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Keywords

encoding, study, human, fmri, administration, tetrahydrocannabinol, 9, effects, pharmacological, function, memory, recall

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Effects of Δ9-Tetrahydrocannabinol Administration on Human Encoding and Recall Memory Function: A Pharmacological fMRI Study

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Abstract

■ Deficits in memory function are an incapacitating aspect of various psychiatric and neurological disorders. Animal studies have recently provided strong evidence for involvement of the endocannabinoid (eCB) system in memory function. Neuropsychological studies in humans have shown less convincing evidence but suggest that administration of cannabinoid substances affects encoding rather than recall of information. In this study, we examined the effects of perturbation of the eCB system on memory function during both encoding and recall. We performed a pharmacological MRI study with a placebo-controlled, crossover design, investigating the effects of Δ9-tetrahydrocannabinol (THC) inhalation on associative memory-related brain function in 13 healthy volunteers. Performance and brain activation during associative memory were assessed using a pictorial memory task, consisting of separate encoding and recall conditions. Administration of THC caused reductions in activity during encoding in the right insula, the right inferior frontal gyrus, and the left middle occipital gyrus and a network-wide increase in activity during recall, which was most prominent in bilateral cuneus and precuneus. THC administration did not affect task performance, but while during placebo recall activity significantly explained variance in performance, this effect disappeared after THC. These findings suggest eCB involvement in encoding of pictorial information. Increased precuneus activity could reflect impaired recall function, but the absence of THC effects on task performance suggests a compensatory mechanism. These results further emphasize the eCB system as a potential novel target for treatment of memory disorders and a promising target for development of new therapies to reduce memory deficits in humans. ■

INTRODUCTION

Learning and memory are critical in our daily lives. Deficits in memory function are associated with various psychiatric and neurological disorders, such as Alzheimer disease, schizophrenia, and mood disorders, and can be severely incapacitating.

Recently, animal studies have provided strong evidence for the involvement of the endocannabinoid (eCB) system in memory (Wise, Thorpe, & Lichtman, 2009; Wegener, Kuhnert, Thuns, Roese, & Koch, 2008; Yim, Hong, Ejaredar, McKenna, & McDonald, 2008; Hampson & Deadwyler, 2000; Mallet & Beninger, 1998; Lichtman & Martin, 1996; Lichtman, Dimen, & Martin, 1995). The eCB system, consisting of cannabinoid receptors and accompanying endogenous ligands, is a retrograde messenger system that regulates both excitatory and inhibitory neurotransmission (Piomelli, 2003; Wilson & Nicoll, 2002). As such, the eCB system may act to "fine tune" the control of important brain functions, including learning and memory (Ranganathan & D'Souza, 2006).

Modulation of the eCB system by systemic administration of exogenous cannabinoids, such as Δ9-tetrahydrocannabinol (THC), the main psychoactive component in cannabis and partial agonist of the CB1 receptor, impairs performance on various learning and memory paradigms in animals (Wise et al., 2009; Wegener et al., 2008; Yim et al., 2008; Hampson & Deadwyler, 2000; Mallet & Beninger, 1998; Lichtman & Martin, 1996; Lichtman et al., 1995). This suggests that the eCB system may be an important target for the development of novel therapies for memory dysfunction in psychiatric disorders. However, animal findings may not directly translate to humans, and there is a need to study the specific role of the eCB system in humans.

In humans, cannabinoids produce a diverse range of acute effects (Hall & Solowij, 1998), with increases in heart rate and subjective effects such as "feeling high" as the strongest and most consistently reported measures (Zuurman,

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Ippel, Moin, & van Gerven, 2009). Despite the consistent findings of memory impairments in animals after cannabinoid administration and the robust cannabinoid-induced human subjective and physiological effects, the evidence for impact of cannabinoid intoxication on learning and memory performance is less convincing. A large number of neuropsychological studies have reported no acute effects of cannabinoid administration on learning and memory paradigms (Hart et al., 2002, 2010; Zuurman et al., 2009; McDonald, Schleifer, Richards, & de Wit, 2003; Hart, van Gorp, Haney, Foltin, & Fischman, 2001; Chait & Perry, 1994; Block & Wittenborn, 1984; Darley, Tinklenberg, Roth, Vernon, & Kopell, 1977). Recall of items acquired before cannabis use is also generally not affected (Dornbush, 1974; Darley, Tinklenberg, Roth, Hollister, & Atkinson, 1973; Abel, 1971). Effects of cannabinoids on memory performance have, however, been reported in the free recall of information that is previously learned under the influence of cannabinoids (D'Souza et al., 2004; Curran, Brignell, Fletcher, Middleton, & Henry, 2002; Miller & Cornett, 1978). This suggests that cannabinoids influence encoding but not recall of information. Notwithstanding reported effects on memory in humans, the effect size is typically surprisingly small.

Assuming that the eCB system does play an important role in memory in both humans and animals, neuropsychology results may be affected by the ability of the human brain to reduce the effects of perturbations of the eCB system on behavior by functional compensation. A more effective method to measure the role of eCB in memory function in humans can be provided by direct visualization of brain activity during performance of a memory task in a pharmacological fMRI study.

In this study, we applied this approach and measured the effect of THC administration on encoding and recall brain function in an fMRI study. On the basis of neuropsychological findings, we tested the hypothesis that THC administration affects encoding, resulting in reduced encoding-related brain activity in a memory network including (para)hippocampal and prefrontal areas (Jager et al., 2007; Henke, Buck, Weber, & Wieser, 1997). In addition, in line with neuropsychological findings, we did not expect direct effects of THC on recall processes, although compensatory mechanisms for the affected encoding function may lead to increases in activity during recall. These hypotheses were tested in an fMRI study with a double-blind, randomized, placebo-controlled, crossover design, using a pictorial associative memory task, containing separate encoding and recall conditions (Jager et al., 2007; Henke et al., 1997).

