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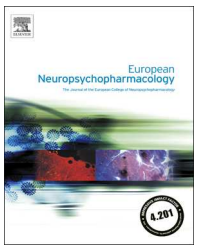

The endocannabinoid system and emotional processing: A pharmacological fMRI study with Δ9-tetrahydrocannabinol

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Abstract
Various psychiatric disorders such as major depression are associated with abnormalities in emotional processing. Evidence indicating involvement of the endocannabinoid system in emotional processing, and thus potentially in related abnormalities, is increasing. In the present study, we examined the role of the endocannabinoid system in processing of stimuli with a positive and negative emotional content in healthy volunteers. A pharmacological functional magnetic resonance imaging (fMRI) study was conducted with a placebo-controlled, cross-over design, investigating effects of the endocannabinoid agonist Δ9-tetrahydrocannabinol (THC) on brain function related to emotional processing in 11 healthy subjects. Performance and brain activity during matching of stimuli with a negative ('fearful faces') or a positive content ('happy faces') were assessed after placebo and THC administration. After THC administration, performance accuracy was decreased for stimuli with a negative but not for stimuli with a positive emotional content. Our task activated a network of brain regions including amygdala, orbital frontal gyrus, hippocampus, parietal gyrus, prefrontal cortex, and regions in the occipital cortex. THC interacted with emotional content, as activity in this network was reduced for negative content, while activity for positive content was increased. These results indicate that THC administration reduces the negative bias in emotional processing. This adds human evidence to support the hypothesis that the endocannabinoid system is involved in modulation of emotional processing. Our findings also suggest a possible

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role for the endocannabinoid system in abnormal emotional processing, and may thus be relevant for psychiatric disorders such as major depression.

1. Introduction

Accurate processing of emotional information is an essential aspect of appropriate social interactions and interpersonal relationships. Abnormalities in emotional processing are among the most important characteristics of psychiatric disorders such as major depression, bipolar disorder and schizophrenia, with significant consequences for social functioning and subjective well-being of patients (Leppanen, 2006; Phillips et al., 2008). Evidence is accumulating for involvement of the endocannabinoid (eCB) system in emotional processing (Lafenetre et al., 2007). Additionally, a possible role for the eCB system in abnormalities in emotional processing related to psychiatric disorders has been suggested (Ashton and Moore, 2011; Hill et al., 2009).

The eCB system is a retrograde messenger system that regulates both excitatory and inhibitory neurotransmission, and consists of cannabinoid receptors and accompanying endogenous ligands (Heifets and Castillo, 2009). Modulation of the eCB system changes emotional responses and processing of emotional information. In humans, for example, smoking cannabis can produce a euphoriant effect, with feelings of intoxication and decreased anxiety, alertness and tension (Ashton, 2001). Administration of Δ9-tetrahydrocannabinol (THC), the main psychoactive component in cannabis and partial agonist of the cannabinoid CB1 receptor, has been shown to reduce perception of fearful facial emotions in healthy volunteers (Ballard et al., 2012), whereas both acute and long-term administration of the eCB antagonist rimonabant appear to induce a bias away from positive emotions on a memory recognition task (Horder et al., 2009, 2012). In animals, low doses of cannabinoid agonists or drugs that enhance levels of endogenous cannabinoids reduce anxiety-like behavior (Kathuria et al., 2003; Marco et al., 2004; Valjent et al., 2002; see for a review Hill et al. (2009)), while disruption of eCB-mediated synaptic regulation produces anxiety- or depressive-like states (Griebel et al., 2005; Martin et al., 2002; see for a review Lafenetre et al. (2007)).

Cannabinoid receptors are highly expressed in many of the key regions for emotional processing (Herkenham et al., 1991; Katona et al., 2001), such as the occipital and temporal lobes, which are involved in perceptual emotional processing, the amygdala and orbital frontal cortex, which are involved in emotion recognition and generation of emotional reactions (LeDoux, 2003), and the anterior cingulate and prefrontal cortex, which are involved in regulation of emotional reactions (Adolphs, 2002; Phillips et al., 2008). Based on this widespread involvement, the purpose of the present study was to examine network-wide interaction effects of the eCB system with emotional content of stimuli. For this purpose, we conducted a pharmacological functional MRI (fMRI) study with healthy volunteers, measuring the effects of THC administration on brain function related to stimuli with either a negative ('fearful faces') or positive ('happy faces') emotional content.

So far, the role of the eCB system in human emotional processing has been investigated in a limited number of functional neuroimaging studies with administration of THC (Fusar-Poli et al., 2009; Phan et al., 2008). Specifically examining the effects of THC in the amygdala region with an identical task as used in the present study, Phan et al. (2008) found reduced amygdala reactivity for processing of stimuli with a negative emotional content. Fusar-Poli et al. (2009) reported less consistent effects, as THC increased activity in precuneus and primary motor cortex, and reduced activity in bilateral middle frontal gyrus and posterior cingulate cortex during a gender discrimination task with stimuli with a negative emotional content.

On the basis of recent neural models, we expected that processing of emotional stimuli would activate a wide network of brain regions, including amygdala, orbital frontal gyrus, prefrontal cortex, anterior cingulate cortex, and temporal and occipital lobes (Adolphs, 2002; Phillips et al., 2008). Based on both the study of Phan et al. (2008), in which a similar design was used as in the present study, as well as on the reported alterations in processing of emotional information after administration of cannabinoids (Ballard et al., 2012; Horder et al., 2009; Horder et al., 2012), it was hypothesized that THC would reduce the negative bias in emotional processing, and shift it towards a positive bias. We expected this to be reflected in reduced brain activity after THC administration when stimuli with a negative emotional content are processed, and increased activity after THC administration when stimuli with a positive emotional content are processed.

