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Differences in Regional Blood Volume During a 28-Day Period of Abstinence in Chronic Cannabis Smokers

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Abstract

Cerebral blood volume (CBV) studies have provided important insight into the effects of illicit substances such as cannabis. The present study examined changes in regional blood volume in the frontal and temporal lobe, and the cerebellum during 28 days of supervised abstinence from cannabis. Dynamic susceptibility contrast MRI (DSCMRI) data were collected on 15 current, long-term cannabis users between 6 and 36 hours after the subjects' last reported cannabis use (Day 0), and again after 7 and 28 days of abstinence. Resting state CBV images were also acquired on 17 healthy comparison subjects. The present findings demonstrate that at Day 7, cannabis users continued to display increased blood volumes in the right frontal region, the left and right temporal regions, and the cerebellum. However, after 28 days of abstinence, only the left temporal area and cerebellum showed significantly increased CBV values in cannabis users. These findings suggest that while CBV levels begin to normalize with continued abstinence from cannabis, specifically in frontal areas, other temporal and cerebellar brain regions show slower CBV decreases.

Keywords

Marijuana; MRI; cerebral blood volume; cerebellum; abstinence

Cannabis research remains an important area of investigation since it is the most widely used illicit drug in the United States (NIDA, 2005; SAMHSA, 2004). One area of interest has focused on the effects of cannabis on the neurovascular system. Specifically, the quantitative measurement of cerebral perfusion is critical for the study of both normal and impaired human brain function; cerebral blood volume (CBV) and cerebral blood flow (CBF) studies have provided important insight into the acute and chronic neurobiologic effects of illicit drugs. Previously, we reported that recently abstinent cannabis users demonstrated significantly increased blood volumes relative to comparison subjects in the right frontal region, left temporal region and cerebellum (Sneider et al., 2006). However, no studies, to our knowledge,

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have examined the effects of chronic heavy cannabis use on the cerebral vasculature after an extended period of abstinence.

A number of prior investigators have focused on the acute effects of cannabis on cerebral blood flow. These studies used either ^{133}Xe inhalation or ^{15}O -water and positron emission tomography (PET) to measure cerebral blood flow both before and after intravenous infusion of delta-9-tetrahydrocannabinol ($\Delta^9\text{-THC}$) or after smoking marijuana cigarettes or placebo (for review, see Quickfall et al., 2006). In particular, work from Mathew and colleagues found increased CBF, predominantly in frontal regions, the insula, cingulate gyrus and the right hemisphere, after an acute challenge with marijuana or $\Delta^9\text{-THC}$ (e.g., Mathew et al., 1997; Mathew et al., 1992; Mathew et al., 2002). In addition, alterations in the neurovascular system have been shown to be associated with behavioral changes in chronic cannabis users. For instance, Mathew and colleagues (1992) found increased ratings of tension and anger after smoking marijuana but not placebo cigarettes as measured by the Profile of Mood States. Another relevant study (Volkow et al., 1996) examined regional brain glucose metabolism; it found that at baseline cannabis users demonstrated lower cerebellar metabolic values than comparison subjects. While cerebellar metabolism was increased in both cannabis users and comparison subjects during $\Delta^9\text{-THC}$ intoxication, only the cannabis users exhibited increased glucose metabolism in the prefrontal cortex, orbitofrontal cortex, and basal ganglia after the acute $\Delta^9\text{-THC}$ challenge. These findings suggest that chronic cannabis users demonstrate differential regional brain metabolic responses in response to an acute cannabis challenge (Volkow et al., 1996); however the duration of these effects was not assessed.

Blood flow changes have been examined during cognitive challenge tasks. Specifically, PET studies have demonstrated decreased regional blood flow in the prefrontal cortex, increased CBF in the cerebellum, and altered lateralization in the hippocampus during performance of an episodic memory task in recently abstinent cannabis users (Block et al., 2002). The effect of smoking cannabis on CBF during auditory attention task was also examined using PET (O'Leary et al., 2002). The findings showed decreased CBF in areas associated with sensory processing and attention (e.g., auditory areas of the temporal lobe, visual cortex, parietal lobe, frontal lobe, and thalamus); however CBF increased in paralimbic regions, which may be associated with the mood-enhancing effects of cannabis. Overall, these findings suggest that cannabis use may affect brain activation in brain regions important for memory, attention and cognition (Block et al., 2002; O'Leary et al., 2002).

