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ORIGINAL INVESTIGATION

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Spatial working memory in heavy cannabis users: a functional magnetic resonance imaging study

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Abstract *Rationale:* Many neuropsychological studies have documented deficits in working memory among recent heavy cannabis users. However, little is known about the effects of cannabis on brain activity. *Objective:* We assessed brain function among recent heavy cannabis users while they performed a working memory task. *Methods:* Functional magnetic resonance imaging was used to examine brain activity in 12 long-term heavy cannabis users, 6–36 h after last use, and in 10 control subjects while they performed a spatial working memory task. Regional brain activation was analyzed and compared using statistical parametric mapping techniques. *Results:* Compared with controls, cannabis users exhibited increased activation of brain regions typically used for spatial working memory tasks (such as prefrontal cortex and anterior cingulate). Users also recruited additional regions not typically used for spatial working memory (such as regions in the basal ganglia). These findings remained essentially unchanged when re-analyzed using subjects' ages as a covariate. Brain activation showed little or no significant correlation with subjects' years of education, verbal IQ, lifetime episodes of cannabis use, or urinary cannabinoid levels at the time of scanning. *Conclusions:* Recent cannabis users displayed greater and more widespread brain activation than normal subjects when attempting to perform a spatial working memory task. This observation suggests that recent cannabis users may experience subtle neurophysiological deficits, and that they compensate for these deficits by "working harder"—calling upon additional brain regions to meet the demands of the task.

Keywords Cannabis · Substance dependence · fMRI · Working memory · Cognitive function

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Introduction

Cannabis is the most widely used illicit drug in the United States (Substance Abuse and Mental Health Services Administration 2002), yet the cognitive effects of long-term cannabis use remain largely unknown. Several lines of evidence suggest that long-term cannabis use may produce working memory impairments and attentional dysfunction (Block and Ghoneim 1993; Pope and Yurgelun-Todd 1996; Fletcher et al. 1996; Pope et al. 2001; Solowij et al. 2002; Bolla et al. 2002). These deficits seem to persist for at least several days after the drug is stopped (Pope et al. 2001). For example, we have previously reported evidence of impaired performance on spatial working memory—short-term memory used to maintain and manipulate spatial information for a brief period—in chronic, heavy cannabis smokers compared with normal control subjects (Pope et al. 2001). These findings complement our previous reports of cognitive deficits in heavy cannabis smokers on other tests sensitive to frontal functions.

However, little is known about the functional changes in cerebral activation that underlie these deficits. Several studies have examined cerebral blood volume, cerebral blood flow, and/or glucose metabolism in recently abstinent chronic cannabis users, or in subjects acutely intoxicated with either cannabis or its active component, Δ-9-tetrahydrocannabinol (Δ^9-THC) (Mathew et al. 1989, 1992, 1997; Volkow et al. 1996; Yurgelun-Todd et al. 2001; see also review by Loeber and Yurgelun-Todd 1999). These investigations have reported that abstinence from cannabis results in depressed cerebral metabolism among chronic users, whereas very recent or acute

cannabis exposure increases cerebral activation as measured by increased blood flow. These studies, however, examined intoxicated cannabis users at a resting state rather than during the performance of a specific cognitive task. Two studies by O'Leary et al. (2000, 2002), utilizing an auditory attention task, found increased regional cerebral blood flow in orbital and mesial frontal lobes, insula, temporal poles, anterior cingulate, and cerebellum, but decreased blood flow in temporal lobe auditory regions, visual cortex, and regions associated with attention (parietal lobe, frontal lobe, and hypothalamus). The same group (O'Leary et al. 2003) found increased forebrain and cerebellar blood flow, but decreased frontal lobe blood flow, in acutely intoxicated cannabis users performing a counting task. In a pilot study in our laboratory using functional magnetic resonance imaging (fMRI) (Yurgelun-Todd et al. 1998), we found increased anterior cingulate activity but decreased dorsolateral prefrontal cortex (DLPFC) activity in eight recent cannabis users compared with eight control subjects during the performance of a working memory task. This pilot investigation represents the only study, to our knowledge, that has used fMRI to measure blood oxygenation level in specific brain regions of cannabis users. Images in this pilot study were analyzed by manually identifying brain regions of interest and then averaging the MR signal of all pixels in that region—a less sophisticated method than the statistical parametric mapping (SPM) method used in the present study and most other modern studies.

