Modulation of effective connectivity during emotional processing by Δ⁹-tetrahydrocannabinol and cannabidiol

Paolo Fusar-Poli¹,², Paul Allen¹, Sagnik Bhattacharyya¹, José A. Crippa¹,³, Andrea Mechelli¹, Stefan Borgwardt¹,³, Rocio Martin-Santos¹, Marc L. Seal¹,³, Colin O’Carrol⁴, Zerrin Atakan⁴, Antonio W. Zuardi³, and Philip McGuire¹

¹ Neuroimaging Section, Division of Psychological Medicine, Institute of Psychiatry, King’s College London, UK
² Section of Psychiatry, Department of Health Sciences, University of Pavia, Italy
³ Department of Neuroscience and Behaviour, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil and INCT Translational Medicine, Ribeirão Preto, Brazil
⁴ Psychiatric Outpatient Department, University Hospital Basel, Switzerland
⁵ Melbourne Neuropsychiatry Centre, The University of Melbourne, Australia

Abstract

Cannabis sativa, the most widely used illicit drug, has profound effects on levels of anxiety in animals and humans. Although recent studies have helped provide a better understanding of the neurofunctional correlates of these effects, indicating the involvement of the amygdala and cingulate cortex, their reciprocal influence is still mostly unknown. In this study dynamic causal modelling (DCM) and Bayesian model selection (BMS) were used to explore the effects of pure compounds of C. sativa [600 mg of cannabidiol (CBD) and 10 mg Δ⁹-tetrahydrocannabinol (Δ⁹-THC)] on prefrontal-subcortical effective connectivity in 15 healthy subjects who underwent a double-blind randomized, placebo-controlled fMRI paradigm while viewing faces which elicited different levels of anxiety. In the placebo condition, BMS identified a model with driving inputs entering via the anterior cingulate and forward intrinsic connectivity between the amygdala and the anterior cingulate as the best fit. CBD but not Δ⁹-THC disrupted forward connectivity between these regions during the neural response to fearful faces. This is the first study to show that the disruption of prefrontal-subcortical connectivity by CBD may represent neurophysiological correlates of its anxiolytic properties.

Introduction

Cannabis sativa is the most widely used illicit drug with about 20% of young people now reporting regular or heavy use (Moore et al. 2007). There is substantial evidence that cannabis can be classified as an independent risk factor for the development of psychosis that may lead to a worse outcome of the disease in already diagnosed patients (Murray et al. 2007). The neurobiological substrates of cannabis use, including modulated activity of dopaminergic, GABAergic, and glutamatergic neurons (Chen et al. 1993; Pistis et al. 2002), are consistent with abnormalities described in people with psychotic disorders.

The evidence that cannabis use leads to affective disturbance outcomes is less strong than for psychosis as literature on cannabis and emotion has abundantly reported contradictory results (Moreira & Lutz, 2008). For example, it is paradoxical that while individuals report reduced anxiety as the motivation for using cannabis, acute anxiety is one of the most common adverse effects of cannabis use. These conflicting statements may be reconciled through the observation that the effects of cannabis on anxiety appear to be dose-dependent, with low doses producing an anxiolytic-like effects in laboratory rodents and higher doses
producing anxiogenic behaviour (Crippa et al. 2009). Another possible reason for this inconsistency could rely in the diverse number of substances present in the plant. Although Δ9-tetrahydrocannabinol (Δ9-THC) is commonly regarded as the main factor responsible for the psychoactive effects of cannabis, several reports have demonstrated that other components of the plant influence its pharmacological activity (Ashton, 2001; Crippa et al. 2009).

One of these compounds is cannabidiol (CBD). Although CBD may constitute up to 40% of cannabis extracts, it is not associated with the psychological and cognitive effects of cannabis use and has anxiolytic properties (Crippa et al. 2009). In line with this hypothesis, in a previous functional magnetic resonance imaging (fMRI) study we showed that CBD but not Δ9-THC attenuated the neural response to the presentation of fearful faces (Fusar-Poli et al. 2009). The neurophysiological effect of CBD was observable in limbic system structures such as the amygdala and the anterior cingulate cortex (ACC) – which are core regions of the ‘emotional brain’ (Pessoa, 2008) – and was correlated with a concurrent electrophysiological effect (Fusar-Poli et al. 2009). The amygdala is normally activated when subjects are presented with negative stimuli (Breiter et al. 1996; Morris et al. 1996, 1998, 1999; Philips et al. 1997, 1998), while the cingulate cortex is critically involved in processing emotional information both in animals (Hadland et al. 2003; Rudebeck et al. 2006) and in humans (Killgore & Yurgelun-Todd, 2004). However, the integration of these neural systems during the presentation of fearful faces and how these interact with Δ9-THC and CBD manipulation is still mostly unknown.

