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Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report

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Abstract

Animal and human studies indicate that cannabidiol (CBD), a major constituent of cannabis, has anxiolytic properties. However, no study to date has investigated the effects of this compound on human pathological anxiety and its underlying brain mechanisms. The aim of the present study was to investigate this in patients with generalized social anxiety disorder (SAD) using functional neuroimaging. Regional cerebral blood flow (rCBF) at rest was measured twice using (99m)Tc-ECD SPECT in 10 treatment-naïve patients with SAD. In the first session, subjects were given an oral dose of CBD (400 mg) or placebo, in a double-blind procedure. In the second session, the same procedure was performed using the drug that had not been administered in the previous session. Within-subject between-condition rCBF comparisons were performed using statistical parametric mapping. Relative to placebo, CBD was associated with significantly decreased subjective anxiety ($p < 0.001$), reduced ECD uptake in the left parahippocampal gyrus, hippocampus, and inferior temporal gyrus ($p < 0.001$, uncorrected), and increased ECD uptake in the right posterior cingulate gyrus ($p < 0.001$, uncorrected). These results suggest that CBD reduces anxiety in SAD and that this is related to its effects on activity in limbic and paralimbic brain areas.

Keywords

Cannabidiol, CBD, social anxiety, regional cerebral blood flow, SPECT

Introduction

Generalized Social Anxiety Disorder (SAD) is one of the most common and impairing anxiety conditions. However, the pharmacological treatment of SAD remains problematic since it is poorly controlled by the currently available drugs, with only about 30% of the subjects achieving true recovery or remission (Blanco et al., 2002). Therefore, there is a clear need to develop and explore novel therapeutic agents for the management of SAD.

Although it is well recognized that cannabis use can cause adverse effects, including anxiety, there is consistent evidence that many individuals use the drug to obtain relief from anxiety symptoms (Crippa et al., 2009). Moreover, it has been suggested that individuals with SAD are more likely to use cannabis than those with other anxiety disorders to 'self-medicate' anxiety reactions (Buckner et al., 2008). These apparently conflicting statements may partly reflect the fact that low doses of the best-known constituent of the plant, Δ^9-tetrahydrocannabinol (Δ^9-THC), have anxiolytic-like effects,

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whereas higher doses produce anxiogenic reactions (Crippa et al., 2010; Fusar-Poli et al., 2009; Viveros et al., 2005).

In addition, *Cannabis sativa* contains multiple compounds which may have different psychoactive properties (Zuardi et al., 2006). In particular, cannabidiol (CBD), one major non-psychotomimetic compound from the *Cannabis sativa* plant, has shown anxiolytic effects both in animals and in human studies (Zuardi et al., 1993b; Zuardi et al., 2006). Using functional neuroimaging in healthy volunteers, we have recently observed that CBD has anxiolytic properties and that these effects are associated with an action on limbic and paralimbic brain areas (Crippa et al., 2004; Fusar-Poli et al., 2009).

We have thus hypothesized that CBD may be effective in SAD. However, no study to date has investigated the effects of CBD on human pathological anxiety and its underlying brain mechanisms. Therefore, in the present study, we applied functional neuroimaging to investigate the neurophysiological basis of the effects of CBD in patients with SAD. Based on previous Single Photon Emission Computed Tomography (SPECT) (Crippa et al., 2004) and functional Magnetic Resonance Imaging (fMRI) (Fusar-Poli et al., 2009) studies of CBD effects, we predicted that, relative to placebo, CBD would reduce anxiety in subjects with SAD and that this effect would be associated with the modulation of the functional activity of temporo-limbic structures (amygdala-hippocampus complex and parahippocampal gyrus) and paralimbic regions, including the cingulate cortex. To the best of our knowledge, this is the first study to directly investigate the neural and/or behavioral effects of CBD in patients with an anxiety disorder.

Methods

Subjects

Ten right-handed men with generalized SAD, recruited from an epidemiological sample of 2320 university students, were selected by the screening procedure described elsewhere (Crippa et al., 2008; De Lima Osório et al., 2007). They had the SAD diagnosis confirmed by the SCID for the DSM-IV. Participants were aged between 20 and 33 years (mean age 24.2 years; SD 3.7), were treatment-naïve and did not have any comorbid psychiatric disorders. Their mean age of onset of illness was 9.1 years (SD 1.53), and the mean illness duration was 14.8 years (SD 3.12).

