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ARTICLE

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Modulation of effective connectivity during emotional processing by Δ^9-tetrahydrocannabinol and cannabidiol

Paolo Fusar-Poli[1,2], Paul Allen[1], Sagnik Bhattacharyya[1], José A. Crippa[1,3], Andrea Mechelli[1], Stefan Borgwardt[1,4], Rocio Martin-Santos[1], Marc L. Seal[1,5], Colin O'Carrol[1], Zerrin Atakan[1], Antonio W. Zuardi[3] and Philip McGuire[1]

[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 Δ^9-tetrahydrocannabinol (Δ^9-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 Δ^9-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-subocrtical connectivity by CBD may represent neurophysiological correlates of its anxiolytic properties.

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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

Address for correspondence: Dr P. Fusar-Poli, Neuroimaging Section, Division of Psychological Medicine PO67, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, UK.
Tel.: +44 (0)77 8666 6570 *Fax*: +44 (0)20 7848 0976
Email: p.fusar@libero.it

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 Δ^9-tetrahydrocannabidiol (Δ^9-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 Δ^9-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 Δ^9-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 *C. sativa* constituents. To address this latter point we explored the effect of pure compounds of the plant such as CBD and Δ^9-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 Δ^9-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 $\leqslant$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, Δ^9-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 Δ^9-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 Δ^9-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 Δ^9-THC content of a typical cannabis cigarette ('joint', the equivalent dose is around 10 mg) (WHO, 1997). CBD, Δ^9-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 (CBD, Δ^9-THC as measured in the whole blood by Tricho-Tech, UK). 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 Δ^9-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 Δ^9-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, Δ^9-THC). A further comparison contrasted all fearful faces (50% and 100% fearful faces) against neutral faces for each drug treatment (placebo, CBD, Δ^9-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 Δ^9-THC on regional brain function during fearful processing (Fusar-Poli *et al*. 2009).

We found that Δ^9-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 Δ^9-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.

Δ^9-THC mainly modulated activation in frontal and parietal areas. We concluded that Δ^9-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 Δ^9-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 Δ^9-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

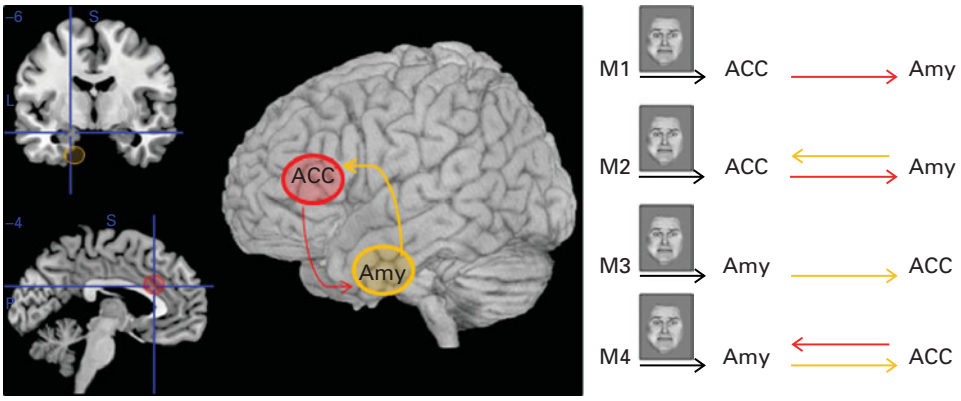

Fig. 1. Competing dynamic causal models of effective connectivity between the left amygdala (Amy) and the anterior cingulate cortex (ACC). Model 1 (M1) = driving inputs via ACC and forward only intrinsic connection. Model 2 = model 1 with bi-directional intrinsic connections. Model 3 = driving inputs via Amygdala and forward only intrinsic connection. Model 4 = model 3 with bi-directional intrinsic connections.

were defined as 6-mm spheres and regional activities were extracted in terms of principal eigenvariates. 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 network via interconnections (model 4).

Although simple, these models allow for interesting mechanistic inferences by testing competing hypotheses. Using BMS (see below) we were able to test these competing models of amygdala–ACC effective connectivity. Finally we were able to assess whether CBD and Δ^9-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 separately.

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 indicates 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 underlying 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 evidence', i.e. the probability $p(y|m)$ of the data y given a particular model m. For details of BMS as implemented in SPM5, see Penny *et al*. 2004.

For each subject we first performed pairwise comparisons 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 comparison. 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 connections 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 $p<0.05$).

