

Regional Brain Abnormalities Associated With Long-term Heavy Cannabis Use

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Context: Cannabis is the most widely used illicit drug in the developed world. Despite this, there is a paucity of research examining its long-term effect on the human brain.

Objective: To determine whether long-term heavy cannabis use is associated with gross anatomical abnormalities in 2 cannabinoid receptor-rich regions of the brain, the hippocampus and the amygdala.

Design: Cross-sectional design using high-resolution (3-T) structural magnetic resonance imaging.

Setting: Participants were recruited from the general community and underwent imaging at a hospital research facility.

Participants: Fifteen carefully selected long-term (>10 years) and heavy (>5 joints daily) cannabis-using men (mean age, 39.8 years; mean duration of regular use, 19.7 years) with no history of polydrug abuse or neurologic/mental disorder and 16 matched nonusing control subjects (mean age, 36.4 years).

Main Outcome Measures: Volumetric measures of the hippocampus and the amygdala combined with mea-

asures of cannabis use. Subthreshold psychotic symptoms and verbal learning ability were also measured.

Results: Cannabis users had bilaterally reduced hippocampal and amygdala volumes ($P = .001$), with a relatively (and significantly [$P = .02$]) greater magnitude of reduction in the former (12.0% vs 7.1%). Left hemisphere hippocampal volume was inversely associated with cumulative exposure to cannabis during the previous 10 years ($P = .01$) and subthreshold positive psychotic symptoms ($P < .001$). Positive symptom scores were also associated with cumulative exposure to cannabis ($P = .048$). Although cannabis users performed significantly worse than controls on verbal learning ($P < .001$), this did not correlate with regional brain volumes in either group.

Conclusions: These results provide new evidence of exposure-related structural abnormalities in the hippocampus and amygdala in long-term heavy cannabis users and corroborate similar findings in the animal literature. These findings indicate that heavy daily cannabis use across protracted periods exerts harmful effects on brain tissue and mental health.

Arch Gen Psychiatry. 2008;65(6):694-701

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THERE IS CONFLICTING evidence regarding the long-term effects of regular cannabis use. Although growing literature suggests that long-term cannabis use is associated with a wide range of adverse health consequences,¹⁻⁴ many people in the community, as well as cannabis users themselves, believe that cannabis is relatively harmless and should be legally available. With nearly 15 million Americans using cannabis in a given month, 3.4 million using cannabis daily for 12 months or more, and 2.1 million commencing use every year,⁵ there is a clear need to conduct robust investigations that elucidate the long-term sequelae of long-term cannabis use.

The strongest evidence against the notion that cannabis is harmless comes from the animal literature⁶⁻⁹ in which long-

term cannabinoid administration has been shown to induce neurotoxic changes in the hippocampus, including decreases in neuronal volume, neuronal and synaptic density, and dendritic length of CA3 pyramidal neurons. Although such work suggests that exposure to cannabinoids may be neurotoxic in animals, much less is known about the neurobiologic consequences of long-term cannabis exposure in humans.

Only a handful of brain imaging studies have been conducted in human cannabis users, with inconsistent findings reported. Early cannabis research using pneumoencephalography¹⁰ reported cerebral atrophy in a small sample ($N = 10$) of cannabis users, but further studies using computed tomography¹¹⁻¹³ did not detect any abnormalities, despite the potential confounds of polydrug use, comorbid neurologic/psychiatric diagnoses, and a lack of appropriate comparison groups. More

recent structural magnetic resonance imaging (MRI) studies have also reported contradictory findings, ranging from no global or regional changes in brain tissue volume or composition¹⁴⁻¹⁶ to gray and white matter density changes, either globally¹⁷ or in focal regions, most notably in the hippocampal and parahippocampal areas.^{18,19} However, these previous studies used imaging techniques with relatively coarse spatial and anatomical resolution and typically focused on samples with multiple substance use or comorbid psychiatric disorders and on only moderate levels of cannabis use (ie, <2 joints per day). Indeed, despite strong evidence of neurotoxicity in the animal literature,⁶⁻⁹ to our knowledge, no neuroimaging study has examined the neurobiologic sequelae of long-term heavy cannabis use while controlling for the important confounds of polydrug abuse and co-occurring psychiatric disorders.

In this study, we used high-resolution 3-T MRI to assess volumetric changes in 2 cannabinoid-rich regions of the brain (the hippocampus and the amygdala) known to be susceptible to the neurotoxic effects of cannabis exposure in a sample of long-term heavy users carefully screened for polysubstance abuse and mental disorders. Given the growing literature regarding an association between cannabis use and the development of psychosis²⁰ and cognitive impairment,^{16,21} we also assessed for sub-threshold psychotic symptoms and verbal learning ability in this otherwise psychologically healthy sample.

METHODS

PARTICIPANTS

Male cannabis users with long histories of regular and heavy cannabis use (n=15) and nonusing healthy male volunteers (n=16) matched on age, estimated premorbid intelligence (National Adult Reading Test),²² years of education, and state and trait anxiety (Spielberger State-Trait Anxiety Inventory)²³ were recruited from the general community via a variety of advertisements (**Table**). Cannabis users had lower Global Assessment of Functioning scale scores and greater depressive symptoms (as measured using the Hamilton Depression Rating Scale)²⁴ than the comparison group; however, there were no current or lifetime histories of diagnosable medical, neurologic, or psychiatric conditions as assessed using the *Structured Clinical Interview for DSM-IV Axis I Disorders, Patient Edition*.²⁵ All the control subjects also underwent a *Structured Clinical Interview for DSM-IV Axis I Disorders, Non-Patient Edition*.²⁵ Subthreshold psychotic symptoms were probed using the Scale for the Assessment of Positive Symptoms²⁶ and the Scale for the Assessment of Negative Symptoms.²⁷ Regarding alcohol use, the groups did not differ in levels of current consumption, lifetime use, or history of abuse or dependence; and no participant drank more than 24 standard alcoholic drinks per week. Significantly more cannabis users were also tobacco smokers ($\chi^2=22.9, P<.001$) (**Table**). For all users, cannabis was the primary drug of abuse, with only limited experimental use of other illicit drugs (generally <10 lifetime episodes).