In order to extend these findings, the present investigation used fMRI to examine brain function of long-term cannabis users while they performed a spatial working memory task. Evidence from imaging studies of normal subjects indicates that the functional processes involved in spatial working memory are mediated by a neural network linking the prefrontal cortex (PFC), including both dorsal PFC (Brodmann's area 46/9) and ventral PFC (BA 44, 45, 47), and the parietal cortex, temporal cortex, and anterior cingulate cortex (BA 24/32) (Goldman-Rakic 1995; D'Esposito et al. 1995, 1999; Rowe and Passingham 2001; Wagner et al. 2001; Munk et al. 2002). In particular, working memory tasks appear to activate Brodmann's area 46/9, as well as ventral PFC (BA 44, 45, 47), and supplementary motor and premotor cortices (BA 6) (Smith et al. 1996; Braver et al. 1997; Courtney et al. 1997, 1998; Rowe and Passingham 2001). Working memory studies have suggested that BA6 is activated regardless of the type of information being processed (Baker et al. 1996; D'Esposito et al. 1998).

Based on findings from healthy control subjects, we designed a protocol to extend our understanding of cannabis-induced working memory deficits by examining cerebral activation in specific frontal cortical regions of heavy cannabis users. We hypothesized that, in response to a spatial working memory task, heavy cannabis users would demonstrate significantly lower activation than normal controls, particularly in the DLPFC (BA 46/9), ventral PFC (BA 44, 45, 47), and more posterior regions such as Brodmann's area eight and premotor cortex (BA 6).

Methods

Subjects

We enrolled 12 heavy cannabis users and 10 control subjects. The cannabis users were recruited in the course of a larger study of the residual neuropsychological effects of cannabis use; full details of subject selection criteria and study procedures have been published previously (Pope et al. 2001). Briefly, the heavy cannabis users were 30–55 years old, had all smoked cannabis at least 5000 times and were currently smoking at least seven times per week at the time of entry into the study. Imaging data were collected between 6 h and 36 h after the subject's last reported cannabis use. Subjects were excluded if they reported lifetime use of any category of illicit drugs [such as cocaine, hallucinogens, or 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy")] more than 100 times or any lifetime history of DSM-IV alcohol abuse or dependence (American Psychiatric Association 1994). We also excluded subjects reporting any current DSM-IV Axis-I disorder, as determined by the Structured Clinical Interview for DSM-IV Axis-I Disorders (SCID; First et al. 1996); a history of head injury with loss of consciousness requiring hospitalization; current use of any psychoactive medication; or any other medical condition that might affect cognitive function. We estimated the verbal IQ of each subject using the vocabulary subscale of the Wechsler adult intelligence test as described previously (Pope et al. 2001).

At the time of imaging, we also obtained subjects' urine samples, collected under observation, which we screened by immunoassay (EMIT II, Behring Diagnostics, Cupertino, CA) for 11-nor-9-carboxy-delta 9-tetrahydrocannabinol (THCCOOH), creatinine, cocaine metabolites, benzodiazepines, barbiturates, phencyclidine, opioids, and amphetamines, and by enzymatic assay (EA) for ethanol. We obtained quantitative THCCOOH and creatinine concentrations by gas chromatography–mass spectroscopy; we then used urinary creatinine levels to adjust for differences in the concentration of subjects' urine samples. In the results below, we report subjects' THCCOOH levels normalized to an assumed uniform urinary creatinine concentration of 100 ng/ml. These normalized urinary THCCOOH levels likely provided a better reflection of subjects' recent cannabis use than the simple measure of hours since last use, since subjects smoked cannabis of widely varying potency in widely varying patterns. Thus, a subject who smoked a large amount of potent cannabis 30 h prior to testing might have higher cannabinoid levels than a subject who smoked a small amount of lower potency cannabis 6 h prior to testing.

The control subjects were individuals who reported no history of cannabis abuse or dependence, no history of abuse of or dependence on any other illicit drug or alcohol, and no current or past DSM-IV Axis-I disorder on the SCID. Controls were neither tested for verbal IQ nor required to undergo urinary drug testing. As with the cannabis users, controls were excluded if they reported a history of head injury with loss of consciousness, medical or neurological conditions likely to affect cognitive functioning, or usage of psychotropic medication(s).

Task paradigm

While undergoing imaging, all subjects completed a spatial working memory paradigm that included two tasks: a perception task and a short-delay working memory task. This paradigm was adapted from one previously used in a positron emission tomography (PET) study of spatial working memory by Jonides et al. (1993). The tasks were as follows.