2. Experimental procedures

This study is part of the Pharmacological Imaging of the Cannabinoid System (PhICS) project, the design and objectives of which are provided in a methodological paper (van Hell et al., 2011).

2.1. Subjects

Fourteen healthy male right-handed subjects were recruited through flyers, posters and internet advertisements. 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 and physical examination, and were screened for axis I psychiatric disorders using the Dutch version of the Mini International Neuropsychiatric Interview for DSM-IV clinical disorders. Subjects were asked to refrain from cannabis for at least two weeks before the first study day until study completion. Illicit drug use other than cannabis was not allowed within six months prior to inclusion. Compliance was tested by means of a urine sample at the beginning of each test day. For further details on inclusion and exclusion criteria we refer to van

Table 1 Subject characteristics (n=11).

Characteristic	*Mean ± SD*	*Range*
Age (years)	21.5 ± 2.5	18-26
IQ	105.2 ± 5.5	98-113
Height (cm)	183.4 ± 6.5	175-195
Weight (kg)	74.1 ± 7.6	65-87
BMI (kg/m^2)	22.0 ± 1.2	20.1-23.6
Cannabis use (Occasions/year)	20.0 ± 9.4	4-30
Tobacco smoking (Cigarettes/week)	0.3 ± 0.7	0-2
Alcohol consumption (Units/week)	12.0 ± 5.9	5-20
Coffee consumption (Units/week)	12.7 ± 11.6	0-35
Illicit drug use (Occasions lifetime)	1.0 ± 1.8	0-5

Use of cannabis, tobacco, alcohol and coffee was given for the year before inclusion in the study. Subjects refrained from cannabis for at least two weeks before the first study day until study completion and from alcohol for 48 h 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.

Hell et al. (2011). All volunteers gave written informed consent before entry into the study. The study was approved by the Independent Ethics Committee of the University Medical Center Utrecht, the Netherlands, in accordance to the Declaration of Helsinki 2008.

Results are reported on 11 out of the 14 included subjects. One subject did not complete the study procedure due to a strong disruptive response to inhalation of medication during one of the scanning sessions. Two other subjects were excluded because of an absence of elevated THC plasma levels and movement-related errors during scanning, respectively. Subject characteristics are summarized in Table 1.

2.2. Design and procedure

In a double-blind, randomized, placebo-controlled, crossover pharmacological fMRI study, subjects underwent two scanning sessions after administration of placebo and of THC. Study days were scheduled at least 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.

On the beginning of each study day, a catheter was placed intravenously in the left arm for the withdrawal of blood samples. Subsequently, subjects performed three cognitive paradigms, during which functional MRI scans were obtained. One of these paradigms was the emotional processing task. Paradigm sequence was randomized between subjects, but remained unchanged within subjects across sessions. Results of other assessments are reported elsewhere (Bossong et al., 2012a; Bossong et al., 2012b; van Hell et al., 2011). Although there is some overlap in subjects participating in our current and previous studies, none of the published studies have identical experimental groups.

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). The first THC dose was 6 mg, followed by three doses of 1 mg each to maintain stable levels of CNS effects. See van Hell et al. (2011) for detailed study procedures.

2.3. 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, and were processed according to Zuurman et al. (2008). Subjective effects were determined with two sets of visual analog scales (Bond and Lader, 1974; Bowdle et al., 1998), which were performed consecutively at baseline and before and after task performance, and analyzed as described previously (Bossong et al., 2009). Heart rate was monitored continuously during scanning (van Buuren et al., 2009). VAS data and heart rate were corrected for baseline values, and analyzed with repeated measures MANOVA (factors drug and time) and a paired t test, respectively.

2.4. Task paradigm

Emotional processing was assessed with an emotional faces task consisting of two conditions involving processing of facial expressions of emotion (fearful ('FF') and happy faces ('HF'), respectively) and a sensorimotor control condition ('CT') (Figure 1) (Hariri et al., 2002; Phan et al., 2008). During FF and HF, subjects viewed a trio of unfamiliar faces and selected one of the two bottom faces that expressed the same facial emotion as the target face on top. The target and congruent probe face displayed either a fearful or happy expression, while the incongruent probe face displayed a neutral expression. The identity of all three faces was always different. FF and HF were interspersed with a sensorimotor control condition in which subjects viewed a trio of simple geometric shapes (circles, vertical and horizontal ellipses) and selected one of the two bottom shapes identical to the target shape on top. Subjects responded by pressing one of two buttons with their right thumb.

The emotional faces task consisted of 17 experimental blocks of 24 s: four each for FF and HF, interleaved with nine control blocks, for a total task length of 7 min. The order of blocks was counterbalanced. All blocks were preceded by a 4 s instruction (in Dutch): "Match Faces" or "Match Shapes", followed by four different trios of images presented sequentially for 5 s each, randomized for all conditions. Trios of faces were balanced for gender. All facial images were derived from a standard set of pictures of facial affect (Ekman and Friesen, 1976).

Outcome measures included reaction time for correct responses and the mean percentage of correctly identified targets. Group differences in reaction time and performance accuracy between placebo and THC were analyzed using repeated measures MANOVA with drug (two levels: placebo and THC) and condition (two levels: FF and HF) as factors. Post hoc paired t tests were performed to further investigate effects of THC on individual task conditions.

