Methods of the Pharmacological Imaging of the Cannabinoid System (PhICS) study: towards understanding the role of the brain endocannabinoid system in human cognition

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Abstract

Various lines of (pre)clinical research indicate that cannabinoid agents carry the potential for therapeutic application to reduce symptoms in several psychiatric disorders. However, direct testing of the involvement of cannabinoid brain systems in psychiatric syndromes is essential for further development. In the Pharmacological Imaging of the Cannabinoid System (PhICS) study, the involvement of the endocannabinoid system in cognitive brain function is assessed by comparing acute effects of the cannabinoid agonist Δ9-tetrahydrocannabinol (THC) on brain function between healthy controls and groups of psychiatric patients showing cognitive dysfunction. This article describes the objectives and methods of the PhICS study and presents preliminary results of the administration procedure on subjective and neurophysiological parameters. Core elements in the methodology of PhICS are the administration method (THC is administered by inhalation using a vaporizing device) and a comprehensive use of pharmacological magnetic resonance imaging (phMRI) combining several types of MRI scans including functional MRI (fMRI), Arterial Spin Labeling (ASL) to measure brain perfusion, and resting-state fMRI. Additional methods like neuropsychological testing further specify the exact role of the endocannabinoid system in regulating cognition. Preliminary results presented in this paper indicate robust behavioral and subjective effects of THC. In addition, fMRI paradigms demonstrate activation of expected networks of brain regions in the cognitive domains of interest. The presented administration and assessment protocol provides a basis for further research on the involvement of the endocannabionoid

systems in behavior and in psychopathology, which in turn may lead to development of therapeutic opportunities of cannabinoid ligands. *Copyright © 2011 John Wiley & Sons, Ltd.*

Introduction

The present paper describes the objectives and methods of a large Dutch pharmacological magnetic resonance imaging (MRI) project investigating the neurophysiological role of the brain endocannabinoid (eCB) system in cognitive disorders, impulse control and addiction. The current project was designed in line with the recommendations of the World Health Organization's (WHO's) Priority Medicines project (Kaplan and Laing, 2004), which identifies "pharmaceutical gaps": diseases that pose high burdens to society, but where effective pharmacological treatment either does not exist or is inadequate. Against this background, Top Institute (TI) Pharma was founded in the Netherlands, in 2006. TI Pharma is a public private partnership (PPP) consisting of industrial and academic research teams and conducts cross-disciplinary research that addresses a large number of the diseases mentioned in the WHO's Priority Medicines project. Among these diseases are several brain diseases, such as cognitive decline in Alzheimer's disease and several psychiatric disorders with a neurobiological basis, including depression, schizophrenia and addiction. One of the projects funded by TI Pharma addresses the role of the brain eCB-system in the regulation of neurotransmission and the therapeutic opportunities of cannabinoid ligands. The presently described Pharmacological Imaging of the Cannabinoid System (PhICS) study is part of this broader TI Pharma project on the neurophysiological role of the eCB-system.

The eCB-system is ubiquitously present in the brain and is involved in many physiological functions, such as pain, food intake, and cognitive processing (Lupica *et al.*, 2004; Witkin *et al.*, 2005; Ranganathan and D'Souza, 2006). It consists of cannabinoid receptors and endocannabinoids ligands that work on these receptors. There are at least two different cannabinoid receptors, but in the brain CB1-receptors are the most important and they are widely distributed throughout the brain (see for extensive reviews on the eCB-system, Ameri, 1999; Wilson and Nicoll, 2002; Piomelli, 2003). The two most important and best studied endogenous cannabinoid ligands are anandamide and 2-arachidonoylglycerol (2-AG). Endocannabinoids are synthesized on demand, and act as retrograde messengers, which means that when

necessary, they are released post-synaptically and work on pre-synaptic receptors, thereby regulating the release of both inhibitory and excitatory neurotransmitters (Wilson and Nicoll, 2002; Piomelli, 2003). As such, the eCB-system acts as a modulating system which is involved in the control of many brain functions including learning and memory, emotion and reward (Lupica *et al.*, 2004; Witkin *et al.*, 2005; Ranganathan and D'Souza, 2006).

Modulation of the eCB-system by administering exogenous cannabinoids such as Δ9-tetrahydrocannabinol (THC), the main psychoactive constituent of cannabis (Gaoni and Mechoulam, 1964), produces a diverse range of acute effects by activating the CB1-receptor. Apart from the euphoriant effect or "high" (D'Souza *et al.*, 2004; Ilan *et al.*, 2004; Bossong *et al.*, 2009), THC also induces impairments in working memory (D'Souza *et al.*, 2004; Ilan *et al.*, 2004, 2005), episodic memory (Curran *et al.*, 2002; see for a review Ranganathan and D'Souza, 2006), and attention (Casswell and Marks, 1973; Marks and MacAvoy, 1989; Ramaekers *et al.*, 2009). THC also affects impulse control (McDonald *et al.*, 2003; Ramaekers *et al.*, 2006). High-dose intoxication with cannabis can result in acute psychosis, usually of a transient nature (Chopra and Smith, 1974; Thomas, 1996).

THC possesses rewarding properties: it is self-administered by monkeys (Tanda *et al.*, 2000) and enhances striatal dopamine levels in both animals (Tanda *et al.*, 1997) and humans (Bossong *et al.*, 2009; see for a review Lupica *et al.*, 2004).