In addition to blood flow studies, cerebrovascular perfusion has been examined by measuring blood flow velocity (e.g., systolic velocity and pulsatility index) with transcranial Doppler sonography in light, moderate, and heavy cannabis users after 3 days, and again after 28 to 30 days of supervised abstinence (Herning et al., 2005). Both systolic velocity and pulsatility index, a measure of cerebrovascular resistance, were increased in cannabis users compared with control subjects. Further, this increase remained essentially unchanged in heavy cannabis users for the entire month of abstinence, yet began to decrease in the light users. The authors suggested that these persistent alterations in vascular hemodynamics might possibly be due to changes in the blood vessels or the density of CB1 receptors in the brain (Herning et al., 2005).

Neuroimaging data have provided evidence of alterations in patterns of brain activity after an extended period of abstinence in heavy marijuana users (e.g., Bolla et al., 2005; Eldreth et al., 2004). For example, Bolla and colleagues (2005) demonstrated deficits in decision-making ability during the Iowa Gambling Task, as well as alterations in brain activity using Positron Emission Tomography (PET), in heavy marijuana users abstinent for 25 days. Results from this study demonstrated that marijuana users exhibited less activation in the right lateral orbitofrontal cortex and the right dorsolateral prefrontal cortex and greater activation in the

left cerebellum compared to the control group. Further analyses dividing the marijuana group into heavy and moderate users revealed more pronounced deficits in performance and brain activity in the heavy marijuana group. In a PET investigation, Eldreth and colleagues (2004) examined 25-day abstinent marijuana smokers during the performance of a modified Stroop test; they found hypoactivity of the ACC and lateral PFC and increase activity in the hippocampus relative to control subjects despite a lack of performance differences between the groups (Eldreth et al., 2004). The authors suggested that marijuana smokers may recruit alternative networks as a compensatory strategy for the completion of their version of the Stroop task. More recently, Jager and colleagues (2006), using functional magnetic resonance imaging (fMRI), found no overall differences in the patterns of brain activity between 7-day abstinent moderate users of cannabis and controls during a working memory task and a selective attention task. However, these authors did report altered brain activity in the left superior parietal cortex in the cannabis users during the working memory task suggesting possible region-specific effects of cannabis on brain function after 1 week of abstinence. Further, a study from the same investigators used fMRI to examine the effects of frequent cannabis use on a hippocampal-dependent associative memory task (Jager et al., 2007). The findings revealed hypoactivity in 7-day abstinent cannabis users in the left and right parahippocampal regions and the right dorsolateral prefrontal cortex specifically during the associative learning component of the task. Thus, despite similar behavioral performance, activational differences are evident in abstinent cannabis users during hippocampal-associative memory. Chang and colleagues (2006) examined activation using BOLD (blood oxygenation-level dependent) fMRI during a visual-attention task in abstinent chronic marijuana smokers (the majority abstinent for less than 2 months), active marijuana smokers and control subjects. Both marijuana groups exhibited altered activation patterns during the visual-attention tasks. The investigators found that marijuana users demonstrated decreased activation in the right prefrontal, medial and dorsal parietal areas, and medial cerebellar regions, but greater activation in frontal, parietal, and occipital regions. Notably, there was a positive correlation between duration of abstinence and BOLD activation in the right prefrontal area and the cerebellum suggesting normalization of brain activation with continued abstinence (Chang et al., 2006). Overall, the majority of the above studies suggest that altered neural activity patterns still persist even after a week of abstinence from cannabis. However, after longer durations of abstinence (i.e., over 1 month) brain activation patterns may begin to normalize.

Findings from the above neuroimaging studies, along with the converging data from neurocognitive and neuromorphology studies provide additional evidence of deficits even after an extended washout period from cannabis. Neurocognitive studies have provided evidence that cognitive deficits persist for at least a week after abstinence from cannabis most notably in verbal memory (e.g., Bolla et al., 2002; Pope et al., 2001; Rodgers et al., 2000). Furthermore, studies have used voxel-based morphometry to examine differences in gray and white matter tissue density between abstinent heavy marijuana users and controls. Specifically, twenty-day abstinent heavy marijuana users displayed altered brain tissue composition in gray matter areas such as the parahippocampal gyrus, precentral gyrus, and thalamus and in white matter areas such as the parietal lobe, fusiform gyrus, parahippocampal gyrus, and brainstem compared to controls (Matochik et al., 2005). These findings suggest alterations in brain integrity even after a period of abstinence in heavy marijuana smokers.