2.5. 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 3D PRESTO-SENSE pulse sequence (Neggers et al., 2008) (parameters: scan time 0.6075 s; TR 22.5 ms (in contrast to EPI, for PRESTO the TR is much shorter than the time to scan one volume, see Neggers et al., 2008); TE 33.2 ms; flip angle=10°; FOV 224 × 256 × 160; matrix 56 × 64 × 40; voxel size 4 mm isotropic; 40 slices (sagittal orientation); 700 volumes). A high-contrast volume with a flip angle 27° was scanned for

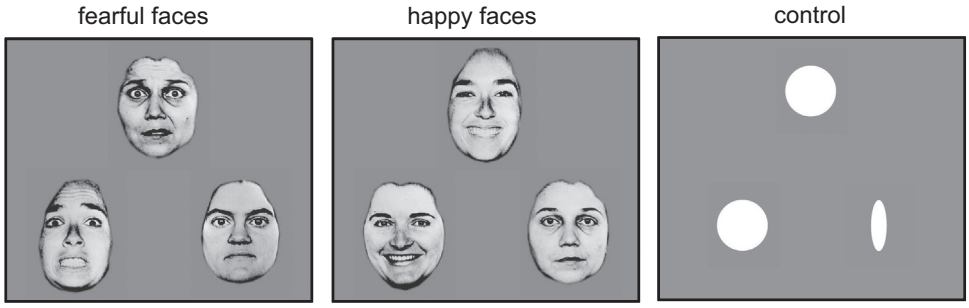

Figure 1 Schematic outline of the task used to assess effects of THC on processing of facial expressions of emotion. The task consists of two experimental conditions (fearful faces (left) and happy faces (middle), respectively), during which subjects viewed a trio of unfamiliar faces and selected one of the two bottom faces that expressed the same facial emotion as the target face on top. Experimental conditions were interspersed with a sensorimotor control condition (right), during which subjects viewed a trio of simple geometric shapes (circles, vertical and horizontal ellipses) and selected one of the two bottom shapes identical to the target shape on top. Each block consisted of four different trios of images presented sequentially for 5 s each. See for detailed information the experimental procedures section.

Table 2 Subjective effects of Δ9-tetrahydrocannabinol (THC) ($n=11$).

Assessment	*Drug effect* ($F(1,10)$)	*Mean placebo score* ($\pm SD$)	*Mean THC score* ($\pm SD$)
VAS feeling high	11.06, $p=0.008$*	1.14 ± 4.79	34.77 ± 32.76
VAS internal perception	6.21, $p=0.032$*	-0.32 ± 1.06	5.14 ± 6.86
VAS external perception	11.97, $p=0.006$*	0.68 ± 2.25	10.23 ± 7.93
VAS alertness	8.19, $p=0.017$*	-5.63 ± 3.80	-18.86 ± 14.30
VAS contentedness	6.96, $p=0.025$*	-2.36 ± 6.19	-9.73 ± 10.38
VAS calmness	7.72, $p=0.020$*	5.11 ± 10.90	-11.25 ± 20.23
VAS anxiety	3.60, $p=0.087$**	-1.59 ± 3.92	7.50 ± 13.69

Statistical analysis was performed with baseline corrected values using repeated measures ANOVA with drug and time as factors. VAS, Visual Analogue Scale.
*Significant difference ($p<0.05$).
**Trend towards significant difference ($p<0.10$) between placebo and THC.

registration purposes. A T1-weighted structural image was obtained for anatomical registration (parameters: TR 9.5 ms; TE 4.7 ms; flip angle=8°; FOV 220.8 × 240 × 159.6; matrix 368 × 400 × 266; voxel size 0.6 mm isotropic, 266 slices (sagittal orientation)).

2.6. Functional MRI analysis

Functional MRI data were preprocessed and analyzed using SPM5 (Wellcome Trust Centre for Neuroimaging, London, UK). Preprocessing included realignment of functional images, co-registration with the anatomical volume using the flip angle of 27° volume, spatial normalization into standard MNI space, and smoothing (FWHM=8 mm), as described previously (van Hell et al., 2011; Bossong et al., 2012a; Bossong et al., 2012b). There were no significant differences between sessions in scan quality in terms of the average standard deviation of time series.

First level single subject analysis included a general linear model regression analysis using a factor matrix with factors for the FF and HF condition, as well as the instructions that were presented during the task and factors to correct for slow drifts in the signal up to 0.006 Hz. Group activity maps were created for both the placebo and THC condition for the contrasts FF-CT and HF-CT.

We chose to perform ROI analyses, because we expected regions involved in emotional processing to act as a connected network. In addition, this analysis (unlike voxel-wise whole brain analysis) allows for both calculation and presentation of effect sizes and follow-up analysis, and has sufficient power for smaller samples (Friston et al., 2006; Zandbelt et al., 2008). We preselected 'task' voxels that showed a significant signal increase associated with the experimental paradigm (thresholded at $t>4.1$, $p<0.001$). To prevent session bias in voxel selection, voxels were included if they exceeded threshold in at least one of the four group activity maps. Regions of interest (ROIs) were identified by clustering groups of at least 10 neighboring active voxels (640 mm^3). We chose a lenient threshold for voxel selection to ensure that we included most regions showing signal changes related to the task. Notably, the threshold for voxel selection is not related to the tested experimental hypotheses (Friston et al., 2006). 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 the Marsbar SPM tool (Brett et al., 2002).