METHODS

This study is part of the Pharmacological Imaging of the Cannabinoid System study, of which design and objectives are provided in a methods paper (van Hell, Bossong, Jager, Kahn, & Ramsey, 2011).

Subjects

Fourteen healthy male right-handed subjects were recruited through advertisements on the Internet and in local newspapers. All subjects used cannabis on an incidental basis, defined as having used cannabis at least four times but at most once a week in the year before inclusion in the study. All subjects were in good physical health as assessed by medical history, physical examination, electrocardiogram (ECG), and routine laboratory tests. Subjects were asked to refrain from cannabis for at least 2 weeks before the first study day until study completion. A maximum use of five cigarettes per day was allowed. Illicit drug use other than cannabis was restricted to a maximum of five times lifetime and not within 6 months before inclusion. Urine screening for cannabis, cocaine, amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine, morphine, methadone, tricyclic antidepressants, barbiturates, and benzodiazepines was performed at screening and on both study days. Subjects with a positive drug test were excluded from the study. Subjects were also asked to abstain from alcohol for 48 hr before each study day. Smoking was not allowed from the moment of arrival until the end of a study day. Alcohol and nicotine use was assessed by self-report. Subjects were asked to fast for at least 4 hr before arrival. On the beginning of each test day, they were served a standard meal. For further details on inclusion and exclusion criteria, we refer to van Hell et al. (2011). All volunteers gave written informed consent before entry into the study and were compensated for their participation. The study was approved by the University Medical Center Utrecht Independent Ethics Committee.

Results are reported on 13 of the 14 included subjects. One subject did not complete the second scanning session because of anxiety. See Table 1 for subject characteristics.

Design and Procedure

In a double-blind, randomized, placebo-controlled, crossover pharmacological MRI study, subjects underwent two scanning sessions after either administration of placebo or THC. Study days were scheduled 2 weeks apart to allow for complete clearance of drugs. Two weeks before the first study day, participants were familiarized with the scanner environment using a mock scanner. Verbal intelligence was estimated with the Dutch Adult Reading Test, the Dutch version of the National Adult Reading Test (Schmand, Bakker, Saan, & Louman, 1991).

At the beginning of each study day, a catheter was placed percutaneously in the left arm for the withdrawal of blood samples. Subsequently, subjects performed three cognitive paradigms, during which fMRI scans were obtained. One of these paradigms was the associative memory task. Paradigm sequence was randomized between subjects but remained unchanged within subjects across sessions. Results of the other two paradigms are reported elsewhere.

Table 1. Subject Characteristics (n = 13)

Characteristic	*Mean ± SD*	*Range*
Age (years)	21.6 ± 2.1	18–27
IQ	104.8 ± 5.6	94–111
Height (cm)	185.9 ± 5.3	176–196
Weight (kg)	78.7 ± 9.1	64–96
BMI (kg/m^2)	22.7 ± 2.3	18.6–27.8
Cannabis use (occasions/year)	17.0 ± 12.4	5–52
Tobacco smoking (cigarettes/week)	2.7 ± 7.7	0–28
Alcohol consumption (units/week)	16.7 ± 8.7	2–30
Coffee consumption (units/week)	11.2 ± 9.9	0–28
Illicit drug use (occasions lifetime)	1.3 ± 1.6	0–4

Use of cannabis, tobacco, alcohol, and coffee was given for the year before inclusion in the study. Subjects refrained from cannabis for at least 2 weeks before the first study day until study completion and from alcohol for 48 hr before each study day. Caffeine intake and smoking were not allowed from the moment of arrival until the end of a study day. Illicit drug use other than cannabis was at least more than 6 months before the first study day. All subjects showed negative urine screening at both study days.

On study days, subjects received subsequent doses of THC or placebo with 30-min intervals. Drugs were administered before each fMRI task using a Volcano vaporizer (Storz-Bickel GmbH, Tuttlingen, Germany) according to a method described earlier (Bossong et al., 2009; Zuurman et al., 2008; Hazekamp, Ruhaak, Zuurman, van Gerven, & Verpoorte, 2006). The first THC dose was 6 mg, followed by three doses of 1 mg each to maintain stable levels of CNS effects throughout the scanning procedure. Doses were based on pharmacokinetic/pharmacodynamic modeling of CNS effects induced by THC (Strougo et al., 2008; see van Hell et al., 2011, for detailed study procedures).

Drug Levels and Behavioral Measurements

Venous blood samples were collected to determine plasma concentrations of THC and its two most important metabolites, 11-OH-THC and 11-nor-9-carboxy-THC. Blood samples were processed according to Zuurman et al. (2008). Subjective and psychedelic effects were determined with two sets of visual analogue scales (VAS; Bowdle et al., 1998; Bond & Lader, 1974). Both rating scales were performed consecutively at baseline and before and after performance of the associative memory task. VAS were analyzed as described previously (Zuurman et al., 2008). Correlations between THC peak concentration and behavioral changes (THC vs. placebo) were determined using Pearson's correlation coefficient. Heart rate and respiration were monitored continuously during scanning, as described by van Buuren et al. (2009). Mean heart rate was calculated by dividing the total number of heart beat trigger signals by the duration of the associative memory task. Data were corrected for baseline values and analyzed with paired t tests.