Dynamic susceptibility contrast magnetic resonance imaging (DSC MRI) can detect changes in cerebral blood volume and metabolism by measuring magnetic resonance (MR) signal loss as the contrast agent passes through the vessels (Belliveau et al., 1990; Levin et al., 1995; Levin et al., 1996). This method involves injection of an individual with a bolus of paramagnetic contrast agent, gadolinium; the gadolinium disrupts the magnetic field around the vessels as it passes through the intravascular space, thus causing a reduction in the T2-weighted MR signal intensity (Levin et al., 1996). Subsequently, this signal change can be

mapped using a concentration time curve to measure relative cerebral blood volume (rCBV) (Belliveau et al., 1990; Levin et al., 1996; Rosen et al., 1990).

The objective of the present study was to use dynamic susceptibility contrast magnetic resonance imaging (DSCMRI) to examine if there were differences in relative cerebral and cerebellar blood volume (rCBV) between long-term cannabis users and a non-using comparison group with continued abstinence from cannabis. We hypothesized that during an extended washout period there would be differences between chronic cannabis smokers and comparison subjects in regional rCBV measures involving the cerebellum, right and left temporal regions, and frontal regions. However, we hypothesized that after approximately a month of abstinence rCBV levels would begin to normalize.

Experimental Procedures

Subjects

Fifteen current, long-term daily cannabis users (32-47 years of age) and 17 comparison subjects (21-31 years of age) participated in the study. All cannabis users had smoked at least 5000 times and were smoking daily up to the time of evaluation. Lifetime cannabis use was estimated by reviewing the average number of weekly episodes of smoking by the subject on a year-by-year basis, and then totaling lifetime episodes of use at the end. In addition, cannabis use in the past 30 days and years of use were collected. Comparison subjects reported no cannabis use within the last month and no history of cannabis abuse or dependence in their lives (see Table 1 for further details). Subjects were excluded from both groups if they reported (1) current use of psychotropic medications; (2) a history of head injury with loss of consciousness requiring hospitalization; (3) a history of alcohol dependence; (4) a history of abuse of or dependence upon any other illicit substance; (5) medical or neurological illness that might affect cognition; or (6) a current Diagnostic and Statistical Manual of Mental Disorders 4th ed. (DSM-IV) (American Psychiatric Association, 1994) Axis I disorder other than simple phobia or social phobia, as determined by the Structured Clinical Interview for DSM-IV (First et al., 1996). Further details about selection criteria for the heavy cannabis users have been described previously (Pope et al., 2001).

Cannabis users underwent a 28-day period of supervised abstinence from the drug, as described previous (Pope et al., 2001). Briefly, all users were required to provide daily observed urine samples that were assessed for levels of 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) and for creatinine. Levels of THC-COOH were normalized to a urinary creatinine level of 100 ng/ml to allow for differences in urinary concentration among the subjects. Levels were then monitored to ascertain that they decreased over the abstinence period in a manner consistent with residual drug excretion in the absence of any new cannabis use (see Huestis et al., 1998). All subjects were allowed to consume caffeine and tobacco, and drink up to 2 alcoholic drinks per day (a drink was defined as: 4 oz. of wine, 12 oz. of beer, or 11/2 oz. of distilled liquor). Further details about these procedures have been presented previously (Pope et al., 2001). The Hamilton Depression Rating Scale (HAM-D) and Hamilton Anxiety Scale (HAM-A) were administered to all cannabis users after 6 to 36 hours of abstinence ("Day 0"), and again at Day 7 and at Day 28 of the abstinence period. In addition, blood pressure was measured on Day 0 in the cannabis group (14/15 subjects). All subjects signed an informed consent that was approved by McLean Hospital's institutional review board.

