Role of the endocannabinoid system in human brain functions relevant for psychiatric disorders

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Role of the endocannabinoid system in human brain functions relevant for psychiatric disorders

De rol van het endocannabinoïde systeem bij humane hersenfuncties relevant voor psychiatrische aandoeningen

(met een samenvatting in het Nederlands)

Proefschrift

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Contents

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Introduction

General introduction

Smoking cannabis produces a diverse range of acute effects. The best known effect and the main reason to use cannabis is the feeling of euphoria, better known as 'feeling high' or 'being stoned'. Further, people may have alterations in the perception of their surroundings, or may experience episodes of increased laughter and appetite. Cannabis use can also induce acute changes in feelings of anxiety, acute impairments in memory, reduced impulse control, and mild hallucinatory effects. All these effects are predominantly caused by Δ9-tetrahydrocannabinol (THC), the main psychoactive component in cannabis. THC exerts its effects through action on the cannabinoid receptors in the brain. However, the main role of these receptors is obviously not to facilitate acute effects induced by an exogenous substance such as THC. They have an important biological function in binding cannabis-like molecules that are produced in our brain.

Some acute effects of cannabis show overlap with symptoms of psychiatric disorders. For example, patients with schizophrenia typically have feelings of anxiety, impairments in memory, altered impulse control and hallucinations. This is not only true for schizophrenia, also symptoms of depression, attention-deficit hyperactivity disorder (ADHD) or addiction show similarities with the effects of cannabis. This suggests that the cannabinoid receptors and the cannabis-like molecules in the brain, collectively referred to as the endocannabinoid system, could play a role in symptoms of psychiatric disorders. When it is understood if and how the endocannabinoid system is involved, new research could focus on the relief of symptoms by manipulating this system.

This chapter describes the general scope of this thesis, provides an introduction on the endocannabinoid system, describes the research questions that are addressed in this thesis, and explains the neuroimaging techniques that are used to answer these questions. In addition, there is an introduction on brain functions in which the role of the endocannabinoid system has been assessed, and an outline of the current thesis is given.

Scope of this thesis

All studies described in this thesis are part of the Pharmacological Imaging of the Cannabinoid System (PhICS) project, a comprehensive research project on the role of the endocannabinoid system in the regulation of brain function in healthy volunteers and patients with a psychiatric disorder.

The aim of this thesis is to gain novel insights into the role of the endocannabinoid system in several human brain functions, including associative memory, working memory, executive function and emotional processing. These cognitive functions are also affected in psychiatric disorders such as schizophrenia, depression or ADHD. In addition, endocannabinoid involvement in regulation of dopamine release in the striatum is addressed, as this is a robust pathophysiological feature of both schizophrenia and addiction.

The role of the endocannabinoid system in cognitive domains that are associated with symptoms of addiction, such as reward processing and response inhibition, is described in a related thesis by Erika van Hell, entitled: "Endocannabinoid involvement in reward and impulsivity in addiction".
All described studies use neuroimaging techniques to measure and visualize brain function, in combination with challenging of the endocannabinoid system of healthy volunteers with administration of THC. Similarities in brain function between healthy volunteers after THC administration and psychiatric patients would provide an argument for possible involvement of the endocannabinoid system in symptoms of psychiatric disorders.

The endocannabinoid system

The endocannabinoid system is ubiquitously present in the brain and is involved in many brain functions, such as memory, mood and reward processing. It consists of cannabinoid receptors and endocannabinoid ligands that work on these receptors[1,2]. At least two types of cannabinoid receptors have been identified, being the CB_1 and CB_2 receptor[3,4]. CB_2 receptors are mainly found in the peripheral tissue[4], whereas CB_1 receptors are abundantly present in the central nervous system. CB_1 receptors are widely distributed throughout the brain with the highest densities in the basal ganglia, cerebellum, hippocampus and cortex[5,6]. Most of the psychoactive effects of cannabinoid substances are mediated through activation of CB_1 receptors[7,8]. The two most important endocannabinoid ligands binding to these receptors are anandamide and 2-arachidonylglycerol (2-AG)[9-11]. They act as retrograde messengers, which means that they are synthesized and released postsynaptically and bind to presynaptic receptors, thereby regulating the release of both inhibitory and excitatory neurotransmitters (see Figure 1.1). This signaling works according to an 'on-demand' principle: endocannabinoids are released when and where they are needed[1,2,12]. As such, the endocannabinoid system acts as a 'fine tuning' system that is involved in the control of learning and memory, emotion, reward, movement and pain relief[13-18].

Challenging the endocannabinoid system

In the studies described in this thesis, the endocannabinoid system is challenged using the partial CB_1 agonist THC. The main reason for using this compound is because other pharmacological agents that challenge the endocannabinoid system are either still under investigation in preclinical studies (for instance, indirect agonists altering levels of endogenous cannabinoids) or withdrawn from the market due to the occurrence of severe side effects (the CB_1 antagonist rimonabant). A validated method to administer THC in humans is by using an intrapulmonary route (inhalation), in which purified THC is dissolved in a small amount of alcohol, and vaporized into a balloon with a Volcano® Vaporizer[19,20]. This produces significant and dose-dependent physiological responses, which allows for the use of this method in clinical studies.

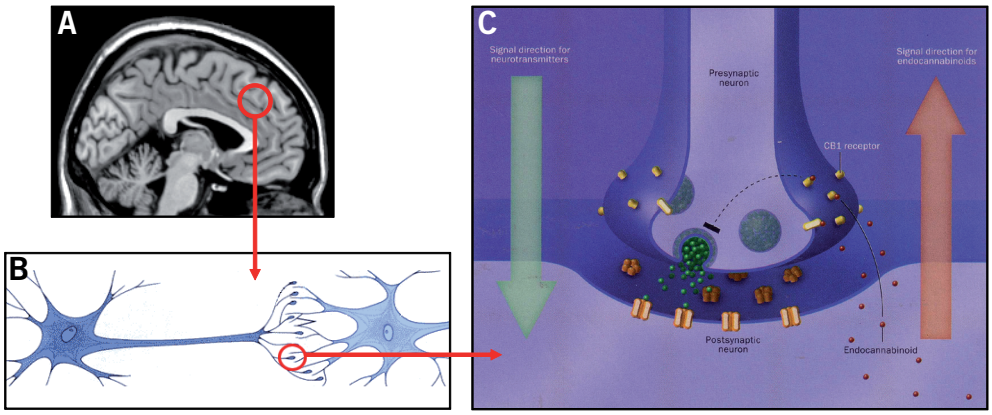

Figure 1.1 The human brain from macro- to microscopic level. **A**, Sagittal view of the brain. **B**, Two neighboring neurons in an arbitrary brain region (adapted from www.dancesafe.org). **C**, Communication between two neurons. Information is transferred from the presynaptic to the postsynaptic neuron through neurotransmitters that cross the synaptic cleft (green arrow). The endocannabinoid system controls neurotransmitter release in a retrograde manner: endocannabinoids are released postsynaptically and bind to presynaptically located cannabinoid receptors (red arrow) (from Kraft U. (2006) Natural high. *Scientific American Mind*, 17, 60 - 65).

Neuroimaging techniques

The aim of this thesis is to elucidate the role of the endocannabinoid system in human brain functions that are relevant for psychiatric disorders. Methods used to address this aim are Positron Emission Tomography (PET) and functional Magnetic Resonance Imaging (fMRI). We use PET in combination with a pharmacological challenge (**Chapter 2**), whereas fMRI is used with a simultaneous pharmacological and cognitive challenge (**Chapter 4 - 7**).

Positron Emission Tomography (PET)

The study described in **Chapter 2** has used PET to measure striatal dopamine levels. PET is a quantitative nuclear medicine imaging technique that visualizes neurophysiological processes in the body with the use of radioactive tracers. These tracers are radioactive isotopes that are incorporated either into a biologically active molecule such as glucose or water, or into molecules that bind to receptors or other sites of drug action. Once introduced into the body, positron emission by the tracer induces pairs of gamma rays that are recorded by the PET scanner. PET recordings show the total radioactivity concentration in living tissue. For example, using this technique, differences in metabolism or receptor density can be investigated between groups or scanning sessions[21-24].

Functional Magnetic Resonance Imaging (fMRI)

The studies described in **Chapter 4 - 7** have used fMRI to measure brain activity. fMRI is a non-invasive measurement of brain activity. It is not an absolute measure of brain activity, but does provide a reliable measure of acute changes in brain activity with a high spatial resolution.

fMRI is sensitive to the so-called Blood Oxygenation Level-Dependent (BOLD) contrast[25]. The BOLD contrast is based on changes in blood oxygenation in the brain. This pattern of changes in blood oxygenation has good correspondence with underlying changes in neuronal activity[26,27]. It can be reliably reproduced between sessions if averaged over several subjects[28].

fMRI is typically used with a cognitive challenge. A local signal change that is correlated in time with the cognitive challenge, for instance with performance of a cognitive task versus performance of a control task, is interpreted as an indication that that region of the brain is involved in that task.

When fMRI is used in combination with a pharmacological challenge it is referred to as pharmacological fMRI (phMRI). Task-related brain activity changes after administration of a certain drug can be compared to those after placebo. Hence phMRI can offer a non-invasive technique to assess neurophysiological processes caused by that specific drug[29]. In particular, phMRI is a very useful technique to study the effects of a pharmacological manipulation in a specific cognitive domain, as fMRI is often combined with a cognitive task[30]. This makes this technique highly suitable for use in the studies described in this thesis, as the role of the endocannabinoid system in specific cognitive domains is investigated through challenging the system with THC.

Due to the complex nature of fMRI data, different approaches are applicable in terms of statistical analysis. First, a whole-brain analysis gives information about the experimental condition in the entire brain, but, as there are at least 20,000 voxels to be compared to each other, a very stringent correction for multiple comparisons is needed to reduce the chance of a false positive finding. Second, a region of interest (ROI) analysis can be performed. This allows for a more powerful analysis, by only testing hypotheses in brain areas of interest. Basically, there are two valid ROI approaches. ROIs can be chosen on the basis of the underlying brain function, which is often referred to as 'functionally defined ROIs'[31]. The other way of performing an ROI analysis is the 'anatomically defined ROI analysis', which means that there is a specific hypothesis about a particular brain area. In the fMRI studies described in this thesis, data are analyzed using an ROI analysis based on functionally defined ROIs, i.e. all areas specifically involved in a particular task. The choice for an ROI approach was primarily driven by the advantages of this approach with respect to specificity and sensitivity. In addition, it allows for calculation and presentation of effect sizes. We chose to perform analyses on functionally defined ROIs because of the complexity of the design, and the exploratory and broad character of the studies.

Brain functions

Striatal dopamine release

Research Question 1

Does the endocannabinoid system regulate dopamine release in the human striatum?

A common feature of rewarding experiences in life is that they induce release of dopamine in a brain region called the striatum. This is known for food and sex, but also rewarding properties of addictive drugs are thought to be mediated by enhancing synaptic dopamine levels in the striatum[32,33]. Using neuroimaging techniques, increased dopamine levels have been found in the human striatum after the administration of amphetamine[21,34-37], cocaine[38], alcohol[39], and nicotine[40,41]. In animals, it has been demonstrated that cannabinoid substances such as THC enhance neuronal firing of mesolimbic dopamine neurons[42-44] and elevate striatal dopamine levels[45-49], both through activation of cannabinoid CB_1 receptors[42-44,47,48].

In addition, increased striatal dopamine function is one of the most robust pathophysiological features of schizophrenia. This has been acknowledged for years, as therapeutic effects of antipsychotic drugs directly relate to the blockade of striatal dopamine receptors[50,51], and dopamine enhancing drugs are able to induce direct psychotic effects[52,53]. Neuroimaging studies have consistently shown that baseline levels of synaptic dopamine and striatal dopamine release in response to amphetamine are increased in schizophrenia patients[34,35,54]. This effect is directly related to the severity of amphetamine-induced psychotic symptoms and the response to subsequent antipsychotic treatment[54,55].