In the present study we initially tested the hypothesis that ACC and amygdala were functionally coupled during the neural response to fearful stimuli. There is evidence for anatomical connections between ACC and amygdala (Ghashghaei et al. 2007), and previous neuroimaging studies in humans have already suggested that the ACC is usually engaged with the amygdala in response to fear and anxiety (Bush et al. 2000; Das et al. 2007; Pissiota et al. 2003). We also examined the direction of this coupling by testing a series of competing forward and backward models. Formal analyses of effective connectivity, which indicates the contributory influence of one connected region on another (Friston et al. 2003) allow heightened understanding of the network processing signals of fear in humans. Furthermore, dynamic causal modelling (DCM), a method that can be used to assess effective connectivity in functional neuroimaging data and how this may be modulated by experimental manipulation, allowed us to examine the manner in which coupling between limbic regions during fear processing was modulated by the main Cannabis sativa constituents. To address this latter point we explored the effect of pure compounds of the plant such as CBD and Δ9-THC on the putative amygdala–ACC effective connectivity. On the basis of our previous findings (Bhattacharyya et al. 2009; Fusar-Poli et al. 2009) we predicted that CBD but not Δ9-THC would show a modulatory effect on this network during processing of fearful faces.

Materials and methods

Subjects

Fifteen healthy native English-speaking right-handed males (mean age 26.67 yr, s.d. = 5.7, age range 20–42) who had a lifetime exposure to cannabis of ≤15 times, with no cannabis use in the last month, no personal or family history of psychiatric illness, no alcohol or other drug abuse (see below) or dependence were recruited through advertisement in the local media.

Mean IQ, estimated using the National Adult Reading Test (NART; Willshire et al. 1991) was 98.67 (s.d. = 7.0). All subjects had a negative urinary drug screen (amphetamines, benzodiazepines, cocaine, methamphetamine, opiates, Δ9-THC) prior to all scanning sessions. Illicit substance use was assessed using the Structured Clinical Interview (SCID) and the Addiction Severity Index (McLellan et al. 1980), and subjects were advised to abstain from using illicit drugs throughout the duration of the study and to avoid alcohol intake for 24 h and caffeine intake for 12 h before each study day. The study was approved by the Maudsley Hospital ethical committee and all participants gave their informed consent.

Experimental design

Each participant was scanned three times with a 1-month interval between scans. After at least 8 h fasting, subjects were instructed to have a light, standardized breakfast 2 h before the experiment. Subjects were not allowed to smoke nicotine during the experiment. Each subject was imaged on three separate occasions, with each session preceded by oral administration of Δ9-THC (10 mg), CBD (600 mg) (both approximately 99.6% and 99.9% pure, respectively, and supplied by THC-Pharm, Germany), or a capsule of placebo (flour) in a double-blind, pseudo-randomized, placebo-controlled, repeated-measures, within-subject
design. Order of drug administration was pseudo-randomized across subjects so that an equal number of subjects received any of the drugs during the first, second, or third session. These doses of Δ⁹-THC and CBD were selected on the basis of previous research (Agurell et al. 1981; Chesher et al. 1990; Koethe et al. 2006; Leweke et al. 1999) to produce an effect on regional brain function while having as low a risk of severe adverse effects as possible. The dose was also chosen as to approximately correspond to the Δ⁹-THC content of a typical cannabis cigarette (‘joint’, the equivalent dose is around 10 mg) (WHO, 1997). CBD, Δ⁹-THC and placebo were identical in appearance and taste and neither the experimenters nor the participants knew which tablets were being administered in a double-blind procedure. We also recorded electrodermal skin conductance responses (SCR) (number, amplitude, and rise time of SCR fluctuations) during fMRI scanning as a measure of autonomic arousal via a pair of silver–silver chloride electrodes placed on the distal phalanges of digits. fMRI scans and electrodermal activity (SCR) were taken between 1 and 2 h after administration of the drug. Periodic (at baseline, and 1, 2 and 3 h post-administration) psychopathological ratings [mood, Visual Analogue Mood Scale (VAMS; Folstein & Luria, 1973)]; anxiety, Spielberger State Trait Anxiety Inventory (STAI; Spielberger, 1983); intoxication, Analogue Intoxication Scale (AIS; Mathew et al. 1999), psychotic symptoms, Positive and Negative Symptom Scale (PANSS; Kay et al. 1987; Morrison et al. 2009), were collected in all participants. Prior to the experiment each volunteer had performed a training session completing all the scales. Blood samples were taken at the same time-points from an indwelling intravenous line in the non-dominant arm of each participant to monitor the levels of drugs in blood. Heart rate and blood pressure were monitored continuously throughout the procedure. All these procedures were conducted by a psychiatrist experienced in the clinical effects of Δ⁹-THC and CBD who monitored participant well-being during the entire session. No serious adverse events (death, hospitalization, emergency room visit) occurred during the study. Three subjects from the original samples (n = 18) had a psychotic reaction (as assessed by the PANSS and clinical manifestation) to Δ⁹-THC administration and were excluded, since they were unable to perform the tests (final sample n = 15). These subjects were followed up for 24 h until the psychotic symptoms relieved. They were also monitored monthly and remained well, with no psychiatric or clinical symptom.