The cognitive domains that are affected by THC show overlap with domains typically impaired in psychiatric disorders. PhICS aims at studying intermediate phenotypes, by coupling non-specific cognitive symptoms, i.e. symptoms that go beyond specific disorders, to brain function when manipulated with THC administration. For example, working memory dysfunction is an established cognitive impairment in schizophrenia, but not selectively so. Working memory deficits are also common in substance abuse disorders and obsessive compulsive disorder (OCD). For PhICS, we selected five psychiatric disorders where a link has been established between the eCB-system and cognitive symptoms that characterize these disorders, including schizophrenia, addiction, attention deficit hyperactivity disorder (ADHD), depression and OCD. Figure 1
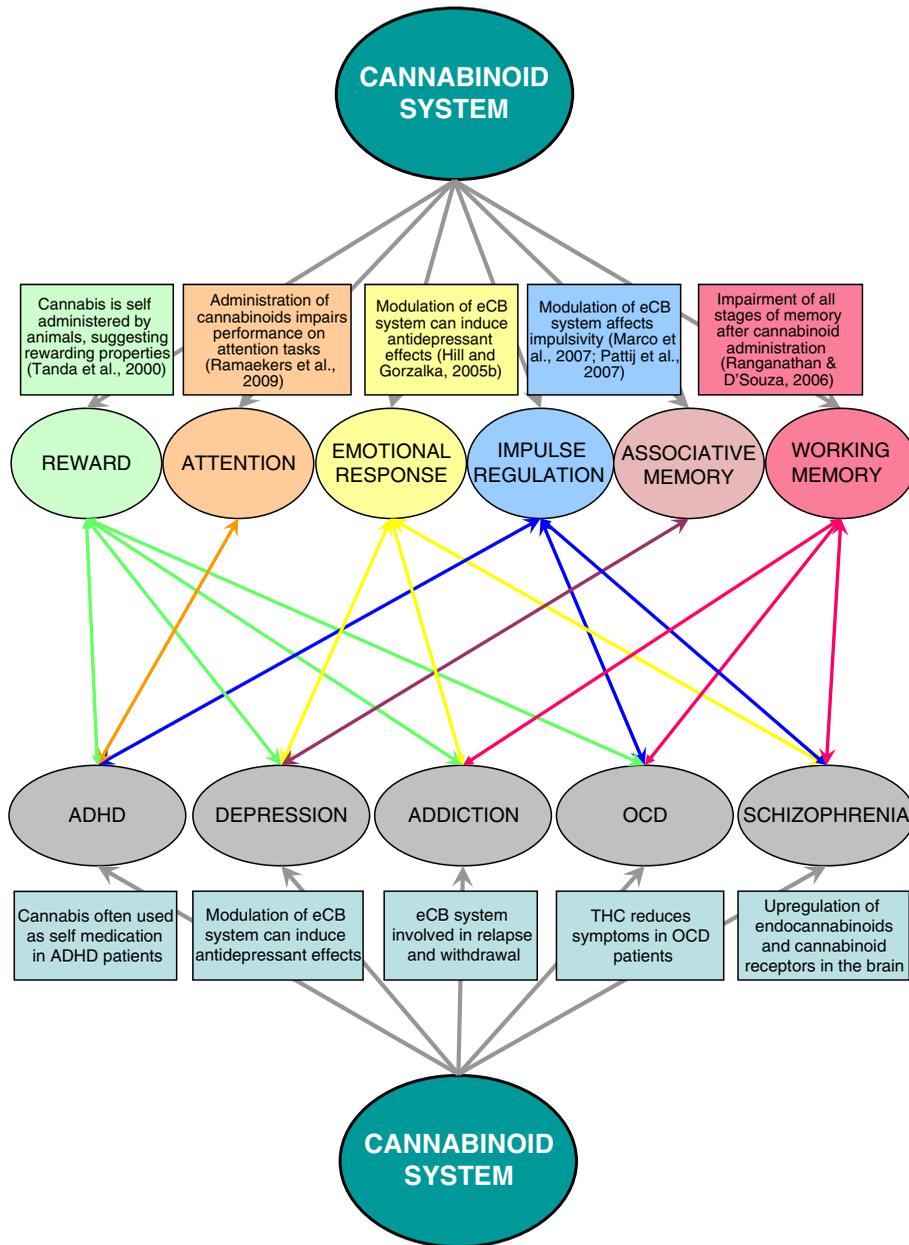
Figure 1 A schematic presentation of the rationale behind the PhICS study. There is evidence for involvement of the eCB system in both psychiatric disorders (lower part) and different domains of cognition (upper part). Impairments in cognition are significant symptoms in psychiatric disorders (see also Table 2). Since psychiatric disorders can be considered as a composition of specific symptoms rather than individual disorders, we focus in the PhICS study on the role of the eCB-system in cognitive symptoms. The colored arrows indicate the cognitive domains that are studied in the respective patient groups.

summarizes the relationship between the eCB-system, cognitive domains of interest and these psychiatric disorders.

There is substantial evidence that the eCB-system is involved in schizophrenia. First of all, it is known that cannabis use increases the risk for developing schizophrenia

(Arseneault *et al.*, 2004; Moore *et al.*, 2007) and worsens its clinical outcome (Linszen *et al.*, 1994; D'Souza *et al.*, 2005). Further, patients with schizophrenia demonstrate both enhanced CB1-receptor densities in cortical regions (Dean *et al.*, 2001; Zavitsanou *et al.*, 2004; Newell *et al.*, 2006) and

increased levels of endogenous cannabinoids in cerebral spinal fluid (Leweke *et al.*, 1999; Giuffrida *et al.*, 2004) and plasma (De Marchi *et al.*, 2003). Finally, there is a substantial body of evidence from both pre-clinical and clinical studies that the eCB-system is involved in the cognitive dysfunction in schizophrenia, in particular in attention, learning and memory and inhibitory regulatory mechanisms (see for reviews Lichtman *et al.*, 2002; Solowij and Michie, 2007).

The eCB-system is involved in different aspects of drug addiction, including reward, withdrawal and relapse (see for reviews De Vries and Schoffelmeer, 2005; Maldonado *et al.*, 2006; Fattore *et al.*, 2007). For example, animal studies have shown that addictive properties reflected in behaviors such as self-administration or conditioned place preference of opiates, nicotine and alcohol are absent or attenuated in cannabinoid CB1-receptor knockout mice and after the administration of the CB1-antagonist rimonabant (Maldonado *et al.*, 2006). Further, CB1-agonists reinstate drug seeking behavior of drugs of abuse, whereas rimonabant blocks this effect (De Vries and Schoffelmeer, 2005; Fattore *et al.*, 2007). In humans, clinical trials are performed to investigate the effect of rimonabant on the cessation of smoking nicotine (Cahill and Ussher, 2007) and in the reduction of food intake in obesity (Christensen *et al.*, 2007).

Key symptoms of ADHD are disturbed impulse regulation and attention (Biederman and Faraone, 2005). Pre-clinical studies indicate that the eCB-system is involved in impulse regulation, since CB1-receptor agonists and antagonists, as well as inhibiting fatty acid amide hydrolase (FAAH), the enzyme responsible for the degradation of the endogenous cannabinoid anandamide, affect impulsivity (Marco *et al.*, 2007; Pattij *et al.*, 2007). Impaired performance on attention tasks after administration of cannabinoids to both animals and humans indicates the involvement of the eCB-system in attention (Verrico *et al.*, 2004; Ramaekers *et al.*, 2009). The cognitive deficits in ADHD may be caused by a dysregulation of dopaminergic frontal-subcortical circuits, also affecting the reward system (Scheres *et al.*, 2007; Plichta *et al.*, 2009).

In depression, the role of the eCB-system is less straightforward (see for reviews Witkin *et al.*, 2005; Hill and Gorzalka, 2005a). Pre-clinical studies have demonstrated that both facilitation (Hill and Gorzalka, 2005b; McLaughlin *et al.*, 2007) and inhibition (Shearman *et al.*, 2003; Griebel *et al.*, 2005) of endocannabinoid signaling can induce antidepressant effects. However, this seems at odds with clinical trials testing rimonabant for the treatment of obesity that report depressed mood and anxiety as the most common adverse events (Christensen *et al.*, 2007).

In OCD impairments in working memory, attention and impulse regulation are core symptoms (de Geus *et al.*, 2007). As mentioned earlier, there are several indications that the eCB-system is involved in these symptoms (see for a review Solowij and Michie, 2007). Interestingly, treatment with THC reduces obsessive compulsive symptoms in patients with Gilles de la Tourette-Syndrome (Muller-Vahl *et al.*, 2002) and OCD (Schindler *et al.*, 2008).

In summary, various lines of pre-clinical and clinical research indicate that the eCB-system plays a role in the pathophysiology of cognitive dysfunction in various psychiatric disorders. Hence, cannabinoid agents carry the potential to become novel pharmaceutical agents for treatment of symptoms of psychiatric disorders. However, direct testing of the involvement of cannabinoid brain system in psychiatric symptomatology is essential for further development. Most importantly, we need to systematically assess whether the cannabinoid brain system indeed affects the cognitive symptoms and associated brain functions that are implied on the basis of (pre)clinical research (see Figure 1).

The PhICS study is unique in its multi-disciplinarity and the wide array of convergent methods used. Core methodology in PhICS involves measuring brain function in humans with a neuroimaging technique called pharmacological MRI (phMRI) (see for a review Honey and Bullmore, 2004). phMRI is a powerful tool to map direct modulation of brain function by psychopharmacological agents, in this case the CB1-agonist THC. By comparing acute effects of THC administration on brain function between psychiatric patients with specific cognitive impairments and healthy controls, we explore the role of the eCB-system in the regulation of cognitive brain function in these populations. The purpose of this paper is to present the background and methodology of the PhICS study.