Synaptic dopamine levels in the striatum can be measured with the use of PET and the [^{11}C]raclopride displacement paradigm. This paradigm provides an indirect measure of *in vivo* synaptic dopamine concentration by quantifying the change in dopamine D_2/D_3 receptor availability to the binding of [^{11}C]raclopride. A reduction in striatal [^{11}C]raclopride binding to dopamine D_2/D_3 receptors will reflect an increase in striatal dopamine levels, which is expected after THC administration (see Figure 1.2).

Enhanced dopamine levels in the human striatum after THC administration would suggest endocannabinoid control over striatal dopamine release. This would indicate an important role for the endocannabinoid system in psychiatric disorders such as schizophrenia and addiction. **Chapter 2** describes the effects of THC administration on dopamine release in the striatum of healthy volunteers, as measured with PET and the [^{11}C]raclopride displacement paradigm.

Memory encoding and recall

Research Question 2

How is the endocannabinoid system involved in memory encoding and recall processes?

Impairments in long-term memory can be due to insufficiencies in either of two processes involved: encoding or recall of information. Both memory processes are thought to rely on

functioning of the medial temporal lobe and prefrontal areas[56,57], and deficits in memory function are associated with various psychiatric and neurological disorders, such as Alzheimer's disease, schizophrenia and mood disorders[57,58].

Evidence for impact of cannabinoid intoxication on human learning and memory performance is not univocal. A large number of studies have reported no acute effects of cannabinoid administration on learning and memory paradigms[59-66]. Other studies did indicate memory impairments after administration of cannabinoids[67-74]. However, although these cannabinoid-induced effects are statistically significant, most of them are relatively small. Interestingly, these small effects of cannabinoids on memory are usually reported in the free recall of information that is previously learned under the influence of cannabinoids[67,68,73], whereas recall of items acquired before cannabis use is generally not affected[75-77]. This suggests that cannabinoids influence encoding but not recall of information.

A valid fMRI paradigm to measure memory encoding and recall processes is a pictorial associative memory task[78,79]. This paradigm involves three different task conditions. First, an encoding condition is conducted which requires subjects to remember a specific combination of two pictures. In the second phase, single item pictures must be classified, which serves as a control task. Finally, in a recall condition subjects have to recognize specific combinations of pictures previously presented during the encoding phase. In healthy volunteers, this task reliably reveals brain activity in a memory network including (para)hippocampal and prefrontal areas[78,79].

Effects of THC administration on encoding and recall brain function could further indicate how the endocannabinoid system is involved in both memory processes. **Chapter 4** shows task accuracy and brain activity patterns after THC administration during performance of a pictorial associative memory paradigm.

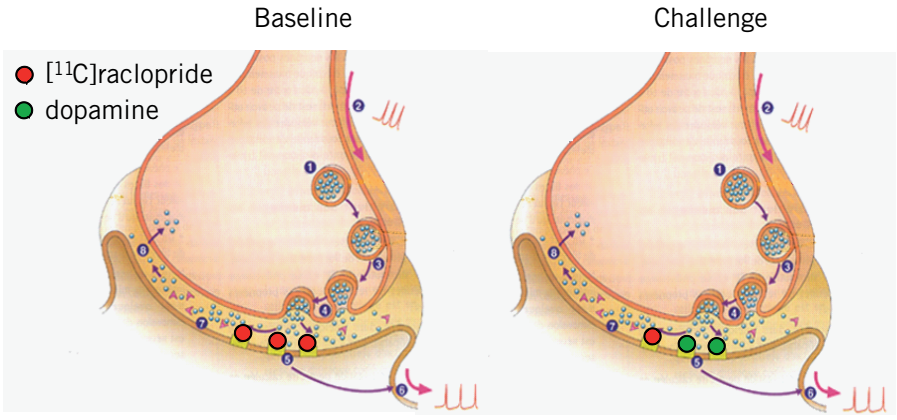

Figure 1.2 Schematic representation of the [^{11}C]raclopride displacement paradigm. **Left**, At baseline, [^{11}C]raclopride binds to available striatal dopamine D_2/D_3 receptors. **Right**, A reduction in the binding of [^{11}C]raclopride reflects an increase in striatal dopamine levels (adapted from http://droguesetcerveau.free.fr).

Research Question 3

What is the role of the endocannabinoid system in working memory efficiency?

Working memory is the ability for short term storage and manipulation of information[80], and depends on functioning of a network of brain regions including the prefrontal and parietal cortex[56,81]. Impairment of working memory has been described for psychiatric disorders such as addiction and ADHD[82,83], and is considered a core feature of schizophrenia[84].

Working memory can be measured with a Sternberg item-recognition paradigm (SIRP) containing increasing levels of difficulty[85,86]. In the version of the SIRP described in **Chapter 5**, participants are instructed to memorize alternating sets of one, three, five, seven or nine consonants. After presentation of a memory set, eight single consonants are displayed in sequence, and subjects have to indicate whether these probes were present in the preceding memory set.

It has been shown that during SIRP performance, brain activity increases linearly with increasing working memory load, tapering off until a maximum is reached (Figure 1.3a)[87-89]. In addition, performance is high until task load causes a gradual increase in errors (Figure 1.3b). Neuroimaging studies have shown that schizophrenia patients often exhibit a reduced load-dependent increase in brain activity, together with enhanced brain activity for tasks with low working memory load (Figure 1.3a, gray line)[90-94]. This has led to the theoretical notion that impaired cognitive function in schizophrenia is related to neurophysiologically inefficient working memory function[95,96]. According to this working memory inefficiency hypothesis, both brain activity and performance levels that are normally related to a higher working memory load will already occur at a lower load (Figure 1.3).

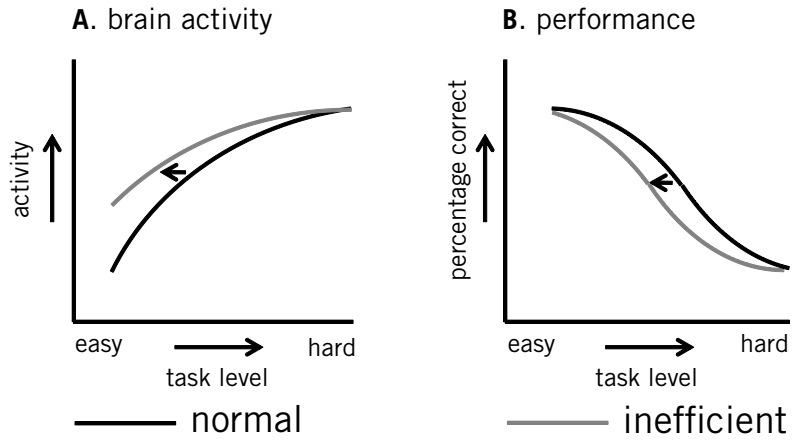

Figure 1.3 Effect of working memory inefficiency on profile of brain activity and performance in a parametric design. **A**, A shift of the load-response curve to the left will effectively reduce the load-dependent increase in brain activity, while increasing activity for easy tasks. **B**, A shift of the performance curve to the left will effectively cause a drop off in performance at a lower load.

Inefficiency refers to the disproportionate magnitude of brain activity in relation to workload, and compromised performance as a result. Similarities in working memory-related performance and brain activity patterns between healthy volunteers after THC administration and patients with schizophrenia would provide an argument for involvement of the endocannabinoid system in cognitive symptoms of these patients. **Chapter 5** shows THC-induced profiles of brain activity and SIRP performance in healthy subjects.

Executive function

Research Question 4

How is the endocannabinoid system involved in executive function?

The term executive function describes a set of high level cognitive functions that are essential for goal-directed behavior. They influence more basic functions such as attention, memory and motor skills. Executive function includes the ability to monitor and change behavior as needed, and to plan behavior in novel tasks and situations. Impaired executive function is considered a fundamental cognitive deficit in neurological and psychiatric disorders such as schizophrenia, Alzheimer's disease and ADHD[82,97-100].

Modulation of the endocannabinoid system by administration of exogenous cannabinoids such as THC impairs performance on various executive function paradigms in both animals[101,102] and humans[103-110]. Two major mechanisms can be distinguished through which the endocannabinoid system could influence executive function. First, it may directly affect brain processes involved in task performance. These effects are expected to be reflected in activity changes in brain regions involved in the central executive system[111,112]. Second, the endocannabinoid system could influence task performance through involvement in regulation of activity in a set of brain regions called the default mode network[113]. It has been shown that goal-directed behavior is associated with reduced activity in this network[114,115], and that failure to reduce default mode activity is related to task errors[116-121].

Executive function can be assessed with the use of a continuous performance task with identical pairs (CPT-IP)[122-124]. Performance of this task requires processing of a continuously changing stream of data[122,125], and is characterized by a heavy reliance on executive function while short-term memory load is relatively small[80,126]. In this paradigm, participants are presented with a series of four-digit numbers, and are instructed to press a button when two consecutive numbers are identical. Previous neuroimaging studies using CPT paradigms have shown activation of an executive system predominantly consisting of frontal and parietal regions[123,124].

Determining executive function after THC administration in terms of task performance and brain activity in both activated and deactivated networks could further elucidate the role of the endocannabinoid system in this cognitive domain. **Chapter 6** shows task accuracy and brain activity patterns after THC administration during performance of a CPT-IP.

Emotional processing

Research Question 5

What is the role of the endocannabinoid system in emotional processing?

Impaired processing of emotions is an important characteristic of psychiatric disorders such as major depression[127], bipolar disorder[128] and schizophrenia[129-131], with significant consequences for social functioning and subjective well-being of patients. Although imaging studies suggest an important role for the amygdala in emotional processing[132-135], it has also been posited that the amygdala is part of a large network of brain regions that regulates processing of emotions, including orbital frontal cortex, prefrontal cortex, anterior cingulate, and occipital and temporal lobes[128,136-138].

There is ample evidence for an important role for the endocannabinoid system in processing of emotions. For example, recreational cannabis users describe the euphoriant effect of cannabis as a feeling of intoxication with decreased anxiety, alertness, depression and tension[139-141]. In addition, animal studies show that disruption of endocannabinoid-mediated synaptic regulation through genetic deletion or pharmacological blockade of cannabinoid receptors produces anxiety- or depressive-like states[142-147]. Administration of low doses of cannabinoid agonists or drugs that enhance levels of endogenous cannabinoids reduces anxiety-like behavior[145,148-154].

Processing of emotional expressions can be assessed with a widely applied emotional faces task[155-157]. This paradigm consists of two conditions involving processing of fearful and happy facial expressions of emotion, respectively. Subjects are instructed to view a trio of unfamiliar faces and to select one of the two bottom faces that express the same facial emotion as the target face on top. Fearful and happy faces conditions are interspersed with a sensorimotor control condition in which subjects have to match simple geometric shapes. This task has been shown to reliably and robustly engage a network of brain regions involved in emotional processing, including the amygdala[155-157].

Effects of THC administration on brain function related to processing of positive and negative emotions could further indicate how the endocannabinoid system is involved in emotional processing. Furthermore, similarities in brain function between healthy volunteers after THC administration and psychiatric patients would indicate involvement of the endocannabinoid system in impaired processing of emotions in these patients. **Chapter 7** shows THC-induced profiles of task performance and brain activity during matching of stimuli with positive and negative content.