PROCEDURE

Participants were assessed on 2 occasions, usually 1 week apart. In the first test session, participants completed demographic, clinical, and substance use history assessments. In the second

test session, they completed the Rey Auditory Verbal Learning Test (RAVLT) and underwent structural MRI.

Participants were asked to abstain from using substances for at least 12 hours before each test session, and cannabis users reported abstaining from cannabis for a mean of 21.3 hours before the first test session (median, 14 hours; range, 10-72 hours) and a mean of 19.8 hours before the second test session (median, 17 hours; range, 12-48 hours). Urine samples were obtained from users on 4 occasions and from controls on 2 occasions to corroborate self-reported abstinence. Specifically, for cannabis users, samples were obtained on the evening before each test session and on the day of testing. For controls, samples were collected only on the day of testing. Examination of these samples demonstrated that all but 1 cannabis user had cannabinoid metabolites (11-nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid creatinine normalized) detected in urine samples from the first test session, and levels were generally high (evening: median, 467 ng/mg [range, 0-2320 ng/mg]; day of testing: median, 447 ng/mg [range, 0-11 293 ng/mg]). From the second test session, 2 users returned a 0 reading; otherwise, cannabinoid metabolite levels were again high (evening: median, 456 ng/mg [range, 0-3511 ng/mg]; day of testing: median, 389 ng/mg [range, 0-4470 ng/mg]). The levels of urinary cannabinoid metabolites generally corroborate the self-reported patterns of heavy cannabis use in the sample. All but 2 control subjects returned a 0 reading for cannabinoid metabolites across both test sessions. The 2 controls with positive urine samples reported only minimal and very occasional exposure to cannabis. The median level of cannabinoid metabolites in controls at the first test session was 0 ng/mg (range, 0-184 ng/mg) and at the second test session was 0 ng/mg (range, 0-180 ng/mg).

STRUCTURAL MRI

The MRI data were obtained using a 3-T scanner (Intera; Philips Medical Systems NA, Bothell, Washington) at the Symptom Clinical Research Imaging Centre, Prince of Wales Medical Research Institute, Sydney. A 3-dimensional volumetric spoiled gradient-recalled echo sequence generated 180 contiguous coronal slices. The imaging parameters were as follows: echo time, 2.9 milliseconds; repetition time, 6.4 milliseconds; flip angle, 8°; matrix size, 256 × 256; and 1-mm³ voxels. Hippocampal, amygdala, whole brain, and intracranial volumes were measured using established reliable protocols²⁸⁻³¹ and were delineated by a trained rater (S.W.) masked to group information. Specifically, the hippocampal boundaries were as follows: posterior, the slice with the greatest length of continuous fornix; medial, the open end of the hippocampal fissure posteriorly, the uncus fissure in the hippocampal body, and the medial aspect of the ambient gyrus anteriorly; lateral, the temporal horn of the lateral ventricle; inferior, the white matter inferior to the hippocampus; superior, the superior border of the hippocampus; and anterior, the alveus was used to differentiate the hippocampal head from the amygdala. The anterior border was the most difficult to identify consistently and was aided by moving between slices before and after the index slice. The amygdala boundaries were as follows: posterior, the appearance of amygdala gray matter above the temporal horn; superolateral, the thin strip of white matter that separates the amygdala from the claustrum and the tail of the caudate; medial, the angular bundle, which separates the amygdala from the entorhinal cortex; superomedial, the semilunar gyrus; inferior, the hippocampus; inferolateral, the temporal lobe white matter and the extension of the temporal horn; and anterior, the slice anterior to the appearance of the optic chiasm. Whole brain volumes were estimated using the Brain Extraction Tool method³²

Table. Demographic, Clinical, Drug Use, and MRI Volumetric Measures

Measure	Long-term Cannabis Users (n=15)	Nonusing Control Subjects (n=16)	P Value ^a
Age, mean (SD), y	39.8 (8.9)	36.4 (9.8)	.31
IQ, mean (SD)	109.2 (6.3)	113.9 (8.1)	.09
RAVLT score, mean (SD)			
Sum of 5 learning trials	43.8 (8.8)	57.4 (10.1)	<.001
20-min delay	8.9 (4.1)	12.3 (3.7)	.009 ^b
Educational level, mean (SD), y	13.4 (3.2)	14.8 (3.7)	.28
GAF scale score, mean (SD)	72.0 (11.2)	80.8 (9.4)	.02
HAM-D score, mean (SD)	5.87 (3.2)	2.56 (1.9)	<.001 ^b
STAI, mean (SD)			
State anxiety	34.3 (9.8)	32.9 (9.4)	.67
Trait anxiety	39.3 (9.7)	39.0 (8.2)	.92
SAPS score, mean (SD)	8.1 (7.9)	0.6 (1.2)	<.001 ^b
SANS score, mean (SD)	11.7 (8.5)	1.4 (1.4)	<.001 ^b
Cannabis use			
Duration of regular use, mean (SD) [range], y ^c	19.7 (7.3) [10-32]	NA	NA
Age started regular use, mean (SD) [range], y ^c	20.1 (6.9) [12-34]	NA	NA
Current use, mean (SD), d/mo ^d	28 (4.6)	NA	NA
Current use, mean (SD), cones/mo ^{d,e}	636 (565)	NA	NA
Cumulative exposure, past 10 y, mean (SD) ^f	77 816 (66 542)	NA	NA
Cumulative exposure, lifetime, mean (SD) ^f	186 184 (210 022)	12.7 (12.2)	<.001
Estimated episodes of use, median (range)	62 000 (4600-288 000)	11 (0-30)	<.001
Alcohol use, mean (SD), standard drinks/wk	9.6 (6.1)	6.8 (5.0)	.19
Tobacco use, mean (SD), cigarettes/d	16.5 (8.9)	7.5 (9.2)	.20
Brain volumes, mean (SD), mm ³			
Intracranial cavity	1 546 237 (94 018)	1 607 590 (136 386)	.14
Whole brain	1 310 780 (90 778)	1 374 123 (105 673)	.09
Hippocampus			.002 ^g
Left hemisphere	2849 (270)	3240 (423)	
Right hemisphere	2949 (244)	3348 (400)	
Amygdala			.01 ^g
Left hemisphere	1766 (98)	1878 (190)	
Right hemisphere	1601 (143)	1744 (158)	