Design

General design of the PhICS study

To unravel the role of the eCB-system in cognitive symptoms of psychiatric disorders both healthy volunteers and psychiatric patients take part in the PhICS study. Five groups of patients with a specific psychiatric disorder, including schizophrenia, depression, ADHD, OCD, and addiction, are composed. These patient groups are selected based on symptomatology and the indication of involvement of the eCB-system in these symptoms (see Figure 1). Each patient group is compared with a group of matched healthy controls. All subjects participate in a double-blind, randomized,

placebo-controlled, crossover phMRI study and are scanned and tested on two separate study days after the inhalation of either placebo or THC. During scanning participants perform cognitive functional MRI (fMRI) tasks. Using this approach, brain activity patterns in brain networks can be compared between placebo and THC sessions and between healthy controls and psychiatric patients (Latin square design). All measurements take place at the University Medical Center Utrecht, The Netherlands.

Subjects

For each patient group 12 patients are recruited. We include only males due to expected interactions between hormonal cycle and brain activity patterns in women, which will flaw the design. In addition, there is evidence for gender differences in the effects of THC (Craft, 2005). Patients with more than one psychiatric disorder are excluded from the study. Each patient group is analyzed separately and is compared to healthy controls matched on age, IQ, socio-economical status and nicotine and alcohol use. All subjects are current incidental cannabis users, defined as having used cannabis more than four times a year and less then once a week in the year preceding the first MRI scan. During screening and at the beginning of each study day, urine drug screens for cannabis, cocaine, amphetamine, methamphetamine, morphine, benzodiazepines and ecstasy are performed. Subjects with a positive drug test on other drugs than cannabis are excluded from the study. Subjects with a positive cannabis test at screening are tested again, and are required to be negative before the first study day. All subjects undergo a physical examination performed by a physician, to establish good physical health before entering the study. All volunteers give written informed consent before entry into the study and are paid 250 euros for participation. See Table 1 for all criteria of participation.

Procedure

Prior to the first study day subjects are familiarized with the scanner environment using a mock scanner, a replica of a standard MRI scanner. The MRI procedure is fully described to the subjects and the fMRI tasks are practiced.

The actual study consists of two test days, separated by at least two weeks to allow for complete clearance of drugs between both occasions. Subjects have fasted for at least four hours before their arrival at the hospital. Subjects need to refrain from cannabis for at least two weeks before the first study day until study completion and from alcohol for 48 hours before each study day.

Table 1 Inclusion and exclusion criteria for participants

Inclusion criteria
- Male
- Current occasional cannabis use since at least one year (<1/week and ≥4/year) without known negative implications (e.g. bad trip, cannabis-induced psychosis)
- Right-handedness, assessed with the Edinburgh Handedness Inventory (Oldfield, 1971)
- Written informed consent of the subject

For patients:
- Diagnosed with one psychiatric disorder ([1] schizophrenia; [2] depression; [3] ADHD; [4] addiction to nicotine (heavy smokers); [5] OCD) according to DSM-IV criteria, axis I

Exclusion criteria
- Clinical significant abnormalities, except for the predetermined psychiatric disorder
- First degree relatives with a psychiatric disorder according to DSM-IV criteria (healthy controls only)
- Impaired physical health evaluated by medical history and physical (including neurological) examination
- History of alcohol and/or drug abuse (DSM-IV criteria) except for nicotine in the addiction group
- Past but recent diagnosis of abuse of drugs or alcohol other than tobacco, i.e. within 12 months preceding study inclusion
- Body mass index (BMI) <18 kg/m^2 or >28 kg/m^2
- Any subject who received any investigational medication within 90 days prior to the start of the study or who is scheduled to receive an investigational drug
- The use of any medication within three weeks prior to the start of the study, except for paracetamol and medication for the psychiatric disorder
- Blood donation within three months before the start of the study
- Claustrophobia
- Metal objects in or around the body (braces, pacemaker, metal fragments)

Caffeine intake and smoking is not allowed from the moment of arrival until the end of a study day. Use of drugs of abuse, including cannabis, is checked with urine drug screenings and use of alcohol, caffeine, and nicotine is checked by self-report. A standard meal is served and symptomatology is assessed in patients using a disorder-specific symptom scale. An intravenous catheter is placed in the arm for venous blood sampling.

The scan session includes three fMRI scans during a cognitive challenge. Sequence of the tasks is randomized between subjects, but remains unchanged within subjects across sessions. In addition to fMRI, Arterial Spin Labeling (ASL) techniques and resting state fMRI are applied to measure THC-induced effects on cerebral

blood flow and default brain activity respectively. Finally, the scan protocol includes a three-dimensional (3D)-anatomical scan for registration purposes. After the scanning session a neuropsychological test battery is performed outside the scanner. Subjective and psychedelic effects of THC are measured at fixed intervals during the test day using visual analogue scales. Heart rate and respiration are monitored continuously during scanning sessions. See Figure 2 for a schematic outline of a study day. Subjects are allowed to go home when subjective and physiological effects are normalized, and after permission of a psychiatrist.

Drugs and administration

THC or placebo is administered by inhalation using a Volcano® vaporizer (Storz & Bickel GmbH, Tuttlingen, Germany). This is a novel safe, effective and reproducible mode of intrapulmonary THC administration (Hazekamp *et al.*, 2006; Abrams *et al.*, 2007). It overcomes disadvantages of other administration methods, such as the limited and variable bioavailability of oral administration and the inhalation of toxic compounds produced by burning cannabis. In addition, the administration of pure THC prevents co-administration of other psychoactive compounds contained in cannabis. Final pulmonal uptake, plasma concentrations and subjective effects of THC are similar for smoking and vaporizing cannabis (Hazekamp *et al.*, 2006; Abrams *et al.*, 2007), making the Volcano® vaporizer pre-eminently suitable for studies investigating the eCB-system in humans with a pharmacological challenge.

THC is purified from Cannabis sativa according to Good Manufacturing Practice (GMP)-compliant procedures (Farmalyse BV, Zaandam, The Netherlands) and each milligram of THC is dissolved in 100 μl 100 vol% alcohol. The solvent is used as placebo. Five minutes before administration, THC is vaporized at a temperature of 225°C into an opaque polythene bag equipped with a valved mouthpiece, preventing the loss of THC in between inhalations. Subjects inhale the volume of this bag in 2–3 minutes, holding their breath for 10 seconds after each inhalation. They are not allowed to speak during the inhalation process, which is practiced at screening using placebo.

On study days, subjects receive subsequent doses of THC or placebo. The first THC dose is 6 mg, followed by four doses of 1 mg each to maintain equal levels of central nervous system (CNS) effects. These doses are based on pharmacokinetic/pharmacodynamic (PK/PD) modeling of the CNS effects induced by THC (Strougo *et al.*, 2008).

Assessments

Symptomatology, IQ and personality

Since differences in severity of psychiatric symptoms may affect brain activity patterns, symptomatology of patients is assessed on both study days. We determine this using validated disorder-specific symptom scales. For schizophrenia patients, the Positive and Negative Syndrome Scale (PANSS) is used (Kay *et al.*, 1987). For OCD patients the Y-BOX questionnaire is used. The ADHD rating scale is used for assessing symptomatology in ADHD patients (Kooij *et al.*, 2008), the Beck Depression Inventory for depressive patients (Beck *et al.*, 1961), and the Fägerstrom Test for Nicotine Dependence (FTND) for smokers (Heatherton *et al.*, 1991). An estimate of verbal IQ is obtained by the Dutch version of the National Adult Reading Test (DART). Personality questionnaires (the Sensation Seeking Scale [SSS; Zuckerman and Link, 1968] and the Behavioral Inhibition Scale/Behavioral Activation Scale [BIS/BAS; Carver and White, 1994]) are administered to improve interpretation of fMRI results.