fMRI paradigm

Study subjects participated in one 6-min experiment employing event-related fMRI, where they were presented with 10 different facial identities, each expressing 50% (mildly fearful) or 100% (intensely fearful) intensities of fear or a neutral expression (Facial Expressions of Emotion: Stimuli and Tests; Young et al. 2002). There were thus 30 different facial stimuli in total; each stimulus was presented twice for 2 s. Individuals therefore viewed 60 stimuli in total. The order of facial identities and expression type was pseudo-randomized such that there was no successive presentation of the same identity or facial expression type. During the inter-stimulus interval, the duration of which was varied from 3 s to 8 s according to a Poisson distribution with an average interval of 5.9 s, individuals viewed a fixation cross (Surguladze et al. 2005). They were requested to decide on the gender of face stimuli and press one of two buttons accordingly. Throughout image acquisition, accuracy and reaction times were monitored via button press and recorded on a personal computer.

Image acquisition

Images were acquired on a 1.5 T Sigma (GE) system at the Maudsley Hospital, London. T2*-weighted images were acquired with a TR of 2 s, TE 40 ms, flip angle 90° in 16 axial planes (7-mm-thick slices with a 0.7-mm gap), parallel to the AC–PC line. A high-resolution inversion recovery image dataset was also acquired to facilitate anatomical localization of activation.

Image processing and analysis

Functional MRI data were analysed with Statistical Parametric Mapping software (SPM5; Wellcome Department of Cognitive Neurology, London, UK) running under the MATLAB7.1 environment. All volumes were realigned to the first volume, corrected for motion artefacts, mean adjusted by proportional scaling, normalized into standard stereotactic space (template provided by the Montreal Neurological Institute) and smoothed using a 6 mm full-width at half-maximum Gaussian kernel. The time-series were high pass filtered to eliminate low-frequency components (filter width 128 s) and adjusted for systematic differences across trials. The onset times (in seconds) for each trial of neutral, mildly fearful, and intensely fearful faces were convolved with a canonical haemodynamic response function. Each task condition (neutral, mildly fearful, intensely fearful) was then contrasted against the baseline condition (cross...
fixation) for each of the drug treatments (placebo, CBD, Δ⁹-THC). A further comparison contrasted all fearful faces (50% and 100% fearful faces) against neutral faces for each drug treatment (placebo, CBD, Δ⁹-THC), to isolate activation related to processing emotional expression. To test our hypothesis that there were between-group differences, the activation for each task condition was then compared between drugs, using an ANOVA within-subjects test. Small volumes correction (SVC; sphere of 12 mm radius) were used for clusters observed in hypothesized regions of interest (limbic and paralimbic areas). Regional activation was reported at a cluster threshold of p < 0.05 corrected.

Previous fMRI results

In a previously published study employing fMRI and electrophysiological measures of emotional arousal (SCR), we investigated the effects of CBD and Δ⁹-THC on regional brain function during fearful processing (Fusar-Poli et al. 2009).