Abbreviations: GAF, Global Assessment of Functioning; HAM-D, Hamilton Depression Rating Scale; MRI, magnetic resonance imaging; NA, not applicable; RAVLT, Rey Auditory Verbal Learning Test; SAPS, Scale for the Assessment of Positive Symptoms; SANS, Scale for the Assessment of Negative Symptoms; STAI, State-Trait Anxiety Inventory.

^aTwo-tailed *t* test unless otherwise indicated.

^bMann-Whitney test.

^cRegular use was defined as at least twice a month.

^dCannabis users had used at this level for most of their drug-using history.

^eA *cone* is the small funnel into which cannabis is packed to consume through a water pipe in a single inhalation. Without the loss of sidestream smoke, the quantity of tetrahydrocannabinol delivered by this method is estimated as equating 3 cones to 1 cigarette-sized joint. Thus, the cannabis users in this study smoked the equivalent of 212 joints per month, or approximately 7 joints per day.

^fExpressed as cones for users and as episodes for controls. Estimates of lifetime exposure beyond 10 years in these very long-term users became skewed and unreliable; hence, the 10-year estimate was used in correlational analyses.

^gRegion \times group analysis of variance.

to separate brain from nonbrain tissue. After brain/nonbrain segmentation, each voxel was classified into gray matter, white matter, or cerebrospinal fluid using FAST Model statistical software.³³ Only gray and white matter were used in the estimate of whole brain volumes. The intracranial cavity was delineated from a sagittal reformat of the original 3-dimensional data set. The major anatomical boundary was the dura mater below the inner table, which was generally visible as a white line. Where the dura mater was not visible, the cerebral contour was outlined. Other landmarks included the undersurfaces of the frontal lobes, the dorsum sellae, the clivus, and the posterior arch of the craniovertebral junction.

Interrater and intrarater reliabilities were assessed by means of the intraclass correlation coefficient (ICC) (absolute agreement) using 15 brain images from a separate MRI database established specifically for this purpose and that has previously been delineated by another expert rater. For the hippocampus, interrater ICC reliabilities were 0.92 (right) and 0.91 (left)

and intrarater ICC reliabilities were 0.98 (right) and 0.95 (left). For the amygdala, interrater ICC reliabilities were 0.85 (right) and 0.88 (left) and intrarater ICC reliabilities were 0.93 (right) and 0.97 (left). Once reliability was established, the rater (S.W.) delineated the regions of interest for the images acquired from the present study.

STATISTICAL ANALYSES

Whole brain volume, age, educational level, and estimated IQ were not significantly different between the 2 groups and were, therefore, not used as covariates (Table). Regional gray matter volumes for the hippocampus and amygdala were corrected for the effect of the intracranial cavity using a previously described formula³⁴ and were analyzed using analyses of variance, with hemisphere (left or right) and region (hippocampus and amygdala) as within-subject factors and group as the between-subject factor. Main effects and interactions were evalu-

ated using Greenhouse-Geisser-corrected degrees of freedom, with $\alpha = .05$. Effect sizes, expressed as Cohen d , are also reported for pairwise contrasts. Only effects involving group (cannabis users vs nonusers) and associations with cannabis use parameters are reported because this was the primary focus of the present study. Group comparisons of performance on the RAVLT and measures of subthreshold psychotic symptoms (using the Scale for the Assessment of Positive Symptoms and the Scale for the Assessment of Negative Symptoms) were conducted using independent-samples t tests or Mann-Whitney tests for nonnormally distributed data. Pearson product moment correlational analyses were conducted to examine the behavioral (ie, symptom and cognitive) relevance of any identified group differences in regional brain volumes and the association between these brain changes and parameters of cannabis use. These analyses were necessarily exploratory given the limited sample size.