Brain scans

Functional MRI

Image acquisition is performed on a Philips Achieva 3.0 Tesla MR scanner with a Quasar dual gradient set. A 3D-PRESTO-SENSE scan protocol is used for all fMRI tasks,

Figure 2 Outline of a study day. The scanning session comprises three fMRI scans during a cognitive challenge. In addition, an anatomical scan, a resting state scan and ASL scans are acquired. Subjects receive subsequent doses of THC or placebo. The first THC dose is 6 mg, followed by four doses of 1 mg each to maintain equal levels of CNS effects. After the scanning session a neuropsychological test battery is performed outside the scanner.

as well as the resting state scan (Neggers *et al.*, 2008) (scan parameters: TR 22.5 ms; TE 33.2 ms; flip angle = 10°; FOV 224 × 256 × 160; matrix 56 × 64 × 40; voxel size 4.0 mm isotropic; scan time 0.6075 seconds; 40 slices; sagittal orientation). A high-resolution volume with a flip angle of 27° (FA27) is scanned after each task for registration purposes. Before the functional imaging runs, a high-resolution whole brain anatomical image is performed (scan parameters: TR 9.4 ms; TE 4.7 ms; flip angle = 8°; FOV 220.8 × 240 × 159.6; matrix 368 × 400 × 113; voxel size 0.6 mm × 0.6 mm × 0.6 mm, 266 slices; sagittal orientation).

fMRI data are preprocessed and analyzed using SPM5 (Wellcome Trust Centre for Neuroimaging, London, UK). Preprocessing of data includes re-alignment of functional images and coregistration with the anatomical scan using the high-resolution volume. Subsequently, functional scans are normalized into standard MNI space and smoothed (Full width at half maximum [FWHM] = 8 mm). Statistical analysis is performed for every fMRI task separately using a General Linear Model (GLM) repeated measures model, implemented in SPM5.

All subjects perform three fMRI tasks activating different networks of brain regions. These tasks differ between the psychiatric patient groups. For each group of patients and matched controls three relevant fMRI tasks are selected based on the cognitive domains impaired in a specific psychiatric disorder. For example, since impairments in attention, impulse regulation and reward are associated with ADHD (Biederman and Faraone, 2005; Scheres *et al.*, 2007), these patients perform fMRI tasks known to activate brain networks underlying these cognitive domains. Table 2 shows the impaired cognitive domains in each of the psychiatric disorders and the fMRI tasks that are performed. Below are descriptions of the different fMRI tasks that are used in the PhICS study.

Working memory. Working memory is assessed using a modified version of the Sternberg recognition task (Sternberg, 1966). The task involves memorizing sets of consonants and deciding whether subsequently presented letters belong to the set or not. The number of consonants will vary between memory sets (1, 3, 5, 7, and 9 consonants respectively) to create different levels of working memory load. Cognitive processing during this task consistently activates a well-defined fronto-parietal network of brain regions (Jansma *et al.*, 2001; Ramsey *et al.*, 2004; Jager *et al.*, 2006).

Reward. To activate reward circuitry an adapted version of the monetary incentive delay task as developed by Knutson and colleagues is used (Knutson *et al.*, 2001). In this task, subjects need to press a button as fast as possible on seeing a target stimulus. Depending on both the cue that precedes the target stimulus and reaction time, subjects can either win or lose a certain amount of money. Brain activity of both anticipation and outcome of reward and loss is assessed.

Attention. Sustained attention is measured with a continuous performance task, using an identical pairs paradigm. This task is adapted from Strakowski *et al.* (2004) and consists of a continuous stream of four-digit numerals presented every 0.75 seconds. Subjects are instructed to press a button whenever the same four-digit numeral appears twice in succession during the sequence. In healthy volunteers this paradigm activates networks of brain regions including both anterior structures involved in attentional processes (prefrontal cortex, anterior cingulate cortex and insula) and posterior structures involved in integrating sensory information (temporal cortex, parietal cortex and fusiform gyrus) (Adler *et al.*, 2001; Strakowski *et al.*, 2004).

Impulse regulation. As a measure of impulsivity, brain activity underlying inhibitory motor control is assessed with a Stop Signal Task (Li *et al.*, 2006; Chevrier *et al.*, 2007). In this task, subjects need to press a button when they are presented with a visual stimulus. On a subset of trials this go signal is followed by a stop signal, which instructs participants to cancel or withdraw their ongoing response on that particular trial. This inhibition of a response is shown to rely on frontal and striatal brain activation (Vink *et al.*, 2005; Li *et al.*, 2006; Chevrier *et al.*, 2007).

Emotion. Brain activity involved in processing of emotion is assessed with a task adapted from Hariri and colleagues, measuring the neural response to happy and fearful faces (Hariri *et al.*, 2002). Subjects are presented with a trio of faces and select one of the two bottom faces that express the same emotion as the target face on top. The target and congruent probe face display either a fearful or happy expression and the other probe face always displays a neutral expression. Fearful and happy faces are presented in different blocks, interleaved with a control task in which geometric shapes are shown. This task has been shown to reliably and robustly engage a network involved in emotional processing including the amygdala (Hariri *et al.*, 2002; Phan *et al.*, 2008).

Associative memory. Associative memory is assessed with a pictorial task involving three different task conditions.

Table 2 Overview of the psychiatric patient groups involved in the PhICS study, together with the cognitive domains impaired in the respective disorder and the functional MRI and neuropsychological tasks performed to study the cognitive domains

Psychiatric disorder	Cognitive impairment	fMRI task	CANTAB/neuropsychological test
Screening test			Motor screening
ADHD	Impulse regulation	Stop Signal Task	Stop Signal Task
	Attention	Attention Task	Simple and Five-choice Reaction Time Task
	Reward	Monetary Reward Task	Cambridge Gambling Task
	Working memory		One Touch Stockings of Cambridge + Spatial Working Memory
	Associative memory		Paired Associates Learning
OCD	Impulse regulation	Stop Signal Task	Stop Signal Task
	Working memory	Working Memory Task	One Touch Stockings of Cambridge + Spatial Working Memory
	Reward	Monetary Reward Task	Cambridge Gambling Task
Schizophrenia	Impulse regulation	Stop Signal Task	Stop Signal Task
	Working memory	Working Memory Task	One Touch Stockings of Cambridge + Spatial Working Memory
	Emotion	Emotional Faces Task	Affective Go-Nogo Task
	Attention		Simple and Five-choice Reaction Time Task
	Associative memory		Paired Associates Learning
Addiction	Impulse regulation	Stop Signal Task	Stop Signal Task
	Reward	Monetary Reward Task	Cambridge Gambling Task
	Working memory	Working Memory Task	One Touch Stockings of Cambridge + Spatial Working Memory
	Emotion		Affective Go-Nogo Task
	Associative memory		Paired Associates Learning
Depression	Reward	Monetary Reward Task	Cambridge Gambling Task
	Emotion	Emotional Faces Task	Affective Go-Nogo Task
	Associative memory	Associative Memory	Paired Associates Learning
	Attention	Task	Simple and Five-choice Reaction Time Task

Abbreviations: ADHD, attention deficit hyperactivity disorder; OCD, obsessive compulsive disorder.

First, an associative learning phase is conducted which requires subjects to remember a specific combination of pictures and to establish a meaningful connection between the two pictures. In the next phase simple pictures have to be judged, which serves as a control task. Finally, in a retrieval phase subjects have to retrieve specific combinations previously presented during associative learning. In healthy volunteers this task reliably reveals brain activity in the hippocampus and the (para)hippocampal gyrus bilaterally, especially during the associative learning condition (Henke *et al.*, 1997; Jager *et al.*, 2007a).