Severity of SAD was assessed with the Portuguese version (Osório et al., 2006, 2010) of the Brief Social Phobia Scale (BSPS) (Davidson, et al., 1991) and using the Portuguese version (Osório et al., 2009) of the Social Phobia Inventory (SPIN) (Connor et al., 2000). The BSPS is an instrument whose objective is to screen and quantify the different symptoms characteristic of SAD. It consists of 18 items divided into three subscales (fear, avoidance and physiological symptoms) which are scored on a five-point Likert scale which, when summed, produce a total score of 0 to 76 points. Discriminative validity proved to be excellent for a cut-off score of 18 to 19. To improve and standardize the application and reliability of this scale, Osório et al. (2006) proposed an interview guide.

The SPIN is a self-applied instrument derived from the BSPS consisting of 17 items scored on a five-point Likert scale, with a maximum total score of 68. Criterion validity revealed excellent sensitivity and specificity for a cut-off score of 19. Other psychometric properties of both BSPS and SPIN have been consistently considered adequate in different studies and cultures.

All subjects had total scores higher than 52 on the SPIN and higher than 54 on the BSPS, thus classified as suffering from severe social phobia. Their mean weight ranged between 65–91 kg and their body mass index ranged between 21–25 kg/m^2. They were included into the study if they did not have a history of head trauma, neurological illness, electroconvulsive therapy, current or previous drug/alcohol use or abuse or major medical illnesses, and had not received any medication for at least 3 months before the study (Mathew et al., 1992), based on a semi-standardized questionnaire and physical examination. All were non-smokers (tobacco), had not used marijuana more than five times in their lifetime, with no use in the past year. None had ever used any other illicit drug. Although no urine or blood tests were given to ensure absence of marijuana use, we have assessed past and current use of this or other illicit drugs use by means of a semi-structured questionnaire and the Addiction Severity Index (ASI) (McLellan et al., 1980). No subject had previously undergone SPECT or other nuclear medicine procedures. Clinical and demographic information of the patient sample are shown in Table 1. All subjects gave written informed consent after being fully informed of the research procedure, following approval by the local ethical committee (HCRP No. 11559/04).

Drug

CBD (400 mg) approximately 99.9% pure (THC-Pharm; Frankfurt, Germany) was dissolved in corn oil. The same amount of corn oil was used as placebo. The drug and placebo were packed inside identical gelatin capsules. Previous studies showed that the plasma peak of an oral dose of CBD usually occurs 1–2 h after ingestion (Agurell et al., 1981; Borgwardt et al., 2008; Fusar-Poli et al., 2009).

Self-rating scale for subjective states during the SPECT imaging procedure

Subjective states were evaluated by mean of the Visual Analogue Mood Scale (VAMS) of Norris (1971), translated into Portuguese (Zuardi and Karniol, 1981). In this scale,

Table 1. Clinical and demographic characteristics of Social Anxiety Disorder patients ($n = 10$)

Age (years)	24.2 (SD ± 3.7)
Age of onset of illness (years)	9.1 (SD ± 1.53)
Illness duration (years)	14.8 (SD ± 3.12)
Social anxiety scores	
BSPS	61.0 (SD ± 5.4)
SPIN	57.9 (SD ± 4.7)
Weight (kg)	74.1 (65–91 kg; SD ± 8.8)

BSPS: Brief Social Phobia Scale, SPIN: Social Phobia Inventory.

the subject is told to mark a point that identifies his/her present subjective state on a 10-cm straight line placed between two words that describe opposite mood states (e.g. calm–agitated). It consists of 16 items that the factor analysis had grouped into four factors, namely anxiety, mental sedation, physical sedation and other feelings and attitudes (Zuardi et al., 1993b). The anxiety factor composed of three scales (calm–excited; relaxed–tense and tranquil–troubled) instead of the two scales obtained with the factor analysis of the English version (Bond and Lader, 1974). Reported results have shown that the VAMS is more sensitive than the STAI-S for the detection of drug effects on anxiety (Hallak et al., 2010; Zuardi et al., 1993b), provided initial instructions and supervision are given to limit the tendency to extreme choices by the subjects (Guimarães et al., 1989). Therefore, prior to the SPECT experiment each volunteer underwent a rigorous training session completing this scale.

Subjective ratings on the VAMS were made at five different time-points: 30 min before drug ingestion (pre-drug; −30′); at the time of drug ingestion (drug intake; 0); at 60 min (pre-stress; 60′) and at 75 min afterwards (adaptation; 75′); and just after SPECT scanning (post-stress; 140 min after drug intake). The anxiety-evoking procedure consisted of the whole SPECT procedure itself, which involves the insertion of an intravenous cannula and the exposition to a totally uncommon situation (medical environment, large examination apparatus). The terms 'pre-stress', 'adaptation' and 'post-stress', refer to the moments of the scanning session in which the VAMS was completed.