It is at this stage that statistical hypothesis testing was conducted, using SPSS 17. Effects of THC on brain activity were determined using a repeated measures MANOVA over all 12 ROIs with drug (two levels: placebo and THC), condition (two levels: FF-CT and HF-CT) and ROI (12 levels: all regions included) as within-subjects factors. Follow up analyses were performed for separate ROIs with factors drug (two levels: placebo and THC) and condition (two levels: FF-CT and HF-CT). Post hoc paired t tests were performed to further investigate effects of THC on individual task conditions. ROI analyses are presented as a further descriptive exploration of the main hypothesis test, and are, as such, not

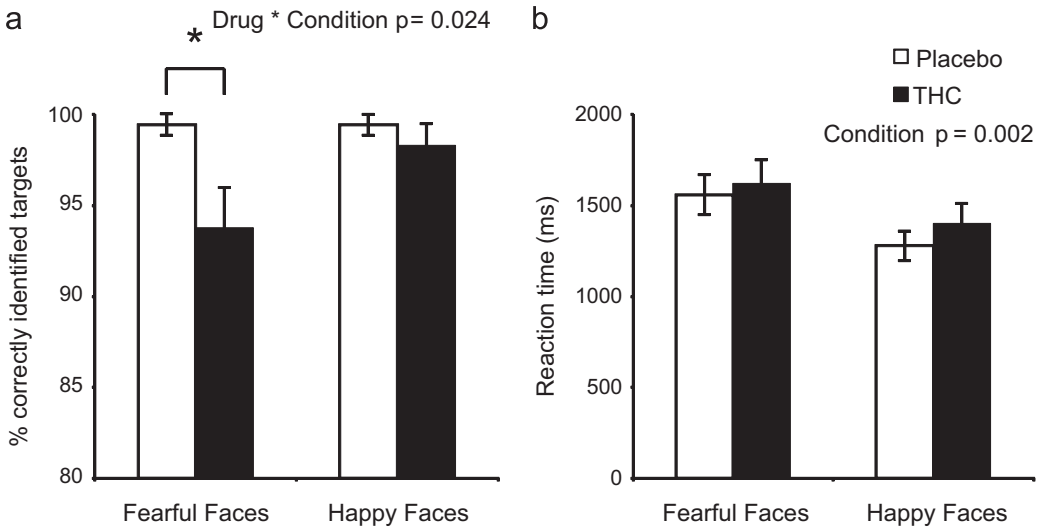

Figure 2 Task performance. (a) Performance accuracy as mean percentage of correctly identified targets after placebo and THC administration. (b) Reaction times of correct responses after placebo and THC administration ($n=11$; mean $\pm$ SEM). * Significant difference between placebo and THC ($p<0.05$). ms, milliseconds.

corrected for multiple comparisons if the omnibus network test proves to be significant.

2.7. Correlations

To assess relationships between effects of THC on anxiety levels, performance accuracy (FF and HF condition) and network activity (FF and HF condition), correlation analyses were performed using Pearson's correlation coefficient (THC vs. placebo, two-sided).

3. Results

3.1. Drug levels and behavioral measurements

Plasma concentrations of THC and its main metabolites were 82.3 $\pm$ 45.9 ng/ml (THC), 4.4 $\pm$ 5.5 ng/ml (11-nor-9-carboxy-THC) and 2.6 $\pm$ 1.3 ng/ml (11-OH-THC), 5 min after inhalation of 6 mg THC.

Analysis of subjective effects before and after performance of the emotional faces task revealed a significant THC-induced increase in VAS score of 'feeling high' ($F(1,10)=11.06$, $p=0.008$), 'internal perception' (reflecting inner feelings that do not correspond with reality) ($F(1,10)=6.21$, $p=0.032$), and 'external perception' (reflecting misperception of external stimuli or changes in the awareness of the environment) ($F(1,10)=11.97$, $p=0.006$) compared to placebo. In addition, THC significantly reduced 'alertness' ($F(1,10)=8.19$, $p=0.017$), 'contentedness' ($F(1,10)=6.96$, $p=0.025$), and 'calmness' ($F(1,10)=7.72$, p=0.020). THC caused a trend towards a significant increase in VAS score of 'anxiety' ($F(1,10)=3.60$, $p=0.087$). Subjective effects are summarized in Table 2.

Heart rate increased significantly after THC compared with placebo (16.0 $\pm$ 13.9 and -1.5 ± 9.9 bpm increase compared to baseline, respectively; $p<0.001$). For a more detailed description of drug levels and behavioral measurements following THC see van Hell et al. (2011).

3.2. Task performance

The effect of THC administration on performance accuracy was significantly different between the two experimental conditions (drug $\times$ condition, $F(1,10)=7.11$; $p=0.024$), with a THC-induced decrease in the mean percentage of correctly identified emotions for FF only (from 99.4 $\pm$ 1.9% to 93.8 $\pm$ 7.4%, $p=0.024$). Reaction times differed significantly between conditions (condition, $F(1,10)=17.53$; $p=0.002$), with the longest response time for FF, but showed no effects of THC administration (drug, $F(1,10)=2.59$; $p=0.139$) (Figure 2).

3.3. Selection of regions of interest

Processing of facial expressions of emotion (pooled FF-CT and HF-CT group activity maps) yielded a network of 12 brain regions, comprising vermis, bilateral prefrontal cortex, hippocampus and occipital cortex, and right amygdala/parahippocampal gyrus, inferior orbital frontal gyrus, supplementary motor area, superior parietal gyrus and middle frontal gyrus (Table 3 and Figure 3).

3.4. Brain activity

Brain activity in the network of ROIs showed a significant interaction effect between drug and condition ($F(1,10)=6.66$; $p=0.027$), indicating that THC administration had a different effect on the processing of FF and HF. There was no significant effect of drug ($F(1,10)=0.14$, $p=0.718$) or condition ($F(1,10)=2.71$, $p=0.131$), and no difference in the effect of THC between ROIs (drug $\times$ condition $\times$ ROI interaction, $F(6,64)= 1.69$, $p=0.133$). Post hoc analysis revealed a significant THC-induced decrease in FF activity (from 0.70 $\pm$ 0.05 to 0.51 $\pm$ 0.06, $p=0.017$). This suggests that the significant interaction effect between drug and condition is mainly reflected in decreased processing of FF (Table 3 and Figure 4).