Imaging Techniques

Imaging data were collected on cannabis users at Days 0, 7, and 28 of abstinence. Dynamic susceptibility contrast magnetic resonance imaging data (DSCMRI) were acquired with a 1.5-Tesla GE Signa scanner retrofitted with an Advance NMR Systems whole-body echo planar

imaging coil. A spin-echo planar imaging sequence was used (TR=2 seconds, TE=100 msec) with a 1.5 mm × 1.5 mm in-plane resolution and a 7 mm slice thickness with a 3 mm skip. All scans were acquired in the axial plane following a bolus of gadolinium contrast agent (0.2 mmol/kg) injected over six seconds through an 18-G angiocatheter in an antecubital vein. Numerical integration of the transformed image intensity data over the initial 16s of contrast inflow was used to determine first-pass rCBV for each contrast bolus (Loeber et al., 1999). Ten functional and T₁-weighted matched structural axial images were collected for each subject to allow for anatomical localization of the regions of interest. The first slice typically began 4cm below the AC-PC line. rCBV data processing was performed using an in house IDL based fMRI processing program (fMRI Analysis Tool (FAT); Maas et al., 1997). This software program displays the functional images and provides a tool for drawing multiple regions within each slice. Mean blood volume data were collected by measuring cortical brain area on each slice and then averaging the slices. Blood volume was measured in the left and right frontal and temporal areas, and the cerebellum. For the majority of subjects, frontal area was drawn on 2 slices, temporal area included 1 slice, and the cerebellum was drawn on 2 slices, with the exception of 12 out of 55 cases in which the cerebellum was captured on 1 slice (see Figure 1).

Statistical Analysis

Analyses were performed with SPSS (Chicago, IL). The differences between cannabis users and comparison subjects in regional blood volume were analyzed by separate one-way ANOVAs for Day 0, Day 7, and Day 28. Blood volume was measured in both the left and right frontal and temporal areas, and the cerebellum. The relationship between rCBV levels and 1) urinary cannabinoid concentrations, 2) lifetime episodes of smoking, and 3) ratings of anxiety and depression in cannabis users was tested by nonparametric correlation analysis (Spearman Correlation).

We did not adjust for age, because there was no overlap in the age distribution between groups (cannabis users: age 32-47, comparison subjects: age 21-31). However, regression analyses were conducted to examine whether age was a predictor of rCBV measures in cannabis users or comparison subjects.

Results

Heavy cannabis users (8 males, 7 females) and comparison subjects (9 males, 8 females) were well matched on gender, but users were significantly older than comparison subjects (mean (SD) age 38.3 (5.6) years vs. 26.4 (3.8) years; $p<0.001$). The cannabis users reported a mean of 20,601.3 (13,540.8) lifetime episodes of smoking cannabis. There were 12 subjects examined on Day 0, 13 subjects on Day 7, and 13 subjects on Day 28. Three subjects were not included on Day 0 due to data corruption, 2 subjects were not included on Day 7 due to data corruption (N=1) or dropout (N=1) and 2 subjects were not included on Day 28 due to dropout.

Previously, we have shown that that cannabis users demonstrated significantly increased blood volumes in the right frontal area ($F(1,28)=5.14$, $p<0.05$), the left temporal area ($F(1,28)=9.67$, $p<0.005$) and the cerebellum ($F(1,28)=10.50$, $p<0.005$) relative to comparison subjects on Day 0 (Sneider et al., 2006). In the current investigation, we carried out a multivariate ANOVA with diagnostic group (cannabis vs. comparison group) and sex as the between group variables. The findings revealed that after 7 days of abstinence, cannabis users continued to display increased blood volumes in the right frontal area ($F(1,29)=4.57$, $p<0.05$), the left ($F(1,29)=11.21$, $p<0.005$) and right temporal area ($F(1,29)=5.99$, $p<0.05$) and in the cerebellum ($F(1,29)=11.69$, $p<0.005$) relative to comparison subjects. There were no significant differences between users and comparison subjects for the left frontal area ($p=0.07$). In addition, a significant main effect of sex regardless of diagnostic group was found for Day 7; data

indicating that overall males had higher CBV values compared to females in the left and right frontal and temporal areas, and cerebellum (all, $p < 0.05$) (see Table 2). After 28 days of abstinence, only rCBV values in the left temporal area ($F(1,29)=9.93$, $p<0.005$) and cerebellum ($F(1,29)=6.33$, $p<0.05$) remained significantly elevated in the cannabis users (see Figure 2). In addition, there was a significant main effect of sex only in the cerebellum region ($F(1,29)=4.35$, $p<0.05$); indicating that males had higher CBV values compared to females (see Table 2). There were no significant interactions between sex and diagnosis after 7 days of abstinence (all, $p > 0.1$) or after 28 days of abstinence (all, $p > 0.1$).