SPECT imaging

Using the SPECT technique, we compared the effects of CBD and placebo on resting regional cerebral blood flow (rCBF) in the SAD patients in a double-blind, randomized, repeated measures, within-subject cross-over design. Each subject was evaluated on two different occasions, 1 week apart, with identical procedures except the drug that was administered. In the experimental session, after a 30-min period of adaptation, subjects were given a single dose of oral CBD (400 mg) or placebo. SPECT image acquisition was performed 110 min after drug ingestion (double-detector SOPHA® DST system (Sophy Medical Vision, USA), 20 min after intravenous injection of 740 MBq of ^{99m}Tc-ECD at rest; high-resolution low-energy collimators; 128 views; 128 × 128 matrix (30 s per view); total acquisition time of 30 min; 75,000 counts/frame/head).

Raw images were pre-filtered with a Butterworth filter (order number 9, cutoff frequency 0.14), and reconstructed by filtered back-projection as transaxial slices parallel to the long axis of the temporal lobe. Attenuation correction was performed considering a pixel size of 2.55 mm and using the first order algorithm of Chang (coefficient 0.12/cm).

Image processing and statistical analysis

Images were analyzed using Statistical Parametric Mapping software (SPM2; Wellcome Department of Imaging Neuroscience, Institute of Neurology, London, UK) in MATLAB 6.1 (MathWorks, MA).

The first processing steps involved the creation of a customized SPECT template. In order to accomplish this, the two SPECT images of each patient (for the CBD and placebo sessions) were normalized using linear 12-parameter affine transformations to the standard SPECT template of SPM2, which is based on the Montreal Neurological Institute (MNI) pool of rCBF SPECT datasets of healthy subjects (Mazziotta et al., 1995). After spatial normalization, all images were averaged and then smoothed with an 8 mm full width at half maximum isotropic Gaussian kernel, creating our customized SPECT template.

The original datasets of all subjects (obtained after CBD or placebo ingestion) were then processed. This included: spatial normalization to the customized SPECT template using linear (translations and rotations) and non-linear transformations ($3 \times 4 \times 3$ non-linear basis functions); re-slicing, using a trilinear interpolation to a final voxel size of $2 \times 2 \times 2\,mm^3$; and smoothing of spatially normalized images with a 12 mm full width at half maximum isotropic Gaussian kernel. The resulting smoothed images were used in the statistical analysis.

Between-condition (CBD versus placebo) comparisons of regional tracer uptake were performed on a voxel-by-voxel basis with the general linear model, based on random Gaussian field theory (Friston et al., 1995), using paired *t*-tests. Before statistical testing, the regional ECD uptake of every voxel in each subject was standardized to the mean global uptake of the image in that subject, using proportional scaling. Only voxels with signal intensities above a threshold of 0.8 of the global mean (calculated using the standardized values) entered the statistical analysis. Results were displayed as statistical parametric maps (SPMs) into standard space. A statistical threshold of $Z = 3.09$ ($p < 0.001$, one-tailed), and a minimum cluster extent of 20 voxels, were employed for the inspection of significant findings in the brain regions where ECD uptake changes had been predicted a priori. The threshold of $p < 0.001$, uncorrected for multiple comparisons, has been empirically shown previously to provide good protection against false positive results when there are clear hypotheses as to the location of findings in former functional imaging studies using the SPM approach (Friston et al., 1996). The selection of our a priori brain regions was based on previous studies using CBD (Crippa et al., 2004; Fusar-Poli et al., 2009), and they included the medial temporal cortex (encompassing the parahippocampal gyrus, amygdala and hippocampus), and the posterior cingulate gyrus. The anatomical localization of clusters of statistical significance shown on the resulting SPM involving these brain regions was conducted using the Anatomical Automatic Labeling (AAL) tool available in the SPM2 package, which provides a predefined set of region-of-interest masks that were manually drawn over the whole brain on high-resolution standardized MNI images (Tzourio-Mazoyer et al., 2002) Other, unpredicted differences between conditions were reported as significant only if they survived family-wise error (FWE) correction for multiple comparisons ($p < 0.05$) over the whole brain (Friston et al., 1996). The MNI coordinates for the voxel of maximal statistical significance for each anatomical brain region included in a given cluster of significance were converted to the Talairach and Tournoux (1988) system using the method described by Brett et al. (2002).

The four VAMS factors were submitted to a two-way repeated-measures analysis of variance (ANOVA) to assess the statistical significance of main effects of time and drug, and drug × time interaction. When the drug × time interaction was significant, the differences between CBD and placebo in each phase of the experimental session were analyzed by paired sample *t*-tests.

