, when compared to those who start smoking after 17. These effects may be related to reported effects of marijuana on hormone secretion, but there may also be neurotoxic effects.

Effects on Gray Matter Volumes

These findings indicate that both males and females who started using marijuana during early adolescence have a reduced percent of cortical gray matter when compared to those who started later, and the difference is greatest in the frontal lobes. This was the case even when the effects of age and current level of marijuana use were removed with covariate analysis. Our analyses failed to find differences related to duration of marijuana use, estimated from current age and age of first use. There was no difference in ventricle volume adjusted for WBV, nor was there an effect on whole brain volume. It seems reasonably safe to conclude that there are changes in gray

matter, because it differs by ROI (particularly in frontal). High densities of THC receptors have been found in hippocampus and cerebral neocortex,[32] and we have reported that THC has a greater effect on the frontal lobes.[11] A potential mechanism of this effect on gray matter is open to debate, but because it is related to age of first use, we suggest that this has more to do with brain development than atrophy. Physical and psychiatric examination of these subjects failed to indicate any gross cognitive dysfunction, but neurocognitive testing that might detect subtle differences was not done.

Our hypothesis of a potential mechanism for the observed effects is based on two issues. First there is a significant degree of brain development going on during adolescence, and second, marijuana has been reported to have effects on gonadal and pituitary hormones.

Reported changes of CBF and CMR during adolescence are associated with other developmental changes ongoing in the brain during this time.[33-36] The study by Reiss et al.[36] has shown ''Prominent age related changes in gray matter, white matter and CSF . . .'', which ''. . . appear to reflect ongoing maturation and remodeling.'' The changes include increases in myelinization and decreases in gray matter. Purves[37] has suggested that due to the rapid somatic growth during adolescence, there may be a need for neural plasticity to persist to accommodate these changes.

Thus, data show that during adolescence there is an active change in brain maturation. To this observation must be added findings concerning the effect of gonadal hormones on brain development and function. It has been shown that gonadal hormones play a role in prenatal brain development;[38-39] it has also been shown that they may play a significant role, postnatally, in sex differences in function and possibly structure. Clark and Goldman-Rakic[40] demonstrated that postnatal treatment of normal rhesus monkeys with testosterone had a significant ''masculinizing'' influence on the maturation of the orbital prefrontal cortex of female monkeys. Clark and Goldman-Rakic concluded that gonadal hormones may play a significant role in modifying the brain at times when there is ''dynamic change'' ongoing in the cortex. It is clear from the work of Jernigan et al.,[31, 35] and Reiss et al.,[36] that adolescence is a time of active brain development with one component of this activity being a significant change in gray matter. This is important because it suggests a possible process where a reduction of gonadal hormones may effect change in development and subsequently function.

It has been reported that THC has an inhibitory effect on serum testosterone levels, and it has been shown to reduce testicular weight,[41] and it has also been shown that a single marijuana cigarette significantly reduces luteinizing hormone (LH) in human males[42] and females.[43] In males LH stimulates the interstitial endocrinocytes in the testes for production and secretion of testosterone. It may be that both males and females who utilize marijuana during

early adolescence alter the course of brain development due to alteration in gonadal and pituitary hormones, and that this is related to global CBF and to the reduction in cortical gray matter.

It is important to note that our finding of significantly greater cortical gray matter (percents) in females is consistent with the findings of Schlaepfer et al.,[44] and Murphy et al.[45] Similarly our findings of a gray/white ratio in the total sample of 1.31% was virtually identical to other reports from a comparable age group of normal subjects.[31]

The first candidate mechanism to account for our observed effects on cortical gray matter is the effect of marijuana on pituitary and gonadal hormones, and their effect on development. However, another possibility is a direct effect on tissue. Initial findings of cerebral atrophy following exposure to marijuana were identified with pneumoencephalography.[6] However, those findings were not confirmed with computed tomography.[7-9] This first report of anatomical changes[6] was conducted on patients who had otherwise been identified for evaluation for neurological disorder or cognitive impairment, making the interpretation of these data difficult. The three studies to use computed tomography (CT) scans[7-9] in heavy marijuana users only examined ventricle measurements, and none could confirm differences from controls. It should be noted that in our study the lateral ventricle as a percent of WBV was 2.2% for the group, which is consistent with other reports.[10]

Animal data support possible neurotoxic effects, perhaps permanent changes in brain neurochemistry, morphology and function following chronic exposure to cannabinoids. It has been reported that exposure to cannabinoids alters dopaminergic system development.[46,47] Klausner and Dingell[48] reported that exposure to cannabinoids during critical periods of development led to changes in dopamine neurons in adulthood, and that there were clear sex differences in these effects with greater effects in males. Chronic administration of relatively large doses of marijuana has been reported to lead to chronic changes in behaviors believed to be related to hippocampal function[49] and structure.[30,49,50] Importantly, the degree of histological change was greater in exposed peripubertal animals than in young adults. However, other investigators have reported that a one-year exposure to inhalation of marijuana smoke in adolescent rhesus monkey did not lead to long term changes of CA3 region neurons in the hippocampus.[51] Our data also failed to show differences in the hippocampus gray matter volumes.