RESULTS

GROUP CONTRASTS

In the analysis of regional gray matter volumes, there was a significant main effect of group ($F_{1,29} = 12.98$, $P = .001$) and a region \times group interaction ($F_{1,29} = 6.25$, $P = .02$). This result and the post hoc pairwise analyses demonstrated reduced hippocampal volumes in cannabis users ($F_{1,29} = 11.14$, $P = .002$ corrected; a reduction of 12.1% in the left and 11.9% in the right hippocampus relative to controls), with a very large effect size (Cohen d : left hippocampus, 1.17; and right hippocampus, 1.27) (**Figure 1**). Cannabis users also had smaller amygdala volumes ($F_{1,29} = 7.31$, $P = .01$ corrected; a reduction of 6.0% in the left amygdala and 8.2% in the right amygdala relative to controls), with large effect sizes (Cohen d : left amygdala, 0.80; and right amygdala, 0.99). The region \times group interaction reflects that the overall reduction in hippocampal volume was relatively (and significantly) greater than the reduction in amygdala volume (12.0% in the hippocampus vs 7.1% in the amygdala). In the analysis of subthreshold psychotic symptoms, cannabis users reported significantly higher positive symptoms (Scale for the Assessment of Positive Symptoms; $z = -3.57$, $P < .001$) and negative symptoms (Scale for the Assessment of Negative Symptoms; $z = -3.66$, $P < .001$) than nonusing controls. Regarding verbal learning, cannabis users displayed significantly poorer performance than controls on the RAVLT measures (sum of words recalled across the 5 learning trials: $z = -3.97$, $P < .001$; and free recall after a 20-minute delay: $z = -2.61$, $P = .009$).

CORRELATIONAL ANALYSES

There was a significant inverse association between left hippocampal volume and cumulative cannabis exposure during the previous 10 years ($r = -0.62$, $P = .01$; accounting for 38% of the variance in left hippocampal volume) (**Figure 2A**). When 1 participant with relatively higher cumulative cannabis exposure and small hippocampal volume was excluded, 22% of the variance was still accounted for despite falling short of significance in the reduced sample ($r = -0.47$, $P = .09$). There was also an

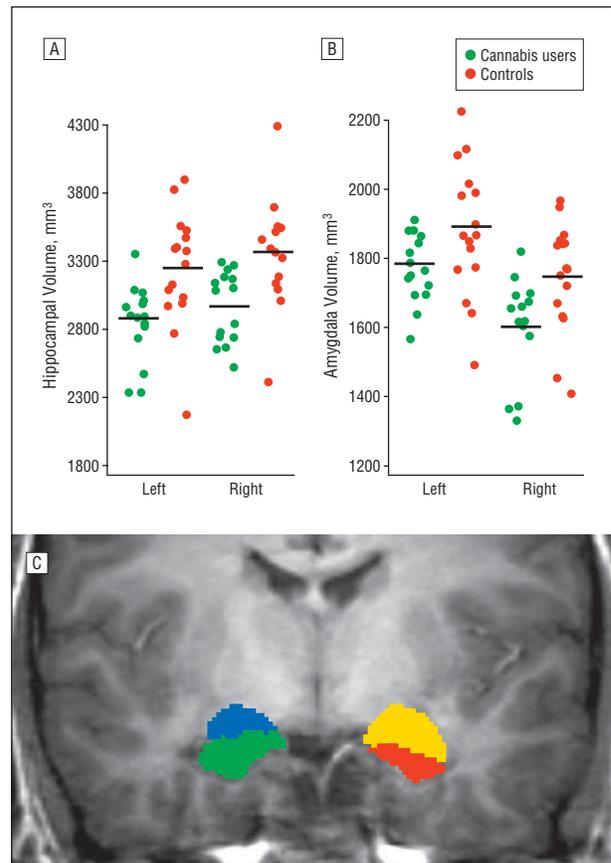


Figure 1. Brain regions of interest and individual volumetric measures. The scattergraphs illustrate hippocampal (A) and amygdala (B) volumes of cannabis users and nonusing control subjects. The horizontal lines represent the group means. Tracings of left (yellow) and right (blue) amygdalae and left (red) and right (green) hippocampi are also illustrated (C).

association between left hippocampal volume and positive symptoms ($r = -0.77$, $P < .001$) (Figure 2B) and between positive symptoms and cumulative cannabis exposure ($r = 0.52$, $P = .048$) (Figure 2C). The associations between left hippocampal volume and cumulative cannabis exposure and between left hippocampal volume and positive symptoms remained after controlling for the effects of global functioning (Global Assessment of Functioning scale) and depressive symptoms (Hamilton Depression Rating Scale). No other associations were found between other brain volumetric measures, cannabis use, and psychotic symptoms, and they did not vary as a function of alcohol or tobacco use. Measures of RAVLT performance did not correlate with hippocampal or amygdala volumes in either controls or cannabis users.

COMMENT

To our knowledge, this is the first human study of long-term heavy cannabis users to demonstrate marked exposure-related hippocampal volume reductions. These findings corroborate previous animal research,^{6,9} suggesting that long-term heavy cannabis use is associated with significant and localized hippocampal volume reductions that relate to increasing cumulative cannabis exposure. In addition, the present findings are consis-