Outline of this thesis

The aim of this thesis is to gain novel insights into the role of the endocannabinoid system in human brain functions that are relevant for psychiatric disorders. This is achieved by

investigating brain function in healthy volunteers after acute administration of Δ9-tetrahydrocannabinol (THC), a partial agonist of the CB_1 receptor. **Chapter 2** provides results of a PET study in which the effects of THC administration on striatal dopamine release were investigated. On the basis of animal studies that showed THC-induced elevated striatal dopamine levels, it was hypothesized that THC administration would also cause dopamine release in the human striatum. In **Chapter 3**, the objectives and methods of the Pharmacological Imaging of the Cannabinoid System (PhICS) study are described. In addition, behavioral, subjective and physiological effects of the THC challenge are shown. PhICS is a comprehensive research project aimed at elucidating the role of the endocannabinoid system in symptoms of psychiatric disorders in a multidisciplinary manner. Studies addressed in this thesis were performed within the framework of PhICS. **Chapter 4** shows the role of the endocannabinoid system in human encoding and recall memory function. Based on neuropsychological studies that suggested impaired encoding of information after THC administration, reductions in encoding-related brain activity were expected. In **Chapter 5**, it is demonstrated how the endocannabinoid system is involved in working memory function. Given the ample evidence for endocannabinoid involvement in both working memory function in healthy subjects and the pathophysiology of schizophrenia, it was hypothesized that working memory function between healthy volunteers after THC administration and patients with schizophrenia would show similarities. **Chapter 6** describes the role of the endocannabinoid system in human executive function. Effects of THC administration are shown on task performance and on brain activity patterns in regions of both the (activated) central executive network and the (deactivated) default mode network. **Chapter 7** shows how the endocannabinoid system is involved in the processing of emotions. Based on the recreational effects of cannabis and findings in animal studies, it was expected that THC administration would have opposite effects on brain function related to processing of positive versus negative emotions, with a THC-induced increase in activity for positive emotions and a decrease in activity for negative emotions. Finally, in **Chapter 8**, the results, conclusions and limitations of the studies presented in the previous chapters are discussed, together with their future implications.

References

1. Wilson R.I., Nicoll R.A. (2002) Endocannabinoid signaling in the brain. Science. 296 (5568), 678-682.
2. Piomelli D. (2003) The molecular logic of endocannabinoid signalling. Nat Rev Neurosci. 4 (11), 873-884.
3. Matsuda L.A., Lolait S.J., Brownstein M.J., Young A.C., Bonner T.I. (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature. 346 (6284), 561-564.
4. Munro S., Thomas K.L., Abu-Shaar M. (1993) Molecular characterization of a peripheral receptor for cannabinoids. Nature. 365 (6441), 61-65.
5. Herkenham M., Lynn A.B., Johnson M.R., Melvin L.S., De Costa B.R., Rice K.C. (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci. 11 (2), 563-583.
6. Glass M., Dragunow M., Faull R.L. (1997) Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neuroscience. 77 (2), 299-318.
7. Huestis M.A., Gorelick D.A., Heishman S.J., Preston K.L., Nelson R.A., Moolchan E.T., Frank R.A. (2001) Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. Arch Gen Psychiatry. 58 (4), 322-328.
8. Huestis M.A., Boyd S.J., Heishman S.J., Preston K.L., Bonnet D., Le Fur G., Gorelick D.A. (2007) Single and multiple doses of rimonabant antagonize acute effects of smoked cannabis in male cannabis users. Psychopharmacology (Berl). 194 (4), 505-515.
9. Devane W.A., Hanus L., Breuer A., Pertwee R.G., Stevenson L.A., Griffin G., Gibson D., Mandelbaum A., Etinger A., Mechoulam R. (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science. 258 (5090), 1946-1949.
10. Sugiura T., Kondo S., Sukagawa A., Nakane S., Shinoda A., Itoh K., Yamashita A., Waku K. (1995) 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun. 215 (1), 89-97.
11. Stella N., Schweitzer P., Piomelli D. (1997) A second endogenous cannabinoid that modulates long-term potentiation. Nature. 388 (6644), 773-778.
12. Heifets B.D., Castillo P.E. (2009) Endocannabinoid signaling and long-term synaptic plasticity. Annu Rev Physiol. 71, 283-306.
13. Lupica C.R., Riegel A.C., Hoffman A.F. (2004) Marijuana and cannabinoid regulation of brain reward circuits. Br J Pharmacol. 143 (2), 227-234.
14. Ranganathan M., D'Souza D.C. (2006) The acute effects of cannabinoids on memory in humans: a review. Psychopharmacology (Berl). 188 (4), 425-444.
15. Hill M.N., Hillard C.J., Bambico F.R., Patel S., Gorzalka B.B., Gobbi G. (2009) The therapeutic potential of the endocannabinoid system for the development of a novel class of antidepressants. Trends Pharmacol Sci. 30 (9), 484-493.
16. Fernandez-Ruiz J., Gonzales S. (2005) Cannabinoid control of motor function at the basal ganglia. Handb Exp Pharmacol. (168), 479-507.
17. Riedel G., Davies S.N. (2005) Cannabinoid function in learning, memory and plasticity. Handb Exp Pharmacol. (168), 445-477.
18. Walker J.M., Hohmann A.G. (2005) Cannabinoid mechanisms of pain suppression. Handb Exp Pharmacol. (168), 509-554.
19. Hazekamp A., Ruhaak R., Zuurman L., van Gerven J., Verpoorte R. (2006) Evaluation of a vaporizing device (Volcano) for the pulmonary administration of tetrahydrocannabinol. J Pharm Sci. 95 (6), 1308-1317.
20. Zuurman L., Roy C., Schoemaker R.C., Hazekamp A., den Hartigh J., Bender J.C., Verpoorte R., Pinquier J.L., Cohen A.F., van Gerven J.M. (2008) Effect of intrapulmonary tetrahydrocannabinol administration in humans. J Psychopharmacol. 22 (7), 707-716.
21. Martinez D., Narendran R., Foltin R.W., Slifstein M., Hwang D.R., Broft A., Huang Y., Cooper T.B., Fischman M.W., Kleber H.D., Laruelle M. (2007) Amphetamine-induced dopamine release: markedly blunted in cocaine dependence and predictive of the choice to self-administer cocaine. Am J Psychiatry. 164 (4), 622-629.
22. McCann U.D., Szabo Z., Scheffel U., Dannals R.F., Ricaurte G.A. (1998) Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. Lancet. 352 (9138), 1433-1437.

23. Volkow N.D., Chang L., Wang G.J., Fowler J.S., Ding Y.S., Sedler M., Logan J., Franceschi D., Gatley J., Hitzemann R., Gifford A., Wong C., Pappas N. (2001) Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. Am J Psychiatry. 158 (12), 2015-2021.
24. Howes O.D., Montgomery A.J., Asselin M.C., Murray R.M., Valli I., Tabraham P., Bramon-Bosch E., Valmaggia L., Johns L., Broome M., McGuire P.K., Grasby P.M. (2009) Elevated striatal dopamine function linked to prodromal signs of schizophrenia. Arch Gen Psychiatry. 66 (1), 13-20.
25. Ogawa S., Tank D.W., Menon R., Ellermann J.M., Kim S.G., Merkle H., Ugurbil K. (1992) Intrinsic signal changes accompanying sensory stimulation: functional brain mapping with magnetic resonance imaging. Proc Natl Acad Sci U S A. 89 (13), 5951-5955.
26. Logothetis N.K., Pauls J., Augath M., Trinath T., Oeltermann A. (2001) Neurophysiological investigation of the basis of the fMRI signal. Nature. 412 (6843), 150-157.
27. Hermes D., Miller K.J., Vansteensel M.J., Aarnoutse E.J., Leijten F.S., Ramsey N.F. (2011) Neurophysiologic correlates of fMRI in human motor cortex. Hum Brain Mapp.
28. Aron A.R., Gluck M.A., Poldrack R.A. (2006) Long-term test-retest reliability of functional MRI in a classification learning task. Neuroimage. 29 (3), 1000-1006.
29. Honey G., Bullmore E. (2004) Human pharmacological MRI. Trends Pharmacol Sci. 25 (7), 366-374.
30. Stein E.A. (2001) fMRI: a new tool for the in vivo localization of drug actions in the brain. J Anal Toxicol. 25 (5), 419-424.
31. Poldrack R.A. (2007) Region of interest analysis for fMRI. Soc Cogn Affect Neurosci. 2 (1), 67-70.
32. Wise R.A. (2004) Dopamine, learning and motivation. Nat Rev Neurosci. 5 (6), 483-494.
33. Hyman S.E., Malenka R.C., Nestler E.J. (2006) Neural mechanisms of addiction: the role of reward-related learning and memory. Annu Rev Neurosci. 29, 565-598.
34. Laruelle M., Abi-Dargham A., van Dyck C.H., Gil R., D'Souza C.D., Erdos J., McCance E., Rosenblatt W., Fingado C., Zoghbi S.S., Baldwin R.M., Seibyl J.P., Krystal J.H., Charney D.S., Innis R.B. (1996) Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. Proc Natl Acad Sci U S A. 93 (17), 9235-9240.
35. Breier A., Su T.P., Saunders R., Carson R.E., Kolachana B.S., De Bartolomeis A., Weinberger D.R., Weisenfeld N., Malhotra A.K., Eckelman W.C., Pickar D. (1997) Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. Proc Natl Acad Sci U S A. 94 (6), 2569-2574.
36. Drevets W.C., Gautier C., Price J.C., Kupfer D.J., Kinahan P.E., Grace A.A., Price J.L., Mathis C.A. (2001) Amphetamine-induced dopamine release in human ventral striatum correlates with euphoria. Biol Psychiatry. 49 (2), 81-96.
37. Martinez D., Slifstein M., Broft A., Mawlawi O., Hwang D.R., Huang Y., Cooper T., Kegeles L., Zarahn E., bi-Dargham A., Haber S.N., Laruelle M. (2003) Imaging human mesolimbic dopamine transmission with positron emission tomography. Part II: amphetamine-induced dopamine release in the functional subdivisions of the striatum. J Cereb Blood Flow Metab. 23 (3), 285-300.
38. Schlaepfer T.E., Pearlson G.D., Wong D.F., Marenco S., Dannals R.F. (1997) PET study of competition between intravenous cocaine and [11C]raclopride at dopamine receptors in human subjects. Am J Psychiatry. 154 (9), 1209-1213.
39. Boileau I., Assaad J.M., Pihl R.O., Benkelfat C., Leyton M., Diksic M., Tremblay R.E., Dagher A. (2003) Alcohol promotes dopamine release in the human nucleus accumbens. Synapse. 49 (4), 226-231.
40. Brody A.L., Olmstead R.E., London E.D., Farahi J., Meyer J.H., Grossman P., Lee G.S., Huang J., Hahn E.L., Mandelkern M.A. (2004) Smoking-induced ventral striatum dopamine release. Am J Psychiatry. 161 (7), 1211-1218.
41. Brody A.L., Mandelkern M.A., Olmstead R.E., Scheibal D., Hahn E., Shiraga S., Zamora-Paja E., Farahi J., Saxena S., London E.D., McCracken J.T. (2006) Gene variants of brain dopamine pathways and smoking-induced dopamine release in the ventral caudate/nucleus accumbens. Arch Gen Psychiatry. 63 (7), 808-816.
42. French E.D., Dillon K., Wu X. (1997) Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. Neuroreport. 8 (3), 649-652.
43. French E.D. (1997) Delta9-tetrahydrocannabinol excites rat VTA dopamine neurons through activation of cannabinoid CB1 but not opioid receptors. Neurosci Lett. 226 (3), 159-162.
44. Gessa G.L., Melis M., Muntoni A.L., Diana M. (1998) Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB1 receptors. Eur J Pharmacol. 341 (1), 39-44.