Arterial Spin Labeling (ASL)

Since fMRI measures the BOLD (blood oxygen level dependent) signal, THC-induced global changes in cerebral blood flow may affect the fMRI findings. ASL permits the non-invasive quantification of global and regional brain perfusion (see for a review Petersen *et al.*, 2006). As such, ASL provides additional physiological data, that facilitate interpretation of fMRI findings and enables us to correct for THC-induced effects on blood flow.

ASL scans are acquired before and after administration of both placebo and THC. Pseudo-continuous labeling is performed by employing a train of Hanning-shaped RF pulses (tip angle 18°, duration 0.5 ms) with an interpulse pause of 0.5 ms in combination with a balanced gradient scheme. The duration of labeling is 1650 ms. The control situation is achieved by adding 180° to the phase of every other RF pulse. ASL imaging is performed combined with background suppression (a saturation pulse immediately before labeling and inversion pulses at 1680 and 2830 ms after the saturation pulse). We use single-shot echo planar imaging (EPI) in combination with parallel imaging

(SENSE factor 2.5). In total, 17 slices of 7 mm slice thickness are acquired in ascending fashion with an in-plane resolution of 3×3 mm^2. Imaging is performed 1525 ms after labeling stops. The total scan time for a pair of control and label images is eight seconds. For measurement of the magnetization of arterial blood (M_0) and also for segmentation purposes, an inversion recovery sequence is acquired with the same geometry and resolution as the ASL sequence (inversion times 100–1900 ms with 200-ms intervals, preceded by a saturation pulse at ~1680 ms) (van Osch *et al.*, 2009).

Resting state fMRI

Obviously, the brain is not inactive during rest, and a resting state network has been identified representing the state of the human brain in the absence of goal-directed neuronal action or external input (Damoiseaux *et al.*, 2006). Effects of THC on this resting state activity may affect the fMRI findings. We obtain resting state fMRI data to assess if and how THC affects brain activity patterns during rest.

Subjective effects

Subjective and psychedelic effects are measured regularly throughout study days. A rating scale consisting of 16 visual analogue scales is used to determine subjective effects. From these analogue scales three factors are calculated, corresponding to alertness, contentedness and calmness (Bond and Lader, 1974). Psychedelic effects are assessed using an adapted version of a 13-item visual analog rating scale, originally described by Bowdle and colleagues (Bowdle *et al.*, 1998; Zuurman *et al.*, 2008). The visual analog scale "Feeling High" is analyzed individually and composite scores of "External Perception" and "Internal Perception" are calculated. Changes in external perception reflect a misperception of an external stimulus or a change in the awareness of the subject's surroundings. Internal perception reflects inner feelings that do not correspond with reality (Zuurman *et al.*, 2008). A computerized version of both rating scales is performed consecutively.

Physiological measurements

Heart rate and respiration are monitored continuously during scanning. Before and after scanning blood pressure and heart rate are measured regularly at fixed intervals.

Pharmacokinetics

Venous blood samples are collected to determine plasma concentrations of THC and its two most important metabolites, 11-OH-THC and 11-nor-9-carboxy-THC. Blood samples are processed according to Zuurman *et al.* (2008).

Neuropsychological tests

After scanning, neuropsychological tests are applied to measure the acute behavioral effects of THC on cognitive task performance. The results of these tests are related to the fMRI results. This provides insight in the behavioral correlates of brain activity findings and improves our understanding of the neurophysiological basis of the CB1-mediated behavioral effects of THC. Testing is done using a comprehensive set of eight subtests of the Cambridge Neuropsychological Test Automated Battery (CANTAB®), including motor screening (MOT), Spatial Working Memory (SWM), paired associative learning (PAL), One Touch Stockings of Cambridge (OTS), reaction time (RTI), Stop Signal Task (SST), Cambridge Gambling Task (CGT) and an Affective Go-Nogo Task (AGN) (for details on task formats see www.cantab.com). Each patient group and matched healthy control group perform those neuropsychological tests that match the cognitive domains of the fMRI tasks they have performed (see Table 2).

Statistics

Power analysis

In fMRI studies, a sample size of 12 subjects is considered to be sufficient for reliable measurement of cognition-related functional brain activity patterns (Zandbelt *et al.*, 2008). Previous studies with THC administration show significant effects between THC and placebo on response inhibition and emotional processing with groups of 15 and 16 subjects respectively (Borgwardt *et al.*, 2008; Phan *et al.*, 2008). However, in these studies THC was administered orally. Since THC plasma concentrations are much higher after intrapulmonary compared to oral administration (Bossong *et al.*, 2009; Zuurman *et al.*, 2008), we expect to detect the same degree of THC-induced effects with a sample size of 12 subjects. In addition, groups of 12 subjects provide ample power to demonstrate significant subjective and psychedelic effects of THC (Bossong *et al.*, 2009; Zuurman *et al.*, 2008) and to detect differences in cerebral blood flow between patients and healthy controls using ASL (O'Gorman *et al.*, 2008). To ensure a minimum sample size of 12 subjects per group, inclusion will continue until 12 complete and qualitatively good datasets per group have been acquired (i.e. patient dropout or data loss due to movement and/or technical malfunction will not affect eventual sample size).

Statistical analyses

All obtained parameters are compared between psychiatric patient groups and matched healthy controls using analysis of variance (ANOVA) with group (patient versus control) and drug manipulation (THC versus placebo) as within-subject factors. *Post hoc t*-tests are performed for further exploration of significant effects.

Analysis of fMRI data consists of three steps (see Table 3). First, in fMRI paradigms a specific cognitive process is switched on and off within minutes: periods involving the cognitive process of interest alternate with periods of rest and/or a control task. Using a subtraction method, contrasting activation during task performance with activation during rest (on versus off) results in a measure of brain activity that reflects the pattern of activity specific for the cognitive process of interest. For each subject, both the pattern and the magnitude of brain activity during the cognitive process under investigation are computed. Second, this on versus off contrast is compared between THC and placebo sessions to determine the effect of THC administration on brain activity. Third, the effect of THC is compared between patients and healthy controls.

Ethical considerations

The PhICS study is approved by the Medical Ethics Committee of the University Medical Center Utrecht, The Netherlands. To rule out any suggestion that we approve or stimulate the use of cannabis, the consent form, which patients and healthy volunteers have to sign, states that participation is voluntary, that cannabis is potentially harmful and that the researchers do not have the intention to stimulate the use of cannabis. To minimize the risk of an adverse reaction to THC-administration, we only include subjects with previous experience with cannabis (incidental users), who have not experienced negative effects (e.g. bad trip, panic attack, cannabis-induced psychosis) (see Table 1).

According to Good Clinical Practice guidelines, data monitoring is performed by an independent monitor. In addition, an independent psychiatrist acts as a patient safety monitor, and evaluates the safety of both patients and healthy controls.

Results

We present data supporting proof of concept of the PhICS study in terms of the effect of the THC challenge procedure on physiological and subjective measures. Results from the involved patient groups and their matched controls will be published in due time in peer-reviewed international journals. The effects of the THC-challenge indicate robust effects on the CNS level. In addition, imaging results from placebo sessions in healthy volunteers display the (expected) networks of brain areas involved in three (out of six) fMRI task paradigms applied in PhICS.

Physiological and subjective effects

This section describes THC-induced physiological and subjective effects in a group of 13 healthy subjects (all male, age 21.6 ± 2.1 [standard deviation]).

THC plasma concentrations

THC plasma concentration reached a maximum of 58.1 ± 31.3 (standard error of the mean [SEM]) ng/ml five minutes after inhalation of 6 mg THC and decreased rapidly thereafter. Subsequent doses of 1 mg THC induced peaks in THC plasma concentration of 13.7 ± 7.7, 13.0 ± 3.8 and 13.8 ± 6.0 ng/ml five minutes after each respective dose. 11-Nor-9-carboxy-THC showed a stable plasma concentration over time with a maximum of 5.4 ± 1.8 ng/ml 87 minutes after the first THC administration. Plasma concentration of 11-OH-THC peaked at five minutes after the first inhalation (2.8 ± 3.0 ng/ml) (see Figure 3).