Voxel-wise correlations between regional tracer uptake indices and each of the VAMS factor scores were also investigated with SPM2, at the same statistical significance levels as described above for the between-condition rCBF comparisons ($p < 0.01$, uncorrected). The mean change from the baseline scores and the last time point in which the VAMS was applied before image acquisition (75′ after drug intake) were chosen for these correlations, given the proximity of this time point to the injection of the SPECT tracer, and due to the fact that this is the point where CBD is expected to have its maximum anxiolytic effect among all the time points chosen for assessment during the experimental session (Borgwardt et al., 2008; Crippa et al., 2004; Fusar-Poli et al., 2009; Zuardi et al., 1993a, 1993b). Finally, the choice for this time point was also based on previous pharmacological studies that have shown that the plasma peak of an oral dose of CBD usually occurs between 1 and 2 h after ingestion (Agurell et al., 1981; Borgwardt et al., 2008; Fusar-Poli et al., 2009).

Results

VAMS

The ANOVA for the anxiety factor showed significant main effects of time ($F(4,36) = 21,4$; $p < 0.001$) and drug ($F(1,9) = 6.6$; $p < 0.03$), as well as a significant time by drug interaction ($F(4,36) = 10.3$, $p < 0.001$) (Figure 1a; Table 2). Post hoc between-condition analyses at each time-point indicated that CBD was associated with significantly decreased VAMS anxiety scores at the phases of venous cannula insertion (60′ after drug intake, $t = 2.74$, $p = 0.02$), pre-SPECT resting (75′ after drug intake, $t = 3.61$, $p = 0.006$) and post-SPECT imaging (140′ after drug intake, $t = 3.94$, $p = 0.003$), as compared with placebo (Figure 1a; Table 2). The results of the ANOVAs for the factors: physical sedation (time $F(4,36) = 0.99$; drug $F(1,9) = 3,39$ and interaction $F(4,36) = 2.34$), mental sedation (time $F(4,36) = 1.68$; drug $F(1,9) = 1.90$ and interaction $F(4,36) = 1.4$), and other feelings and attitudes (time $F(4,36) = 2.66$; drug $F(1,9) = 1.46$ and interaction $F(4,36) = 0.05$) were not significant (Table 2).

Between-condition rCBF comparisons

Between-condition tracer uptake comparisons revealed that relative to placebo, CBD attenuated ECD uptake in a cluster encompassing the left parahippocampal gyrus and hippocampus ($p < 0.001$, uncorrected for multiple comparisons; Table 3; Figure 1b). Conversely, there was a cluster of significantly increased ECD uptake in the CBD relative to the placebo condition ($p < 0.001$, uncorrected) in the right posterior cingulate gyrus (BA23/31) (Table 3; Figure 2). There were no other clusters of differential activation in either predicted or unpredicted brain regions.

Correlations between rCBF indices and subjective status ratings

No correlations were found between subjective ratings in the VAMS and ECD uptake in any brain areas, predicted a priori or not, either in the CBD or in the placebo condition.