45. Ng Cheong Ton J.M., Gerhardt G.A., Friedemann M., Etgen A.M., Rose G.M., Sharpless N.S., Gardner E.L. (1988) The effects of delta 9-tetrahydrocannabinol on potassium-evoked release of dopamine in the rat caudate nucleus: an in vivo electrochemical and in vivo microdialysis study. Brain Res. 451 (1-2), 59-68.
46. Chen J.P., Paredes W., Li J., Smith D., Lowinson J., Gardner E.L. (1990) Delta 9-tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely-moving rats as measured by intracerebral microdialysis. Psychopharmacology. 102 (2), 156-162.
47. Tanda G., Pontieri F.E., Di Chiara G. (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. Science. 276 (5321), 2048-2050.
48. Malone D.T., Taylor D.A. (1999) Modulation by fluoxetine of striatal dopamine release following Delta9-tetrahydrocannabinol: a microdialysis study in conscious rats. Br J Pharmacol. 128 (1), 21-26.
49. Fadda P., Scherma M., Spano M.S., Salis P., Melis V., Fattore L., Fratta W. (2006) Cannabinoid self-administration increases dopamine release in the nucleus accumbens. Neuroreport. 17 (15), 1629-1632.
50. Seeman P., Lee T. (1975) Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurons. Science. 188 (4194), 1217-1219.
51. Creese I., Burt D.R., Snyder S.H. (1976) Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science. 192 (4238), 481-483.
52. Angrist B., Van Kammen D.P. (1984) CNS stimulants as tools in the study of schizophrenia. Trends in Neurosciences. 7, 388-390.
53. Lieberman J.A., Kane J.M., Alvir J. (1987) Provocative tests with psychostimulant drugs in schizophrenia. Psychopharmacology (Berl). 91 (4), 415-433.
54. Abi-Dargham A., Rodenhiser J., Printz D., Zea-Ponce Y., Gil R., Kegeles L.S., Weiss R., Cooper T.B., Mann J.J., Van Heertum R.L., Gorman J.M., Laruelle M. (2000) Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. Proc Natl Acad Sci U S A. 97 (14), 8104-8109.
55. Laruelle M., Abi-Dargham A. (1999) Dopamine as the wind of the psychotic fire: new evidence from brain imaging studies. J Psychopharmacol. 13 (4), 358-371.
56. Cabeza R., Nyberg L. (2000) Imaging cognition II: An empirical review of 275 PET and fMRI studies. J Cogn Neurosci. 12 (1), 1-47.
57. Dickerson B.C., Eichenbaum H. (2009) The Episodic Memory System: Neurocircuitry and Disorders. Neuropsychopharmacology.
58. Cirillo M.A., Seidman L.J. (2003) Verbal declarative memory dysfunction in schizophrenia: from clinical assessment to genetics and brain mechanisms. Neuropsychol Rev. 13 (2), 43-77.
59. Zuurman L., Ippel A.E., Moin E., van Gerven J.M. (2009) Biomarkers for the effects of cannabis and THC in healthy volunteers. Br J Clin Pharmacol. 67 (1), 5-21.
60. Block R.I., Wittenborn J.R. (1984) Marijuana effects on semantic memory: verification of common and uncommon category members. Psychol Rep. 55 (2), 503-512.
61. Chait L.D., Perry J.L. (1994) Acute and residual effects of alcohol and marijuana, alone and in combination, on mood and performance. Psychopharmacology (Berl). 115 (3), 340-349.
62. Darley C.F., Tinklenberg J.R., Roth W.T., Vernon S., Kopell B.S. (1977) Marijuana effects on long-term memory assessment and retrieval. Psychopharmacology (Berl). 52 (3), 239-241.
63. Hart C.L., van Gorp W., Haney M., Foltin R.W., Fischman M.W. (2001) Effects of acute smoked marijuana on complex cognitive performance. Neuropsychopharmacology. 25 (5), 757-765.
64. Hart C.L., Ward A.S., Haney M., Comer S.D., Foltin R.W., Fischman M.W. (2002) Comparison of smoked marijuana and oral Delta(9)-tetrahydrocannabinol in humans. Psychopharmacology (Berl). 164 (4), 407-415.
65. Hart C.L., Ilan A.B., Gevins A., Gunderson E.W., Role K., Colley J., Foltin R.W. (2010) Neurophysiological and cognitive effects of smoked marijuana in frequent users. Pharmacol Biochem Behav. 96 (3), 333-341.
66. McDonald J., Schleifer L., Richards J.B., de Wit H. (2003) Effects of THC on behavioral measures of impulsivity in humans. Neuropsychopharmacology. 28 (7), 1356-1365.
67. Curran H.V., Brignell C., Fletcher S., Middleton P., Henry J. (2002) Cognitive and subjective dose-response effects of acute oral Delta 9-tetrahydrocannabinol (THC) in infrequent cannabis users. Psychopharmacology. 164 (1), 61-70.
68. D'Souza D.C., Perry E., MacDougall L., Ammerman Y., Cooper T., Wu Y.T., Braley G., Gueorguieva R., Krystal J.H. (2004) The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. Neuropsychopharmacology. 29 (8), 1558-1572.

69. Liem-Moolenaar M., Te Beek E.T., de Kam M.L., Franson K.L., Kahn R.S., Hijman R., Touw D., van Gerven J.M. (2010) Central nervous system effects of haloperidol on THC in healthy male volunteers. J Psychopharmacol. 24 (11), 1697-1708.
70. Miller L., Cornett T., Brightwell D., McFarland D., Drew W.G., Wikler A. (1976) Marijuana and memory impairment: the effect of retrieval cues on free recall. Pharmacol Biochem Behav. 5 (6), 639-643.
71. Miller L.L., Cornett T.L., Brightwell D.R., McFarland D.J., Drew W.G., Wikler A. (1977) Marijuana: effects on storage and retrieval of prose material. Psychopharmacology (Berl). 51 (3), 311-316.
72. Miller L.L., McFarland D., Cornett T.L., Brightwell D. (1977) Marijuana and memory impairment: effect on free recall and recognition memory. Pharmacol Biochem Behav. 7 (2), 99-103.
73. Miller L.L., Cornett T.L. (1978) Marijuana: dose effects on pulse rate, subjective estimates of intoxication, free recall and recognition memory. Pharmacol Biochem Behav. 9 (5), 573-577.
74. Miller L.L., Cornett T.L., Wikler A. (1979) Marijuana: effects on pulse rate, subjective estimates of intoxication and multiple measures of memory. Life Sci. 25 (15), 1325-1330.
75. Abel E.L. (1971) Marihuana and memory: acquisition or retrieval? Science. 173 (4001), 1038-1040.
76. Darley C.F., Tinklenberg J.R., Roth W.T., Hollister L.E., Atkinson R.C. (1973) Influence of marijuana on storage and retrieval processes in memory. Memory & Cognition. 1, 196-200.
77. Dornbush R.L. (1974) Marijuana and memory: effects of smoking on storage. Trans N Y Acad Sci. 36 (1), 94-100.
78. Henke K., Buck A., Weber B., Wieser H.G. (1997) Human hippocampus establishes associations in memory. Hippocampus. 7 (3), 249-256.
79. Jager G., van Hell H.H., De Win M.M., Kahn R.S., van den Brink W., van Ree J.M., Ramsey N.F. (2007) Effects of frequent cannabis use on hippocampal activity during an associative memory task. Eur Neuropsychopharmacol. 17 (4), 289-297.
80. Baddeley A. (1986) Working memory. Claredon Press, Oxford.
81. Curtis C.E., D'Esposito M. (2003) Persistent activity in the prefrontal cortex during working memory. Trends Cogn Sci. 7 (9), 415-423.
82. Biederman J., Faraone S.V. (2005) Attention-deficit hyperactivity disorder. Lancet. 366 (9481), 237-248.
83. Robbins T.W., Ersche K.D., Everitt B.J. (2008) Drug addiction and the memory systems of the brain. Ann N Y Acad Sci. 1141, 1-21.
84. Elvevag B., Goldberg T.E. (2000) Cognitive impairment in schizophrenia is the core of the disorder. Crit Rev Neurobiol. 14 (1), 1-21.
85. Sternberg S. (1966) High-speed scanning in human memory. Science. 153 (736), 652-654.
86. Jansma J.M., Ramsey N.F., Slagter H.A., Kahn R.S. (2001) Functional anatomical correlates of controlled and automatic processing. J Cogn Neurosci. 13 (6), 730-743.
87. Manoach D.S., Schlaug G., Siewert B., Darby D.G., Bly B.M., Benfield A., Edelman R.R., Warach S. (1997) Prefrontal cortex fMRI signal changes are correlated with working memory load. Neuroreport. 8 (2), 545-549.
88. Altamura M., Elvevag B., Blasi G., Bertolino A., Callicott J.H., Weinberger D.R., Mattay V.S., Goldberg T.E. (2007) Dissociating the effects of Sternberg working memory demands in prefrontal cortex. Psychiatry Res. 154 (2), 103-114.
89. Jansma J.M., Ramsey N.F., de Zwart J.A., van Gelderen P., Duyn J.H. (2007) fMRI study of effort and information processing in a working memory task. Hum Brain Mapp. 28 (5), 431-440.
90. Manoach D.S., Press D.Z., Thangaraj V., Searl M.M., Goff D.C., Halpern E., Saper C.B., Warach S. (1999) Schizophrenic subjects activate dorsolateral prefrontal cortex during a working memory task, as measured by fMRI. Biol Psychiatry. 45 (9), 1128-1137.
91. Callicott J.H., Bertolino A., Mattay V.S., Langheim F.J., Duyn J., Coppola R., Goldberg T.E., Weinberger D.R. (2000) Physiological dysfunction of the dorsolateral prefrontal cortex in schizophrenia revisited. Cereb Cortex. 10 (11), 1078-1092.
92. Manoach D.S., Gollub R.L., Benson E.S., Searl M.M., Goff D.C., Halpern E., Saper C.B., Rauch S.L. (2000) Schizophrenic subjects show aberrant fMRI activation of dorsolateral prefrontal cortex and basal ganglia during working memory performance. Biol Psychiatry. 48 (2), 99-109.
93. Jansma J.M., Ramsey N.F., van der Wee N.J., Kahn R.S. (2004) Working memory capacity in schizophrenia: a parametric fMRI study. Schizophr Res. 68 (2-3), 159-171.
94. Potkin S.G., Turner J.A., Brown G.G., McCarthy G., Greve D.N., Glover G.H., Manoach D.S., Belger A., Diaz M., Wible C.G., Ford J.M., Mathalon D.H., Gollub R., Lauriello J., O'Leary D., van Erp T.G., Toga A.W., Preda A., Lim K.O. (2009) Working memory and DLPFC inefficiency in schizophrenia: the FBIRN study. Schizophr Bull. 35 (1), 19-31.