Table 3 The three steps of fMRI data analysis in the PhICS study. For each subject, brain activation during a cognitive process is compared with a period of rest. Then, brain activity after placebo administration is contrasted with that after THC administration. Finally, the effect of THC administration on brain activation during a cognitive process is compared between patients and controls

|	What effect?	Comes from?	What is compared?
1.	Effect of cognitive process	fMRI paradigm	On versus off
2.	Effect of THC	THC administration	THC versus placebo
3.	Effect on symptom	Psychiatric disorders	Patient versus control

Heart rate

Heart rate was measured at 20 timepoints during the test days. Per subject, heart rate scores (beats per minute [bpm]) were mean corrected, for placebo and THC sessions separately. Figure 4 depicts average mean corrected heart rate curves (±SEM; $N = 13$) over time during placebo and THC sessions. GLM repeated

Plasma Concentration THC
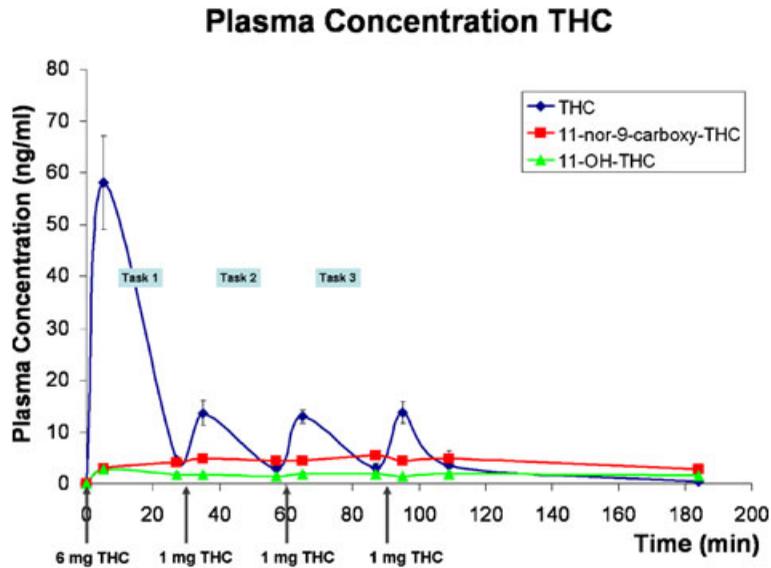
Figure 3 Plasma concentrations of THC and its main metabolites 11-OH-THC and 11-nor-9-carboxy-THC after inhalation of 6, 1, 1 and 1 mg THC with 30 minutes intervals (mean ± standard error of mean [SEM]; $n = 13$). At the X-axis vertical arrows indicate the time points of THC administration. The task blocks in the figure indicate time blocks when fMRI tasks were administered in randomized order.
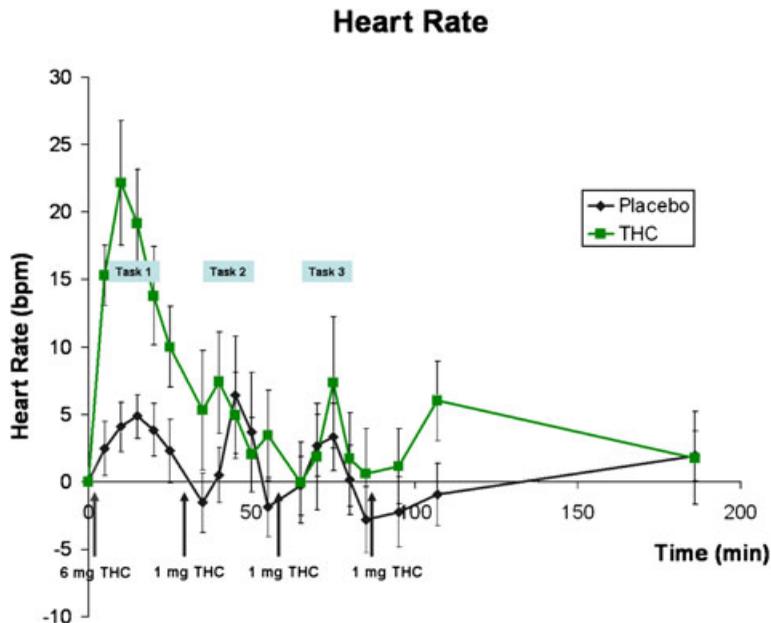
Heart Rate

Figure 4 Mean corrected heart rate in beats per minute (bpm) over time. Error bars denote standard errors of mean (SEM). At the X-axis vertical arrows indicate the time points of THC administration. The task blocks in the figure indicate time blocks when fMRI tasks were administered in randomized order.

measures analysis showed that heart rate was significantly increased in response to the THC-challenge compared to placebo ($F(1,11) = 10.2$, $p < 0.01$).

**Subjective effects (Visual Analogue Scales)**

As expected, THC administration induced a significant increase in the psychedelic effect modality "Feeling High" ($F(1,12) = 12.6$, $p < 0.01$) (see Figure 5). Administration of subsequent doses of THC with 30 minutes intervals maintained equal levels of CNS effects, as indicated by an absence of a significant effect of time on VAS "Feeling High" across the three fMRI sessions ($p = 0.25$). No effect was found in the psychedelic modality internal perception (reflecting inner feelings that do not correspond with reality). External perception (reflecting misperception of external stimuli or changes in the awareness of the environment) showed a marginal increase after THC administration compared to placebo ($F(1,12) = 3.4$, $p = 0.091$). Also, as expected "Alertness" was significantly reduced after THC ($F(1,12) = 6.9$, $p < 0.05$) and showed an interaction effect of drug $\times$ time ($F(2,11) = 4.0$, $p = 0.05$), indicating subjects were feeling less alert throughout the scanning procedure under the influence of THC. Contentedness and calmness did not show significant effects of THC administration. Together, the

subjective effects indicate a robust effect of THC administration on subjects feeling intoxicated, but not to an extent that they were no longer able to perform the cognitive tasks.

Imaging data

Figure 6 displays an overview of brain regions activated in healthy volunteers during placebo sessions for the Associative Memory Task ($N = 13$), the Working Memory Task ($N = 19$) and the Stop Signal Task ($N = 11$) respectively.