Perception task Subjects were presented with a fixation cross in the center of the screen for 0.2 s; the cross was then supplanted by three dots appearing on the circumference of an imaginary circle centered on the cross, presented for 4.3 s. This was followed by an interval of 1.5 s, during which the three dots remained present and a probe for

location memory was added. The probe consisted of a single open circle that either did or did not encircle the location of one of the dots (probability was set at 0.5). Subjects pressed a button once or twice to indicate whether the probe encircled a dot.

Short-delay response task Subjects focused on a cross in the center of the screen for 0.2 s. This cross was then supplanted by three dots. The dots remained on the screen for 1.3 s and were again replaced by the fixation cross alone for a 3-s delay period (with fixation). The probe circle then appeared for 1.5 s, and subjects were asked to press a button once or twice to indicate whether or not the probe circle marked the location where a dot had previously been present.

Five alternating rest/activation cycles (off–on–off–on–off) comprised each condition. An activation cycle contained five trials of 6 s. Thus, for both the perception and short-delay tasks, the time required was 30 s/cycle and 150 s for the full condition. The visual stimuli were presented by a Macintosh controlled video display. Task instructions were presented on the computer screen and were also explained to subjects by trained administrators before scanning.

Imaging techniques

Functional scanning Scanning was performed on a 1.5-Tesla GE whole-body scanner using a quadrature head coil. Head movement was minimized by padding and restraints. After acquisition of high-resolution T1-weighted images for fMRI anatomic localization, 50 sequential gradient-echo echoplanar images (EPIs) sensitive to blood oxygenation level-dependent (BOLD) signal were collected in contiguous slices of 6-mm thickness, with 3000-ms repetition time (TR), 40-ms echo time (TE), 20×20-cm field of view (FOV), a 64×64 image matrix, 70° flip angle, and an in-plane resolution of 3×3 mm. The slices were oriented to cover the entire frontal cortex, and parts of the temporal and parietal lobes, but not the cerebellum.

Image processing and statistical analysis The functional imaging and statistical analyses were performed using SPM99 (Wellcome Department of Cognitive Neurology, London, UK). Functional images were realigned to correct for motion-related variance components, normalized to the standard Montreal Neurological Institute (MNI) EPI template (Talairach and Tournoux 1988; Friston et al. 1995a) and spatially smoothed with an 8-mm full width at half maximum (FWHM) isotropic Gaussian kernel to allow for anatomical variation among subjects. The statistical parametric maps were generated using the general linear model in SPM99 (Friston et al. 1995b). Low-frequency noise was removed with a high-pass filter with a cutoff of 127 s applied to the fMRI time series at each voxel. Data from each subject for each task condition were first analyzed with a fixed box-car function convolved with a model hemodynamic response function. For individual subjects, regions formed by more than ten contiguous voxels with significant activations ($P<0.001$ uncorrected for multiple comparisons) were considered to represent areas of significant response. Predetermined condition effects at each voxel were calculated by the fixed model, creating a single image of mean activation for the short-delay task minus the perception control task in each subject for the group analysis. The group data were then analyzed using a random-effects model on a second level to account for interindividual variance. Comparisons within groups were performed on a voxel-wise basis using a one-sample *t*-test ($P<0.001$ uncorrected), and comparisons between groups were performed using a two-sample *t*-test ($P<0.005$ uncorrected).

Finally, because of age differences between the cannabis and control groups (see below), we repeated our comparison between groups while using age as a covariate. We also tested correlations between brain activation and (1) age and (2) years of education within the cannabis group and the comparison group. In addition, within the cannabis group alone, we assessed correlations between brain activation and (3) verbal IQ, (4) lifetime episodes of cannabis use, and (5) normalized urinary THCCOOH levels (as described above) at the time of imaging. (As noted above, we examined urinary THCCOOH levels, rather than hours since last use, since the former measure appeared likely to be a better reflection of recent cannabis use). These correlations were also performed using activation in the short-delay task minus the perception task as the outcome variable, the same 8-mm FWHM Gaussian kernel, and a significance level of $P<0.005$ uncorrected.

Results

Subject characteristics

The 12 cannabis users were older than the 10 controls; however, the groups did not differ significantly on other demographic measures (Table 1). Cannabis users reported that they had smoked cannabis on a mean of 19,200 occasions in their lives (range 5100–54,000). Their mean ±SD urinary THCCOOH level at the time of scanning (normalized to a urinary creatinine concentration as described above) was 497±515 ng/ml (range 35–1470 ng/ml). All subjects in both groups performed the perception task without errors; on the short-delay task, subjects displayed only a few performance errors, with no significant difference between groups (correct performance in 86±25% of trials among the cannabis users and 93±16% of trials among control subjects; $P=0.46$).