We found that Δ⁹-THC increased anxiety, as well as levels of intoxication, sedation, and psychotic symptoms, whereas there was a trend for a reduction in anxiety following administration of CBD. The number of SCR fluctuations during the processing of intensely fearful faces increased following administration of Δ⁹-THC but decreased following administration of CBD. More importantly in the current study, CBD attenuated the blood oxygenation level-dependent signal in the left amygdala and the left anterior and right posterior cingulate cortex while subjects were processing intensely fearful faces. These changes in activation were accompanied by changes in SCR that are typically seen with increased anxiety (Phelps et al. 2001). Thus, suppression of the left amygdala and left ACC responses by CBD was correlated with the concurrent reduction in SCR fluctuations.

Δ⁹-THC mainly modulated activation in frontal and parietal areas. We concluded that Δ⁹-THC and CBD had clearly distinct effects on the neural, electrodermal, and symptomatic response to fearful faces. The effects of CBD on activation in limbic and paralimbic regions contributed to its ability to reduce autonomic arousal and subjective anxiety, whereas the anxiogenic effects of Δ⁹-THC were related to effects in other brain regions.

DCM

We used DCM (Friston et al. 2003) as implemented in SPM5 software. The aim of DCM is to estimate and make inferences about, the influence that one neural system exerts over another and the extent to which this is affected by the experimental context. In DCM, a reasonably realistic but simple neuronal model of interacting neural regions is constructed. DCM uses a previously validated biophysical model of fMRI measurements (Friston et al. 2000) to predict haemodynamic responses from modelled neural population activity. Importantly, DCM models how the neural dynamics are shaped by experimentally controlled manipulations such as stimulus presentation or task instruction, i.e., external inputs u, that enter the model in two different ways. Inputs can elicit responses through direct influences on specific regions (‘driving inputs’) or they can change the strength of coupling in regions (‘modulatory inputs’). The estimated underlying neural activity is then used to derive the connectivity parameters, as described elsewhere (Friston et al. 2003). Two sets of parameters are of particular interest: (i) ‘intrinsic connections’ that characterize the fixed (context-invariant) coupling strength between regions and (ii) ‘bilinear terms’ that characterize changes in activity associated with experimental manipulations (in this case response congruency and constraint). The general goal of DCM is to explain regional effects (as detected by a conventional general linear model) in terms of connectivity and its experimentally induced modulation. In the present study, we aimed to investigate whether the different effects of Δ⁹-THC on brain activity during emotional processing were associated with differences in functional integration between left amygdala and ACC. Neuroanatomically there are dense reciprocal connections between the ACC and amygdala (Ghashghaei et al. 2007).

Informed by the previous fMRI and electrophysiological results (see above) (Fusar-Poli et al. 2009), we first tested the hypothesis that there would be coupling between the left amygdala and the left ACC under the placebo condition. Since the analysis of regional responses revealed a left-lateralized network (Fusar-Poli et al. 2009), in the present investigation we focused on the left hemisphere for computational expediency. We extracted time-series (VOIs) based on group maxima derived from the results reported in Fusar-Poli et al. (2009) that included the left amygdala (x = −18, y = −4, z = −18, determined from the contrast of intensely fearful faces > baseline), and the left ACC (BA 24/32: x = −4, y = 34, z = 24, determined from the contrast of intensely fearful faces > baseline). In order to account for individual variation in activation we derived the exact coordinates of the regions from the local maxima of the subject-specific statistical parametric maps within 12 mm of the group maxima; regions
were defined as 6-mm spheres and regional activities were extracted in terms of principal eigenvaria-
tes. Then, in a first step we modelled the peripheral
stimulus presentation by allowing all stimuli (fearful
faces) to directly induce activity in the amygdala–ACC
model regardless of task modulation. Driving inputs
entered the model via the ACC (model 1). Three
alternative DCMs were then specified (see Fig. 1). In
model 2 both forward and backward connections
were specified allowing driving inputs to propagate
throughout the network via interconnections between
the left amygdala and ACC. Similarly, we built two
further models allowing the stimuli (fearful faces) to
directly induce activity in the left (driving inputs)
(model 3) and then to propagate throughout the net-
work via interconnections (model 4).