Task Paradigm

Associative memory was assessed with a pictorial memory task (PMT) involving three different task conditions (Figure 1; Jager et al., 2007; Henke et al., 1997). First, an encoding condition (EN) was conducted in which subjects were presented with two pictures, one of a person and one of a house. Subjects were asked to decide whether the person might either be an inhabitant or a visitor of the house and to memorize the combination of pictures. There was no correct or incorrect answer. The purpose of the instruction

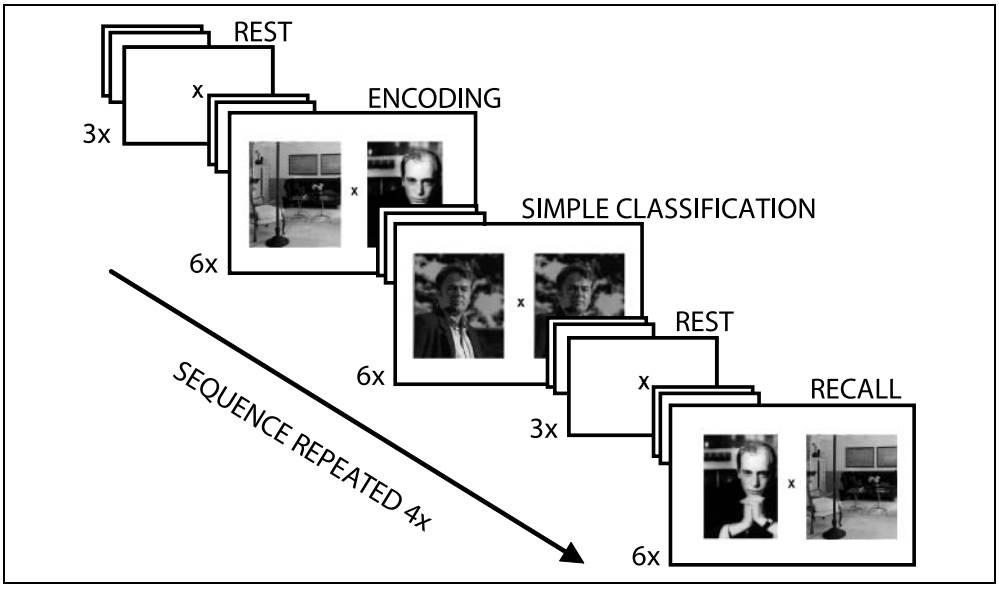

Figure 1. Schematic outline of the PMT used to assess associative memory. First, an encoding condition ("ENCODING") was conducted, in which subjects were presented with two pictures: one of a person and one of a house. In the second condition, identical pictures had to be classified as a house or a person ("SIMPLE CLASSIFICATION"). This condition was the control condition. The third condition was a recall task ("RECALL"), which required subjects to recognize specific combinations of pictures previously presented during ENCODING. Half of the stimuli were new combinations, and half were combinations previously presented.

was to engage subjects in a semantic evaluation of the two pictures which was expected to lead to a deep level of encoding of the paired pictures, irrespective of the decision. In the second condition, single item pictures had to be classified (denoted as SC). Two identical pictures were shown and subjects had to indicate whether a house or a person was presented. This condition was chosen as a control task. SC requires identical amount of perceptual processing and motor response as the two experimental conditions, but without a memory component. The third condition was a recall task (RE), which required subjects to recognize specific combinations of pictures previously presented during EN. Half of the stimuli were new combinations, and half were combinations previously presented during EN. For all conditions, subjects were instructed to press one of two buttons according to the instruction in the respective task condition, with emphasis on accuracy without stressing speed of response.

Each task condition was presented in an epoch consisting an instruction slide of 4000 msec followed by six stimuli. Each stimulus contained two pictures on a white background and was presented for 4000 msec, followed by an 850-msec fixation cross. Rest periods of half the epoch duration were also included. Altogether, a fixed order sequence of all task conditions was repeated four times, resulting in total task duration of 9 min. The PMT task contained different stimuli on both study days. Performance accuracy was assessed for SC and RE and was calculated as the mean percentage of correctly identified stimuli.

Image Acquisition

Image acquisition was performed on a Philips Achieva 3.0-T scanner (Philips Medical Systems, Best, the Netherlands). Functional images were obtained using a 3-D PRESTO-SENSE pulse sequence (Neggers, Hermans, & Ramsey, 2008) with shimmed background and the following parameters: repetition time = 22.5 msec, echo time = 33.2 msec, flip angle = 10°, field of view = 224 × 256 × 160, matrix = 56 × 64 × 40, voxel size = 4 mm isotropic, scan time = 0.6075 sec, 40 slices (sagittal orientation). A total of 900 functional images were acquired. Immediately after the PMT task, one volume with a flip angle of 27° was acquired for image coregistration. A T1-weighted structural image was obtained for anatomical registration with the following parameters: repetition time = 9.5 msec, echo time = 4.7 msec, flip angle = 8°, field of view = 220.8 × 240 × 159.6, matrix = 368 × 400 × 266, voxel size = 0.6 mm isotropic, 266 slices (sagittal orientation).

fMRI Analysis

After reconstruction, imaging data were preprocessed and analyzed using SPM5 (Wellcome Trust Centre for Neuroimaging, London, United Kingdom). Preprocessing of data included realignment of functional images and coregistration with the anatomical scan using the volume with a flip angle of 27°. Subsequently, functional scans were spatially normalized into MNI space (Collins, Neelin, Peters, & Evans, 1994) and smoothed (FWHM = 8 mm).

For each individual subject, regression coefficients for each voxel (*b* values) were obtained from a general linear model regression analysis using a factor matrix that contained factors modeling the EN, SC, and RE conditions (four blocks each) as well as the instructions that were presented during the task. To correct for drifts in the signal, a high-pass filter with a cutoff frequency of 0.005 Hz was applied to the data.