For associative memory (upper panel Figure 6), activated areas were based on group activation maps (thresholded at $T = 4.5$, $p < 0.05$, corrected for multiple comparisons) in the associative learning condition. The network activated (not all areas shown in Figure 6) comprised areas in the cerebellum, fusiform and parahippocampal gyrus, lingual gyrus, middle occipital gyrus, the inferior frontal gyrus and insula (all bilateral), and in the left supplementary motor area and the right putamen. These regions corresponded to the network we expected to be activated during this task, and were similar to the network of brain regions found in previous fMRI studies from our laboratory using the same task paradigm in different groups of subjects (Jager *et al.*, 2007a, 2007b, 2008).
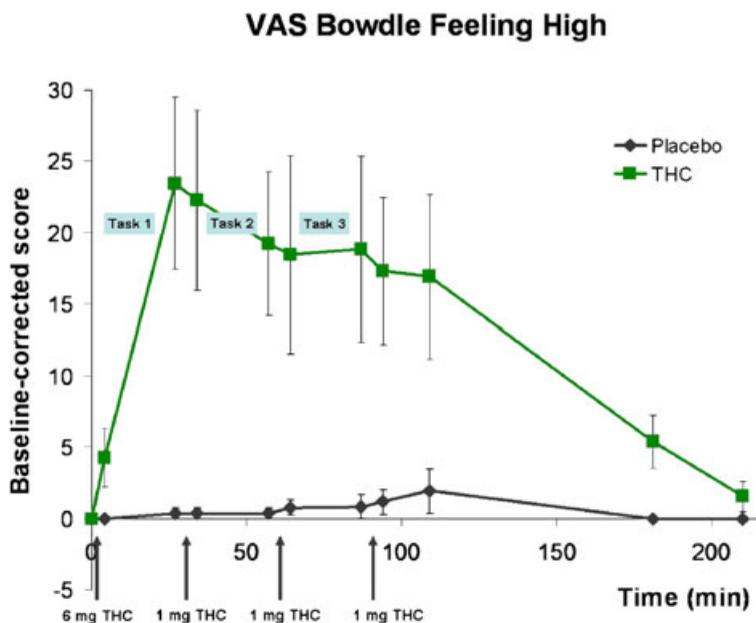
Figure 5 Mean corrected VAS scores of Feeling High over time in minutes. Error bars denote standard errors of mean (SEM). At the X-axis vertical arrows indicate the time points of THC administration. The task blocks in the figure indicate time blocks when fMRI tasks were administered in randomized order.
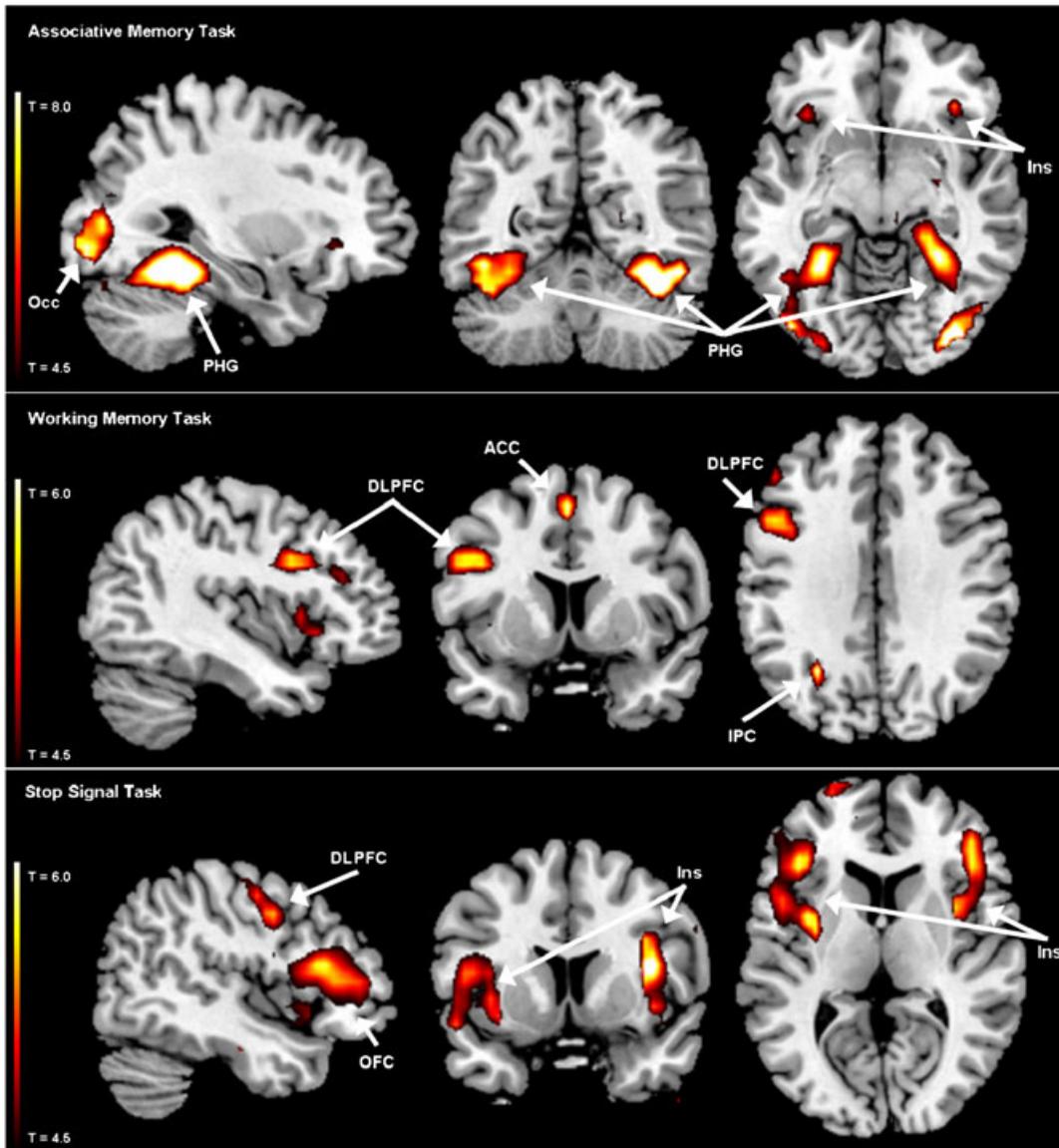
Figure 6 Overview of brain regions critically involved in associative memory, working memory and impulse regulation as measured with the task paradigms used in PhICS. All activation maps reflect supra-threshold ($T > 4.5$, $p < 0.05$, corrected for multiple comparisons) group brain activity during placebo conditions. Maps are presented in neurological orientation (left side is left hemisphere). Upper panel: Group activation map ($N = 13$) of the Associative Memory Task in the associative learning condition. PHG = parahippocampal gyrus, Ins = insula, Occ = occipital gyrus. Middle panel: Group activation map of the Working Memory Task, contrasting working memory load seven (memory set of seven consonants) with load one. DLPFC = dorsolateral prefrontal cortex, ACC = anterior cingulate cortex, IPC = inferior parietal cortex. Lower panel: Group activation map of the Stop Signal Task, contrasting go trials with successful stop trials. DLPFC = dorsolateral prefrontal cortex, Ins = insula, OFC = orbitofrontal cortex.

For working memory, the network of activated regions shown (middle panel Figure 6) was based on group activation contrast maps (threshold value $T = 4.5$, $p < 0.05$, corrected for multiple comparisons), contrasting brain activation during a high working memory load (memory set of seven consonants) with activity during the control condition (memory set of one consonant; no working memory load). This yielded a network including areas in the dorsolateral prefrontal cortex, the inferior parietal cortex, the insula (all bilateral) and the anterior cingulate

cortex. These areas are well known to play a role in working memory brain function using similar task paradigms, as has been shown in previous studies (Rypma and D'Esposito, 1999; Jager *et al.*, 2006; Ramsey *et al.*, 2004).

Brain activity during the Stop Signal Task (measuring inhibition and impulse regulation) was defined as brain activation during go trials contrasted with activity during successful stop trials. Group activation contrast maps (threshold value $T = 4.5$, $p < 0.05$, corrected for multiple comparisons) yielded a network of regions including the dorsolateral prefrontal cortex, the orbitofrontal cortex, and the insula bilaterally (see Figure 6, lower part). These regions have been shown to be critically involved in aspects of impulse regulation (Li *et al.*, 2006, 2009).

Discussion

The PhICS study is a randomized, double-blind, crossover, placebo-controlled phMRI study that investigates the involvement of the eCB-system in cognitive brain function and whether alterations in endocannabinoid signaling may be involved in cognitive dysfunction in patients with a psychiatric disorder.