Although simple, these models allow for interesting
mechanistic inferences by testing competing hypo-
theses. Using BMS (see below) we were able to test these
competing models of amygdala–ACC effective con-
nectivity. Finally we were able to assess whether CBD
and Δ⁹-THC were associated with altered effective
connectivity between these two regions relative to
placebo. This was done by extracting connectivity
parameter estimates from each drug condition sepa-
rateley.

In DCM, the estimated parameters describe the rate
of change (in units of 1/s) in the target region as a
linear function of activity in the source region: a strong
connection means an influence that is expressed
quickly or with a large rate constant (see Friston et al.
2003 for details). A positive (i.e. >0) connection in-
dicates that ‘high’ activity in the source region is
associated with an increase in activity in the target
region. A negative (i.e. <0) connection indicates that
high activity in the source region is associated with a
decrease in activity in the target region. The under-
lying biophysical model of fMRI measurements links
rates of change in the target to the level of activity in
the source (Friston et al. 2003).

Bayesian Model selection (BMS)

We used BMS as implemented in SPM5 to decide
which DCM was optimal. BMS not only takes into
account the relative fit of competing models but also
their relative complexity (number of free parameters,
functional form). It rests on the so-called ‘model evi-
dence’, i.e. the probability \( p(y|m) \) of the data \( y \) given a
particular model \( m \). For details of BMS as imple-
mented in SPM5, see Penny et al. 2004.

For each subject we first performed pairwise com-
parisons between all models. Individual Bayes factors
>1 indicate evidence in favour of the first model in the
pair whereas a Bayes factor <1 indicates evidence in
favour of the second model in the pairwise compari-
son. We then computed the group Bayes factors (GBF;
Stephan & Penny, 2007) by calculating the product of
subject Bayes factors for each model. In our study, the
subject-specific parameter estimates of intrinsic con-
nexions during emotional processing were analysed
for the four models with one-sample \( t \) tests in the three
drug condition separately. Then paired \( t \) tests were
used to compare intrinsic connection strength for
the best model between drug conditions (threshold of
\( p<0.05 \)).
Results

Construction of DCMs based on statistical parametric maps

Based on the observed effects of CBD on the neuro-functional and SCR response in the left amygdala and left ACC, we constructed a simple amygdala–ACC model in the left hemisphere. This model was then tested in the placebo condition against four competing models using BMS (models 2–4, Fig. 1).

Placebo condition

Comparing model 1 against the three variants of the two-area model in each of the 15 subjects using BMS, the GBF indicates that model 1 was optimal (Table 1). In this model the driving inputs enter through the left ACC and the induced activity was then allowed to spread along forward intrinsic connections between this region and the left amygdala. The results from the statistical group analysis are summarized in Table 2(a–c) (first column). The mean group parameters \( \mu = 0.084 \), S.D. = 0.151 were significantly different from the null hypothesis (zero) \( p = 0.048 \) indicating the forward intrinsic connection between ACC and amygdala during emotional processing.

CBD

We then tested model 1 in the CBD conditions and found no significant evidence of effective connectivity between ACC and amygdala. Comparing parameters between placebo and CBD conditions (mean \( = -0.021 \), S.D. = 0.061), we found a significant difference in the forward intrinsic connection between ACC and amygdala \( p = 0.035 \) (Table 2a).

\( \Delta^9\)-THC

Under the \( \Delta^9\)-THC condition we found no significant evidence of effective connectivity for model 1 between ACC and amygdala (mean \( \Delta^9\)-THC = 0.004, S.D. = 0.151). However, when we compared the parameters between placebo and \( \Delta^9\)-THC condition no significant differences in the intrinsic connection between the two areas were elicited \( p > 0.05 \), Table 2b).

\( \Delta^9\)-THC vs. CBD

When we directly compared the model 1 parameters between CBD and \( \Delta^9\)-THC condition, no significant differences in the intrinsic connection between ACC and amygdala were observed \( p > 0.05 \), Table 2c).

Table 1. Subject-specific Bayes factors for comparing the optimal two-area anterior cingulate cortex–amygdala model 1 against all other three models (M2, M3, M4) under placebo condition

|      | S1   | S2   | S3   | S4   | S5   | S6   | S7   | S8   | S9   | S10  | S11  | S12  | S13  | S14  | S15  | GBF    |
|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|-------|
| M3   | 1    | 19.532| 1    | 4.205| 0.892| 0.965| 0.861| 1.208| 0.851| 0.450| 4.042| 0.748| 0.907| 1.005| 1.240| 96.066 |
| M4   | 0.368| 2.718| 4.454| 0.643| 2.424| 2.623| 2.339| 3.481| 2.313| 1.222| 0.675| 2.032| 2.464| 2.732| 3.369| 13038.537 |

GBF, Group Bayes factor.