Perception task

During the simple perception task (in which no working memory was involved), both the control subjects and cannabis users activated the inferior frontal gyrus and middle frontal gyrus bilaterally (BA9 and 44; Table 2). The cannabis subjects also showed activation in several other areas, including right superior frontal gyrus, right caudate, and bilateral anterior cingulate.

Short-delay response task

In the short-delay task, control subjects again displayed prominent activation bilaterally in the middle and inferior

Table 1 Demographic features of cannabis users and control subjects

	Heavy cannabis users $N=12$	Control subjects $N=10$	P^a
Male, *N* (%)	10 (83)	6 (60)	0.35
Caucasian, *N* (%)	10 (83)	10 (100)	0.48
Age (years), mean (SD)	37.9 (7.4)	27.8 (7.9)	0.006
Education (years), mean (SD)	14.8 (2.1)	15.9 (1.9)	0.22

[a]Significance of differences calculated using Fisher's exact test, two-tailed and *t*-test, two-tailed

Table 2 Foci of maximally activated brain regions for the perception task. *L* left hemisphere, *M* midline, *R* right hemisphere. Atlas coordinates are from the MNI standard atlas, such that *x* reflects the distance (mm) to the right or left of midline, *y* reflects the distance anterior or posterior to the anterior commissure, and *z* reflects the distance superior or inferior to the horizontal plane through the AC–PC line. Coordinates and *t*-values are reported for the clusters in each lateral region with the largest number of activated voxels and *t*-values significant beyond $P<0.001$.

Regions of activation	Laterality	Brodmann's area	*x*	*y*	*z*	*t*-value
Control subjects						
Inferior frontal gyrus	L	44	−62	12	12	4.04
	R	9	46	12	22	3.41
Middle frontal gyrus	R	9	46	16	36	3.74
	L	9	−52	8	36	3.41
Heavy cannabis users						
Inferior frontal gyrus	R	38	50	22	−12	5.59
Caudate	R		14	−2	14	5.35
Middle frontal gyrus	R	9	50	22	32	5.11
	L	46	−48	36	28	5.43
Superior frontal gyrus	R	8	−30	36	50	3.92
Anterior cingulate gyrus	L	32	−2	22	42	4.38
	R	32	2	52	50	3.38

frontal gyri (BA 46/9 and BA 47, respectively), with additional areas of activation in the right anterior cingulate (BA 32) and bilateral caudate (Table 3). Cannabis users also showed activation of middle frontal and inferior frontal gyrus, left anterior cingulate, and right caudate together with additional foci of less prominent activation in left superior frontal and right parahippocampal gyrus.

Short-delay response task minus perception task

In order to determine areas of activation specific to the working memory process, we examined activation in the short-delay task minus that in the perception task. In the control group, this subtraction exercise demonstrated widespread activation bilaterally in the middle frontal gyrus (BA 46/9 and BA6), bilateral inferior frontal gyrus (BA47), and right anterior cingulate (BA32). The cannabis users showed activation generally in these same regions, but it was more prominent than in the controls, with larger numbers of foci of activation. Additionally, cannabis users showed activation in three areas of the right lentiform nucleus and one area of right superior frontal gyrus.

Figure 1 illustrates the more prominent and widespread brain activation of the cannabis users relative to the controls during the working memory task. The differences between groups are visible both on rendered brain images (top of figure) and on sagittal, coronal, and axial slices displaying the regions of maximal mean activation in each study group (bottom of figure).

Activation in cannabis subjects minus control subjects

The above observations suggest that contrary to our hypothesis, the cannabis users exhibited more pronounced and more widespread activation than the control subjects in response to the working memory task. To further characterize these differences, we subtracted the results of the short-delay response task minus perception task in controls from that of the cannabis users (Table 4). This comparison demonstrated that, during the working memory task, the cannabis users showed significantly greater

Table 3 Foci of maximally activated brain regions for the short-delay response task. *L* left hemisphere, *M* midline, *R* right hemisphere. Atlas coordinates are from the MNI standard atlas, such that *x* reflects the distance (mm) to the right or left of midline, *y* reflects the distance anterior or posterior to the anterior commissure, and *z* reflects the distance superior or inferior to the horizontal plane through the AC-PC line. Coordinates and *t*-values are reported for the clusters in each lateral region with the largest number of activated voxels and *t*-values significant beyond $P<0.001$