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
M2	2.720	7.187	2.718	2.718	2.718	2.718	2.718	2.718	2.717	2.718	2.718	2.718	2.718	2.718	2.718	8644559.240
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.

Results

Construction of DCMs based on statistical parametric maps

Based on the observed effects of CBD on the neurofunctional 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 (mean = 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 2*a*).

Δ⁹-THC

Under the Δ⁹-THC condition we found no significant evidence of effective connectivity for model 1 between ACC and amygdala (mean Δ⁹-THC = 0.004, S.D. = 0.151). However, when we compared the parameters between placebo and Δ⁹-THC condition no significant differences in the intrinsic connection between the two areas were elicited ($p > 0.05$, Table 2*b*).

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

	M1		
Subjects	Placebo	CBD	Difference
S1	0.006	0.044	−0.038
S2	0.013	0.015	−0.002
S3	0.401	−0.056	0.457
S4	0.016	0.047	−0.030
S5	0.001	−0.001	0.001
S6	0.031	−0.044	0.075
S7	0.004	−0.011	0.015
S8	0.059	−0.143	0.202
S9	0.130	0.060	0.069
S10	0.001	−0.009	0.010
S11	0.186	−0.143	0.330
S12	−0.058	0.020	−0.079
S13	0.028	−0.059	0.087
S14	0.451	−0.005	0.455
S15	−0.003	−0.026	0.023
Mean	**0.084**	**−0.021**	**0.105**
SD	0.151	0.061	0.175
t test[a]	0.048[a]	0.210[a]	0.035[b]

ACC, Anterior cingulate cortex; Amy, amygdala; S.D., standard deviation.
[a] 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.

Δ⁹-THC vs. CBD

When we directly compared the model 1 parameters between CBD and Δ⁹-THC condition, no significant differences in the intrinsic connection between ACC and amygdala were observed ($p > 0.05$, Table 2*c*).

Table 2*b*. Effect of Δ⁹-tetrahydrocannabinol (Δ⁹-THC) on ACC→Amy effective connectivity

	M1		
Subjects	Placebo	Δ⁹-THC	Difference
S1	0.006	−0.041	0.047
S2	0.013	−0.039	0.052
S3	0.401	0.003	0.398
S4	0.016	0.016	0.001
S5	0.001	0.111	−0.111
S6	0.031	0.001	0.031
S7	0.004	0.083	−0.079
S8	0.059	−0.144	0.203
S9	0.130	−0.450	0.580
S10	0.001	0.146	−0.144
S11	0.186	0.040	0.146
S12	−0.058	0.220	−0.277
S13	0.028	0.034	−0.010
S14	0.451	0.008	0.443
S15	−0.003	0.067	−0.070
Mean	**0.084**	**0.004**	**0.081**
SD	0.151	0.152	0.237
t test[a]	0.048[a]	0.922[a]	0.208[b]

ACC, Anterior cingulate cortex; Amy, amygdala; S.D., standard deviation.
[a] 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 cannabidiol conditions was tested.

Table 2*c*. Differential effect of Δ⁹-tetrahydrocannabinol (Δ⁹-THC) and cannabidiol (CBD) on ACC→Amy effective connectivity

	M1		
Subjects	Δ⁹-THC	CBD	Difference
S1	−0.041	0.044	−0.085
S2	−0.039	0.015	−0.053
S3	0.003	−0.056	0.059
S4	0.016	0.047	−0.030
S5	0.111	−0.001	0.112
S6	0.000	−0.044	0.044
S7	0.083	−0.011	0.094
S8	−0.144	−0.143	−0.001
S9	−0.450	0.060	−0.511
S10	0.146	−0.009	0.155
S11	0.040	−0.143	0.184
S12	0.220	0.020	0.200
S13	0.038	−0.059	0.097
S14	0.008	−0.005	0.012
S15	0.067	−0.026	0.093
Mean	**0.004**	**−0.021**	**0.025**
SD	0.152	0.061	0.171
t test[a]	0.922[a]	0.210[a]	0.583[b]

ACC, Anterior cingulate cortex; Amy, amygdala; S.D., standard deviation.
[a] 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 Δ⁹-THC and CBD conditions was tested.

Other models

Models 2–4 were tested under Δ⁹-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*, Δ⁹-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

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 as 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 Δ^9-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-HT_{1A}) receptors (Mishima *et al*. 2005; Russo *et al*. 2005). The ACC has highest 5-HT_{1A} but lowest $GABA_A$ 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-HT_{1A} 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 Δ^9-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 reveled 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 Δ^9-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 Δ^9-THC–CBD effects of the street cannabis (Murray *et al*. 2007) in people with a chronic history of drug abuse.