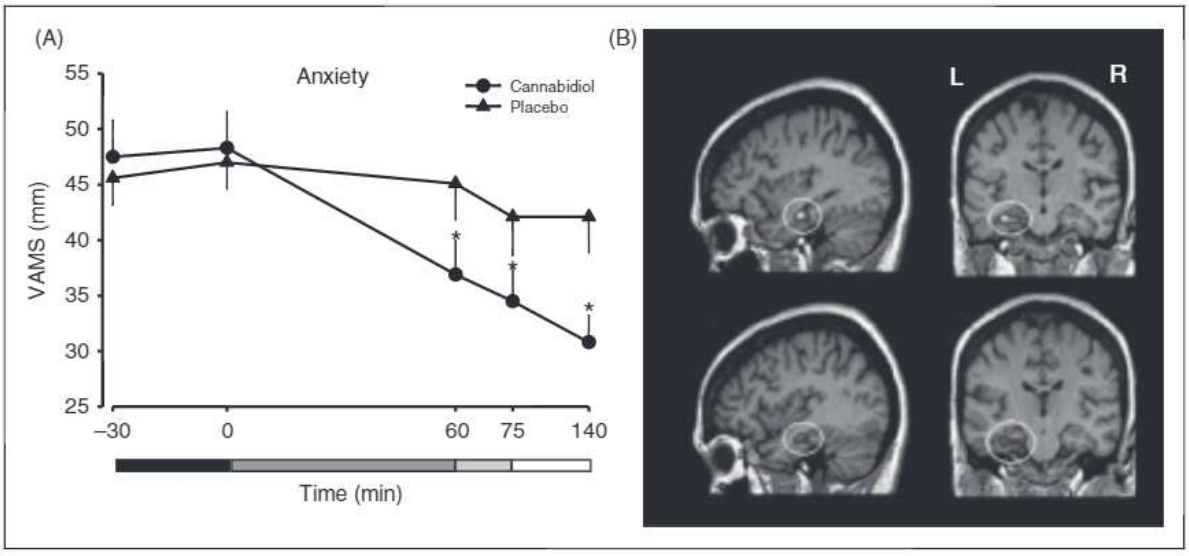

Figure 1. (A) Effect of CBD and placebo (PLCB) on the Anxiety factor of the VAMS. Points are means (±SEM) of the ratings of 10 social Anxiety disorder subjects in the phases of the experiment: pre-drug (■), pre-stess (▩), adaptation (□) and post-stress(□). Asterisks (*) indicates significant difference from placebo in each phase. (B) The brain focus (circled) of significantly decreased rCBF in Social Anxiety Disorder subjects ($n = 10$) during CBD *vs* placebo on sagittal sections (left side of the figure) and coronal sections (right side of the figure) located in the left hippocampal area.

Discussion

This study demonstrated that acute administration of CBD, one of the main psychoactive constituents of *Cannabis sativa*, can reduce subjective anxiety in patients clinically diagnosed with an anxiety disorder, in this case SAD. Furthermore, the present study indicates that this behavioral response is associated with changes in the functional activity of brain areas implicated in the processing of anxiety.

As the SPECT neuroimaging procedure itself can be interpreted as an anxiogenic situation, it allows the evaluation of the anxiolytic action of pharmacological agents (Crippa et al., 2004; Fusar-Poli et al., 2009; Giordani et al., 1990; Grey et al., 2000; Gur et al., 1987; Malizia, 1999). Similar to our previous findings in healthy subjects (Crippa et al., 2004), the present results show that, as compared with placebo, a single dose of CBD induced significant decreases in state anxiety before SPECT scanning in patients with SAD. These anxiolytic effects were detected before (60′ and 75′ after drug intake) the anxiety-provoking situation (the tracer injection and scanning procedure), suggesting that CBD facilitates habituation of anticipatory anxiety in social phobia. Of note, both the avoidance and anticipatory anxiety are considered key features in SAD, and constitute important targets for the clinical management of the disorder (Davidson, 2003). We also detected anxiolytic effects of CBD in the post-SPECT (140′ after drug intake) phase as compared with placebo. This finding is consistent with the results of a study (Zuardi et al., 1993b) in which the anxiolytic effect of CBD and ipsapirone persisted after the stress of public speaking. Our present data thus confirm that this cannabinoid compound has anxiolytic properties, consistent with results from previous studies in both laboratory animals (Campos and Guimarães, 2008; Guimarães et al., 1990; Moreira et al., 2006; Musty et al., 1984; Onaivi et al., 1990; Zuardi and Karniol, 1983) and humans (Crippa et al., 2004; Fusar-Poli et al., 2009; Zuardi et al., 1982, 1993b).

As in our former SPECT study in healthy volunteers using the same dose of CBD (400 mg) (Crippa et al., 2004), the drug was well tolerated by SAD patients and no side-effects were reported. This is consistent with previous studies in humans that have shown that CBD is a safe and tolerable compound, without major side effects when administered acutely by oral route, inhalation or intravenous injection (Zuardi et al., 2006). In addition, CBD chronically administered in various oral daily dosages (from 10–1280 mg) to healthy volunteers (Cunha et al., 1980), and to patients with schizophrenia (Zuardi et al., 1995, 2006), bipolar affective disorder (Zuardi et al., 2010a), Parkinson's disease (Zuardi et al., 2009) and Huntington's disease (Consroe et al., 1991), among other conditions (Zuardi, 2008), did not induce any significant side effects nor lead to the emergence of any neurological, psychiatric or general clinical manifestations (Cunha et al., 1980).

The anxiolytic effects observed with CBD are in contrast to the anxiogenic effects induced by high doses of Δ^9-THC (Fusar-Poli et al., 2009; Mathew et al., 1999; Zuardi et al., 1982, 2010b), and may help to explain why many subjects

Table 2. Effect of cannabidiol and placebo on the four factors of the Visual Analogue Mood Scale (means (SD)) for Social Anxiety Disorder subjects ($n = 10$) over the five phases of the experiment