Results from the regression analysis revealed that age was not a significant predictor for rCBV measures for any brain region for the cannabis group for Day 7 nor Day 28 (all, $p > 0.1$). Similarly, within the comparison group, age was not a significant predictor for rCBV measures across regions for Day 7 nor Day 28 (all, $p > 0.1$), suggesting that age was not a significant confounder in these analyses. Given the significant age differences between the groups, a median split of age calculated for each group and a subsequent $2 (\text{age}) \times 2 (\text{diagnostic group})$ ANOVA was completed. This analysis found no significant effect of age on CBV values. Specifically, there were no significant interactions between age and diagnostic group for CBV values measured on Day 7 (all, $p > 0.1$) or Day 28 (all, $p > 0.1$).

In the cannabis users, there was no significant association between normalized urinary THC levels and rCBV for left frontal, right frontal, left temporal, right temporal and cerebellar regions for Day 7 or Day 28 ($p > 0.1$ for all comparisons) (see Table 3). Additionally, in the cannabis users, we found no significant association between lifetime episodes of smoking and rCBV for any of the five regions (all, $p > 0.1$) for Day 7 or Day 28 (all, $p > 0.1$ except left temporal, where $p = 0.09$ and cerebellum, where $p = 0.06$).

Table 3 reports the mean and SD for cannabis smokers' scores on the HAM-D and HAM-A on Days 0, 7, 28. Clinical scores for cannabis users varied over the course of the washout, with smokers showing increased anxiety and depression on Day 7. However, no significant relationship was seen between ratings of anxiety or depression and any CBV measures on Day 0, 7, 28 (Spearman Correlation; $p > 0.1$ for all comparisons).

Discussion

The present study applied dynamic susceptibility contrast MRI to examine differences in regional blood volume between comparison subjects and cannabis users after an extended period of abstinence. Overall, after a 7-day abstinence, cannabis users continued to display greater blood volumes relative to comparison subjects in the right frontal area, left and right temporal area, and in the cerebellum bilaterally. In contrast, after 28 days of abstinence from cannabis, only rCBV values in the left temporal and cerebellum area remained elevated in the cannabis users. Among the cannabis users, we found no significant correlations between regional blood volumes and (1) total lifetime episodes of smoking; (2) normalized urinary THC-COOH concentrations; or (3) HAM-D and HAM-A scores on any of the three testing days. This last finding contrasts with previous studies of non-drug using subjects that have found a relationship between CBF and depression and/or worry (e.g., Hoehn-Saric et al., 2005; Murata et al., 2000). In addition, conditions associated with increased arousal, such as moderate degrees of anxiety, have been accompanied by increased CBF, especially in frontal areas in cannabis users (Mathew et al., 1993).

While the majority of other studies have studied changes in CBF after recent Δ^9 -THC intoxication, the present study focused on differences in CBV after prolonged abstinence from cannabis. As discussed in the introduction, findings from Mathew and colleagues (1997) and Volkow and colleagues (1996) suggest that the frontal region and cerebellum are areas sensitive

to cannabis exposure. Further, these findings could be related to the high densities of cannabinoid receptors that have been demonstrated using autoradiography in the basal ganglia, cerebellum and frontal areas (Herkenham et al., 1990; Iversen, 2003).

The hippocampus is also an area associated with a high density of cannabinoid receptors (Herkenham et al., 1990; Iversen, 2003) thus it is not surprising that the temporal region is an area sensitive to cannabis exposure. As discussed in the introduction, Jager and colleagues (2007) reported hypoactivity in 7-day abstinent cannabis users in the left and right parahippocampal regions and the right dorsolateral prefrontal cortex during performance on a hippocampal-dependent associative memory task. These authors suggest that decreased brain activation may reflect changes in a physiological response (e.g., cerebral perfusion) due to frequent cannabis use rather than neurobiological changes associated with neurocognitive impairment (Jager et al., 2007).