We chose to perform ROI analyses including areas that were involved in the task, as this analysis provides a good balance between power and information and allows for calculation and presentation of effect sizes (Kriegeskorte, Simmons, Bellgowan, & Baker, 2009; Poldrack, 2007). Group activation maps were created for the contrasts EN–SC and RE–SC for both the placebo and THC condition. All four maps were thresholded ($t = 4.5$, $p < .05$, corrected for multiple comparisons) and placebo and THC maps were pooled, resulting in two group activation maps (EN–SC and RE–SC). For both the EN–SC and RE–SC contrasts, clusters of at least 10 neighboring voxels were defined as ROIs, thus resulting in two sets of ROIs. Constructing the ROIs based on the highest values in either the THC or the placebo session prevents bias toward either the placebo or THC session (Mehta & O'Daly, 2011; Kriegeskorte et al., 2009). Mean signal change for each ROI, each subject, and each session (placebo and THC) was based on regression coefficients (*b* values) averaged over voxels in each ROI, extracted using Marsbar (Brett, Anton, Valabregue, & Poline, 2002).

To measure THC effects on encoding, a repeated measures MANOVA was performed on ROIs based on the EN–SC contrast with drug (2 levels: THC and placebo), condition (2 levels: EN and SC), and ROI (10 levels) as within-subject factors. Post hoc paired *t* test analyses were performed in comparison with SC to further investigate effects in individual ROIs. To measure effects of THC on recall activity, a repeated measures MANOVA was performed on ROIs based on the RE–SC contrast with Drug (two levels), Condition (two levels: RE and SC), and ROI (seven levels) as within-subject factors. Follow-up paired *t* test analyses were again performed for every ROI.

To assess relationships between brain activity and performance and to determine whether activity patterns within involved networks predicted performance, regression analyses were conducted with ROIs as independent variables and accuracy as dependent variable. This was done for each set of ROIs (encoding and recall) and for each session (placebo, THC). If the overall general linear model was significant, individual follow-up correlation analyses were performed between performance and ROIs.

Post hoc paired *t* tests were not corrected for multiple comparisons if the main MANOVA effect was significant,

Table 2. Subjective and Psychedelic Effects of THC (n = 13)

Assessment	*Drug Effect*	*Placebo Score (Mean ± SD)*	*THC Score (Mean ± SD)*
VAS feeling high	$F(1, 12) = 9.98, p = .008$[a]	0.38 ± 1.39	17.31 ± 19.16
VAS internal perception	$F(1, 12) = 3.79, p = .075$	−0.35 ± 1.41	1.69 ± 3.78
VAS external perception	$F(1, 12) = 3.46, p = .087$	0.35 ± 0.72	6.76 ± 12.43
VAS alertness	$F(1, 12) = 13.95, p = .003$[a]	−2.09 ± 7.00	−13.57 ± 9.38
VAS contentedness	$F(1, 12) = 1.09, p = .318$	−2.77 ± 3.64	−4.85 ± 6.69
VAS calmness	$F(1, 12) = 2.44, p = .144$	3.56 ± 8.97	−2.21 ± 11.12

Statistical analysis was performed with baseline-corrected values using repeated measures ANOVA with Drug and Time as factors.

[a]Significant difference between placebo and THC.

as they were considered as a further exploration of an already significant effect. All hypothesis tests were performed using SPSS 17.

RESULTS

Drug Levels and Behavioral Measurements

THC plasma concentration reached a maximum of 58.1 ± 31.3 ng/ml 5 min after inhalation of 6 mg THC and decreased rapidly thereafter. Subsequent doses of 1 mg THC induced peaks in THC plasma concentration of 13.7 ± 7.7, 13.0 ± 3.8, and 13.8 ± 6.0 ng/ml 5 min after each respective dose.

Analysis of subjective and psychedelic effects before and after performance of PMT revealed a significant THC-induced increase in VAS score of "feeling high" ($F(1, 12) = 9.98, p = .008$) and a decrease on "alertness" ($F(1, 12) = 13.95, p = .003$) compared with placebo. In addition, THC caused a trend toward both increased internal perception (reflecting inner feelings that do not correspond with reality) and external perception (reflecting misperception of external stimuli or changes in the awareness of the environment; $F(1, 12) = 3.79, p = .075$ and $F(1, 12) = 3.46, p = .087$, respectively). Subjective and psychedelic effects are summarized in Table 2. Peak THC concentration was positively correlated with alterations (THC vs. placebo) in "feeling high" ($r = .620$; $p = .031$) and negatively with changes in "alertness" ($r = -.746, p = .005$).

Heart rate increased significantly after THC compared with placebo (8.5 ± 10.2 and 2.1 ± 4.9 bpm increase compared with baseline, respectively; $p = .046$). For a more detailed description of drug levels and behavioral measurements following THC, see van Hell et al. (2011).

Task Performance

Performance accuracy on the PMT task did not differ between THC and placebo sessions for both SC (99.4 ± 0.4% for both sessions; $p = 1.000$) and RE (91.4 ± 3.3 and 89.4 ± 2.5%, respectively; $p = .430$; Figure 2).

Selection of ROIs

The EN–SC contrast yielded a network of ten brain regions, comprising bilateral fusiform gyrus/parahippocampal gyrus, inferior frontal gyrus, insula and middle occipital gyrus, right putamen, and left SMA (Table 3).

The RE–SC contrast showed a network of seven regions, comprising bilateral fusiform/parahippocampal gyrus, cuneus/precuneus, middle occipital gyrus, and left superior parietal gyrus (Table 4).