VAMS FACTORS	Drug	Pre-drug (−30′)	Drug intake (0)	Pre-stress (60′)	Adaptation (75′)	Post-stress (140′)
Anxiety	CBD	48.3 (10.4)	47.5 (10.6)	36.9 (9.9)*	34.5 (9.2)*	30.8 (7.7)*
	Placebo	46.9 (7.6)	45.6 (7.6)	45.2 (10.2)	42.1 (11.2)	42.1 (10.3)
Physical sedation	CBD	47.8 (13.3)	47.8 (9.9)	46.7 (9.5)	46.9 (10.7)	43.9 (11.0)
	Placebo	42.6 (6.8)	43.3 (6.9)	43.2 (10.7)	45.2 (10.1)	43.7 (9.8)
Mental sedation	CBD	50.1 (12.2)	44.9 (6.4)	52.2 (7.8)	52.3 (6.2)	58.4 (5.6)
	Placebo	47.8 (7.4)	49.1 (7.7)	46.2 (10.9)	47.5 (6.8)	45.5 (12.0)
Other feelings	CBD	41.2 (11.2)	44.2 (9.4)	41.7 (13.9)	40.8 (13.2)	39.9 (12.7)
	Placebo	38.2 (15.3)	41.7 (13.4)	40.5 (15.2)	39.7 (12.4)	38.4 (14.4)

*Significant difference from placebo in the same phase of the experiment as assessed by paired sample *t*-tests, when the repeated-measures ANOVA showed a significant main effect of drug and a significant interaction between time and drug (see details in the text of the Results section).

Table 3. Areas of significant regional cerebral blood flow change in the cannabidiol condition compared with the placebo condition in subjects with social anxiety disorder ($n = 10$)

Finding	Brain Region	K[a]	*Z*-score[b]	Talairach Coordinates (x, y, z)[c]
CBD > Placebo	Posterior Cingulate Gyrus[R]	28	3.62	8, −26, 31
Placebo > CBD	Parahippocampal Gyrus[L]	31	3.47	−34, −13, −18

[a]Total number of voxels in the cluster.
[b]*Z*-score for the voxel of maximal statistical significance in the cluster.
[c]Coordinates of the voxel of maximal statistical significance according to the atlas of Talairach and Tournoux (1988).
[R]Right hemisphere.
[L]Left hemisphere.

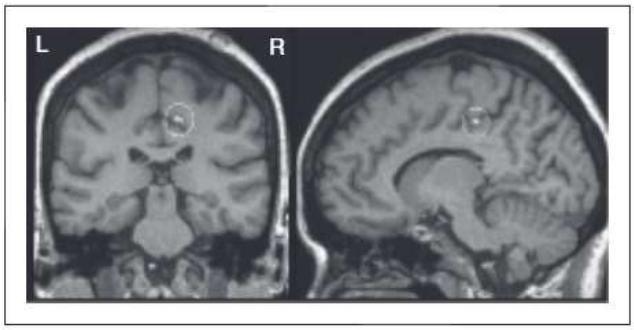

Figure 2. The brain focus (circled) of significantly increased regional cerebral blood flow in Social Anxiety Disorder subjects ($n = 10$) during cannabidiol condition relative to the placebo condition is shown on a coronal section (left side of the figure) and a sagittal section (right side of the figure), located in the right posterior cingulate gyrus.

with generalized SAD use cannabis as a 'self-medication' in order to reduce social anxiety symptoms (Buckner et al., 2006, 2007, 2008).

Consistent with our main hypothesis, and in line with its anxiolytic effect at the behavioral level (Crippa et al., 2004; Fusar-Poli et al., 2009), CBD was significantly associated with functional activity changes in limbic and paralimbic cortical areas that have been consistently implicated in the pathophysiology of anxiety, with effects in the left parahippocampal gyrus and hippocampus, and the right posterior cingulate gyrus (BA23/31) (Crippa et al., 2004; Ferrari et al., 2008; Freitas-Ferrari et al., 2010; Fusar-Poli et al., 2009; Trzesniak et al., 2008). These results are in accordance with our previous SPECT study (Crippa et al., 2004) and a more recent fMRI study (Fusar-Poli et al., 2009) in healthy volunteers. Furthermore, the regional brain localization of the effects of CBD observed in the present study is consistent with results from several previous structural (Ferrari et al., 2008) and functional (Freitas-Ferrari et al., 2010; Trzesniak et al., 2008) imaging studies that have implicated limbic, paralimbic and sensory cortical regions in the brain circuitry underlying the symptoms of anxiety disorders in general and SAD in particular.