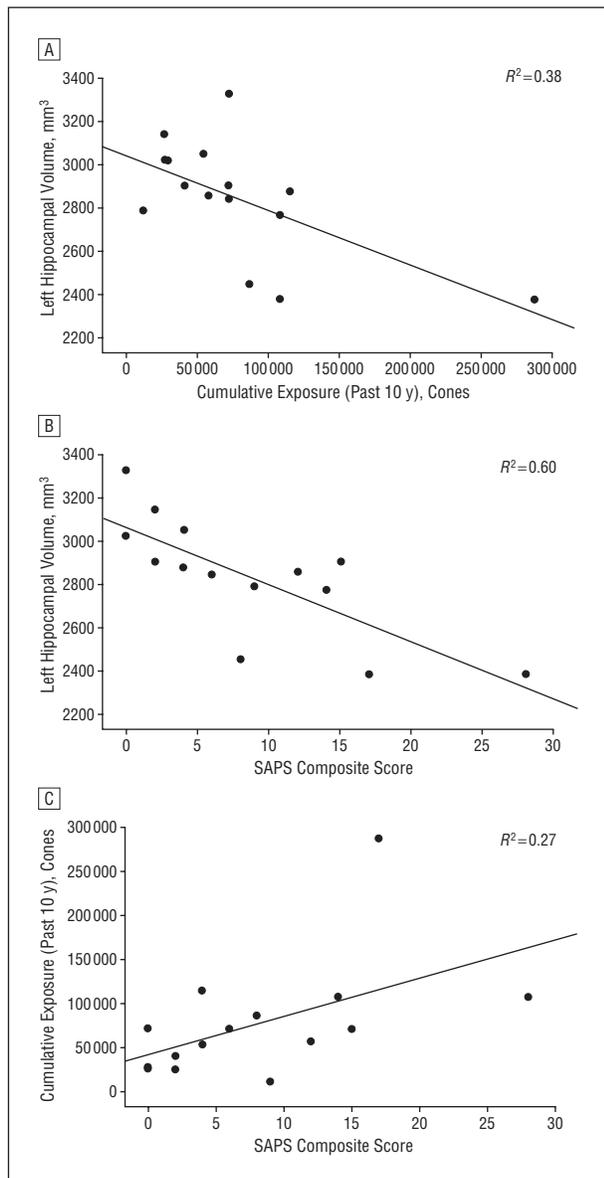


Figure 2. Scatterplots illustrating the pairwise associations between left hippocampal volume and cumulative exposure to cannabis during the past 10 years (A), left hemisphere hippocampal volume and positive symptoms (Scale for the Assessment of Positive Symptoms [SAPS]) (B), and cumulative exposure during the past 10 years and positive symptoms (SAPS) (C).

tent with the view that cannabis use increases the risk of psychotic symptoms and informs the debate concerning the potential long-term hazardous effects of cannabis in this regard. The bilateral reduction in amygdala volume is a novel but not unexpected finding given the dense concentration of cannabinoid receptors in this region.³⁵

Although these findings are consistent with those of a previous study,¹⁸ it is difficult to directly compare these results with those of other human studies given that past work used MRI with lower magnetic field strength and spatial resolution and did not conduct region-of-interest-based analyses (eg, performed whole-brain voxel-based analyses¹⁸). Tzilos et al¹⁴ conducted the only other study, to our knowledge, that investigated cannabis users with

a relatively long history of use (specifically, an average duration of use of 22.6 years, or 18.9 years of daily use) and their study is, therefore, most comparable with the present study. Although they found no effects of long-term cannabis use on hippocampal volume, the authors acquired their images at a lower field strength and with a coarser spatial resolution (1.5 T with 3-mm-thick slices vs 3 T with 1-mm-thick slices in the present study), an important consideration given the size of the brain structures investigated. Moreover, their region of interest was less specific to the hippocampus relative to the present measure because they also included the parahippocampal gyrus. Furthermore, there was a relatively large age discrepancy between their users and controls (38.1 vs 29.5 years), and the minimum duration of exposure to cannabis was considerably lower in their sample (as little as 1 year of cannabis exposure), but, overall, their sample reported an average of 20 100 lifetime episodes of use. In contrast, the minimum duration of exposure to cannabis in the present sample was 10 years, with an average of 62 000 episodes of use. Thus, despite a similar mean duration of use, the present sample used more than 3 times as much cannabis, which may explain the finding of a dose-response relationship between hippocampal volume and cumulative cannabis use. Further high-resolution MRI work is necessary to characterize precisely the dosage of cannabis required for significant brain changes to occur.

The pattern of use in the present sample is consistent with heavy cannabis use patterns that have previously been reported in other Australian studies. For example, Copeland and colleagues³⁶ reported median daily intake of 8 cones (the small funnel into which cannabis is packed to consume through a water pipe in a single inhalation) in an Australian sample of cannabis users seeking treatment for cannabis dependence, ranging up to 125 cones per day in the heaviest user, with 11% reporting cannabis smoking throughout the day. The heaviest user herein reported smoking 80 cones per day (approximately 25 joints smoked throughout the day). This pattern of cannabis use is not dissimilar to the heaviest cannabis users from other studies of non-treatment-seeking samples of Australian cannabis users.^{37,38}

Despite the large magnitude of effects observed, it remains unclear whether these volumetric reductions reflect neuronal or glial loss, a change in cell size, or a reduction in synaptic density (eg, dendritic arborization), all of which have been reported in rodent studies.⁶⁻⁹ For example, Scallet and colleagues⁹ found striking tetrahydrocannabinol-induced residual decreases in the mean volume of hippocampal neurons and their nuclei and a 44% reduction in the number of synapses up to 7 months after the last exposure to tetrahydrocannabinol. Moreover, Landfield and colleagues⁷ administered tetrahydrocannabinol 5 times a week for 8 months (approximately 30% of the rat lifespan, and comparable in frequency and duration to the present sample) and found significant tetrahydrocannabinol-induced decreases in neuronal density in the hippocampus. Such findings may help explain the mechanisms underlying gross hippocampal and amygdala volume loss seen in this sample of long-term heavy cannabis users.