95. Callicott J.H., Mattay V.S., Verchinski B.A., Marenco S., Egan M.F., Weinberger D.R. (2003) Complexity of prefrontal cortical dysfunction in schizophrenia: more than up or down. Am J Psychiatry. 160 (12), 2209-2215.
96. Manoach D.S. (2003) Prefrontal cortex dysfunction during working memory performance in schizophrenia: reconciling discrepant findings. Schizophr Res. 60 (2-3), 285-298.
97. Nuechterlein K.H., Dawson M.E. (1984) Information processing and attentional functioning in the developmental course of schizophrenic disorders. Schizophr Bull. 10 (2), 160-203.
98. Cornblatt B.A., Keilp J.G. (1994) Impaired attention, genetics, and the pathophysiology of schizophrenia. Schizophr Bull. 20 (1), 31-46.
99. Perry R.J., Hodges J.R. (1999) Attention and executive deficits in Alzheimer's disease. A critical review. Brain. 122 (Pt 3), 383-404.
100. Willcutt E.G., Doyle A.E., Nigg J.T., Faraone S.V., Pennington B.F. (2005) Validity of the executive function theory of attention-deficit/hyperactivity disorder: a meta-analytic review. Biol Psychiatry. 57 (11), 1336-1346.
101. Egerton A., Brett R.R., Pratt J.A. (2005) Acute delta9-tetrahydrocannabinol-induced deficits in reversal learning: neural correlates of affective inflexibility. Neuropsychopharmacology. 30 (10), 1895-1905.
102. Hill M.N., Froese L.M., Morrish A.C., Sun J.C., Floresco S.B. (2006) Alterations in behavioral flexibility by cannabinoid CB1 receptor agonists and antagonists. Psychopharmacology (Berl). 187 (2), 245-259.
103. Carlin A.S., Bakker C.B., Halpern L., Post R.D. (1972) Social facilitation of marijuana intoxication: impact of social set and pharmacological activity. J Abnorm Psychol. 80 (2), 132-140.
104. Klonoff H. (1974) Marijuana and driving in real-life situations. Science. 186 (4161), 317-324.
105. Casswell S. (1975) Cannabis intoxication: effects of monetary incentive on performance, a controlled investigation of behavioural tolerance in moderate users of cannabis. Percept Mot Skills. 41 (2), 423-434.
106. Hooker W.D., Jones R.T. (1987) Increased susceptibility to memory intrusions and the Stroop interference effect during acute marijuana intoxication. Psychopharmacology. 91 (1), 20-24.
107. Ramaekers J.G., Robbe H.W., O'Hanlon J.F. (2000) Marijuana, alcohol and actual driving performance. Hum Psychopharmacol. 15 (7), 551-558.
108. Ramaekers J.G., Kauert G., van Ruitenbeek P., Theunissen E.L., Schneider E., Moeller M.R. (2006) High-potency marijuana impairs executive function and inhibitory motor control. Neuropsychopharmacology. 31 (10), 2296-2303.
109. Hart C.L., Haney M., Vosburg S.K., Comer S.D., Foltin R.W. (2005) Reinforcing effects of oral Delta9-THC in male marijuana smokers in a laboratory choice procedure. Psychopharmacology (Berl). 181 (2), 237-243.
110. Morrison P.D., Zois V., McKeown D.A., Lee T.D., Holt D.W., Powell J.F., Kapur S., Murray R.M. (2009) The acute effects of synthetic intravenous Delta9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. Psychol Med., 1-10.
111. Lawrence N.S., Ross T.J., Hoffmann R., Garavan H., Stein E.A. (2003) Multiple neuronal networks mediate sustained attention. J Cogn Neurosci. 15 (7), 1028-1038.
112. Gur R.E., Turetsky B.I., Loughead J., Snyder W., Kohler C., Elliott M., Pratiwadi R., Ragland J.D., Bilker W.B., Siegel S.J., Kanes S.J., Arnold S.E., Gur R.C. (2007) Visual attention circuitry in schizophrenia investigated with oddball event-related functional magnetic resonance imaging. Am J Psychiatry. 164 (3), 442-449.
113. Raichle M.E., MacLeod A.M., Snyder A.Z., Powers W.J., Gusnard D.A., Shulman G.L. (2001) A default mode of brain function. Proc Natl Acad Sci U S A. 98 (2), 676-682.
114. Shulman G.L., Fiez J.A., Corbetta M., Buckner R.L., Miezin F.M., Raichle M.E., Petersen S.E. (1997) Common blood flow changes across visual tasks: II. Decreases in cerebral cortex. J Cogn Neurosci. 9, 648-663.
115. Mazoyer B., Zago L., Mellet E., Bricogne S., Etard O., Houde O., Crivello F., Joliot M., Petit L., Tzourio-Mazoyer N. (2001) Cortical networks for working memory and executive functions sustain the conscious resting state in man. Brain Res Bull. 54 (3), 287-298.
116. Otten L.J., Rugg M.D. (2001) When more means less: neural activity related to unsuccessful memory encoding. Curr Biol. 11 (19), 1528-1530.
117. Drummond S.P., Bischoff-Grethe A., Dinges D.F., Ayalon L., Mednick S.C., Meloy M.J. (2005) The neural basis of the psychomotor vigilance task. Sleep. 28 (9), 1059-1068.
118. Polli F.E., Barton J.J., Cain M.S., Thakkar K.N., Rauch S.L., Manoach D.S. (2005) Rostral and dorsal anterior cingulate cortex make dissociable contributions during antisaccade error commission. Proc Natl Acad Sci U S A. 102 (43), 15700-15705.

119. Weissman D.H., Roberts K.C., Visscher K.M., Woldorff M.G. (2006) The neural bases of momentary lapses in attention. Nat Neurosci. 9 (7), 971-978.
120. Shulman G.L., Astafiev S.V., McAvoy M.P., d'Avossa G., Corbetta M. (2007) Right TPJ deactivation during visual search: functional significance and support for a filter hypothesis. Cereb Cortex. 17 (11), 2625-2633.
121. Eichele T., Debener S., Calhoun V.D., Specht K., Engel A.K., Hugdahl K., von Cramon D.Y., Ullsperger M. (2008) Prediction of human errors by maladaptive changes in event-related brain networks. Proc Natl Acad Sci U S A. 105 (16), 6173-6178.
122. Cornblatt B.A., Risch N.J., Faris G., Friedman D., Erlenmeyer-Kimling L. (1988) The Continuous Performance Test, identical pairs version (CPT-IP): I. New findings about sustained attention in normal families. Psychiatry Res. 26 (2), 223-238.
123. Adler C.M., Sax K.W., Holland S.K., Schmithorst V., Rosenberg L., Strakowski S.M. (2001) Changes in neuronal activation with increasing attention demand in healthy volunteers: an fMRI study. Synapse. 42 (4), 266-272.
124. Strakowski S.M., Adler C.M., Holland S.K., Mills N., DelBello M.P. (2004) A preliminary FMRI study of sustained attention in euthymic, unmedicated bipolar disorder. Neuropsychopharmacology. 29 (9), 1734-1740.
125. Chen W.J., Faraone S.V. (2000) Sustained attention deficits as markers of genetic susceptibility to schizophrenia. Am J Med Genet. 97 (1), 52-57.
126. Norman D.A., Shallice T. (1986) Attention and action: willed and automatic control of behavior. In: Davidson R.J., Schwarts G.E., Shapiro D. (Eds), Consciousness and self-regulation. Plenum, New York, p. 1-18.
127. Leppanen J.M. (2006) Emotional information processing in mood disorders: a review of behavioral and neuroimaging findings. Curr Opin Psychiatry. 19 (1), 34-39.
128. Phillips M.L., Ladouceur C.D., Drevets W.C. (2008) A neural model of voluntary and automatic emotion regulation: implications for understanding the pathophysiology and neurodevelopment of bipolar disorder. Mol Psychiatry. 13 (9), 829, 833-829, 857.
129. Pinkham A.E., Penn D.L., Perkins D.O., Lieberman J. (2003) Implications for the neural basis of social cognition for the study of schizophrenia. Am J Psychiatry. 160 (5), 815-824.
130. Phillips M.L., Drevets W.C., Rauch S.L., Lane R. (2003) Neurobiology of emotion perception II: Implications for major psychiatric disorders. Biol Psychiatry. 54 (5), 515-528.
131. Aleman A., Kahn R.S. (2005) Strange feelings: do amygdala abnormalities dysregulate the emotional brain in schizophrenia? Prog Neurobiol. 77 (5), 283-298.
132. Stein M.B., Goldin P.R., Sareen J., Zorrilla L.T., Brown G.G. (2002) Increased amygdala activation to angry and contemptuous faces in generalized social phobia. Arch Gen Psychiatry. 59 (11), 1027-1034.
133. Phan K.L., Fitzgerald D.A., Nathan P.J., Tancer M.E. (2006) Association between amygdala hyperactivity to harsh faces and severity of social anxiety in generalized social phobia. Biol Psychiatry. 59 (5), 424-429.
134. Etkin A., Wager T.D. (2007) Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. Am J Psychiatry. 164 (10), 1476-1488.
135. Sergerie K., Chochol C., Armony J.L. (2008) The role of the amygdala in emotional processing: a quantitative meta-analysis of functional neuroimaging studies. Neurosci Biobehav Rev. 32 (4), 811-830.
136. Haxby J.V., Hoffman E.A., Gobbini M.I. (2000) The distributed human neural system for face perception. Trends Cogn Sci. 4 (6), 223-233.
137. Adolphs R. (2002) Recognizing emotion from facial expressions: psychological and neurological mechanisms. Behav Cogn Neurosci Rev. 1 (1), 21-62.
138. Ochsner K.N., Gross J.J. (2005) The cognitive control of emotion. Trends Cogn Sci. 9 (5), 242-249.
139. Ashton C.H. (2001) Pharmacology and effects of cannabis: a brief review. Br J Psychiatry. 178, 101-106.
140. Green B., Kavanagh D.J., Young R.M. (2004) Reasons for cannabis use in men with and without psychosis. Drug Alcohol Rev. 23 (4), 445-453.
141. Hathaway A.D. (2003) Cannabis effects and dependency concerns in long-term frequent users: a missing piece of the public health puzzle. Addiction Research and Theory. 11 (6), 441-458.
142. Navarro M., Hernandez E., Munoz R.M., Del A., I, Villanua M.A., Carrera M.R., Rodriguez de F.F. (1997) Acute administration of the CB1 cannabinoid receptor antagonist SR 141716A induces anxiety-like responses in the rat. Neuroreport. 8 (2), 491-496.
143. Martin M., Ledent C., Parmentier M., Maldonado R., Valverde O. (2002) Involvement of CB1 cannabinoid receptors in emotional behaviour. Psychopharmacology. 159 (4), 379-387.

144. Haller J., Bakos N., Szirmay M., Ledent C., Freund T.F. (2002) The effects of genetic and pharmacological blockade of the CB1 cannabinoid receptor on anxiety. Eur J Neurosci. 16 (7), 1395-1398.
145. Haller J., Varga B., Ledent C., Freund T.F. (2004) CB1 cannabinoid receptors mediate anxiolytic effects: convergent genetic and pharmacological evidence with CB1-specific agents. Behav Pharmacol. 15 (4), 299-304.
146. Uriguen L., Perez-Rial S., Ledent C., Palomo T., Manzanares J. (2004) Impaired action of anxiolytic drugs in mice deficient in cannabinoid CB1 receptors. Neuropharmacology. 46 (7), 966-973.
147. Griebel G., Stemmelin J., Scatton B. (2005) Effects of the cannabinoid CB1 receptor antagonist rimonabant in models of emotional reactivity in rodents. Biol Psychiatry. 57 (3), 261-267.
148. Berrendero F., Maldonado R. (2002) Involvement of the opioid system in the anxiolytic-like effects induced by Delta(9)-tetrahydrocannabinol. Psychopharmacology (Berl). 163 (1), 111-117.
149. Valjent E., Mitchell J.M., Besson M.J., Caboche J., Maldonado R. (2002) Behavioural and biochemical evidence for interactions between Delta 9-tetrahydrocannabinol and nicotine. Br J Pharmacol. 135 (2), 564-578.
150. Kathuria S., Gaetani S., Fegley D., Valino F., Duranti A., Tontini A., Mor M., Tarzia G., La Rana G., Calignano A., Giustino A., Tattoli M., Palmery M., Cuomo V., Piomelli D. (2003) Modulation of anxiety through blockade of anandamide hydrolysis. Nat Med. 9 (1), 76-81.
151. Hill M.N., Gorzalka B.B. (2004) Enhancement of anxiety-like responsiveness to the cannabinoid CB(1) receptor agonist HU-210 following chronic stress. Eur J Pharmacol. 499 (3), 291-295.
152. Marco E.M., Perez-Alvarez L., Borcel E., Rubio M., Guaza C., Ambrosio E., File S.E., Viveros M.P. (2004) Involvement of 5-HT1A receptors in behavioural effects of the cannabinoid receptor agonist CP 55,940 in male rats. Behav Pharmacol. 15 (1), 21-27.
153. Patel S., Hillard C.J. (2006) Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: further evidence for an anxiolytic role for endogenous cannabinoid signaling. J Pharmacol Exp Ther. 318 (1), 304-311.
154. Rubino T., Sala M., Vigano D., Braida D., Castiglioni C., Limonta V., Guidali C., Realini N., Parolaro D. (2007) Cellular mechanisms underlying the anxiolytic effect of low doses of peripheral Delta9-tetrahydrocannabinol in rats. Neuropsychopharmacology. 32 (9), 2036-2045.
155. Hariri A.R., Mattay V.S., Tessitore A., Kolachana B., Fera F., Goldman D., Egan M.F., Weinberger D.R. (2002) Serotonin transporter genetic variation and the response of the human amygdala. Science. 297 (5580), 400-403.
156. Bertolino A., Arciero G., Rubino V., Latorre V., De C.M., Mazzola V., Blasi G., Caforio G., Hariri A., Kolachana B., Nardini M., Weinberger D.R., Scarabino T. (2005) Variation of human amygdala response during threatening stimuli as a function of 5'HTTLPR genotype and personality style. Biol Psychiatry. 57 (12), 1517-1525.
157. Phan K.L., Angstadt M., Golden J., Onyewuenyi I., Popovska A., de W.H. (2008) Cannabinoid modulation of amygdala reactivity to social signals of threat in humans. J Neurosci. 28 (10), 2313-2319.