Effects of THC on Encoding Activity

For the 10 encoding ROIs, a significant interaction effect was found between condition, drug, and ROI ($F(9, 108) = 2.20, p = .028$). This indicates that THC induced a change in the pattern of activity during encoding. There was a trend toward a significant effect of drug ($F(1, 12) = 4.15, p = .064$) but no significant difference in the effect of THC on conditions (Drug × Condition, $F(1, 12) = 2.47$; $p = .142$). To elucidate which ROIs were involved in the

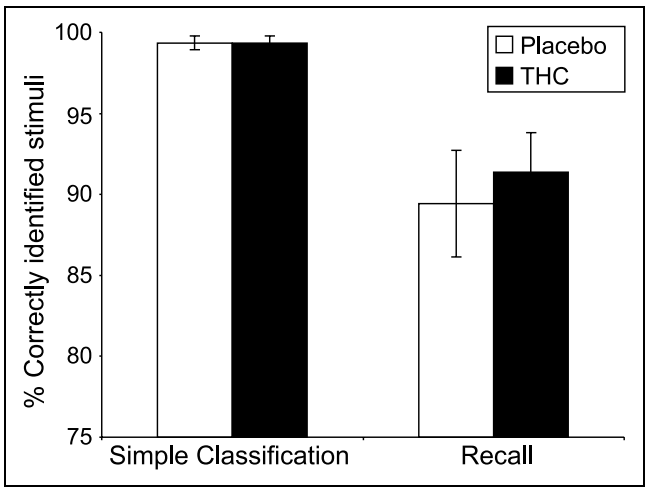

Figure 2. Performance accuracy on the PMT task during simple classification (left) and recall (right) in placebo session (white bars) and THC session (black bars). There was no significant difference in performance between sessions (n = 13; mean ± *SEM*).

Table 3. Significantly Activated Brain Regions during Encoding (n = 13)

Encoding—Single Classification						
Activated Brain Regions	*Brodmann's Area*	*Number of Voxels*	*x*	*y*	*z*	*Maximum t Value*
Fusiform/parahippocampal gyrus L	37	291	−28	−52	−12	13.10
Fusiform/parahippocampal gyrus R	37	330	36	−60	−20	15.44
Inferior frontal gyrus L	44	13	−56	24	28	5.30
Inferior frontal gyrus R	48	19	40	16	28	5.97
Insula L	47	28	−32	28	−4	6.25
Insula R	47	22	40	24	−4	7.45
Middle occipital gyrus L	19	271	−28	−80	16	11.96
Middle occipital gyrus R	39	244	40	−80	24	9.82
Putamen R	48	17	20	8	16	5.57
SMA L	6	37	4	16	52	6.25

Group activation maps for placebo and THC were thresholded at $t = 4.5$, $p < .05$, corrected for multiple comparisons, cluster size ≥ 10 voxels. x, y, and z are MNI coordinates and represent the highest t value in a cluster. Brodmann's areas are obtained from the location in the AAL atlas indicated by the MNI coordinates. L = left; R = right.

significant interaction, post hoc analyses (not corrected for multiple comparisons) were performed on each ROI. These demonstrated significantly reduced brain activity after THC administration (relative to placebo) in the right insula (from 0.53 ± 0.07 to 0.33 ± 0.06, $p = .019$), right inferior frontal gyrus (from 0.54 ± 0.09 to 0.22 ± 0.17, $p = .031$), and left middle occipital gyrus (from 0.54 ± 0.06 to 0.39 ± 0.07, $p = .033$). The mean b values are shown in Figure 3.

Effects of THC on Recall Activity

Repeated measures analysis showed no significant effect of Drug ($F(1, 12) = 1.14$, $p = .306$) in the seven recall ROIs, but THC affected the RE and SC conditions significantly differently (Drug × Condition, $F(1, 12) = 5.92$; $p = .032$). A significant interaction effect between Condition, Drug, and ROI ($F(6, 72) = 3.02$; $p = .011$) indicated that these Drug × Condition effects differed between ROIs. Post hoc analysis (not corrected for multiple comparisons) showed a significant THC-induced increase in brain activity relative to placebo in the left (from 0.37 ± 0.11 to 0.76 ± 0.09, $p = .014$) and right precuneus (from 0.33 ± 0.09 to 0.78 ± 0.10, $p = .004$; for mean b values, see Figure 4).

Brain Activity versus Performance

Overall THC administration did not affect performance. To assess whether activity patterns within involved networks predicted performance, regression analyses were

Table 4. Significantly Activated Brain Regions during Recall (n = 13)

Recall—Single Classification						
Activated Brain Regions	*Brodmann's area*	*Number of Voxels*	*x*	*y*	*z*	*Maximum t Value*
Cuneus/precuneus L	19	182	−12	−72	40	11.21
Cuneus/precuneus R	23	167	12	−68	24	8.27
Fusiform/parahippocampal gyrus L	37	180	−32	−44	−12	10.83
Fusiform/parahippocampal gyrus R	37	210	40	−56	−20	8.54
Middle occipital gyrus L	19	14	−28	−84	24	6.12
Middle occipital gyrus R	19	94	32	−72	20	7.90
Superior parietal gyrus L	19	179	−12	−72	40	11.21

Group activation maps for placebo and THC were thresholded at $t = 4.5$, $p < .05$, corrected for multiple comparisons, cluster size ≥ 10 voxels. x, y, and z are MNI coordinates and represent the highest t value in a cluster. Brodmann's areas are obtained from the location in the AAL atlas indicated by the MNI coordinates. L = left; R = right.