Activation area	Laterality	Brodmann's area	*x*	*y*	*z*	*t*-value
Control subjects						
Middle frontal gyrus	R	46	54	20	32	6.66
	L	9	−48	8	36	5.60
	L	6	−36	8	56	5.09
Anterior cingulate gyrus	R	32	2	12	52	6.36
Inferior frontal gyrus	R	47	50	24	−14	4.65
Caudate	R		12	−4	14	4.04
	L		−12	−6	14	3.77
Heavy cannabis users						
Middle frontal gyrus	R	9	54	22	32	9.14
	L	9	−46	16	34	6.21
Anterior cingulate gyrus	L	32	−2	18	46	8.77
Caudate	R		12	−2	12	7.16
Inferior frontal gyrus	L	47	−42	20	−4	5.40
Superior frontal gyrus	L	6	−26	10	60	4.70
Parahippocampus	R		32	−22	−4	3.65

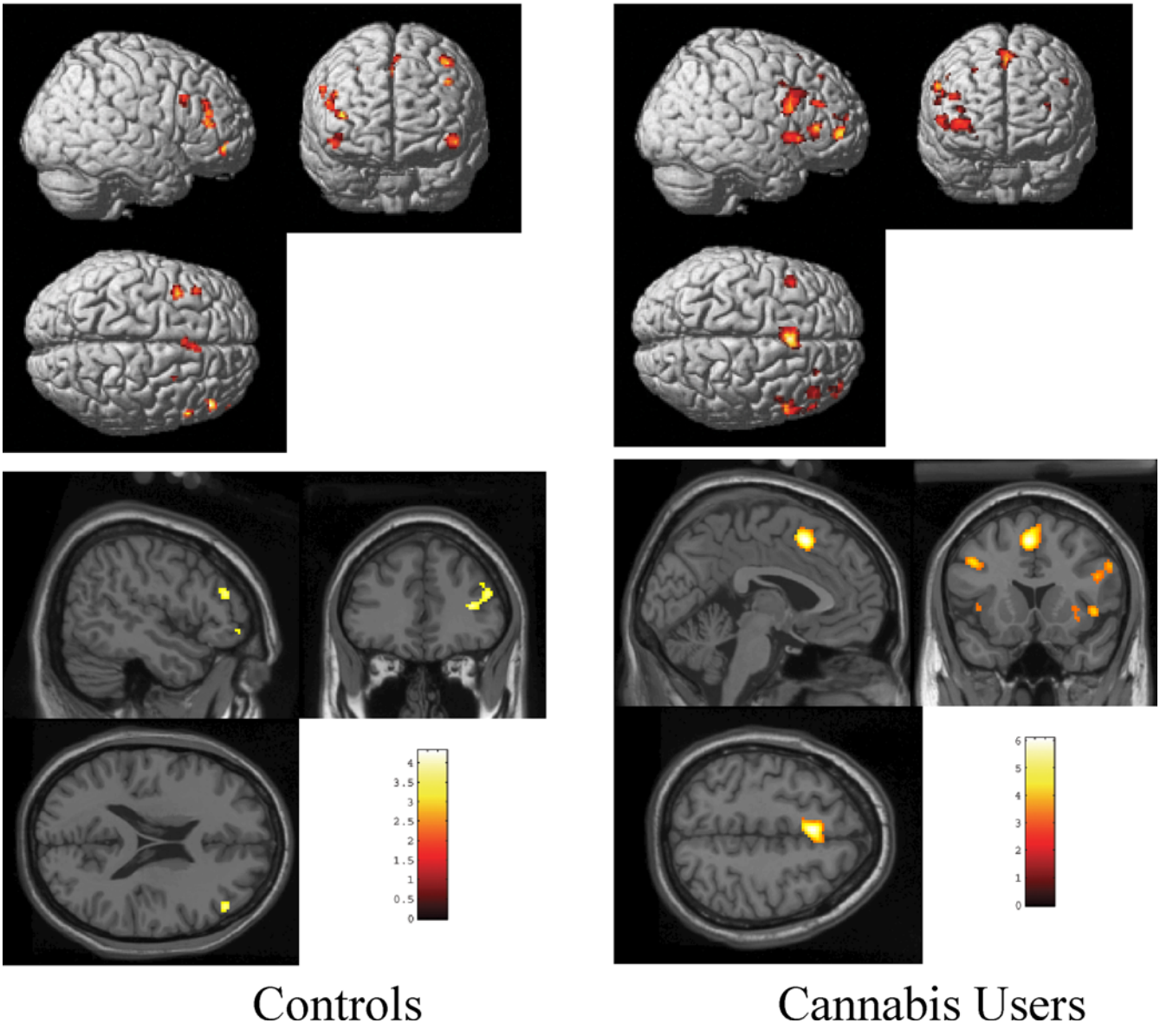

Fig. 1 Foci of maximal activation for the short-delay response task minus the perception task in control subjects (*left*) and long-term heavy cannabis users (*right*). The more prominent and widespread brain activation of the cannabis users is visible both on rendered brain images (*top*) and on sagittal, coronal, and axial slices displaying the regions of maximal mean activation in each study group (*bottom*). Note that the color scale for levels of activation differs between study groups; e.g., a *yellow* color corresponds to a *T* score of about 3.5 for the controls, but represents a *T* score of about 5.0 for the cannabis users. Thus, the difference between cannabis users and controls is actually greater than it first appears

activation than controls in a number of regions, including superior, middle, and inferior frontal gyrus; right superior temporal gyrus; anterior cingulate gyrus bilaterally; right precentral gyrus; and regions of caudate and putamen. By contrast, activation in the controls exceeded that of the cannabis users in only two small regions of the middle frontal cortex.

Secondary analyses

A possible limitation of the above analyses is the difference in mean age between the groups (Table 1). To address this issue, we repeated the analysis in Table 4 while introducing subjects' ages as a covariate This analysis demonstrated that age produced very little effect on the differences between cannabis and control subjects; after adjustment for age, the regions of increased activation among the cannabis users remained virtually the same, and the magnitude of the differences between the

Table 4 Foci of maximally activated brain regions for short-delay response minus perception. *L* left hemisphere, *M* midline, *R* right hemisphere. Atlas coordinates are from the MNI standard atlas, such that *x* reflects the distance (mm) to the right or left of midline, *y* reflects the distance anterior or posterior to the anterior commissure, and *z* reflects the distance superior or inferior to the horizontal plane through the AC–PC line. Coordinates and *t*-values are reported for the clusters in each lateral region with the largest number of activated voxels and *t*-values significant beyond $P < 0.005$

Regions of activation	Laterality	Brodmann's area	*x*	*y*	*z*	*t*-value
Heavy cannabis users minus controls						
Superior temporal gyrus	R	38	34	12	−26	3.49
Anterior cingulate gyrus	R	24	16	6	28	3.38
	L	32	−18	16	34	2.96
Inferior frontal gyrus	R	47	36	26	8	3.35
	R		42	32	4	3.28