The parahippocampal gyrus and hippocampus are thought to play a key role in mediating fear and anxiety. However, we found in SAD subjects that CBD relative to placebo led to significant decrements in the parahippocampal activity, rather than rCBF increases as we previously observed in healthy individuals using exactly the same experimental paradigm reported herein (Crippa et al., 2004). These discrepant findings may reflect differences in the activity of this medial temporal structure in healthy subjects and patients with clinical anxiety disorders (Hou et al., 2007; Liotti et al., 2000). Patients with social phobia have demonstrated increased parahippocampal–hippocampal activity and anxiety symptoms during a stressful public speaking task, which was reversed after citalopram (40 mg/day) treatment (Furmark et al., 2005). In addition, functional neuroimaging studies have also shown increased activity in the hippocampus in association with anxiety in obsessive–compulsive disorder (OCD) (McGuire et al., 1994), panic disorder (PD) (Boshuisen et al., 2002; Bystritsky et al., 2001), post-traumatic stress disorder (PTSD) (Osuch et al., 2001), SAD (Schneider et al., 1999) and simple phobia (Schienle et al., 2005; Veltman et al., 2004). Taken together, these studies suggest that pathological anxiety can be associated with increased functional activity of the hippocampus and parahippocampal gyrus, and the CBD-related reduced activity in this brain region observed in the present study would be consistent with an anxiolytic effect of CBD in SAD sufferers.

In the present study, the only brain region that showed significantly increased activity following administration of CBD was the right posterior cingulate cortex. This region, strongly connected to temporolimbic structures (Afifi and Bergman, 1998; Maddock, 1999; Vogt et al., 1992), is also thought to be critically involved in the processing of emotional information both in animals and humans (MacLean, 1993; Maddock, 1999). Functional activity changes in the posterior cingulate gyrus have been found in healthy individuals (Doronbekov et al., 2005; Fischer et al., 1996), PTSD (Bremner et al., 1999), specific phobia (Straube et al., 2004), PD subjects (Bystritsky et al., 2001; Maddock et al., 2003), and in patients with OCD (Maltby et al., 2005; McGuire et al., 1994; Saxena et al., 2004; Szeszko et al., 2005). In addition, anxiety induction in phobic patients has been associated with deactivation of the posterior cingulate region (Wik et al., 1993). These findings are consistent with our observations of an anxiolytic effect of CBD in patients with SAD and a CBD-related reduction in activity in this brain region. However, both increased and no posterior cingulate activity changes have also been reported in functional neuroimaging studies of anxiety and clinical anxiety disorders (Cannistraro and Rauch, 2003; Eser et al., 2009; Etkin and Wager, 2007; Rauch et al., 2001, 2002; Trzesniak et al., 2008).

The mechanisms of action at the molecular level whereby CBD produces its anxiolytic effects remain unknown. A recent animal study showed that the anxiolytic effect of CBD in the Vogel test does not depend on activation of benzodiazepine receptors (Moreira et al., 2006). Although CBD does not act by means of the known cannabinoid receptors (CB1 or CB2), the stereospecificity previously observed may indicate that this cannabinoid binds to another type of receptor in the brain (Mechoulam and Hanus, 2002; Zuardi, 2008). Moreover, experimental evidence has shown that CBD is capable of antagonizing CB1/CB2 receptor agonists at reasonably low concentrations, although it is well known that this compound has low affinity for CB receptors (Thomas et al., 2007). Such findings raise the possibility that this antagonism is non-competitive in nature, a hypothesis that was recently put forward (Pertwee, 2008). It is possible that the anxiolytic effects of CBD could be due to the action of the endogenous cannabinoid anandamide in the brain. Anxiogenic situations may lead to the release of anandamide in the amygdala (Marsicano et al., 2002), and this endocannabinoid may in turn influence emotional states by regulating outputs from this to other brain regions (Freund et al., 2003; Gaetani et al., 2003). In addition, the hydrolysis of anandamide (Bisogno et al., 2001; Rakhshan et al., 2000; Watanabe et al., 1996) in mouse brain microsomes and the

carrier-mediated cellular uptake of anandamide in mast cells (Bisogno et al., 2001) are both inhibited by CBD. These findings suggest that administration of this cannabinoid may enhance endogenous anandamide activity. Overall, the production of anandamide by amygdalar activation in response to fear could be part of a negative feedback system that limits anxiety and participates in the control of anxious states, and it has been suggested that anandamide hydrolysis may be a new target for anti-anxiety drugs (Gaetani et al., 2003). Finally, another possibility is that CBD acts as an agonist at the human 5-HT1A receptor in a concentration-dependent manner (Campos and Guimarães, 2008; Resstel et al., 2009; Russo et al., 2005; Zanelati et al., 2010), an action that may also be involved in the anxiolytic-like effects of CBD. As mentioned above, both CBD and ipsapirone, a well-known 5-HT1A receptor agonist, have been shown to have acute anxiolytic action in subjects submitted to the simulated public speaking test (Zuardi et al., 1993b).