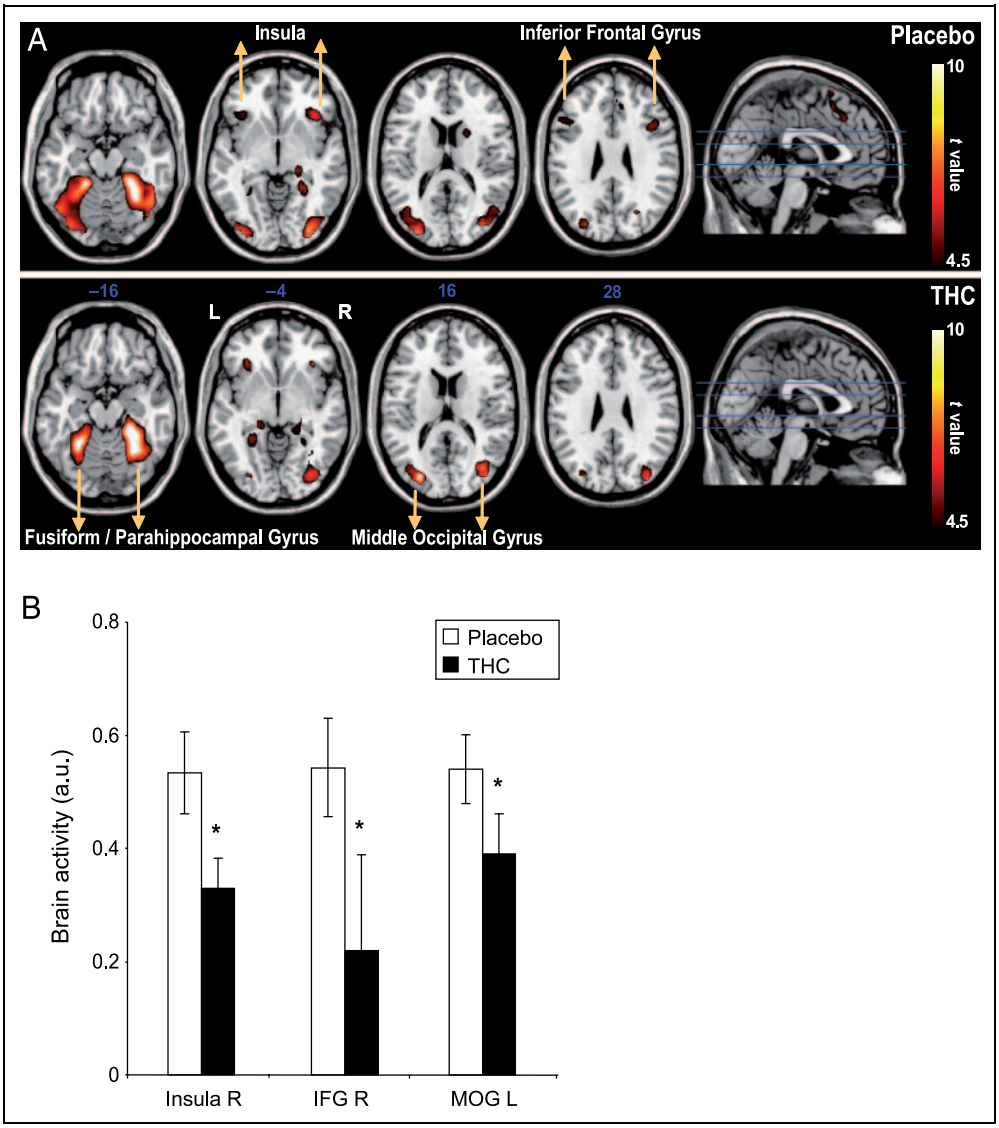

Figure 3. Brain activity during encoding (EN–SC). (A) Group activation maps after placebo (top) and THC (bottom) administration ($n = 13$; $t > 4.5$, $p < .05$, corrected for multiple comparisons, clusters ≥ 10 voxels). L = left; R = right. (B) Effect of THC administration on brain activity in the right insula, the right inferior frontal gyrus, and the left middle occipital gyrus (mean ± *SEM*). *$p < .05$. a.u. = arbitrary units; IFR = inferior frontal gyrus; MOG = middle occipital gyrus.

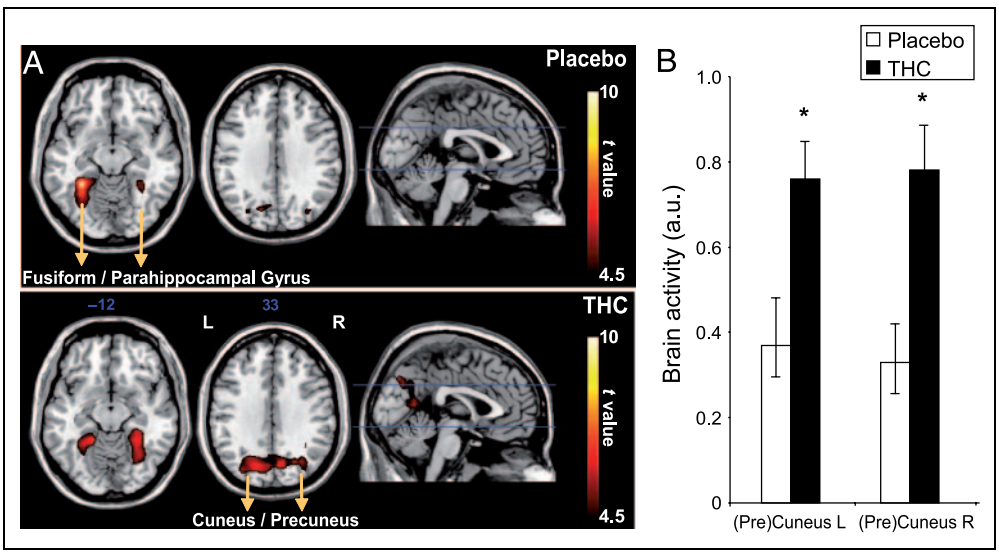

Figure 4. Brain activity during recall (RE–SC). (A) Group activation maps after placebo (top) and THC (bottom) administration ($n = 13$; $t > 4.5$, $p < .05$, corrected for multiple comparisons, cluster size ≥ 10 voxels). L = left, R = right. (B) Effect of THC administration on brain activity in the bilateral cuneus/precuneus (mean ± *SEM*). *$p < .05$. a.u. = arbitrary units.