Caudate	R		14	24	12	3.29
	L		−6	18	6	2.84
Middle frontal gyrus	L		−22	38	14	3.26
	R	10	16	38	−8	2.97
Superior frontal gyrus	R		18	46	−12	3.11
Precentral gyrus	R	6	54	6	18	3.00
Lentiform nucleus	R	Putamen	26	8	18	2.94
Controls minus heavy cannabis users						
Middle frontal cortex	L	6	−42	14	50	3.33
	R	9	44	42	34	2.97

groups actually increased slightly in most of these regions (details available from the authors on request). As a further check on the effects of age, we also assessed the correlation between age and activation in the short-delay minus perception conditions among both the cannabis users and comparison subjects; we again found virtually no significant associations between age and activation. Finally, we repeated the analysis in Table 4 while eliminating the two oldest cannabis users and the four youngest control subjects, leaving ten cannabis users and six controls closely matched on age (mean±SD age 35.1 ±3.6 years versus 31.7±8.2 years, respectively). This analysis again produced findings very similar to those of the primary analysis (details available from the authors on request).

In a series of correlational analyses, we found few significant correlations between years of education and brain activation in either group, and again none of these was associated with the regions of interest activated by the working memory task. Also, within the cannabis group alone, we found virtually no significant correlations between activation and verbal IQ, lifetime episodes of cannabis use, or normalized urinary THCCOOH levels (details available from the authors on request).

Discussion

Using the fMRI BOLD technique, we measured cortical activation in response to a spatial working memory task in 12 recent heavy cannabis users who had last smoked between 6 h and 36 h prior to study. We also measured activation in 10 control subjects with no recent cannabis use and no history of cannabis abuse or dependence. The two groups exhibited no performance errors on the perception task, and few errors on the short-delay task, with no significant difference between groups; this phenomenon may have represented a ceiling effect due to the relative simplicity of the tasks. When examining the fMRI results, however, we found that cannabis users displayed greater and more widespread brain activation than controls during task performance. This finding remained essentially unchanged (and indeed was slightly reinforced) when we repeated our analysis while introducing subjects' ages as a covariate. We found very few significant correlations between brain activation and age or years of education in either group, nor between activation and verbal IQ or lifetime episodes of cannabis use within the cannabis group. Furthermore, none of the clusters showing significant correlations in these exercises was located in the regions of interest activated by the working memory task. The absence of such correlations supports the conclusion that the differences between the cannabis and comparison groups are indeed an effect of recent cannabis use, rather than an artifact caused by confounding variables.