Table 3 Effects of Δ9-tetrahydrocannabinol (THC) on brain activity related to matching of facial expressions of emotion (n=11).

ROI	Activated brain region	Cluster size (mm^3)	MANOVA effects (F(1,10))			Condition effects	
			Drug	Condition	Drug*condition	Fearful faces	Happy faces
Network		155,704	0.14, p=0.718	2.71, p=0.131	**6.66, p=0.027***	**p=0.017***	p=0.285
1	Vermis	832	0.40, p=0.544	1.92, p=0.196	**13.70, p=0.004***	p=0.066	p=0.454
2	Occipital cortex L	59,064	0.56, p=0.472	1.91, p=0.197	**10.12, p=0.010***	**p=0.015***	p=0.179
3	Occipital cortex R	74,496	1.14, p=0.312	1.72, p=0.218	**5.79, p=0.037***	**p=0.026***	p=0.540
4	Amygdala / Parahippocampal gyrus R	896	0.84, p=0.382	0.01, p=0.910	0.14, p=0.716	p=0.716	p=0.133
5	Inferior orbital frontal gyrus R	2944	2.93, p=0.118	0.49, p=0.500	3.18, p=0.105	p=0.636	**p=0.024***
6	Hippocampus L	1728	0.16, p=0.698	1.41, p=0.263	**8.06, p=0.018***	p=0.128	p=0.280
7	Hippocampus R	2176	3.46, p=0.092	1.16, p=0.307	2.87, p=0.121	**p=0.022***	p=0.946
8	Prefrontal cortex L	1536	0.55, p=0.477	**6.05, p=0.034***	4.64, p=0.057	**p=0.032***	p=0.349
9	Prefrontal cortex R	8384	0.01, p=0.932	2.92, p=0.118	**5.26, p=0.045***	p=0.281	p=0.195
10	Superior parietal gyrus R	1408	2.47, p=0.147	1.33, p=0.276	**7.94, p=0.018***	**p=0.012***	p=0.413
11	Middle frontal gyrus R	960	2.56, p=0.141	2.87, p=0.121	2.69, p=0.132	p=0.110	p=0.544
12	Supplementary motor area R	1280	4.09, p=0.071	0.63 p=0.447	**6.52, p=0.029***	p=0.522	**p=0.001***

Group activity maps for placebo and THC were thresholded at t>4.1, p<0.001, cluster size ≥10 voxels (640 mm^3). Overall effects were determined with repeated measures MANOVA, with drug and condition as factors. Condition effects were assessed with paired t tests. ROI numbers correspond to those shown in Figure 3. ROI, region of interest; L, left; R, right.

*Significant effect (p<0.05).

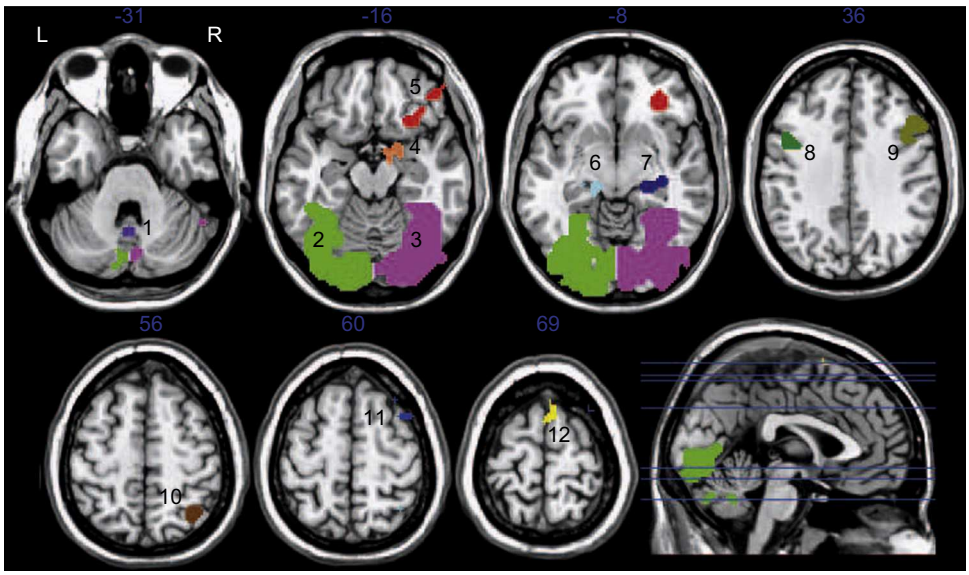

Figure 3 Regions of interest (ROIs) used to assess effects of THC administration on brain activity. ROIs are defined in group activity maps that were pooled for the placebo and THC condition of both the contrasts FF-CT and HF-CT (n=11; t>4.1, p<0.001, uncorrected for multiple comparisons, clusters ≥10 voxels). Numbers above slices indicate MNI z coordinates. ROI numbers correspond to those shown in Table 3. L, left; R, right.

Analysis of individual ROIs showed a significant interaction effect between drug and condition in the vermis ($F(1,10)=13.70$; $p=0.004$), left occipital cortex ($F(1,10)=10.12$; $p=0.010$), right occipital cortex ($F(1,10)=5.79$; $p=0.037$), left hippocampus ($F(1,10)=8.06$; $p=0.018$), right prefrontal cortex ($F(1,10)=5.26$; $p=0.045$), right superior parietal gyrus ($F(1,10)=7.94$; $p=0.018$), and right supplementary motor area ($F(1,10)=6.52$; $p=0.029$), while there was a trend in left prefrontal cortex ($F(1,10)=4.64$, $p=0.057$) (not corrected for multiple comparisons). A significant effect of condition was demonstrated in the left prefrontal cortex ($F(1,10)=6.05$; $p=0.034$), but no significant drug effects were shown in individual ROIs. ROI results are summarized in Table 3 and Figure 5.