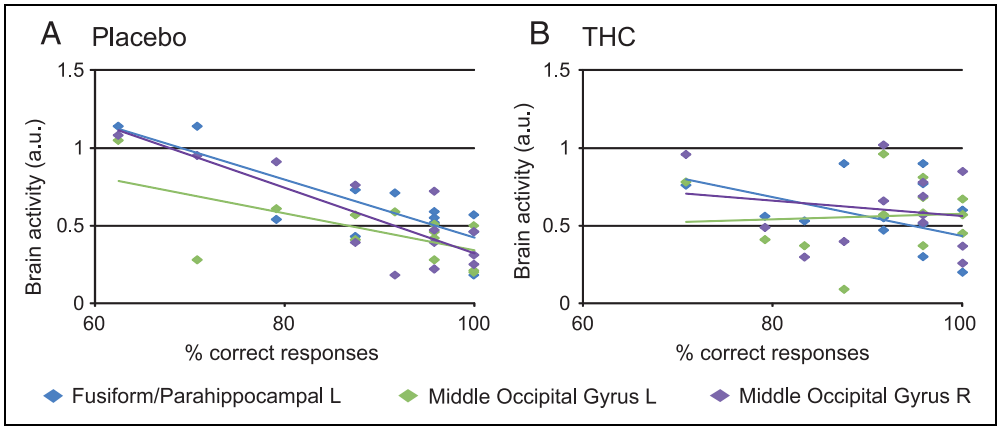

Figure 5. Correlation between recall brain activity and performance accuracy during placebo (A) and THC (B). The left fusiform/parahippocampal and bilateral middle occipital gyrus showed a significant inverse correlation with performance in the placebo session ($p < .05$), whereas there was no significant correlation with performance in the THC session nor in any of the other ROIs. a.u. = arbitrary units.

conducted with ROIs as independent variables and accuracy as dependent variable for each set of ROIs (encoding and recall) and for each session (placebo, THC). This revealed that during the placebo session a significant part of the variance in performance was explained by recall activity ($F = 17.37, p = .003$), but not during the THC session ($F = 0.65, p = .71$). Encoding activity patterns contained no predictive value for performance during placebo ($F = 0.54, p = .79$) or THC ($F = 0.90, p = .63$). A closer look at individual recall ROIs for the placebo session indicated that three of seven were negatively correlated with performance: left fusiform/parahippocampal gyrus ($r = -.83, p < .001$) and left and right middle occipital gyrus ($r = -.63, p = .02$ and $r = -.82, p = .001$, respectively; Figure 5). This shows that, under normal circumstances, good performance is associated with low activity in these regions during recall whereas this association disappears after THC.

DISCUSSION

This study tested the hypothesis that a cannabinoid challenge affects associative memory processes in humans. Activity in the network of regions involved in encoding of paired pictorial stimuli was significantly affected by THC administration, with reduced levels of activity in the right insula, right inferior frontal gyrus, and left middle occipital gyrus. During recall, THC administration was associated with a network-wide increase in activity, which was strongest in a bilateral region comprising cuneus and precuneus. Recall performance was not affected by THC administration. However, during the placebo session recall activity significantly explained variance in performance, with a strong inverse correlation in fusiform/parahippocampal and middle occipital gyrus, indicating that good performance was associated with low activity. This association disappeared after THC. Our interpretation is that under normal circumstances some subjects were able to use a very efficient recognition strategy for recall if information was sufficiently deep encoded. After THC, these subjects were mostly affected, as they could not apply this efficient recall strategy anymore. Hence, the inverse correlation between performance and recall activity disappeared after THC, although average performance itself was not reduced.

Although the design of the study does not provide conclusive evidence concerning the stage of memory processing that is most affected by THC, several arguments can be made for encoding as being more directly affected by THC, whereas the changes during recall are more likely to reflect a form of compensation for the affected encoding. First, THC induced opposite activity changes in encoding and recall. The interpretation that THC reduced encoding depth, indicated by less activity, while subjects could compensate during recall, at the expense of more activity, fits these differential effects. Second, behavioral studies in humans have indicated impairments in the free recall of information that is previously learned under the influence of cannabinoids (D'Souza et al., 2004; Curran et al., 2002; Miller & Cornett, 1978), but recall of items acquired before cannabis use is generally not affected (Dornbush, 1974; Darley et al., 1973; Abel, 1971), which indicates that cannabinoids influence encoding but not recall of information. Third, as task performance did not reach a ceiling during placebo, it was optimally sensitive to detect any changes in performance. Still, no performance effects were found, which suggests that subjects were able to compensate for the effects of THC. An alternative explanation could be that THC did also directly affect the recall process, for instance, by disturbing the retrieval process of previously encoded information. However, this interpretation would be in contrast with the mentioned previous findings that have indicated that THC does not affect recall of material encoded before drug intake.

In the absence of effects of THC on associative memory performance, it could be argued that the reported effects of THC may not be related to associative memory but are rather caused by nonspecific effects of THC intoxication. There are, however, several reasons to argue that the effects are indeed related to associative memory.

First, as mentioned earlier, the opposite effect of THC on encoding and recall activity suggests differential effects of THC that are specific for each process and not task independent. Second, the reduced correlation between performance and recall activity after THC indicates a direct effect of THC on the association between brain activity and task performance. Third, the reported effects of THC on brain activity reflect differences between the control and experimental task. These differences lie predominantly in the addition of an associative memory component in the experimental task. Thus, the effects of THC on brain activity are most likely associated with processes that directly or indirectly affect associative memory. Intoxicating, task-independent effects of THC can be expected to be present in both the control and experimental task.