Conclusions

CBD but not Δ^9-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.

References

Agurell S, Carlsson S, Lindgren J, Ohlsson A, *et al.* (1981). Interactions of delta 1-tetrahydrocannabinol with cannabinol and cannabidiol following orala dministration in man. Assay of cannabinol and cannabidiol by mass fragmentography. *Experientia* **37**, 1090–1092.

Ashton C (2001). Pharmacology and effects of cannabis: a brief review. *British Journal of Psychiatry* **178**, 101–106.

Banks SJ, Eddy KT, Angstadt M, Nathan PJ, Phan KL (2007). Amygdala-frontal connectivity during emotion regulation. *Society for Cognitive Affective Neuroscience* **2**, 303–312.

Barbas H, Ghashghaei H, Rempel-Clower N, Xiao D (2002). Anatomic basis of functional specialization in prefrontal cortices in primates. In: Grafman J (Ed.), *Handbook of Neuropsychology* (pp. 1–27), Amsterdam: Elsevier Science.

Berkowitz RL, Coplan JD, Reddy DP, Gorman JM (2007). The human dimension: how the prefrontal cortex modulates the subcortical fear response. *Reviews in Neurosciences* **18**, 191–207.

Bhattacharyya S, Crippa JA, Martin-Santos R, Winton-Brown T, Fusar-Poli P (2009). Imaging the neural effects of cannabinoids: current status and future opportunities for pyschopharmacology. *Current Pharmaceutical Design* **15**, 2603–2614.

Bishop SJ (2007). Neurocognitive mechanisms of anxiety: an integrative account. *Trends in Cognitive Sciences* **11**, 307–316.

Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, *et al.* (2001). Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *British Journal of Pharmacology* **134**, 845–852.

Bonelli RM, Cummings JL (2007). Frontal-subcortical circuitry and behavior. *Dialogues in Clinical Neurosciences* **9**, 141–151.

Breiter H, Etcoff N, Walen W (1996). Response and habituation of the human amygdala during visual processing of facial expression. *Neuron* **17**, 875–887.

Bush G, Luu P, Posner MI (2000). Cognitive and emotional influences in anterior cingulate cortex. *Trends in Cognitive Sciences* **4**, 215–222.

Campos AC, Guimaraes FS (2008). Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. *Psychopharmacology* (*Berlin*) **199**, 223–230.

Carmichael ST, Price JL (1995). Limbic connections of the orbital and medial prefrontal cortex in macaque monkeys. *Journal of Comparative Neurology* **363**, 615–641.

Chen J, Marmur R, Pulles A, Paredes W, Gardner EL (1993). Ventral tegmental microinjection of delta 9-tetrahydrocannabinol enhances ventral tegmental somatodendritic dopamine levels but not forebrain dopamine levels: evidence for local neural action by marijuana's psychoactive ingredient. *Brain Research* **621**, 65–70.

Chesher G, Bird K, Jackson D, Perrignon A, Starmer G (1990). The effects of orally administered delta 9-tetrahydrocannabinol in man on mood and performance measures: a dose–response study. *Pharmacology Biochemistry and Behavior* **35**, 861–864.

Crippa J, Zuardi A, Martín-Santos R, Bhattacharyya S, *et al.* (2009). Cannabis and anxiety: a critical review of the evidence. *Human Psychopharmacology: Clinical and Experimental*. Published online: 19 August 2009. doi:10.1002/hup.1048.

Das P, Kemp AH, Flynn G, Harris AW, *et al.* (2007). Functional disconnections in the direct and indirect amygdala pathways for fear processing in schizophrenia. *Schizophrenia Research* **90**, 284–294.

Delgado MR, Olsson A, Phelps EA (2006). Extending animal models of fear conditioning to humans. *Biological Psychology* **73**, 39–48.

Eggan SM, Lewis DA (2007). Immunocytochemical distribution of the cannabinoid CB1 receptor in the primate neocortex: a regional and laminar analysis. *Cerebral Cortex* **17**, 175–191.

Etkin A, Egner T, Peraza DM, Kandel ER, Hirsch J (2006). Resolving emotional conflict: a role for the rostral anterior cingulate cortex in modulating activity in the amygdala. *Neuron* **51**, 871–882.