3.5. Correlations

Anxiety levels showed a significant negative correlation with both FF and HF performance accuracy ($r=-0.79$, $p=0.004$ and $r=-0.74$, $p=0.009$, respectively), but not network activity ($r=-0.44$, $p=0.179$ and $r=0.14$, $p=0.677$). Task performance was not significantly correlated with network activity ($r=0.24$, $p=0.482$ and $r=-0.25$, $p=0.461$ for FF and HF, respectively), suggesting that effects of THC on performance accuracy do not fully account for differences in brain activity patterns.

4. Discussion

A pharmacological fMRI study with a THC challenge was performed in healthy volunteers to examine involvement of the eCB system in emotional processing. After THC administration, performance accuracy was decreased for matching stimuli with a negative, but not for matching stimuli with a positive emotional content. Our task activated a network of brain regions including the amygdala, orbital frontal gyrus, hippocampus, prefrontal cortex, parietal gyrus and occipital cortex. We found an interaction between THC and emotional content of processed stimuli, in that activity associated with processing of positive stimuli was reduced and activity associated with processing of negative stimuli was increased after THC administration. Network-wide, this effect was mainly driven by a significant reduction in activity while processing stimuli with a negative emotional content.

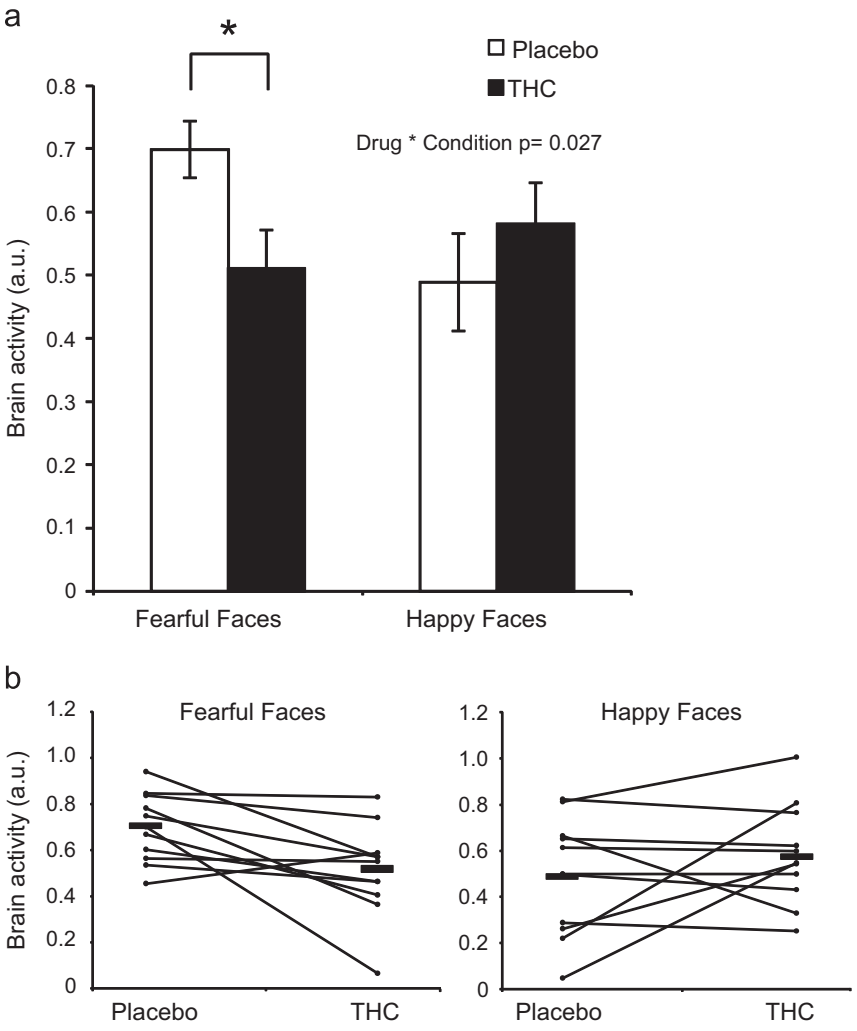

Figure 4 Activity in the network of ROIs during matching of 'fearful faces' or 'happy faces' stimuli ($n=11$). (a) Mean network activity after placebo and THC administration (mean $\pm$ SEM). (b) Network activity after placebo and THC administration presented for individual subjects. * Significant difference between placebo and THC ($p<0.05$). a.u., arbitrary units.