Perhaps surprisingly, we also found no significant correlations between brain activation and normalized urinary THCCOOH concentrations at the time of imaging. However, urinary THCCOOH concentrations may be only weakly associated with brain cannabinoid levels, and brain cannabinoid levels, in turn, may not be tightly correlated with levels of brain activation. Further studies will be required to explore these issues.

Looking at the results in more detail, the findings in our control subjects indicated that the regions activated in the perception condition were generally similar to those observed in previous studies using visual perception tasks (Calhoun et al. 2001). When the perception condition was subtracted from the short-delay response condition in order to isolate activation specific to spatial working memory itself, the results in controls were again generally similar to those found in many recent neuroimaging studies (fMRI or PET) that have examined working memory processes in the frontal lobes (D'Esposito et al. 1998; Smith and Jonides 1999). For example, Rowe and Passingham (2001) used a spatial memory task similar to ours to examine prefrontal activation in six normal

subjects using event-related fMRI. In the experimental task, subjects were required to remember the location of three red dots, presented on three successive trials. Following a delay of 8.5–17.5 s, subjects were shown the number of one of the three dots and asked to indicate where the dot had been located. In agreement with their previous study (Rowe et al. 2000), these investigators found that when the subject was asked to select the location from memory, area 46 in the DLPFC was activated bilaterally, together with the neighboring area 9/46 in the mid-DLPFC. Areas in ventrolateral PFC and anterior cingulate cortex were also activated. Our findings in normal controls are consistent with these results.

Heavy cannabis users, however, in contrast to our predictions, exhibited greater and more widespread activation than controls in response to the perception task, the short-delay task, and in the analysis of short-delay minus perception. In particular, the cannabis users responded to the working memory task with widespread activation of numerous regions, including not only regions typically used for spatial working memory, but other regions as well (Table 4). These findings suggest that cannabis users generate greater activation in the usual regions but also recruit ancillary regions to meet the demands of the task. It is of note that the anterior cingulate, a region known to be involved in attentional monitoring (Luks et al. 2002; Gruber et al. 2002), was more strongly activated in the cannabis users, due perhaps to an attempt to coordinate activity from the unusually wide range of regions recruited for the task.

The notion that increased activation of the anterior cingulate among cannabis users may be attributable to attentional dysfunction is consistent with other studies of cognitive effects of cannabis. Several studies have detected attentional dysfunction in long-term cannabis users (Block and Ghoneim 1993; Pope and Yurgelun-Todd 1996; Fletcher et al. 1996; Pope et al. 2001; Solowij et al. 2002; Bolla et al. 2002), and others have shown activation of the anterior cingulate in tasks requiring attentional function (Posner and Petersen 1990; Corbetta et al. 1991; Luks et al. 2002). In addition, the recent PET studies of O'Leary et al. (2000, 2002) found increased regional cerebral blood flow to the anterior cingulate during an auditory attention task. Therefore, the increased activation of the anterior cingulate of the users of this study may reflect an increased effort to overcome a cannabis-induced attentional impairment.

Our fMRI findings of widely increased activation among very recently abstinent cannabis users appear consistent with previous studies that have found increased cerebral blood flow and glucose metabolism in acutely intoxicated or very recently abstinent users (Loeber and Yurgelun-Todd 1999). However, with the exception of the studies of O'Leary et al. (2000, 2002, 2003), these previous studies are not directly comparable to ours in that they examined subjects at rest, rather than during the performance of a cognitive task. Our own preliminary fMRI investigation of working memory (Yurgelun-Todd et al. 1998) found increased cingulate activation in cannabis users relative to controls, in agreement with the present study, but decreased activity among cannabis users in the DLPFC, in disagreement. However, as previously mentioned, our previous study used an older and imprecise method, where we manually examined only four pixels in the relatively large region of the DLPFC. The more sophisticated SPM method, used in the present paper, is likely to be more reliable, since it corrects for temporal and spatial autocorrelations in the fMRI data using multivariate regression analysis.