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Δ9-Tetrahydrocannabinol induces dopamine release in the human striatum

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Abstract

The influence of cannabis on mental health receives growing scientific and political attention. An increasing demand for treatment of cannabis dependence has refueled the discussion about the addictive potential of cannabis. A key feature of all addictive drugs is the ability to increase synaptic dopamine levels in the striatum, a mechanism involved in their rewarding and motivating effects. However, it is currently unknown if cannabis can stimulate striatal dopamine neurotransmission in humans. Here we show that Δ9-tetrahydrocannabinol (THC), the main psychoactive component in cannabis, induces dopamine release in the human striatum. Using the dopamine D_2/D_3 receptor tracer [^{11}C]raclopride and positron emission tomography in seven healthy subjects, we demonstrate that THC inhalation reduces [^{11}C]raclopride binding in the ventral striatum and the precommissural dorsal putamen but not in other striatal subregions. This is consistent with an increase in dopamine levels in these regions. These results suggest that THC shares a potentially addictive property with other drugs of abuse. Further, it implies that the endogenous cannabinoid system is involved in regulating striatal dopamine release. This allows new directions in research on the effects of THC in neuropsychiatric disorders, such as schizophrenia.

Introduction

The debate whether cannabis can be characterized as an addictive drug has been ongoing for many years[1]. Recently, a substantial increase in the demand for treatment of cannabis dependence has intensified this discussion[2]. The rewarding properties of addictive drugs are thought to be mediated by their action on the mesolimbic dopamine system[3,4]. This dopamine system originates in the ventral tegmental area and projects to the ventral striatum, which predominantly comprises the nucleus accumbens. Addictive drugs probably induce their rewarding effects by enhancement of synaptic dopamine levels in the ventral striatum[3,4]. In the human striatum, increased dopamine levels have been found with the use of neuroimaging techniques after the administration of amphetamine[5-9], cocaine[10], alcohol[11] and nicotine[12,13]. In animals, it has been demonstrated that cannabinoid substances such as Δ9-tetrahydrocannabinol (THC), the main psychoactive component in cannabis[14], also stimulate striatal dopamine neurotransmission[15-17]. Cannabinoids enhance neuronal firing of mesolimbic dopamine neurons[18-20] and elevate striatal dopamine levels[21-25], both through activation of cannabinoid CB_1 receptors[18-20,23,24]. However, whether THC affects the human striatal dopamine system is currently unknown.

The purpose of the present study was to investigate whether THC can induce dopamine release in the striatum of healthy human subjects. This was assessed using positron emission tomography (PET) and the dopamine D_2/D_3 receptor ligand [^{11}C]raclopride. With this method, an increase in striatal synaptic dopamine concentrations can be determined by a reduction in [^{11}C]raclopride binding[6,26]. Based on findings from animal studies, our hypothesis was that

THC should reduce [^{11}C]raclopride binding in the human striatum, consistent with striatal dopamine release.

Materials and methods

Subjects

Healthy male subjects were recruited through advertisements on the Internet. All subjects had a history of mild cannabis use for at least one year, defined as using cannabis more than four times a year and at most once a week. In addition, it was required that they never experienced psychotic effects after cannabis use and did not meet criteria for "Paranoid Ideation" or "Psychoticism" on the self report symptom checklist SCL-90[27]. Mild cannabis users were selected as they *a priori* could be expected to tolerate the THC challenge used in this experiment while not having long-term effects associated with frequent cannabis use. All subjects were in good physical health as assessed by medical history, physical examination, electrocardiogram and routine laboratory tests. Urine screening for cannabis, amphetamine, cocaine and morphine was performed at screening and on both study days. Subjects with a positive drug test on other drugs than cannabis were excluded from the study. Subjects with a positive cannabis test at screening were tested again, and were required to be negative before the first study day. Subjects were excluded from participation in case of history of alcohol or drug abuse and in case of major current psychiatric diagnosis. In addition, subjects were excluded if they, or a first- or second-degree relative, had a lifetime history of a clinically significant psychiatric or neurological illness. Use of medication at the time of the study was not allowed. All volunteers gave written informed consent before entry into the study. The study was approved by the Medical Research Ethics Committee of the University Medical Center Utrecht, the Netherlands.

Design and procedure

In a double-blind, randomized, placebo-controlled, cross-over study, subjects had two PET-scans after either administration of THC or placebo. Scanning sessions were separated by at least two weeks to allow for complete clearance of drug between both occasions. Subjects arrived two hours before the start of the scanning procedure at the Department of Nuclear Medicine & PET Research of the VU University Medical Center in Amsterdam, the Netherlands, having fasted for at least 4 hours prior to their arrival. Subjects refrained from cannabis for at least two weeks prior to the first study day until study completion and from alcohol for 24 hours before each study day. Caffeine intake and smoking were not allowed on study days. Use of drugs of abuse, including cannabis, was checked with urine drug screenings, which had to be negative on the day of the PET scans. Use of alcohol, caffeine and nicotine was checked by self report. A standard breakfast or lunch was served and venous catheters were placed in each arm, one for [^{11}C]raclopride infusion and the other for venous blood sampling.

Drugs and administration

Preparation and administration of drugs was performed according to Zuurman et al. (2008)[28]. THC was purified from *Cannabis sativa* according to GMP-compliant procedures (Farmalyse B.V., Zaandam, the Netherlands) and was dissolved in 200 µl 100 vol% alcohol. The solvent was used as placebo. Drugs were administered using a Volcano ® vaporizer (Storz-Bickel GmbH, Tuttlingen, Germany). Five minutes before administration, 8 mg of THC was vaporized into an opaque polythene bag equipped with a valved mouthpiece, preventing the loss of THC in between inhalations. Subjects inhaled the volume of this bag in three or four subsequent breaths, holding their breath for 10 seconds after each inhalation. They were not allowed to speak during the inhalation process, which was practiced at screening using placebo.

Production of [^{11}C]raclopride

[^{11}C]Raclopride was synthesized via methylation of O-desmethyl raclopride (obtained from ABX, Radeberg, Germany) with $^{11}CH_3I$ in dimethylsulfoxide at 80°C for 5 minutes, utilizing a Nuclear Interface methylation synthesis module. The resulting product was purified from the reaction mixture by HPLC (µbondapak 7.8x300; 0,01M H_3PO_4/MeCN 70/30 5 ml/min, UV at 254 nm). The collected fraction containing [^{11}C]raclopride was diluted with 40 ml of 1 mM NaOH in water and this mixture was subsequently passed over a tC18 SepPak. After washing the SepPak with 20 ml of water for injection, [^{11}C]raclopride was eluted from the SepPak with 1.2 ml of sterile ethanol and a sterile solution of NaH_2PO_4 in saline (7.1 mM, pH 5.4). The final solution was transferred to a sterile product vial via a sterile 0.22 µm MIllex GV filter, yielding a sterile, pyrogen free solution of [^{11}C]raclopride with a (radio)chemical purity of >98% while the specific activity ranged from 26 to 104 GBq/µmol at time of injection. The complete production procedure was performed in accordance with the EU guideline Eudralex volume 4: Good Manufacturing Practices and were approved by the Dutch health authorities (license nr 107627A).

Positron Emission Tomography

PET scans were performed on an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, TN, USA), which is located at the Department of Nuclear Medicine & PET Research of the VU University Medical Centre in Amsterdam, the Netherlands. This scanner enables the acquisition of 63 transaxial planes over a 15.5 cm axial field of view, thus allowing the whole brain to be imaged. Five minutes after inhalation of placebo or THC, [^{11}C]raclopride was given as a bolus plus constant infusion. [^{11}C]Raclopride was delivered in a 50 ml volume and administered by a computer operated infusion pump (Med-Rad, Beek, the Netherlands). First, a bolus dose of 28 ml was given over 3.1 minutes, followed by constant infusion of 22 ml at 0.15 ml/hour for 88 minutes. Thus, the bolus to infusion ratio (K_{bol}) was 112 minutes[29]. A 40 minute scanning period with 8 successive frames of 5 minutes was initiated 40 minutes after the start of [^{11}C] raclopride administration. Finally, a transmission scan of 10 minutes was performed in order to correct for photon attenuation. Correction for emission contamination was performed using the dwell profile method[30].

Image reconstruction

All PET sinograms were corrected for dead time, decay, randoms, scatter and tissue attenuation. All PET emission scans were reconstructed with FORE + 2D FBP using a 0.5 Hanning filter, resulting in a trans-axial spatial resolution of ~7 mm in the centre of the field of view. Images were then transferred to workstations (Sun Microsystems, Santa Clara, CA, USA) for further analysis.

Behavioral, subjective and physiological measurements

Behavioral ratings were assessed with the 18-item Brief Psychiatric Rating Scale (BPRS)[31]. This structured interview was performed at baseline and 21 and 102 minutes after THC administration. Total BPRS scores were analyzed together with scores for the factors thinking disorder (BPRS items conceptual disorganization, hallucinatory behavior and unusual thought content), withdrawal-retardation (BPRS items blunted affect and emotional withdrawal), anxiety-depression (BPRS items anxiety, guilt feelings and depressive mood) and hostility-suspiciousness (BPRS items hostility, suspiciousness and uncooperativeness)[32].

A rating scale consisting of 16 visual analogue scales was used to determine subjective effects. From these analogue scales three factors were calculated, corresponding to alertness, contentedness and calmness[33]. Psychedelic effects were assessed using an adapted version of a 13-item visual analogue rating scale, originally described by Bowdle and colleagues[28,34]. The visual analogue scale "Feeling High" was analyzed individually and composite scores of "External Perception" and "Internal Perception" were calculated[28]. Both rating scales were performed consecutively at baseline and 7, 12, 17, 32 and 100 minutes after THC administration.

ECG was monitored continuously and blood pressure and heart rate were measured at baseline and 6, 11, 15, 19, 34, 49, 64, 79 and 94 minutes after start of THC administration.

Blood sampling

Venous blood samples were collected to determine plasma concentrations of THC and its two most important metabolites, 11-OH-THC and 11-nor-9-carboxy-THC. Blood samples were withdrawn 5, 10, 20, 35, 55 and 90 minutes after THC administration and processed according to Zuurman et al. (2008)[28]. Additional venous blood samples were withdrawn 40, 60, 70 and 80 minutes after start of [^{11}C]raclopride administration in order to measure [^{11}C]raclopride metabolism. These samples were processed according to Schuit et al. (2007)[35].

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) of all subjects was performed at the Department of Radiology of the University Medical Center in Utrecht, the Netherlands, for anatomical definition. MRI scans were acquired using a 1.5T scanner (Philips Gyroscan; Philips Medical Systems, Best, the Netherlands). T1-weighted, 3D, fast-field echo scans with 160–180 1.2mm contiguous coronal slices (echo time, 4.6 ms; repetition time, 30 ms; flip angle 301; field of view 256mm)[36] were used.