Together with our past findings of regional brain blood volume differences, these results are of importance in understanding fMRI BOLD studies of cannabis use. Changes in blood flow, blood volume, and blood oxygenation can affect MRI signals (van Zijl et al., 1998) and physiological measures such as CBV and CBF are known to be related to changes in BOLD signal (Ogawa et al., 1990; van Zijl et al., 1998). As postulated by the central volume principle there is a relationship between CBV and CBF (Rosen et al., 1990; van Zijl et al., 1998), and in turn these physiological measures are highly correlated with one another (Grubb et al., 1974). Therefore, it is imperative to recognize that differences reported in neural activation between chronic cannabis users and healthy controls in studies using both fMRI BOLD and PET likely represent the effects of differences in physiological measures such as CBV (Eldreth et al., 2004; Kanayama et al., 2004; Pillay et al., 2004).

There are two plausible mechanisms that may contribute to the reported increases in CBV and CBF. Evidence from animal studies suggests a possible direct effect of cannabis on the vasculature (e.g., Ellis et al., 1995). For example, direct administration of Δ^9 -THC or the endogenous cannabinoid anandamide to rabbit cerebral arterioles produces vasodilation of the vessels (Ellis et al., 1995). Alternatively, there may be an interaction between Δ^9 -THC and CB₁ receptors, which consequently produces an overall change in neural activity at the neurotransmitter level (Dewey, 1986; Grotenhermen, 2003; Loeber and Yurgelun-Todd, 1999), and changes in the vasculature may appear as a secondary phenomena.

Furthermore, by Day 7 and Day 28 concentrations of Δ^9 -THC are minimally detectable. Given the narrow range of values, therefore, it is not surprising there was not a significant association between increases rCBV and measures of cannabis use, such as urinary THC-COOH level or lifetime episodes of smoking. The results suggest that regional blood volumes during abstinence are not associated with total lifetime marijuana use, nor with the amount of recent marijuana use.

There are several possible limitations of our study that need to be addressed. The first is that the cannabis users and the comparison group did not overlap with age, and the cannabis users were older. However, there was no significant association found between age and CBV values in either group, which suggests that the findings are not an artifact of age. It should be noted that others have found some association between age and CBV measures, but this effect was small, with only a 3% to 6% decline in white and gray matter per decade of increasing age (Wenz et al., 1996). The cannabis users in the current study were older than the comparison group and therefore might be expected to exhibit lower rCBV levels. Therefore, our present finding of *higher* rCBV levels in the cannabis group cannot reasonably be attributed to age.

A second limitation is the fact that the comparison group did not receive urine testing for cannabis; thus recent cannabis use was not definitively excluded at the time of testing.

However, if there were comparison subjects with undisclosed and undetected cannabis use, this possibility would only bias the results in a conservative direction, since it would narrow the difference between the comparison group and cannabis users, rather than widening it. A third limitation is the fact that 2-3 subjects were not examined on each testing day. However, these subjects were eliminated either due to corruption of the data file or dropout since gadolinium can produce adverse effects such as nausea. Since there is no reason to suspect that missing data would be associated with rCBV status, it seems implausible that missing data could have biased the findings. Furthermore, nine cannabis subjects had data available on each of the three testing days. A repeated measures ANOVA conducted for these 9 subjects revealed a significant within subject decline in rCBV in the cerebellum ($F(2,16)=4.68$, $p<0.05$). This supports the findings of reduced rCBV with length of washout period. This limited sample size may have minimized the ability to observe within subject differences in the regions of interest studied.

A fourth limitation is the lack of more detailed information regarding both alcohol and nicotine use. This issue needs to be recognized since both alcohol and nicotine use could have affected cerebral perfusion measures such as CBV in the study. However, all subjects were thoroughly screened to ensure that none showed any obvious signs of alcohol abuse or dependence. Last, decreases in blood pressure and postural hypotension have been associated with cannabis use (Jones, 2002; Sidney, 2002), which can indirectly affect cerebral perfusion measures such as CBV. However, in the current study, the blood pressures of the cannabis users were normal (systolic < 140 mm Hg; diastolic < 90 mm Hg) with the exception of one hypertensive individual with a blood pressures of 145/105 mm Hg. Thus it would appear unlikely that abnormalities of blood pressure would contribute to the present findings.