Fattore L, Altea S, Fratta W (2008). Sex differences in drug addiction: a review of animal and human studies. *Women's Health* (*London*) **4**, 51–65.

Folstein MF, Luria R (1973). Reliability, validity, and clinical application of the Visual Analogue Mood Scale. *Psychological Medicine* **3**, 479–486.

Friston KJ, Harrison L, Penny W (2003). Dynamic causal modelling. *Neuroimage* **19**, 1273–1302.

Friston KJ, Holmes AP, Worsley KJ (1999). How many subjects constitute a study? *Neuroimage* **10**, 1–5.

Friston KJ, Mechelli A, Turner R, Price CJ (2000). Nonlinear responses in fMRI: the Balloon model, Volterra kernels, and other hemodynamics. *Neuroimage* **12**, 466–477.

Fusar-Poli P, Crippa J, Bhattacharyya S, Borgwardt S, *et al.* (2009). Distinct effects of d9-tetrahydrocannabinol and cannabidiol on neural actiation during emotional processing. *Archives of General Psychiatry* **66**, 95–105.

Ghashghaei HT, Hilgetag CC, Barbas H (2007). Sequence of information processing for emotions based on the anatomic dialogue between prefrontal cortex and amygdala. *Neuroimage* **34**, 905–923.

Grotenhermen F (2005). Cannabinoids. *Current Drug Targets CNS Neurological Disorder* **4**, 507–530.

Guimaraes VM, Zuardi AW, Del Bel EA, Guimaraes FS (2004). Cannabidiol increases Fos expression in the nucleus accumbens but not in the dorsal striatum. *Life Sciences* **75**, 633–638.

Hadland KA, Rushworth MF, Gaffan D, Passingham RE (2003). The effect of cingulate lesions on social behaviour and emotion. *Neuropsychologia* **41**, 919–931.

Heidbreder CA, Groenewegen HJ (2003). The medial prefrontal cortex in the rat: evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. *Neuroscience and Biobehavioral Reviews* **27**, 555–579.

Kay SR, Fiszbein A, Opler LA (1987). The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophrenia Bulletin* **13**, 261–276.

Killgore WD, Yurgelun-Todd DA (2004). Activation of the amygdala and anterior cingulate during nonconscious processing of sad versus happy faces. *Neuroimage* **21**, 1215–1223.

Koethe D, Gerth C, Neatby M, Haensel A, *et al.* (2006). Disturbances of visual information processing in early

states of psychosis and experimental delta-9-tetrahydrocannabinol altered states of consciousness. *Schizophrenia Research* **88**, 142–150.

Kotter R, Stephan KE (2003). Network participation indices: characterizing component roles for information processing in neural networks. *Neural Networks* **16**, 1261–1275.

Leweke F, Schneider U, Thies M, Munte T, Emrich H (1999). Effects of synthetic delta9-tetrahydrocannabinol on binocular depth inversion of natural and artificial objects in man. *Psychopharmacology* (*Berlin*) **142**, 230–235.

Mathew RJ, Wilson WH, Chiu NY, Turkington TG, *et al.* (1999). Regional cerebral blood flow and depersonalization after tetrahydrocannabinol administration. *Acta Psychiatrica Scandinavica* **100**, 67–75.

McLellan AT, Luborsky L, Woody GE, O'Brien CP (1980). An improved diagnostic evaluation instrument for substance abuse patients. The Addiction Severity Index. *Journal of Nervous and Mental Disease* **168**, 26–33.

Mishima K, Hayakawa K, Abe K, Ikeda T, *et al.* (2005). Cannabidiol prevents cerebral infarction via a serotonergic 5-hydroxytryptamine1A receptor-dependent mechanism. *Stroke* **36**, 1077–1082.

Moore TH, Zammit S, Lingford-Hughes A, Barnes TR, *et al.* (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet* **370**, 319–328.

Moreira FA, Lutz B (2008). The endocannabinoid system: emotion, learning and addiction. *Addiction and Biology* **13**, 196–212.

Morris J, Friston K, Buchel C (1998). A neuromodulatory role for the human amygdala in processing emotional facial expression. *Brain* **121**, 769–775.

Morris J, Friston K, Perrett D (1996). A differential neural response in the human amygdala to fearful and happy facial expression. *Nature* **383**, 812–815.

Morris J, Ohman A, Dolan R (1999). A subcortical pathway to the right amygdala mediating unseen fear. *Proceedings of the National Academy of Sciences USA* **96**, 1680–1685.