Regions of interest

All MRI scans were rotated to acquire horizontal lines between anterior and posterior commissures (AC-PC line) in the sagittal plane. Then the striatum was divided into five anatomical regions of interest (ROI) (see Figure 2.1 and Table 2.1), according to published criteria[8,37]. These ROIs were delineated on MRI scans oriented in the coronal plane using DISPLAY. ROIs were defined for ventral striatum, precommissural dorsal caudate, precommissural dorsal putamen, postcommisural caudate and postcommissural putamen. These ROIs were classified into three functional subdivisions: limbic striatum (ventral striatum), associative striatum (consisting of precommissural dorsal caudate, precommissural dorsal putamen and postcommisural caudate), and sensorimotor striatum (postcommissural putamen)[8,37]. Cerebellar hemispheres were also defined on the MRI scans. After reconstruction, individual PET frames were co-registered to the first frame in order to correct for motion and summed over all frames. These summed PET images were co-registered to the rotated MRI scan using VINCI software[38]. After projection of the ROIs on the co-registered summed PET images, activity was calculated for each ROI as the volume weighted average of left and right

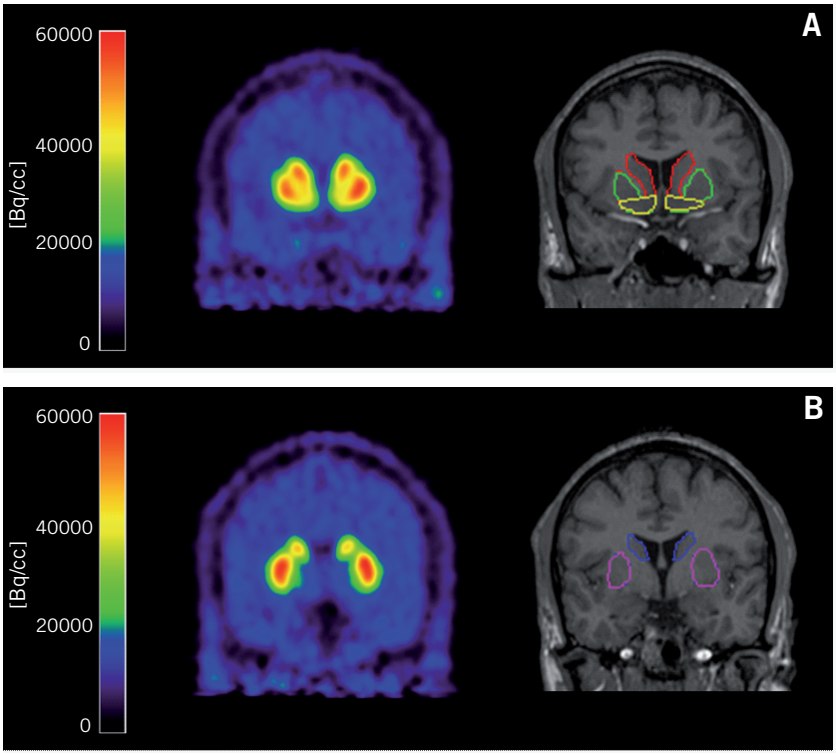

Figure 2.1 Coronal slices of (left) PET [^{11}C]raclopride and (right) co-registered MRI scans at the level of the striatum, (A) anterior and (B) posterior to the AC-plane. Striatal subregions are indicated on the MRI scans: ventral striatum (yellow), precommissural dorsal putamen (green), precommissural dorsal caudate (red), postcommissural putamen (purple) and postcommissural caudate (blue).

regions. Activity in associative striatum was derived as the volume weighted average of precommissural dorsal caudate, precommissural dorsal putamen and postcommisural caudate, whilst activity in striatum as a whole was calculated as the volume weighted average of all five ROIs (Table 2.1).

Outcome measures

Non-displaceable Binding Potential (BP_{ND})[39] was used as measure of dopamine D_2/D_3 receptor availability. BP_{ND} was defined as the distribution volume ratio (DVR) minus 1[40]. As scans were performed during steady state, DVR could be obtained using the average activity concentration in a ROI divided by that of the cerebellum ROI, which was used as reference. In this way, BP_{ND} was calculated for all ROIs for both scanning sessions.

Statistical analysis

Group differences in BP_{ND} between placebo and THC were analyzed using repeated measures ANOVA with ROI and drug as factors. Post-hoc analysis was performed for each ROI using paired t-tests. Behavioral, subjective, psychedelic and physiological effects were corrected for baseline values and also analyzed using repeated measures ANOVA with drug and time as factors. Post-hoc analysis was performed using paired t-tests. Therefore, a mean score was calculated for each parameter and compared between placebo and THC. Differences in PET scan parameters and [^{11}C]raclopride concentrations were measured using paired t-tests. A *p*-value less than 0.05 was considered statistically significant.

Results

Subjects

Nine healthy male subjects gave informed consent for this study. Seven volunteers completed the study procedure. One subject was excluded due to positive urine drug screening on the first study day. Another subject did not complete the second scanning session due to anxiety. Mean age of the seven subjects was 21.9 ± 2.7 years (range 20 - 27). Mean height, weight and BMI were 183 ± 8 cm (range 172 – 196), 80 ± 9 kg (range 72 – 98) and 23.8 ± 0.9 kg/m^2 (range 22.9 – 25.5), respectively. All subjects were familiar with the effects of cannabis. Two subjects used cannabis less than once a month, one subject three times a month, three subjects twice a month and one subject used cannabis once a week. They all showed negative urine screening at both study days.

PET scan parameters

Mean injected dose of [^{11}C]raclopride was similar between placebo (770 ± 30 MBq) and THC sessions (810 ± 90 MBq) ($p = 0.254$). In addition, total mass of administered raclopride (6.1 ± 2.3 µmol and 4.6 ± 1.5 µmol for placebo and THC sessions respectively; $p = 0.181$) was not significantly different between sessions. There were no significant changes in equilibrium

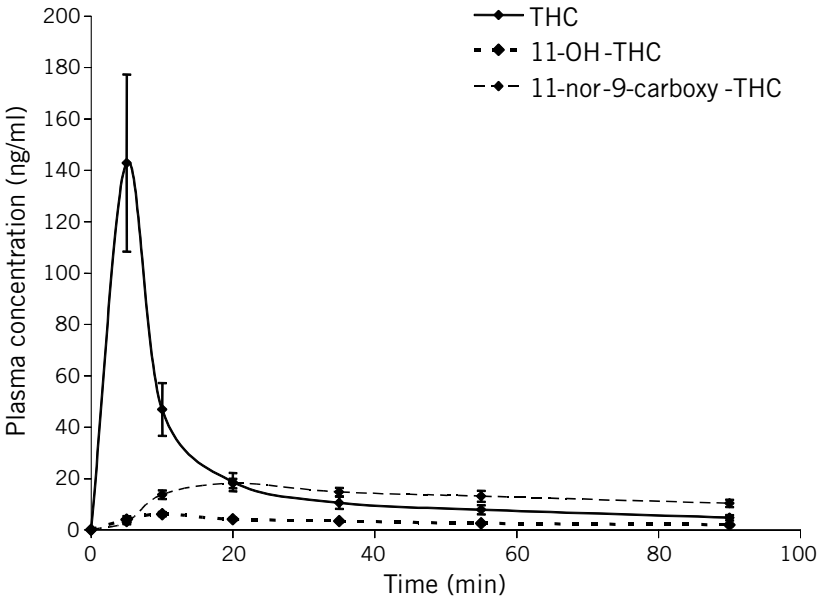

Figure 2.2 Plasma concentrations of Δ9-tetrahydrocannabinol (THC) and its main metabolites 11-OH-THC and 11-nor-9-carboxy-THC after inhalation of 8 mg THC (mean ± SEM; n = 7).

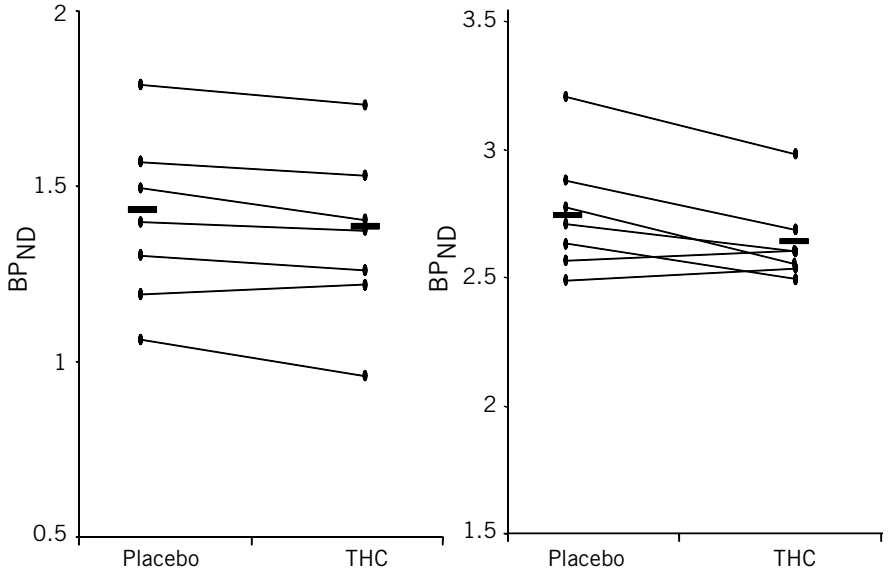

Figure 2.3 Effects of Δ9-tetrahydrocannabinol inhalation (8 mg) on [^{11}C]raclopride binding in (left) ventral striatum and (right) precommissural dorsal putamen of healthy subjects (n = 7). Data are presented as [^{11}C]raclopride non-displaceable Binding Potential (BP_{ND}), reflecting dopamine D_2/D_3 receptor availability.

levels of striatal activity, expressed by percentage change of activity concentration over time, between placebo (-0.10 ± 0.16 %/min) and THC (-0.12 ± 0.08 %/min) sessions ($p = 0.633$). In addition, the slope (i.e. change over time during scanning) of the ratio between striatum and cerebellum [^{11}C]raclopride concentrations was not significantly different between sessions (-0.0011 ± 0.0030 and -0.0010 ± 0.0025 for placebo and THC sessions respectively; $p = 0.967$).

Blood sample analysis

THC plasma concentration reached a maximum of 143 ± 91 ng/ml five minutes after inhalation, decreasing rapidly thereafter. Plasma concentrations of the main metabolites of THC, 11-OH-THC and 11-nor-9-carboxy-THC, peaked at ten minutes (6 ± 2 ng/ml) and twenty minutes (18 ± 5 ng/ml) after inhalation, respectively (Figure 2.2). [^{11}C]Raclopride concentrations were not significantly different between placebo and THC sessions in both whole blood (0.59 ± 0.25 and 0.61 ± 0.28 kBq/g, respectively; $p = 0.717$) and plasma (1.02 ± 0.42 and 1.06 ± 0.43 kBq/g, respectively; $p = 0.687$), normalized to the effectively injected dose. In addition, the fraction of parent [^{11}C]raclopride was not significantly different ($p = 0.191$) between placebo (80.4 ± 5.6%) and THC (77.5 ± 4.3%).

Dopamine D_2/D_3 receptor availability

Non-displaceable Binding Potential (BP_{ND}) of [^{11}C]raclopride, reflecting dopamine D_2/D_3 receptor availability, was significantly reduced in the ventral striatum and the precommissural dorsal putamen after inhalation of THC compared to placebo (-3.43 ± 3.70%, $p = 0.029$ and -3.88 ± 4.07%, $p = 0.042$, respectively) (Figure 2.3). In other subdivisions of the striatum no significant differences were found between THC and placebo (Table 2.1).

Table 2.1 Effects of Δ9-tetrahydrocannabinol (THC) (8 mg) on [^{11}C]raclopride non-displaceable Binding Potential (BP_{ND}), reflecting dopamine D_2/D_3 receptor availability (mean ± SD; n = 7).