In the present study, hippocampal volume in the cannabis-using group was inversely correlated with cumulative exposure to the drug in the left, but not right, hemisphere. Previous functional imaging studies^{16,39} have found reduced left hippocampal activation during cognitive performance in cannabis users, and there is evidence to suggest that hippocampal abnormalities in psychiatric disorders such as schizophrenia are more prominent in the left hemisphere.⁴⁰ These findings converge to suggest that the left hippocampus may be particularly vulnerable to the effects of cannabis exposure and may be more closely related to the emergence of psychotic symptoms. In this context, it is interesting that we found a significant inverse correlation between left hippocampal volume and positive symptoms. Cannabis use was also positively correlated with positive symptoms, suggesting that there are complex associations among exposure to cannabis, hippocampal volume reductions, and psychotic symptoms. Given these relationships, it is possible that the exposure-related hippocampal reduction may reflect heavy cannabis use in response to preexisting or developing psychotic symptoms. However, there is limited empirical support for long-term self-medication of subthreshold psychotic symptoms with cannabis and stronger support for the induction of psychotic symptoms subsequent to cannabis exposure.²⁰ As such, it seems more likely that prolonged heavy use of cannabis induced subthreshold psychotic symptoms and that both of these factors are associated with hippocampal volume loss. These symptoms were subthreshold because these cannabis-using participants were carefully screened for current and past history of mental disorders. Furthermore, the fact that the mean age of the present cannabis-using sample was nearly 40 years suggests that these symptoms are unlikely to reflect a prodrome. One speculation is that the present participants were less genetically vulnerable to developing a psychotic disorder subsequent to cannabis use,^{41,42} allowing them to smoke heavily for many years. Future longitudinal work assessing the emergence of hippocampal reductions and psychotic symptoms with continued exposure to cannabis, and how these are related to polymorphic variations in susceptibility genes for psychotic disorders, will prove useful in better characterizing these relationships.

Given that cannabis users had significantly greater depressive symptom scores than controls and that there is an association between depression and hippocampal volume reduction,⁴³ it could also be argued that depressive symptoms may be another mediating factor in the relationship between cannabis use and hippocampal volume reduction. However, there are a variety of important considerations that make this unlikely. First, there was no significant association between hippocampal volumes and depressive symptom scores. Second, the relationship between left hippocampal volume and quantity of cannabis used was maintained after statistically controlling for depressive symptoms. Finally, the overwhelming evidence suggests that hippocampal reductions in major depressive disorder tend to occur in the more persistent forms of the disorder (eg, multiple episodes, repeated relapses, or long illness duration).^{43,44} This was not the case in the present sample of cannabis us-

ers, who scored less than 6.0 on the Hamilton Depression Rating Scale, had never been diagnosed as having major depression, and did not seek treatment for any depressive disorder.

Cannabis users showed poorer performance on measures of verbal learning, consistent with previous findings.^{21,45,46} Although some functional imaging studies have found reduced left hippocampal blood flow and activation during verbal (and visual) learning tasks in cannabis users,^{16,39} we found no correlation between RAVLT performance measures and hippocampal volume in either controls or cannabis users. It is likely that anatomical volume is a less sensitive measure than brain activation for identifying correlations with behavioral performance. This is a particularly pertinent consideration given that the performance measures on the RAVLT are likely to reflect the operation of numerous cognitive processes not necessarily related to hippocampal function. Future work using experimental tasks designed to more specifically probe memory functions mediated by the hippocampus may be useful in this regard.

The bilateral reduction in amygdala volume is a novel but not unexpected finding given the dense concentration of cannabinoid receptors in this region.³⁵ There were no cognitive, psychotic, or depressive symptom associations with reduced volume in the amygdala. However, this region has been significantly implicated in cannabinoid-associated emotional and reward-related learning and memory processes.^{47,48} Given that these aspects of learning have not been examined in human cannabis users, they would seem to serve as a potentially informative avenue forward to help elucidate the functional relevance of such volumetric reduction in the amygdala.

The relationship between long-term cannabis use and brain abnormalities is complex. Although a limitation of this study may be the residual effects of cannabis in light of the fact that the cannabis users in this study were required to be cannabis free for only 12 to 24 hours before MRI, such issues are likely to be more pertinent for studies examining more dynamic aspects of brain functioning (eg, activations and cognition).⁴⁹ The present structural findings are unlikely to relate to the recent effects of cannabis use because we are unaware of any evidence that suggests that the hippocampus and amygdala can change in volume by 6% to 12% in short periods. However, although we maintain that the present results reflect brain changes associated with long-term heavy cannabis use rather than the consequences of recent exposure, further longitudinal work is required to assess whether such changes are reversible across more protracted periods of abstinence.

Another limitation of this study is the relatively small sample size, although the sample was exceptionally unique in that participants were very long-term and heavy cannabis users (mean of 5-7 joints per day for ≥ 10 years) without polydrug use or co-occurring neurologic or diagnosable mental disorders. As such, we conducted the first, to our knowledge, "pure" examination of the effects of heavy and protracted exposure to cannabis in humans. The large effect sizes of the main findings suggest that these results are robust and reproducible. These findings are further strengthened by the observed dose-

response relationships between hippocampal volume reductions and cumulative cannabis use.

There is ongoing controversy concerning the long-term effects of cannabis on the brain.³⁵ These findings challenge the widespread perception of cannabis as having limited or no neuroanatomical sequelae. Although modest use may not lead to significant neurotoxic effects, these results suggest that heavy daily use might indeed be toxic to human brain tissue. Further prospective, longitudinal research is required to determine the degree and mechanisms of long-term cannabis-related harm and the time course of neuronal recovery after abstinence.

Submitted for Publication: June 29, 2007; final revision received October 29, 2007; accepted January 11, 2008.

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Financial Disclosure: None reported.

Funding/Support: This study was supported by grants from the Clive and Vera Ramaciotti Foundation, the Schizophrenia Research Institute using infrastructure funding from NSW Health, and the University of Wollongong (Dr Solowij); grant 350241 from the National Health and Medical Research Council Program and National Health and Medical Research Council Clinical Career Development Award 509345 (Dr Yücel); the Colonial Foundation (Drs Yücel and Lubman); a J. N. Peters Fellowship and National Health and Medical Research Council C. J. Martin Fellowship 454797 (Dr Fornito); and Neurosciences Victoria.