In the present methodological manuscript results are reported on the physiological and subjective effects of a pharmacological challenge with THC (initial dose 6 mg, followed by three upload doses of 1 mg each, with 30 minute intervals) in healthy volunteers. Our findings of THC-induced effects on heart rate and subjective effects like "Feeling High" confirm the validity of the applied pharmacological manipulation of the eCB-system. Brain imaging data of the placebo sessions demonstrate that brain activation during specified cognitive challenges can be adequately assessed using the proposed paradigms. The PhICS study will progress investigating the effects of a THC challenge on brain activation patterns related to cognitive domains of interest in groups of psychiatric patients showing cognitive dysfunction in one or more domains, as well as in matched healthy volunteers.

Psychiatric disorders are selected based on evidence for a link between the eCB-system and cognitive symptomatology and include schizophrenia, depression, OCD, ADHD, and addiction. Brain activity is measured during tasks that cover six different cognitive domains, including working memory, associative memory, reward, attention, emotion, and response inhibition. Brain activity is also measured in rest, and the influence of THC on brain perfusion is assessed. To investigate the effects of THC on behavioral measures, a neuropsychological test battery is performed.

The PhICS study fits within the recommended research areas for brain disorders, as reported in WHO's Priority Medicines project, and is embedded in the Dutch PPP initiative TI Pharma. PhICS is part of a consortium project consisting of industrial and academic research teams that addresses the role of the brain eCB-system in the regulation of brain functions implicated in psychopathological syndromes. The project involves both pre-clinical and clinical research and combines technologies ranging from *in vitro* approaches to behavioral models matched between animals and humans. It is expected this multi-disciplinary approach will lead to an integrated systems model on the neurophysiological role of the eCB-system. An important challenge within the consortium is translating animal findings on eCB functioning in models that can be applied in humans and vice versa. The PhICS study is designed in such a way that findings can be linked to ongoing or future animal work. For example, phMRI measures the effects of THC, a pharmacological agent, on the BOLD signal – which is a meaningful but indirect measure of brain activity. Knowledge on molecular, electrophysiological and neurochemical mechanisms of action of cannabinoids obtained from animal studies, adds to a meaningful interpretation of phMRI findings in humans. In addition, human pharmacological fMRI studies face the challenge to interpret observed alterations in brain activity and explain their functional relevance. Brain activity as measured with BOLD fMRI is affected by physiological processes, e.g. direct effects of the administered drug on brain vasculature, perfusion, oxygen saturation, heart rate and blood pressure. These effects, either in isolation or synergistically, may also induce changes in the BOLD-signal. An important strength of the multi-disciplinary study design of PhICS is the measurement of many other phsysiological functions besides changes in brain activity. These data will guide the interpretation of potentially increased or decreased brain activity during cognitive processing under the influence of THC, and help determine its functional relevance. Apart from it strengths, the design and methodology of PhICS as presented in this paper has some limitations as well. For one, all subjects will be occasional cannabis users. The choice for incidental cannabis users, as opposed to non-users, is primarily driven by ethical constraints pertaining to patients in that research suggests a role for cannabis use in, for instance, schizophrenia. Even though there is no direct evidence for a causal relationship, it is prudent to limit inclusion for THC administration studies to subjects who have already used cannabis in a recreational context.

Additional motivation for inclusion of incidental canna-bis users as opposed to cannabis-naïve subjects is that they can be expected to tolerate the THC challenge used in this experiment with a minimal risk for adverse reactions. The risk of chronic neuroadaptation due to infrequent use, which would limit generalizability of findings to the population at large, can in our opinion be considered as minimal given the ethical constraints, but needs to be kept in mind. A second limiting factor is that, in the presented study design, the effects of the pharmacological challenge (THC) likely provide feed-back that undermines blinding, and may cause expectancy effects in participants. We try to minimize the influence of expectancy by the use of a randomized crossover design. All subjects receive both THC and placebo on two separate sessions. By randomizing the order of administration of the psychoactive drug and placebo between subjects (50% of the subjects receive THC first, 50% placebo first), expectancy effects will be balanced across sessions. Still, we cannot exclude that expectancy effects may affect the results of the study to some extent and we will report on this in future papers.

Patient groups participating in the PhICS study are selected based on symptomatology and the supposed involvement of the eCB-system in these symptoms (see Figure 1), with a focus on "intermediate phenotypes" (Gottesman and Gould, 2003). That is, we focus on the role of the eCB-system in cognitive symptoms present in psychiatric disorders rather than on the role of this system in the disorders themselves. This is based on the notion that psychiatric disorders are a composition of specific symptoms instead of individual disorders. Where cognitive symptoms overlap, the involved brain systems may share common ground as well. For example, the impaired ability to process emotions is present in both schizophrenia and depression. In both disorders, dysfunction of the limbic areas, amygdala and prefrontal cortex has been postulated (Whalen et al., 2002; Fakra et al., 2008) and in both disorders there is tentative evidence for the involvement of the eCB-system in emotional deregulation. With PhICS, we are the first to systematically explore the effects of a THC-challenge on cognitive brain function both in healthy volunteers and patients with a psychiatric disorder. We search beyond the disorder itself to find a general deficit which may be related to a malfunc-tioning eCB-system.

Examples of the type of questions that can be asked and the type of answers that could be expected from PhICS, thanks to the multi-disciplinary approach and use of convergent methods, include the following. We expect that the THC challenge has differential effects on brain activation, depending on the patient population and the cognitive domain. If we assume that cognitive brain function ranges from normal (in healthy controls) to abnormal (in patients) on a gradual scale, THC-induced effects may vary both in degree and in direction. Regarding the direction of the effect, one option is that THC induces a shift in brain activity in healthy controls in the direction of patients, thus resulting in patient-like abnormalities in cognitive brain function. At the behavioral level, this phenomenon has been observed in healthy volunteers who can experience (temporary) psychotic-like symptoms after use of high doses of cannabis (D'Souza et al., 2004). A similar effect may occur for brain activation. For the patients, a THC-challenge may further aggravate cognitive dys-function, both at the behavioral and the neurophysi-ological level. This has already been observed at the behavioral level, as we know that chronic cannabis use can trigger more severe psychotic symptoms and relapse in schizophrenic patients (D'Souza et al., 2005; Grech et al., 2005). THC may also be beneficial in a specific patient group for a specific symptom, meaning that the patients become more similar to healthy controls. For example, at the behavioral level there is some support that use of cannabis does, at least in the short term, diminish negative symptoms associated with schizo-phrenia, such as anhedonia, apathy and social with-drawal (Compton et al., 2004).

Expanding our knowledge of the eCB-system is highly relevant both from a fundamental scientific perspective as well as from a clinical point of view, because dysfunction of the eCB-system may be one of the factors that can explain specific cognitive symptoms in psychi-atric and neurological disorders. When we know how the eCB-system is involved, the next step may be develop-ment of medication influencing this system to relief these symptoms. Thus, the results from the PhICS study are likely of great interest for research and develop-ment departments of pharmaceutical companies. Other future research directions include confirmation of and expanding the findings of the PhICS study via converg-ing methods. For example, future pharmacological study designs could be applied in humans using direct or indirect endocannabinoid antagonist. In addition, block-ing the degradation of endocannabinoids in humans with a FAAH inhibitor (FAAH is the enzyme that breaks down endocannabinoids once they are released) would be an interesting step forward, since the eCB-system can then be challenged locally and only when it is

activated. Finally, with regard to potential differences in endocannabinoid neurochemistry between psychiatric patients and healthy volunteers, an interesting question regarding cause or consequence arises. Has the system been altered by the illness, or has the illness been altered by the system? It is a challenge to assess these questions, but future studies may consider more longitudinal follow-up designs or (epi)genetics to target research questions like these.

Declaration of interest statement

The authors have no competing interests.

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