In summary, it appears that increases observed in rCBV in recent cannabis users gradually decline over 28 days of abstinence, depending on the region examined. While rCBV values in frontal areas begin to normalize with continued abstinence from cannabis, rCBV levels in areas such as the left temporal and cerebellum region appear to recover from abstinence more slowly, even after 28 days. Thus it would be premature to conclusively state rCBV levels are fully reversible in all areas. It still needs to be determined whether these vascular effects are permanent and/or related to a neurotoxic effect of prolonged cannabis use. Overall, there appears to be persistent changes induced by chronic cannabis users in temporal and cerebellum areas. It is possible with a longer washout period; rCBV values in all areas would normalize. Future research in this area, perhaps using even longer washout periods, will have important implications for understanding the effects of changes in the microvasculature blood volume may have on differences reported in fMRI BOLD studies in chronic cannabis smokers.

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Figure 1. Regions of Interest

The pictures above depict the cerebral brain maps from a comparison subject (A) and a cannabis smoker (B) in the following regions of interest: 1=cerebellum, 2=Left Frontal, 3= Right Frontal, 4=Left Temporal, 5=Right Temporal.

Figure 2. Relative Cerebral Blood Volume (rCBV) During 28-Day Washout

After 7 days of abstinence, cannabis smokers continued to display significantly greater blood volumes in the right frontal area ($p < 0.05$), left ($p < 0.005$) and right ($p < 0.05$) temporal area, and the cerebellum ($p < 0.005$) relative to comparison subjects. However, after 28 days, only the rCBV values in the left temporal area ($p < 0.005$) and cerebellum ($p < 0.05$) remained significantly elevated in the cannabis smokers.

Table 1

Study Demographics and Cannabis Use

	Cannabis Users (n=15)	Comparison subjects (n=17)
Age (years)	38.3 ± 5.6	26.4 ± 3.8
Education (years)	14.1 ± 2.0	16.1 ± 1.4
Handedness	15 R, 0 L	15 R, 2 L
Gender	8M, 7F	9M, 8F
Lifetime Episodes of Cannabis Use	20,601.3 ± 13,540.8	-----
Days of Use in Past 30 days	30 ± 0 *	-----
Years of Use	21.3 ± 5.3	-----

Data represent mean ± SD

* All subjects smoked daily in past 30 days.

Table 2
Mean Relative Cerebral Blood Volume# After 7 and 28 Days of Abstinence by Gender

	Left Frontal	Right Frontal	Left Temporal	Right Temporal	Cerebellum
Day 7					
Males	1.71 ± 0.07*	1.62 ± 0.07*	2.33 ± 0.12*	2.28 ± 0.13*	2.04 ± 0.10*
Females	1.45 ± 0.10	1.35 ± 0.11	1.95 ± 0.13	1.84 ± 0.13	1.71 ± 0.12
Day 28					
Males	1.59 ± 0.07	1.55 ± 0.06	2.22 ± 0.09	2.15 ± 0.13	1.97 ± 0.09*
Females	1.47 ± 0.10	1.34 ± 0.10	2.01 ± 0.14	1.93 ± 0.14	1.67 ± 0.12

Data represent mean ± SEM

* Represents significant main effect of sex whereby males > females and p< 0.05.

Relative CBV values are used based on the relationship postulated by the central volume principle (Rosen et al., 1990; van Zijl et al., 1998).

Table 3

THC levels and Clinical Scales

	Day 0 (n=12)	Day 7 (n=13)	Day 28 (n=13)
Urinary THC-COOH *	206.5 ± 256.3	41.6 ± 59.0	11.0 ± 17.8
HAM-D	1.0 ± 1.1	2.8 ± 3.5	0.5 ± 0.9
HAM-A	1.3 ± 1.2	3.3 ± 3.4	0.4 ± 0.7

Hamilton Depression Rating Scale (HAM-D); Hamilton Anxiety Scale (HAM-A); Data represent mean ± SD

* THC levels were normalized to an assumed urinary creatinine concentration of 100ng/ml.