Additional Contributions: Neuroimaging analysis was facilitated by the Neuropsychiatry Imaging Laboratory managed by Bridget Soulsby, BSc, at the Melbourne Neuropsychiatry Centre; and imaging was performed at the Symbion Clinical Research Imaging Centre, Prince of Wales Medical Research Institute, under the supervision of Ron Shnier, MBBS, FRACR. Scanning protocols were advised by Philip B. Ward, PhD, and Jim Lagopoulos, PhD; and clinical assessments were advised by Brin F. S. Grenyer, PhD.

REFERENCES

- Hall W, Solowij N. Adverse effects of cannabis. *Lancet*. 1998;352(9140):1611-1616.
- Macleod J, Oakes R, Copello A, Crome I, Egger M, Hickman M, Oppenkowski T, Stokes-Lampard H, Davey Smith G. Psychological and social sequelae of cannabis and other illicit drug use by young people: a systematic review of longitudinal, general population studies. *Lancet*. 2004;363(9421):1579-1588.
- Patton GC, Coffey C, Lynskey MT, Reid S, Hemphill S, Carlin JB, Hall W. Trajectories of adolescent alcohol and cannabis use into young adulthood. *Addiction*. 2007;102(4):607-615.
- Murray RM, Morrison PD, Henquet C, Di Forti M. Cannabis, the mind and society: the hash realities. *Nat Rev Neurosci*. 2007;8(11):885-895.
- Substance Abuse and Mental Health Services Administration. *Results From the 2005 National Survey on Drug Use and Health: National Findings*. Rockville, MD: Office of Applied Studies; 2006:1-267. NSDUH series H-30, DHHS publication SMA 06-4194.
- Chan GC, Hinds TR, Impey S, Storm DR. Hippocampal neurotoxicity of Δ^9 -tetrahydrocannabinol. *J Neurosci*. 1998;18(14):5322-5332.
- Landfield PW, Cadwallader LB, Vinsant S. Quantitative changes in hippocampal structure following long-term exposure to Δ^9 -tetrahydrocannabinol: possible mediation by glucocorticoid systems. *Brain Res*. 1988;443(1-2):47-62.
- Lawston J, Borella A, Robinson JK, Whitaker-Azmitia PM. Changes in hippocampal morphology following chronic treatment with the synthetic cannabinoid WIN 55,212-2. *Brain Res*. 2000;877(2):407-410.
- Scallet AC, Uemura E, Andrews A, Ali SF, McMillan DE, Paule MG, Brown RM, Slikker W Jr. Morphometric studies of the rat hippocampus following chronic delta-9-tetrahydrocannabinol (THC). *Brain Res*. 1987;436(1):193-198.
- Campbell AM, Evans M, Thomson JL, Williams MJ. Cerebral atrophy in young cannabis smokers. *Lancet*. 1971;2(7736):1219-1224.
- Co BT, Goodwin DW, Gado M, Mikhael M, Hill SY. Absence of cerebral atrophy in chronic cannabis users: evaluation by computerized transaxial tomography. *JAMA*. 1977;237(12):1229-1230.
- Hannerz J, Hindmarsh T. Neurological and neuroradiological examination of chronic cannabis smokers. *Ann Neurol*. 1983;13(2):207-210.
- Kuehnle J, Mendelson JH, Davis KR, New PF. Computed tomographic examination of heavy marijuana smokers. *JAMA*. 1977;237(12):1231-1232.
- Tzilos GK, Cintron CB, Wood JB, Simpson NS, Young AD, Pope HG Jr, Yurgelun-Todd DA. Lack of hippocampal volume change in long-term heavy cannabis users. *Am J Addict*. 2005;14(1):64-72.
- Block RI, O'Leary DS, Ehrhardt JC, Augustinack JC, Ghoneim MM, Arndt S, Hall JA. Effects of frequent marijuana use on brain tissue volume and composition. *Neuroreport*. 2000;11(3):491-496.
- Jager G, Van Hell HH, De Win MM, Kahn RS, Van Den Brink W, Van Ree JM, Ramsey NF. Effects of frequent cannabis use on hippocampal activity during an associative memory task. *Eur Neuropsychopharmacol*. 2007;17(4):289-297.
- Wilson W, Mathew R, Turkington T, Hawk T, Coleman RE, Provenzale J. Brain morphological changes and early marijuana use: a magnetic resonance and positron emission tomography study. *J Addict Dis*. 2000;19(1):1-22.
- Matochik JA, Eldreth DA, Cadet JL, Bolla KI. Altered brain tissue composition in heavy marijuana users. *Drug Alcohol Depend*. 2005;77(1):23-30.
- Medina KL, Schweinsburg AD, Cohen-Zion M, Nagel BJ, Tapert SF. Effects of alcohol and combined marijuana and alcohol use during adolescence on hippocampal volume and asymmetry. *Neurotoxicol Teratol*. 2007;29(1):141-152.
- Moore TH, Zammit S, Lingford-Hughes A, Barnes TR, Jones PB, Burke M, Lewis G. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet*. 2007;370(9584):319-328.
- Solowij N, Stephens RS, Roffman RA, Babor T, Kadden R, Miller M, Christiansen K, McRee B, Vendetti J; Marijuana Treatment Project Research Group. Cognitive functioning of long-term heavy cannabis users seeking treatment [published correction appears in *JAMA*. 2002;287(13):1651]. *JAMA*. 2002;287(9):1123-1131.
- Nelson HE. *National Adult Reading Test (NART): Test Manual*. Windsor, England: NFER-Nelson; 1992.
- Spielberger CD, Gorsuch RL, Lushene R, Vagg PR, Jacobs GA. *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press; 1983.
- Hamilton M. Rating scale for depression. *J Neurol Neurosurg Psychiatry*. 1960;23(1):56-62.
- First MB, Spitzer RL, Gibbon M, Williams JB. *Structured Clinical Interview for DSM-IV Axis I Disorders*. Washington, DC: American Psychiatric Press; 1997.
- Andreasen NC. *The Scale for the Assessment of Positive Symptoms (SAPS)*. Iowa City: University of Iowa; 1983.
- Andreasen NC. *The Scale for the Assessment of Negative Symptoms (SANS)*. Iowa City: University of Iowa; 1983.
- Velakoulis D, Pantelis C, McGorry PD, Dudgeon P, Brewer W, Cook M, Desmond P, Bridle N, Tierney P, Murrie V, Singh B, Copolov D. Hippocampal volume in first-episode psychoses and chronic schizophrenia: a high-resolution magnetic resonance imaging study. *Arch Gen Psychiatry*. 1999;56(2):133-141.
- Cook MJ, Fish DR, Shorvon SD, Straughan K, Stevens JM. Hippocampal volumetric and morphometric studies in frontal and temporal lobe epilepsy. *Brain*. 1992;115(4):1001-1015.
- Convit A, McHugh P, Wolf OT, de Leon MJ, Bobinski M, De Santi S, Roche A, Tsui W. MRI volume of the amygdala: a reliable method allowing separation from the hippocampal formation. *Psychiatry Res*. 1999;90(2):113-123.
- Eritaia J, Wood SJ, Stuart GW, Bridle N, Dudgeon P, Maruff P, Velakoulis D, Pantelis C. An optimized method for estimating intracranial volume from magnetic resonance images. *Magn Reson Med*. 2000;44(6):973-977.
- Smith SM. Fast robust automated brain extraction. *Hum Brain Mapp*. 2002;17(3):143-155.
- Zhang Y, Brady M, Smith S. Segmentation of brain MR images through a hidden Markov random field model and the expectation maximization algorithm. *IEEE Trans Med Imaging*. 2001;20(1):45-57.
- Free SL, Bergin PS, Fish DR, Cook MJ, Shorvon SD, Stevens JM. Methods for normalization of hippocampal volumes measured with MR. *AJNR Am J Neuroradiol*. 1995;16(4):637-643.
- Iversen L. Cannabis and the brain. *Brain*. 2003;126(6):1252-1270.