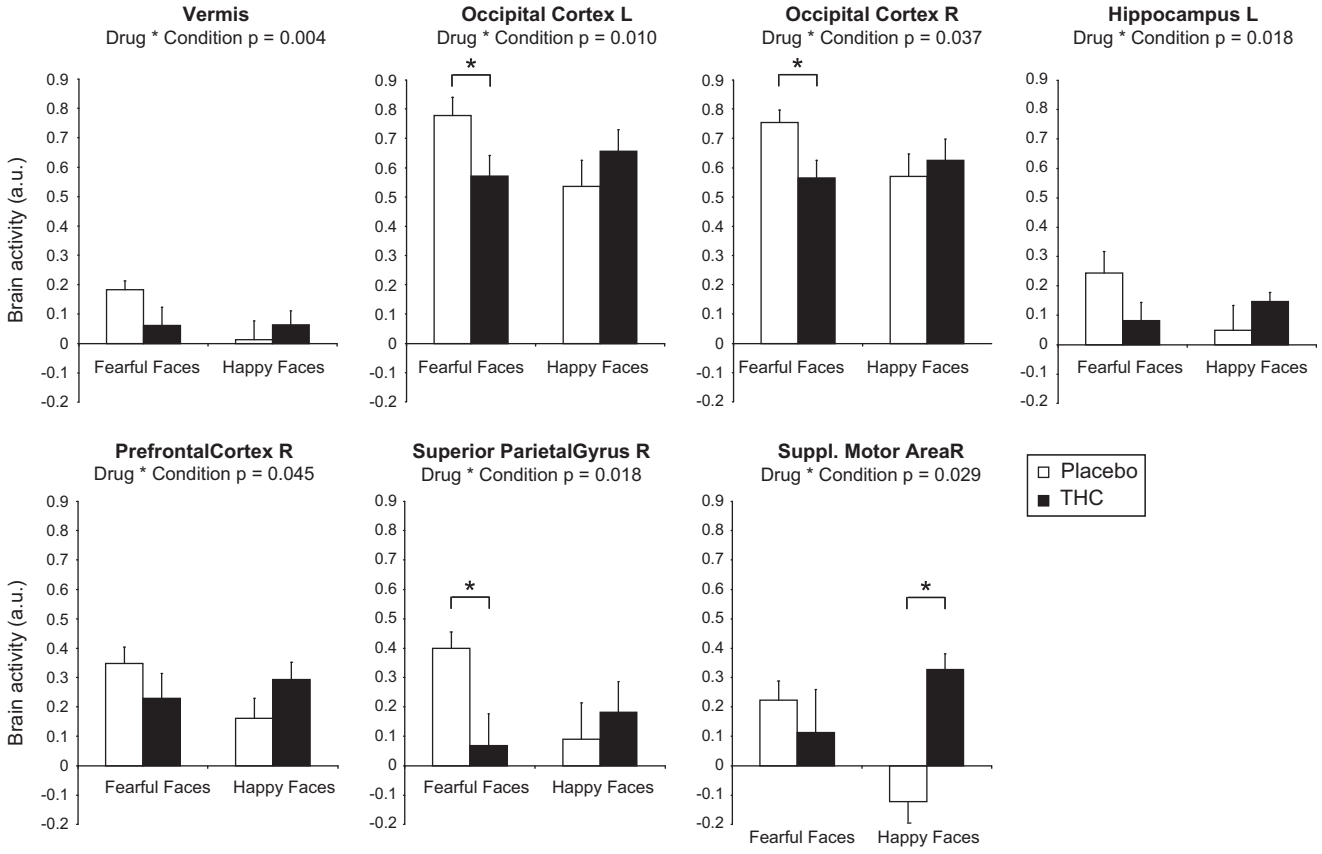

Figure 5 Brain activity during matching of 'fearful faces' or 'happy faces' stimuli in ROIs that demonstrated a significant interaction effect between drug and condition ($n=11$; mean$\pm$SEM). * Significant difference between placebo and THC ($p<0.05$). a.u., arbitrary units; L, left; R, right.

The interaction effect was also present in many regions that showed task-related activity. For the occipital and parietal regions this effect was predominantly a result of reduced activity for negative stimuli, while for the supplementary motor area the effect was mainly reflected in higher activity for positive stimuli. For the right prefrontal cortex, left hippocampus, and vermis, the effect was only present as an interaction.

These results suggest that administration of THC shifts the brain's bias for stimuli that have a negative impact towards a bias for stimuli that have a positive impact. Our findings support the hypothesis of involvement of the eCB system in modulation of emotional processing. It adds important human neuroimaging evidence to a large body of literature that implicates the eCB system in modulation of emotional responses, as it is the first neuroimaging study that shows network-wide opposite effects of THC for processing stimuli with a negative and positive emotional content.

Two previous neuroimaging studies are closely related to the present study. First, this study is in part a reproduction of that of Phan et al. (2008), with the important notion that we examined effects of THC in a network of brain regions, while Phan and colleagues limited their results to the amygdala. Phan et al. reported reduced amygdala reactivity for processing of stimuli with a negative emotional content. Although we did not reproduce this significant effect in the amygdala, we did find similar effects in many other regions involved in processing of emotions. In addition, we detected a decrease in task performance for matching negative stimuli, which was consistent with the neuroimaging results. A second study that is directly relevant to ours is that of Fusar-Poli et al. (2009). After THC administration, they demonstrated increased activity in precuneus and primary motor cortex, and reduced activity in bilateral middle frontal gyrus and posterior cingulate cortex, together with subjective anxiogenic effects. Effects of THC on task performance were not detected. Possibly, differences in results between our study and Fusar-Poli et al. (2009) are related to the nature of the fMRI task. In contrast to the task used in both the present study and that of Phan et al. (2008), Fusar-Poli and colleagues used a gender discrimination task, which did not require explicit processing of the emotional content of the stimuli.