Table 2a. Effect of cannabidiol (CBD) on ACC–Amy effective connectivity

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Placebo</th>
<th>CBD</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0.006</td>
<td>0.044</td>
<td>-0.038</td>
</tr>
<tr>
<td>S2</td>
<td>0.013</td>
<td>0.015</td>
<td>-0.002</td>
</tr>
<tr>
<td>S3</td>
<td>0.401</td>
<td>-0.056</td>
<td>0.457</td>
</tr>
<tr>
<td>S4</td>
<td>0.016</td>
<td>0.047</td>
<td>-0.030</td>
</tr>
<tr>
<td>S5</td>
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<td>-0.001</td>
<td>0.001</td>
</tr>
<tr>
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<td>0.031</td>
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<td>0.075</td>
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<td>S7</td>
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<td>S8</td>
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<td>S9</td>
<td>0.130</td>
<td>0.060</td>
<td>0.069</td>
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<td>S11</td>
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</tr>
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<td>S12</td>
<td>-0.058</td>
<td>0.020</td>
<td>-0.079</td>
</tr>
<tr>
<td>S13</td>
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<td>-0.059</td>
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<td>S15</td>
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<tr>
<td>Mean</td>
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<td>SD</td>
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<tr>
<td>t test*</td>
<td>0.048*</td>
<td>0.210*</td>
<td>0.035b</td>
</tr>
</tbody>
</table>

ACC, Anterior cingulate cortex; Amy, amygdala; S.D., standard deviation.
* One-sample t test was performed, using a statistical threshold of \( p < 0.05 \). We first tested for the forward connection of model 1 the null hypothesis that it was not different from zero across subjects.
 b Second, the difference between the connection parameters in the placebo and CBD conditions was tested.
Other models

Models 2–4 were tested under Δ⁸-THC and CBD but the mean group parameters were non-significantly different from the null hypothesis (p > 0.05).

Discussion

The present study used DCM to examine the modulation of effective connectivity during emotional processing by two main psychoactive constituents of C. sativa, Δ⁸-THC and CBD. To the best of our knowledge this is the first time the effects of such cannabinoids on brain connectivity have been assessed in the same subjects. Using DCM and BMS we were able to test competing models of frontal and subcortical integration associated with emotional processing. As DCM should be used to test specific a-priori hypothesis (Penny et al. 2004), it was therefore important to select regions that were clearly involved in the task and which clear hypotheses could be formulated. Thus, the selection of a simple model was based on previous findings in the same sample showing that CBD attenuates the neurofunctional signal in the amygdala and ACC during the processing of fearful faces (Fusar-Poli et al. 2009). Furthermore, this neural effect was correlated with a concurrent electrophysiological effect, suggesting that the amygdala and the ACC play a key role in the neural network underlying the anxiolytic effect of CBD. This is consistent with evidence indicating strong anatomical connections between these two regions (Ghashghaei et al. 2007).

The first aim of this study was to establish a physiological model of effective connectivity between ACC and amygdala during emotional processing. In line with the assumptions above, we confirmed that the amygdala and the ACC are functionally connected during the neural response to fearful faces. The finding of an amygdala–prefrontal interaction is supported by known reciprocal anatomical connections.

<table>
<thead>
<tr>
<th>Table 2b. Effect of Δ⁸-tetrahydrocannabinol (Δ⁸-THC) on ACC→Amy effective connectivity</th>
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<td><strong>Mean</strong></td>
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<td><strong>SD</strong></td>
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<tr>
<td><em>t</em> test*</td>
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ACC, Anterior cingulate cortex; Amy, amygdala; s.d., standard deviation.
<sup>a</sup> One-sample *t* test was performed, using a statistical threshold of *p* < 0.05. We first tested for the forward connection of model 1 the null hypothesis that it was not different from zero across subjects.
<sup>b</sup> Second, the difference between the connection parameters in the placebo and cannabidiol conditions was tested.