Morrison PD, Zois V, McKeown DA, Lee TD, *et al.* (2009). The acute effects of synthetic intravenous Delta9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. *Psychological Medicine*. Published online: 1 April 2009. doi:10.1017/S0033291709005522.

Murray RM, Morrison PD, Henquet C, Di Forti M (2007). Cannabis, the mind and society: the hash realities. *Nature Reviews Neurosciences* **8**, 885–895.

New AS, Hazlett EA, Buchsbaum MS, Goodman M, *et al.* (2007). Amygdala-prefrontal disconnection in borderline personality disorder. *Neuropsychopharmacology* **32**, 1629–1640.

Palomero-Gallagher N, Vogt BA, Schleicher A, Mayberg HS, Zilles K (2009). Receptor architecture of human cingulate cortex: evaluation of the four-region neurobiological model. *Human Brain Mapping* **30**, 2336–2355.

Penny WD, Stephan KE, Mechelli A, Friston KJ (2004). Comparing dynamic causal models. *Neuroimage* **22**, 1157–1172.

Pessoa L (2008). On the relationship between emotion and cognition. *Nature Reviews Neurosciences* **9**, 148–158.

Petrovic P, Dietrich T, Fransson P, Andersson J, *et al.* (2005). Placebo in emotional processing – induced expectations of anxiety relief activate a generalized modulatory network. *Neuron* **46**, 957–969.

Phelps EA, O'Connor KJ, Gatenby JC, Gore JC, *et al.* (2001). Activation of the left amygdala to a cognitive representation of fear. *Nature Neuroscience* **4**, 437–441.

Philips M, Young A, Scott S (1998). Neural responses to facial and vocal expressions of fear and disgust. *Proceedings of the Royal Society of London* **83**, 1809–1817.

Philips M, Young A, Senior C (1997). A specific neural substrate for perceiving facial expression of disgust. *Nature* **389**, 495–498.

Phillips ML, Drevets WC, Rauch SL, Lane R (2003). Neurobiology of emotion perception I: the neural basis of normal emotion perception. *Biological Psychiatry* **54**, 504–514.

Pissiota A, Frans O, Michelgard A, Appel L, *et al.* (2003). Amygdala and anterior cingulate cortex activation during affective startle modulation: a PET study of fear. *European Journal of Neuroscience* **18**, 1325–1331.

Pistis M, Ferraro L, Pira L, Flore G, Tanganelli S, *et al.* (2002). Delta(9)-tetrahydrocannabinol decreases extracellular GABA and increases extracellular glutamate and dopamine levels in the rat prefrontal cortex: an in vivo microdialysis study. *Brain Research* **948**, 155–158.

Quirk GJ, Beer JS (2006). Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. *Current Opinion in Neurobiology* **16**, 723–727.

Rudebeck PH, Buckley MJ, Walton ME, Rushworth MF (2006). A role for the macaque anterior cingulate gyrus in social valuation. *Science* **313**, 1310–1312.

Russo EB, Burnett A, Hall B, Parker KK (2005). Agonistic properties of cannabidiol at 5-HT1a receptors. *Neurochemical Research* **30**, 1037–1043.

Spielberger C (1983). *Manual of the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press, Inc.

Stephan K, Penny D (2007). Dynamic causal models and Bayesian selection. In: Friston K (Ed.), *Statistical Parametric Mapping* (pp. 577–585). Amsterdam: Elsevier.

Surguladze S, Brammer MJ, Keedwell P, Giampietro V, *et al.* (2005). A differential pattern of neural response toward sad versus happy facial expressions in major depressive disorder. *Biological Psychiatry* **57**, 201–209.

Thomas A, Baillie GL, Phillips AM, Razdan RK, *et al.* (2007). Cannabidiol displays unexpectedly high potency

as an antagonist of CB1 and CB2 receptor agonists in vitro. *British Journal of Pharmacology* **150**, 613–623.

Willshire D, Kinsella G, Prior M (1991). Estimating WAIS-R IQ from the National Adult Reading Test: a cross-validation. *Journal of Clinical and Experimental Neuropsychology* **13**, 204–216.

WHO (1997). Cannabis: a health perspective and research agenda (WHO/MSA/PSA/97.4). Geneva: World Health Organization.

Young A, Perret D, Calder A, Sprengelmeyer R, Ekman P (2002). *Facial Expressions of Emotion: Stimuli and Tests* (*FEEST*). England: Thames Valley Test Company.