36. Copeland J, Swift W, Rees V. Clinical profile of participants in a brief intervention program for cannabis use disorder. *J Subst Abuse Treat*. 2001;20(1):45-52.
37. Reilly D, Didcott P, Swift W, Hall W. Long-term cannabis use: characteristics of users in an Australian rural area. *Addiction*. 1998;93(6):837-846.
38. Swift W, Hall W, Copeland J. Characteristics of long-term cannabis users in Sydney, Australia. *Eur Addict Res*. 1998;4(4):190-197.
39. Block RI, O'Leary DS, Hichwa RD, Augustinack JC, Boles Ponto LL, Ghoneim MM, Arndt S, Hurtig RR, Watkins GL, Hall JA, Nathan PE, Andreasen NC. Effects of frequent marijuana use on memory-related regional cerebral blood flow. *Pharmacol Biochem Behav*. 2002;72(1-2):237-250.
40. Petty RG. Structural asymmetries of the human brain and their disturbance in schizophrenia. *Schizophr Bull*. 1999;25(1):121-139.
41. Caspi A, Moffitt TE, Cannon M, McClay J, Murray R, Harrington H, Taylor A, Arseneault L, Williams B, Braithwaite A, Poulton R, Craig IW. Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biol Psychiatry*. 2005;57(10):1117-1127.
42. Henquet C, Rosa A, Krabbendam L, Papiol S, Fananás L, Drukker M, Ramaekers JG, van Os J. An experimental study of *catechol-O-methyltransferase* Val¹⁵⁸Met moderation of Δ -9-tetrahydrocannabinol-induced effects on psychosis and cognition. *Neuropsychopharmacology*. 2006;31(12):2748-2757.
43. Frodl T, Meisenzahl EM, Zill P, Baghai T, Rujescu D, Leinsinger G, Bottlender R, Schüle C, Zwanzger P, Engel RR, Rupprecht R, Bondy B, Reiser M, Möller HJ. Reduced hippocampal volumes associated with the long variant of the serotonin transporter polymorphism in major depression. *Arch Gen Psychiatry*. 2004; 61(2):177-183.
44. MacQueen GM, Campbell S, McEwen BS, Macdonald K, Amano S, Joffe RT, Nahmias C, Young LT. Course of illness, hippocampal function, and hippocampal volume in major depression. *Proc Natl Acad Sci U S A*. 2003;100(3):1387-1392.
45. Bolla KI, Brown K, Eldreth D, Tate K, Cadet JL. Dose-related neurocognitive effects of marijuana use. *Neurology*. 2002;59(9):1337-1343.
46. Pope HG Jr, Yurgelun-Todd D. The residual cognitive effects of heavy marijuana use in college students. *JAMA*. 1996;275(7):521-527.
47. Azad SC, Monory K, Marsicano G, Cravatt BF, Lutz B, Zieglgänsberger W, Rammes G. Circuitry for associative plasticity in the amygdala involves endocannabinoid signaling. *J Neurosci*. 2004;24(44):9953-9961.
48. Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, Hermann H, Tang J, Hofmann C, Zieglgänsberger W, Di Marzo V, Lutz B. The endogenous cannabinoid system controls extinction of aversive memories. *Nature*. 2002; 418(6897):530-534.
49. Pope HG Jr, Gruber AJ, Yurgelun-Todd D. The residual neuropsychological effects of cannabis: the current status of research. *Drug Alcohol Depend*. 1995; 38(1):25-34.