Several fMRI studies have suggested important roles for some of the brain regions implicated in memory encoding in the current study (Dobbins, Foley, Schacter, & Wagner, 2002; Kelley et al., 1998; Wagner et al., 1998). The insula has been implicated in the process of selecting relevant item information, whereas the inferior frontal gyrus has been implicated in the organization of multiple pieces of information, possibly by building associations among items (Blumenfeld & Ranganath, 2007; Staresina & Davachi, 2006; Summerfield et al., 2006; Simons & Spiers, 2003). The middle occipital gyrus may not only be involved in the visual processing of to-be-remembered stimuli (Ishai, Ungerleider, Martin, & Haxby, 2000), but also in maintenance and imagery of visual information (Johnson, Mitchell, Raye, D'Esposito, & Johnson, 2007). As all these functions include attentional processes and the right insula and inferior frontal gyrus are part of the ventral attention network (Corbetta, Patel, & Shulman, 2008; Corbetta & Shulman, 2002), the decrease in activity in these brain areas after THC administration may be related to disturbed attentional processes, which is in line with the reported THC-induced reduction in alertness.

A possible alternative interpretation for the reduced encoding activity after THC would be that encoding was performed more efficiently under the influence of THC. However, both animal and human behavioral studies argue against this, as previous studies have not indicated increased efficiency of encoding after THC, only impairments (e.g., Wise et al., 2009; Wegener et al., 2008; Yim et al., 2008; D'Souza et al., 2004; Curran et al., 2002; Hampson & Deadwyler, 2000; Lichtman et al., 1995; Miller & Cornett, 1978).

A potential mechanism underlying the THC-induced decreases in brain activity may be found in the regulatory role of the eCB system in neurotransmitter release. As shown in electrophysiological animal studies, activation of cannabinoid receptors reduces both GABA and glutamate release from presynaptic terminals (Heifets & Castillo, 2009; Wilson & Nicoll, 2002). This eCB-mediated inhibition of synaptic transmission is critically involved in learning and memory processes (Heifets & Castillo, 2009) and has been demonstrated in the PFC (Lafourcade et al., 2007), among other brain regions.

In our study, we found an increase in activity in bilateral precuneus after THC during recall. Previous imaging studies have suggested a pivotal role for the bilateral cuneus and precuneus in recall memory (Wiesmann & Ishai, 2008; Gardini, Cornoldi, De Beni, & Venneri, 2006; Lundstrom, Ingvar, & Petersson, 2005; Gilboa, Winocur, Grady, Hevenor, & Moscovitch, 2004; Lundstrom et al., 2003; Burgess, Maguire, Spiers, & O'Keefe, 2001; Henson, Rugg, Shallice, Josephs, & Dolan, 1999; Krause et al., 1999; Fletcher et al., 1995). It is suggested that the (pre)cuneus is particularly involved in recall of context-rich memories, such as memories entailing specific contextual associations (Gardini et al., 2006; Lundstrom et al., 2003, 2005; Gilboa et al., 2004; Henson et al., 1999). Increased involvement of the precuneus has been demonstrated when subjects claimed to recognize items based on conscious recollection of contextual details rather than on feelings of familiarity (Wiesmann & Ishai, 2008; Wheeler & Buckner, 2004; Henson et al., 1999). More specifically, it may signal whether context information should be used to recognize an item correctly (Dorfel, Werner, Schaefer, von Kummer, & Karl, 2009). The enhanced precuneus activity found in the current study after THC administration thus could be related to a change in retrieval strategy, with increased utilization of contextual associations to accurately recall information. One mechanism would be that after THC administration recall relies more on processing of individual features of to-be-remembered items, such as the color of a person's shirt, than on the recognition of the complete composition of the picture, which can be expected to be more efficient. Importantly, increases in precuneus activity during recall memory have also been associated with compensatory mechanisms in individuals with and at risk for mild cognitive impairment or Alzheimer's disease (Schwindt & Black, 2009; Seidenberg et al., 2009; Woodard et al., 2009).

To date, only one other fMRI study has been published that investigated the acute effects of THC administration on learning and memory (Bhattacharyya et al., 2009). A normal linear decrease in activity in the parahippocampal gyrus present over repeated encoding blocks was no longer evident after oral THC administration. As in the current study, task performance was unaffected. Because Bhattacharyya and colleagues presented the same stimuli during four blocks of encoding, thereby investigating the effect of THC on learning activity, only the imaging results for the first presentation of stimuli are comparable to our study. These findings are in line with our results in that a THC-induced reduction in encoding activity was found. However, differences in recall activity in the first session were not reported (Bhattacharyya et al., 2009).

This study has several limitations. First, the sample size was relatively small. We therefore cannot exclude the possibility that subtle effects of THC on brain activity have been missed. Second, inclusion of incidental cannabis

users, as opposed to nonusers, may have hampered interpretation of the results, as previous cannabis use may have affected the eCB system. The choice for incidental cannabis users was based on ethical grounds (van Hell et al., 2011). Third, absence of significant differences between placebo and THC in performance accuracy may suggest that the memory task used in this study was not an appropriate task to assess memory function. However, we have previously shown that performance on this task correlates inversely with the amount of cannabis used in the year before testing, in heavy cannabis users (Jager et al., 2007), indicating that the task is sensitive to impairment. Finally, nonspecific THC-induced changes on CBF may have confounded our results (Iannetti & Wise, 2007). However, we have designed our study to minimize the influence of this effect by comparing brain activity between task-specific conditions and a closely matched control condition, as the nonspecific effect of THC on blood flow can be expected to be present in all conditions. Furthermore, as we found both significant decreases and increases in activity after THC administration, it is highly unlikely that our findings can be explained by such nonspecific effects.

In conclusion, findings reported in this article contribute to the growing body of evidence that suggests the involvement of the eCB system in learning and memory processes. Our results further emphasize the eCB system as a potential novel target for treatment of memory disorders, encouraging further research into novel, eCB-targeting compounds.

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