Our hypothesis of increased utilization of brain reserve among cannabis users is consistent with the findings of studies on other types of patients with neurophysiological and behavioral syndromes. For example, patients with early human immunodeficiency virus (HIV) infection may show little or no deficit on neuropsychological tests of working memory, yet they display significantly greater and more widespread brain activation than controls while performing such tests, suggesting that they recruit additional brain regions to compensate for neural changes (Ernst et al. 2002). Similarly, another study found that alcoholic subjects performed at a comparable level to matched controls on a verbal working memory task, but the alcoholics displayed greater activation in left frontal and superior cerebellar regions—suggesting a compensatory increase in brain activation in order to maintain the same performance as controls (Desmond et al. 2003).

Few studies have examined cortical activation during spatial working memory tasks in subjects with substance abuse, and none, to our knowledge, has examined cannabis users. In a study of alcoholics, Pfefferbaum et al. (2001) employed an experimental design similar to ours, examining brain activation in a somewhat more difficult (match 2-back) spatial working memory task. When compared with the ten controls, the seven detoxified chronically alcoholic men showed diminished activation of frontal cortical systems, but greater activation of posterior and inferior frontal cortex. By contrast, the cannabis users in our study displayed increased activation of frontal cortical systems. However, the cannabis users, like the alcoholics, displayed increased activation of ancillary regions. In a recent imaging study of alcoholic women, Tapert et al. (2001) found significantly lower brain activation in patients than in controls across a variety of frontal and parietal regions with little evidence of compensatory activation elsewhere. Future studies are needed to clarify such contradictory findings.

Several possible limitations of our study should be considered. The first is the difference in mean age between the cannabis users and control subjects. However, our findings remained essentially the same when we repeated our analysis while using subjects' ages as a covariate. Moreover, when we repeated our analyses with a restricted sample of subjects matched for age and gender distribution, the results again remained essentially the same. These observations suggest that our findings are unlikely to represent an artifact of age or gender. A second possible limitation is that the drug use among the control subjects was not assessed as systematically as the drug use among

the cannabis users. Although we excluded controls with a history of alcohol or other substance abuse or dependence, the possibility remains that some control subjects had used substantial amounts of cannabis without qualifying for abuse or dependence. If this were the case, however, it would likely have narrowed the difference between cannabis users and controls, making our results a more conservative estimate of the effects of cannabis. Third, the cannabis users had ingested varying amounts of active drug (depending on the potency of the preparation used and the amount smoked), and had last smoked at varying times (6–36 h) prior to the time of imaging. As noted above, we have attempted to explore this variability by examining the correlation between brain activation and subjects' urinary THCCOOH levels. This analysis found virtually no voxels showing a significant correlation between activation and THCCOOH levels—but we cannot exclude the possibility that there might nevertheless be an association between brain activation and time since last cannabis use within the 6- to 36-h range. At present, speculation on this point must be limited, since we are not aware of any studies that have used fMRI to examine task-related neuronal activation in subjects who were directly administered cannabinoids—or, for that matter, comparable studies with any other drugs of abuse, with the exception of one study showing increased task-related neuronal activity with amphetamine administration (Uftring et al. 2001).

We also cannot exclude the possibility that some confounding variable, rather than cannabis use itself, accounted for the differences between groups. Although the groups were carefully screened to exclude subjects with substantial use of other drugs or alcohol, current psychiatric or medical disorders, and use of psychoactive medications, the possibility remains that the cannabis users possessed some attribute other than cannabis use associated with the greater cortical activation during the working memory task. Notably, however, we found virtually no voxels showing a significant correlation between activation and years of education, suggesting that this variable was not an important confounder.

Finally, we examined cortical activation only within the first 36 h after last cannabis use. Subsequent studies should attempt to examine brain activation during a working memory task in cannabis users after a prolonged period of abstinence to assess whether our findings represent a temporary or more persistent effect. Data from our earlier neuropsychological testing study suggest generally that neuropsychological test performance in cannabis users remains compromised at 7 days, but improves by 28 days of abstinence. It is unclear, however, whether these changes in performance are paralleled by changes in patterns of cerebral activation.

In summary, our findings suggest that long-term, heavy cannabis users, like individuals with alcoholism or early HIV infection, display increased cortical activation and even recruit ancillary brain regions during the performance of a working memory task. This finding raises the possibility that long-term cannabis users suffer from subtle neurophysiological changes, at least during the immediate period after discontinuing cannabis use. Furthermore, this change may be greater than that suggested by studies using conventional neuropsychological tests, since users may superficially perform as well as control subjects but only at the cost of "working harder," activating brain regions more strongly and more broadly than normally required. Such observations highlight the importance of supplementing neuropsychological findings with fMRI imaging data in cannabis users.

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