The brain areas where we found modulatory effects of CBD, as well as being clearly implicated in the mediation of the expression of anxiety in various contexts, have also been associated with the anxiolytic effects of diazepam (Di Piero et al., 2001), citalopram (Carey et al., 2004; Furmark et al., 2005; Van der Linden et al., 2000), escitalopram (Spindelegger et al., 2009), nefazodone (Kilts et al., 2006), sertraline, desipramine (Hoehn-Saric et al., 2005), and cognitive behavioral therapy (CBT) for anxiety disorders (Paquette et al., 2003; Prasko et al., 2004; Roffman et al., 2005). However, although the medial temporal regions that show SPECT changes in this study have been implicated in anxiety, these are not unique for this condition since these areas have also been implicated in psychosis, mood and memory (Reichenberg and Harvey, 2007), and most anxiety imaging studies show changes in the amygdala (Ferrari et al., 2008; Trzesniak et al., 2008), which has not been implicated in this study. The absence of alterations in the amygdala might reflect the intrinsic difficulties in investigating this structure using imaging techniques, namely, it is a small structure in close vicinity of the bony and areal structures of the basal skull and nasal cavities.

In the present study, subjective rating scores indicated that the anxiolytic effects of CBD were not attributable to sedation. Our present results contrast with the sedative effects of CBD observed in animals (Pickens, 1981; Zuardi et al., 1981, 1991), humans (Carlini et al., 1979; Zuardi et al., 1982, 1993a), and in our former SPECT and fMRI studies involving healthy subjects (Crippa et al., 2004; Fusar-Poli et al., 2009). Also in support of the view that there was no interference of sedative effects on the present results, no CBD-related alterations in hypothalamic activity were detected in SAD patients. Reduced activity of the hypothalamus was detected in association with CBD administration in our previous SPECT study with healthy volunteers, as well as in other studies using different sedative compounds (Tung et al., 2001).

The findings reported herein need to be interpreted with caution, given the limitations of the study. First, it would have been desirable to measure plasma levels of CBD and relate such measurements to the magnitude of rCBF changes; without a dose-response curve, there remain doubts about the regional cerebral effects of CBD. However, it should be pointed out that previous investigations have not been able to confirm whether there is a direct relationship between plasma levels of cannabinoids, in particular CBD, and their clinical effects (Agurell et al., 1986). In addition, the subject sample was small and without a sample of control subjects, and the relatively lower sensitivity of the SPECT technique compared with other functional imaging methods limited the statistical power of the study. Also, given the intrinsically limited spatial resolution of SPECT and the smoothing stage included in the SPM processing before the statistical analysis, caution is warranted in the interpretation of findings of large foci of tracer uptake changes in small brain structures (such as the amygdala, hippocampus and parahippocampal gyrus).

Another limitation of the study concerns the absence of absolute quantitative estimates of rCBF when SPECT tracers are used, including the ECD method employed here. As we calculated relative rCBF indices normalized by the mean global tracer uptake in the brain, it is not possible to rule out the possibility that such rCBF measures would have been influenced by differences in global blood flow. Last, it should be noted that differences in regional brain uptake patterns obtained with the 99mTc-ECD-SPECT technique used here have been demonstrated in comparison with the more commonly employed 99mTc-HMPAO method. These differences are probably due to the distinct brain retention characteristics of the two tracers. Differences in resting rCBF estimates obtained with these two SPECT methods in comparison with rCBF measurements using positron emission tomography (PET) with $H_2\ ^{15}O$ occur in a number of brain regions, including the parahippocampal gyrus and posterior cingulate cortex, which were found to be affected by CBD in the present study. However, it is unlikely that the results reported here reflect distinctive brain retention characteristics of ECD rather than true effects of CBD on rCBF. The 99mTc-ECD-SPECT technique has been extensively demonstrated to provide valid measures of resting-state rCBF. Moreover, exactly the same protocol for SPECT imaging with ECD was used in the two scanning sessions for each subject. Between-scanning tracer uptake differences would therefore be attributable to CBD versus placebo effects, rather than to any particular properties of the ECD tracer itself. All of these limitations could be overcome in future studies examining larger SAD samples (and including non-SAD subjects) with fMRI, which would permit the acquisition of larger numbers of imaging sequences, with greater spatial and temporal resolution.

In conclusion, our results support the notion that CBD has anxiolytic effects that are associated with an action on limbic and paralimbic areas of the brain. This suggests a potential usefulness of CBD in ameliorating symptoms of clinically significant anxiety. Since CBD has important advantages in comparison with the currently available pharmacological agents for the treatment of SAD, such as an early onset of action and lack of important side effects, this compound may be a promising novel therapeutic in the management of generalized SAD. However, double-blind, placebo-controlled long-term studies would be necessary to further confirm these observations.

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