<table>
<thead>
<tr>
<th>Table 2c. Differential effect of Δ⁸-tetrahydrocannabinol (Δ⁸-THC) and cannabidiol (CBD) on ACC→Amy effective connectivity</th>
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<tbody>
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<td><em>t</em> test*</td>
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ACC, Anterior cingulate cortex; Amy, amygdala; s.d., standard deviation.
<sup>a</sup> One-sample *t* test was performed, using a statistical threshold of *p* < 0.05. We first tested for the forward connection of model 1 the null hypothesis that it was not different from zero across subjects.
<sup>b</sup> Second, the difference between the connection parameters in the Δ⁸-THC and CBD conditions was tested.
between the amygdala and the frontal regions, and by studies indicating that metabolic rates in the amygdala are positively correlated with those in the ACC (New et al. 2007). The amygdala–prefrontal circuitry has been termed the emotion generation–regulation circuit (Ghashghaei et al. 2007) and is implicated in attention to threat and interpretation of emotional stimuli (Bishop, 2007). Previous studies found that lesions to these areas result in emotional dysregulation (Banks et al. 2007). Thus, the balance of activity within this circuitry seems to be altered in anxiety disorders, creating a bias towards threat-related responses (Berkowitz et al. 2007). Within the prefrontal cortex, the ACC circuit has the strongest anatomical connection with the amygdala (Ghashghaei et al. 2007) and is specifically involved in emotional processing (Bonelli & Cummings, 2007). The key role of the ACC in controlling negative emotions such as fear has been highlighted by paradigms testing fear extinction and anxiety suppression in animals (Delgado et al. 2006) and humans (Petrovic et al. 2005). Some authors have specifically posited a functional distinction between ventral and dorsal ACC, such that ventral regions are thought to cooperate with the amygdala in the appraisal for emotional salience of stimuli while dorsal regions are crucial in the regulation of the affective state (Phillips et al. 2003). A previous study has already explored the within-subject inter-regional connectivity between amygdala and prefrontal cortex in the context of affect regulation (Banks et al. 2007). By using psychophysiological interaction analyses of fMRI data, activity in specific areas of the frontal cortex (dorsolateral, dorsal medial, ACC, orbital) covaried with amygdala activity (Banks et al. 2007), supporting the importance of functional connectivity within limbic-frontal circuitry during emotion regulation.

However, despite evidence of a dynamic coalition between the amygdala and the prefrontal cortex, the directionality of these interactions has not previously been tested, given that the psychophysiological interaction analyses only tests if differences in inter-regional coupling exist as function of task. Previous studies indicate that communication between the amygdala and the ACC is bi-directional (Carmichael & Price, 1995). Consequently there is considerable uncertainty on the organization of the complementary part of the amygdala–prefrontal interaction during emotional processing, namely input and output zones in prefrontal cortices connected with the amygdala. To address this issue we tested several candidate models. We found that a model in which the driving inputs enter via the ACC and the forward connectivity was allowed to spread to the amygdala was the best fit (model 1). Conversely, BMS produced no consistent evidence for the alternative hypothesis that the driving inputs enter via the amygdala (model 3). Models in which the connections between regions were bi-directional were also inferior (models 2 and 4). The current findings taken altogether show that during emotional processing, the ACC modulates the functional response in the amygdala and not vice versa. Although this finding clearly needs to be replicated there is converging evidence demonstrating that the ACC has a top-down effect on the amygdala (Etkin et al. 2006; Ghashghaei et al. 2007; Quirk & Beer, 2006). Prefrontal-subcortical circuits are effector mechanisms that allow the organism to act on its environment (Bonelli & Cummings, 2007) and successful control of affect by prefrontal cortex partly depends on the capacity to modulate emotional responses mediated by the amygdala (Banks et al. 2007). Interestingly, this directionality accords with a recent and detailed anatomical study in monkeys which revealed that ACC areas sent proportionally more projections to the amygdala than they received (Ghashghaei et al. 2007). Based on these features, ACC areas may be considered more as ‘senders’ than ‘receivers’ (Kotter & Stephan, 2003), consistent with their role in affective vocalization in primates, and extinction of fear in rats (Heidbreder & Groenewegen, 2003). As cingulate cortices receive robust projections from other subcortical limbic structures (Barbas et al. 2002), they may also relay information to the amygdala about the internal milieu which evoke emotional arousal. Interestingly, ACC has strong connections with central autonomic structures (Ghashghaei et al. 2007) in line with our previous observation that neural response in this area correlates with electrophysiological measures of autonomic arousal (Fusar-Poli et al. 2009).