Our results are in line with accumulating evidence for involvement of the eCB system in modulation of emotional processing. Animal studies have previously shown reduced anxiety-like behavior after administration of either low doses of exogenous cannabinoid agonists including THC or drugs that enhance levels of endogenous cannabinoids (Kathuria et al., 2003; Marco et al., 2004; Valjent et al., 2002; see for a review Hill et al. (2009)). Elimination of eCB-mediated synaptic transmission through genetic deletion or pharmacological blockade of cannabinoid receptors produces anxiety- or depressive-like states in animals (Griebel et al., 2005; Martin et al., 2002; see for a review Lafenetre et al. (2007)). Human neuropsychological studies

have indicated that THC administration reduces perception of fearful facial emotions in healthy volunteers (Ballard et al., 2012), while both acute and long-term administration of the eCB antagonist rimonabant have been shown to induce a bias away from positive emotions on a memory recognition task (Horder et al., 2009, 2012). A recent neuroimaging study demonstrated that people with a genetic profile associated with increased eCB signaling (carriers of FAAH385A) have decreased fear-related amygdala reactivity (Hariri et al., 2009). Clinical trials testing rimonabant and the inverse agonist taranabant for treatment of obesity have shown depressed mood and anxiety as the most common adverse events (Addy et al., 2008; Christensen et al., 2007). Also in humans, the administration of cannabidiol has been reported to reduce activity in amygdala, anterior and posterior cingulate cortex during processing of intensely fearful faces, while the level of suppression in these regions was correlated with physiological markers of anxiety (Fusar-Poli et al., 2009).

The current study also adds arguments for a possible role of the eCB system in abnormal emotional processing related to psychiatric disorders, as has been suggested previously (Ashton and Moore, 2011; Hill et al., 2009). For example, individuals diagnosed with major depressive disorder exhibit an attentional bias towards negative cues and a bias away from positive cues (Surguladze et al., 2004), together with an increased reactivity towards negative and reduced reactivity towards positive emotions (Fu et al., 2007; Surguladze et al., 2005). Administration of antidepressant medication reduces this bias in patients (Fu et al., 2007; Harmer et al., 2009), and appears to induce a shift in emotional bias in healthy volunteers similar to the one related to THC in the current study (Harmer et al., 2006; Murphy et al., 2009). Thus, a defect in endocannabinoid neurotransmission could contribute to the abnormal emotional reactions as seen in patients with a major depression. This also suggests potential for eCB-mediated medication in the treatment of psychiatric symptoms related to abnormal emotional responses.

A potential mechanism underlying the effects of THC administration on brain activity may be found in the regulatory role of the eCB system in neurotransmitter release. The eCB system is a retrograde messenger system that regulates both excitatory glutamate and inhibitory GABA neurotransmission according to an 'on-demand' principle: endocannabinoids are released when and where they are needed (Heifets and Castillo, 2009). This eCB-mediated regulation of synaptic transmission is a widespread phenomenon in the brain, and is thought to play an important role in higher brain functions, including emotional processing (Heifets and Castillo, 2009; Hill et al., 2009). As emotional responses such as anxiety and fear are associated with increased glutamate and diminished GABA neurotransmission (Millan, 2003), the reduced negative emotional bias as demonstrated in the current study may be the result of a THC-induced reinstatement of the balance between both neurotransmitter systems (Ruehle et al., 2012).

THC plasma concentrations and reported subjective effects in our study indicate that a moderate high dose of THC was used (Huestis et al., 1992; Ramaekers et al., 2006). In line with behavioral animal studies that used high doses of THC (Marco et al., 2004; Valjent et al., 2002), subjective ratings in the present study are more in the direction of anxiety-like effects, with a trend towards a significant THC-induced increase in the VAS score of 'anxiety', and significantly reduced measures of 'contentedness' and 'calmness'. These behavioral findings seem to contradict the effects of THC on brain activity. The circumstances of the experiment, particularly the unfamiliar environment and the fact that subjects were aware that they had to perform a task while possibly under the influence of THC, are likely to have caused an increase in self-reported feelings of anxiety. Another possibility may be that self-reported subjective states related to THC may not always be reflected in brain activity related to emotional processing.

Some limitations have to be taken into account in interpreting the results of this study. First, the sample size of the current study was small. We therefore cannot exclude the possibility that subtle effects of THC on brain activity have been missed. However, the sample is large enough to detect effects on brain activity with an ROI approach (Zandbelt et al., 2008). Second, inclusion of incidental cannabis users, as opposed to non-users, may affect interpretation of results as previous cannabis use may influence the eCB system. The choice for incidental cannabis users was based on ethical grounds (van Hell et al., 2011). Third, although the study was designed to be double-blind, THC induced behavioral effects that were identified by most subjects, possibly causing expectancy effects across sessions. The influence of expectancy was minimized by using a randomized crossover design, thus balancing the effects of expectancy across study days. Still, it cannot be excluded that expectancy effects may have affected our results to some extent. Finally, nonspecific THC-induced changes on cerebral blood flow may have confounded our results (Iannetti and 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 control condition, as the nonspecific effects of THC on blood flow can be expected to be present in all conditions. Furthermore, as we found significant differences in THC effects between task conditions, it is unlikely that our findings are associated with nonspecific effects.

We did not find significant effects of THC on amygdala activity. Possibly, the subjective anxiety-like effects of THC administration may have specifically masked THC-induced effects on the response of the amygdala, as it has been shown that particularly amygdala activity may be involved in the subjective response to pharmacologically induced anxiety (Eser et al., 2009). This view is supported by results of Fusar-Poli et al. (2009), who showed strong subjective anxiety-like effects of THC, but no significant effects of THC administration on the amygdala response.

In conclusion, our study shows that THC administration induced a network-wide shift from a bias for negative emotional content towards a bias for positive emotional content. This was accompanied by a reduced ability to recognize stimuli with a negative emotional content. These findings add to existing evidence that implicate the endocannabinoid system in modulation of emotional reactions, and support a previously suggested role for the endocannabinoid system in abnormal emotional processing associated with various psychiatric disorders.

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Contributors

MB, HvH, GJ and NR designed the study and wrote the protocol. MB, HvH and GJ collected the data. MB undertook the statistical analysis. MB and JMJ managed the literature searches, and MB wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflicts of interest

All authors declare that they have no conflicts of interest.

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