White Matter

In the present study early marijuana users had a greater percent of white matter (adjusted for whole brain volume) than later smokers. It is reported that cannabinoids bind to myelin basic protein with high affinity.[52,53] However, it is unclear why such an effect might lead to greater white matter for

early users. One might argue that because we computed white matter by subtracting gray and ventricle volumes from whole brain, the apparent increase in white is because of a reduction in gray. However, whole brain and ventricle volumes were measured independently of gray in this study, and our findings for these three are consistent with other reports[10,17,44] as is the gray/white ratio.[31]

Effects on CBF

It is generally accepted that females have a higher resting CBF than males,[54-56] and in the present study this expected main effect of sex was confirmed. However, CBF of males who started using marijuana early was not different from females, and was significantly higher than males who started smoking after age 17. It has not been clearly established why females have a higher CBF. One proposal is that it has to do with brain volume. Yoshii et al.[29] reported a sex difference in cerebral metabolic rate (CMR), but when he removed the effects of brain volume from CMR, the sex differences disappeared. Because CBF and CMR are closely correlated within physiologically normal ranges,[57,58] it may be assumed that correcting for brain volume might eliminate the sex difference in CBF. However, in our analysis when corrected for brain volume the CBF difference remained. It should also be noted that the effect of brain volume should not have played a role in our data, because we used a measured attenuation correction in determining CBF.[56] The methods we employed did not make partial volume correction. However, the differences seen between groups showed higher flow in the groups with less cortical gray matter, which is opposite the direction of resolution (partial volume) effect. Application of an appropriate correction for the partial volume effect would boost the women's CBF values more than the men's, making the CBF values even more different. The global CBF for our sample (46.1 ± 8.7) is within the range reported for other non-activation PET ^{15}O-water studies with normal volunteers reviewed by Roland.[59]

A potential role of gonadal hormones on CBF should be considered. CBF studies in children [60-63] do not address the question of sex difference in CBF during childhood, but this difference is generally agreed to be present after puberty. There is a CBF reduction during puberty.[60-63] The difference between males and females may be partly related to gonadal hormones. Studies in rats[64] and humans[65] have shown that the administration of estrogen increases CBF. The study by Ohkura et al. was carried out in postmenopausal women with absolute quantification of CBF before and after treatment with estrogen replacement.[65] It has also been reported that the sex differences in CBF decrease following menopause.[66] Hormones may also differentially affect brain developmental changes during adolescence (discussed below)

which may influence CBF. It should be noted, however, that not all studies have been able to demonstrate an effect of estrogen on CBF.[67]

Effects on Weight and Height

In this study both males and females who started smoking marijuana early were shorter, but the effect was greater in the males. One of the reported effects of THC is a suppression of release of pituitary hormones, including prolactin, growth hormone, and gonadotropin.[41,46,68,69] Thus, it is possible that adolescents who use marijuana early (and particularly if they use it regularly) might blunt their physical growth. Such an effect is consistent with normal growth hormone release patterns in early adolescence. Around age 11 to 12 for females and 13 to 14 for males there is a significant increase in the rate of growth hormone release which is accompanied by an increased rate of growth.[70] Most females achieve most of their adult height by about age 15 while males achieve their adult height about 2 years later.[70,71] In our sample this timing difference may have played a significant role, because the mean age of first use was about 14 for both males and females. Since males tend to start and end their adolescent growth period somewhat later than females, starting to smoke at age 14 would be starting earlier in males' growth period, and would thus expose them to a longer period of the adverse effects of reduced growth hormone release. It has been shown that THC can reduce the pubertal body weight growth in male rats,[72] and can delay sexual maturation.[73] These data are particularly interesting for our results, because they suggest that the timing of exposure to THC is important.

Caveats

There are several issues that this study did not address that require additional consideration. There is no control group of non-drug users matched to the subjects to determine if the observed findings fall within a normal range. We did not include subjects who were not marijuana users because other components of the study involved the infusion of THC to determine acute effects on CBF. The subjects reported trying a variety of other drugs, and the extent that the effects in this study may be attributable to drug use rather than to marijuana alone can not be determined. Duration of use was expressed as current age minus age at first use of marijuana. Such a variable, while correlated with chronic exposure, must be viewed with caution because variation in drug use across life span is very high. Vastly differing patterns of use may be present related to cost, availability, sharing joints with others, etc. Categorizing the subjects on the basis of their median age of first use was an empirical step.

In spite of the shortcomings, this study includes a relatively large sample of minimal to moderate marijuana users, who were physically healthy. The findings are not isolated to a single dimension, but rather manifest themselves in body size, brain morphology and cerebral blood flow. These findings suggest that exposure to marijuana (and possibly other drugs) at certain critical periods such as during early adolescence may alter normal patterns of development.

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