The main goal of our DCM analysis was to investigate whether the neurofunctional effects of two of the principal compounds of C. sativa could be explained through emotion-dependent differences of connectivity between left amygdala and ACC regions. We showed that these components of C. sativa exert different effects on brain connectivity. Thus, CBD but not Δ9-THC was found to disrupt ACC–amygdala effective connectivity during emotional processing. This finding suggests that the effects of CBD extends beyond the local modulation of neural activity and may engage wider neural circuits in the brain.

More importantly, as ACC and amygdala are physiologically connected during processing of stimuli that evoked anxiety, the disruption of the brain connectivity by CBD may underlie its anxiolytic properties. It is possible that the effect of CBD on brain
connectivity is primarily mediated through its action on the ACC, which in turn modulates brain activity in the left amygdala. Although it is possible to speculate that the anxiolytic effects of CBD are mediated by interference with the action of the endogenous cannabinoid anandamide in the brain (Bisogno et al., 2001), the putative mechanism of action of CBD on limbic circuitry is mostly unknown. The highest densities of cannabinoid 1 (CB1) receptors in the human neocortex are present in higher-order association regions, such as the prefrontal cortex, including the cingulate (Eggan & Lewis, 2007). In spite of its low affinity for CB1 and cannabinoid 2 (CB2) receptors, experimental evidence has shown that CBD is capable of antagonizing CB1/CB2 receptor agonists at reasonably low concentrations (Thomas et al., 2007). Consistent with these findings, it was observed that CBD induced Fos immunoreactivity (Fos is the protein product of the early intermediate gene c-fos) in the prefrontal cortex (Guimaraes et al., 2004). Electron microscopy studies of CB1 receptors in human cortex showed that when CB1 immunoreactivity was observed in cell bodies, they had the characteristic features of gamma aminobutyric acid (GABA) neurons (Eggan & Lewis, 2007). Since interaction with the CB1 agonist blocks GABA inhibition, resulting in activation, the antagonist action of CBD on CB1 receptors in the ACC could result in a reduction of their outputs. Another possible explanation could involve the CBD agonistic action on 5-hydroxytryptamine 1A (5-HT1A) receptors (Mishima et al., 2005; Russo et al., 2005). The ACC has highest 5-HT1A but lowest GABA densities (Palomero-Gallagher et al., 2009) and, in rats, the anxiolytic-like effects produced by CBD injected into the dorsolateral periaqueductal grey were prevented by 5-HT1A receptor antagonist (Campos & Guimaraes, 2008).

Limitations

Our group sizes were modest as pseudo-randomized, placebo-controlled, repeated-measures, within-subject design studies are logistically demanding (Friston et al., 1999). We therefore cannot exclude the possibility that we would have detected more extensive effects of Δ9-THC and/or CBD on effective connectivity had the sample size been larger. In addition the present investigation focused on the effective connectivity between the amygdala and ACC as these regions were modulated by the experimental paradigm as revealed by a previous analysis of regional responses (Fusar-Poli et al., 2009). However, we cannot exclude the possibility that these compounds have more widespread effects upon neural connectivity encompassing regions which were not considered in the present investigation. A further caveat is that there is a difference between oral ingestion of pure Δ9-THC or CBD and smoking cannabis which contains a variable mixture of psychoactive compounds. Following oral ingestion, intoxication is typically seen after 1 h and gradually increases to a plateau, which lasts ~3 h. However, effects following inhalation begins almost immediately and reach a peak within 1 h then decline fairly rapidly (Grotenhermen, 2005). Finally, given the experimental nature of this study it is not straightforward to generalize our gender-specific results (Fattore et al., 2008) or speculate on the complex Δ9-THC-CBD effects of the street cannabis (Murray et al., 2007) in people with a chronic history of drug abuse.

Conclusions

CBD but not Δ9-THC reduces effective connectivity between ACC and amygdala during emotional processing of fearful stimuli. This change in neural coupling between these regions may partly represent the neurophysiological correlates of the anxiolytic properties of CBD.

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Statement of Interest

None.

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