Region	BP_{ND} Placebo	BP_{ND} THC	Difference (%)	*p*-values
Ventral striatum	1.40 ± 0.24	1.35 ± 0.24	-3.43 ± 3.70	0.029 *
Precommissural dorsal caudate	2.18 ± 0.25	2.12 ± 0.13	-2.09 ± 6.44	0.355
Precommissural dorsal putamen	2.75 ± 0.24	2.64 ± 0.16	-3.88 ± 4.07	0.042 *
Postcommissural caudate	1.62 ± 0.19	1.55 ± 0.15	-4.12 ± 7.14	0.157
Postcommissural putamen	2.74 ± 0.29	2.69 ± 0.20	-1.50 ± 4.42	0.329
Striatum	2.28 ± 0.22	2.21 ± 0.12	-2.57 ± 4.42	0.153

Striatum values were calculated as the volume weighted averages of all five regions of interest. * BP significantly different between THC and placebo.

Behavioral, subjective and physiological measurements

Analysis of variance revealed a significant drug x time effect on the BPRS total score ($F(2,12) = 11.28$, $p = 0.002$) and on the BPRS composite score withdrawal-retardation ($F(2,12) = 18.23$, $p < 0.001$). THC induced significant increases in VAS scores of "Feeling High"

(F(5,30) = 3.88, $p = 0.008$), "External Perception" (F(5,30) = 2.91, $p = 0.029$) and "Calmness" (F(5,30) = 3.22, $p = 0.019$), and a decrease on "Alertness" (F(5,30) = 3.07, $p = 0.024$). Heart rate increased significantly after THC compared with placebo (F(9,54) = 9.36, $p < 0.001$). Behavioral, subjective and physiological measurements are summarized in Table 2.2 and Table 2.3. No significant associations between measures of dopamine release and behavioral, subjective or physiological effects were demonstrated.

Table 2.2 Behavioral, subjective, and physiological effects of Δ9-tetrahydrocannabinol (n = 7).

Assessment	Drug x time interaction	Paired t-test
BPRS Total Score	F(2,12) = 11.28, $p = 0.002$ *	$p = 0.008$ *
Thinking Disorder	-	-
Withdrawal - Retardation	F(2,12) = 18.23, $p < 0.001$ *	$p < 0.001$ *
Anxiety - Depression	F(2,12) = 0.11, $p = 0.898$	-
Hostility - Suspiciousness	F(2, 12) = 1.64, $p = 0.235$	-
VAS Alertness	F(5, 30) = 3.07, $p = 0.024$ *	$p = 0.038$ *
VAS Contentedness	F(5, 30) = 1.73, $p = 0.157$	-
VAS Calmness	F(5, 30) = 3.22, $p = 0.019$ *	$p = 0.458$
VAS Feeling High	F(5, 30) = 3.88, $p = 0.008$ *	$p = 0.009$ *
VAS Internal Perception	F(5, 30) = 2.51, $p = 0.052$	-
VAS External Perception	F(5, 30) = 2.91, $p = 0.029$ *	$p = 0.048$ *
Heart Rate	F(9, 54) = 9.36, $p < 0.001$ *	$p = 0.040$ *
Systolic Blood Pressure	F(9, 54) = 1.38, $p = 0.240$	-
Diastolic Blood Pressure	F(9, 54) = 0.72, $p = 0.689$	-

Statistical analysis was performed using repeated measures ANOVA with drug and time as factors. Post-hoc analysis was performed with paired t-tests. Therefore, a mean score was calculated for each parameter and compared between THC and placebo.
* Significant difference between THC and placebo. BPRS, Brief Psychiatric Rating Scale; VAS, Visual Analogue Scale.

Table 2.3 Post-hoc analysis performed with paired t-tests of the baseline-corrected behavioral, subjective, and physiological parameters that demonstrated a significant drug x time effect (see Table 2.2) (mean ± SD; n = 7).

Assessment	Mean Placebo Score	Mean THC Score	*p*-values
BPRS Total Score	-0.10 ± 0.16	1.95 ± 1.42	0.008 *
BPRS Withdrawal - Retardation	0.00 ± 0.00	0.62 ± 0.23	< 0.001*
VAS Alertness	0.62 ± 2.23	-6.34 ± 6.31	0.038 *
VAS Calmness	2.11 ± 3.54	4.88 ± 8.63	0.458
VAS Feeling High	0.45 ± 1.50	27.33 ± 17.73	0.009*
VAS External Perception	0.17 ± 0.30	9.77 ± 10.16	0.048*
Heart Rate	-4.71 ± 4.38	16.17 ± 19.87	0.040*

For each parameter a mean score was calculated and compared between THC and placebo.
* Significant difference between THC and placebo. BPRS, Brief Psychiatric Rating Scale; VAS, Visual Analogue Scale.

Discussion

This study examined the effects of THC inhalation on [^{11}C]raclopride specific binding in seven healthy volunteers, finding a reduction in the ventral striatum and precommissural dorsal putamen. The reduction in [^{11}C]raclopride specific binding is consistent with an increase in dopamine levels in these regions.

This is the first study demonstrating THC-induced dopamine release in the human striatum. This result is in line with animal findings, showing enhanced neuronal firing of mesolimbic dopamine neurons after administration of cannabinoids[18-20]. In addition, it is consistent with microdialysis studies demonstrating that cannabinoids induce elevated striatal dopamine levels[21-25]. These effects are dependent on the activation of cannabinoid CB_1 receptors[18-20,23,24].

The ability of THC to induce dopamine release in the human striatum suggests that THC shares addictive properties with other drugs of abuse. Dopamine release in the striatum is a key feature of all addictive drugs, specifically involved in their rewarding effects and in the formation of reward-related associations[3,4]. However, whereas amphetamine[7-9], cocaine[10], alcohol[11] and nicotine[12,13] cause reductions in dopamine D_2/D_3 receptor availability in the range of 10% to 30%, we found a relatively modest THC-induced decrease of 3.4% and 3.9% in the ventral striatum and the precommissural dorsal putamen, respectively. Interestingly, this modest decrease in [^{11}C]raclopride binding is consistent with the moderate increase in striatal dopamine levels measured after administration of THC in animals[21-24]. Assuming a ratio between increase in dopamine levels and reduction in [^{11}C]raclopride binding of approximately 40:1 [6,26], our data indicate an increase in dopamine concentrations in the ventral striatum of 136%. This is in line with the THC-induced increase in striatal dopamine levels as demonstrated in microdialysis studies[21-24].

As THC was dissolved in 100 vol% alcohol and the solvent was used as placebo, we can not exclude that the inhalation of alcohol has caused dopamine release. However, this is very unlikely, as only 200 µl alcohol was administered. Please note that the placebo scan was subtracted from the THC scan and both conditions contained the same amount of alcohol.

THC induced well-known behavioral, subjective and physiological effects[41,42] in our subjects, replicating effects caused by the highest dose of THC administered in previous research using the same vaporizing device[28,43]. In addition, THC plasma concentrations in this study were comparable with or even higher than those obtained after smoking of high-potency cannabis[44,45]. Thus, our results indicate that a relatively high dose of THC induces a moderate degree of dopamine release in the human striatum. This effect may be explained by the indirect effects of THC on striatal dopamine levels through cannabinoid CB_1 receptors on glutamate and GABA neurons in the nucleus accumbens and the ventral tegmental area[16,46]. Other drugs of abuse have more direct effects on the dopamine system[4,47].

As the PET scan was performed around 45 - 85 minutes after inhalation of THC it could be argued that most of the effect of THC on dopamine release had dissipated at the time of the scan. By this time, plasma concentrations of THC were only 2.0 to 4.4% of the maximum

concentration. However, pharmacokinetic/pharmacodynamic (PK/PD) models that have been described recently indicate that central nervous system effects of THC last much longer than suggested by the rapid decline of plasma concentrations[43]. Application of these PK/PD-models to this study showed that 84.5 - 95.9% of the maximum CNS-effects were still present during acquisition of the PET scan. These findings suggest that it is unlikely that striatal dopamine release immediately after THC administration has been much larger than the modest levels of 3.4 - 3.9% that were observed around 45 minutes after administration. Indeed, effects on VAS Feeling High that were reported 101 minutes after inhalation were still significant.

Moreover, it is known that drug-induced effects on dopamine D_2/D_3 receptor availability last longer than changes in synaptic dopamine concentrations[6,26]. In humans, [^{11}C]raclopride Binding Potential was still decreased six hours after amphetamine administration[48]. This is probably due to an internalization of dopamine receptors[49], indicating that a drug-induced effect on striatal dopamine release can be detected for a long time after administration.

Our finding of THC-induced release of dopamine in the striatum suggests that human striatal dopamine release is under control of the endogenous cannabinoid system. The exact mechanism is still unclear, but cannabinoid CB_1 receptors on glutamate and GABA terminals in both the nucleus accumbens and the ventral tegmental area are involved in the regulation of dopamine release in the striatum[16,46]. Interestingly, it is known that cannabis use increases the risk for developing schizophrenia[50,51] and worsens its clinical outcome[52,53]. Schizophrenia is an illness that has consistently been related to increased dopamine function in the striatum[54,55], possibly caused by disinhibition of striatal dopamine transmission[5,6]. Thus, elevated striatal dopamine release after the use of cannabis may explain how cannabis use contributes to the development and pathophysiology of schizophrenia.

In conclusion, we have demonstrated that Δ9-THC, the main psychoactive component of cannabis, induces dopamine release in the human striatum. This finding implies that THC may share a putatively addictive property with other drugs of abuse and that the endogenous cannabinoid system plays a role in regulating striatal dopamine release, possibly explaining some of the detrimental effects of THC in neuropsychiatric disorders such as schizophrenia.

Disclosure/Conflict of interest

The authors declare that no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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References

1. Grinspoon L., Bakalar J.B., Zimmer L., Morgan J.P. (1997) Marijuana addiction. Science. 277 (5327), 749.
2. Hall W. (2006) The mental health risks of adolescent cannabis use. PLoS Med. 3 (2), 159-162.
3. Wise R.A. (2004) Dopamine, learning and motivation. Nat Rev Neurosci. 5 (6), 483-494.
4. Hyman S.E., Malenka R.C., Nestler E.J. (2006) Neural mechanisms of addiction: the role of reward-related learning and memory. Annu Rev Neurosci. 29, 565-598.
5. Laruelle M., Abi-Dargham A., van Dyck C.H., Gil R., D'Souza C.D., Erdos J., McCance E., Rosenblatt W., Fingado C., Zoghbi S.S., Baldwin R.M., Seibyl J.P., Krystal J.H., Charney D.S., Innis R.B. (1996) Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. Proc Natl Acad Sci U S A. 93 (17), 9235-9240.
6. Breier A., Su T.P., Saunders R., Carson R.E., Kolachana B.S., De Bartolomeis A., Weinberger D.R., Weisenfeld N., Malhotra A.K., Eckelman W.C., Pickar D. (1997) Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. Proc Natl Acad Sci U S A. 94 (6), 2569-2574.
7. Drevets W.C., Gautier C., Price J.C., Kupfer D.J., Kinahan P.E., Grace A.A., Price J.L., Mathis C.A. (2001) Amphetamine-induced dopamine release in human ventral striatum correlates with euphoria. Biol Psychiatry. 49 (2), 81-96.
8. Martinez D., Slifstein M., Broft A., Mawlawi O., Hwang D.R., Huang Y., Cooper T., Kegeles L., Zarahn E., Abi-Dargham A., Haber S.N., Laruelle M. (2003) Imaging human mesolimbic dopamine transmission with positron emission tomography. Part II: amphetamine-induced dopamine release in the functional subdivisions of the striatum. J Cereb Blood Flow Metab. 23 (3), 285-300.
9. Martinez D., Narendran R., Foltin R.W., Slifstein M., Hwang D.R., Broft A., Huang Y., Cooper T.B., Fischman M.W., Kleber H.D., Laruelle M. (2007) Amphetamine-induced dopamine release: markedly blunted in cocaine dependence and predictive of the choice to self-administer cocaine. Am J Psychiatry. 164 (4), 622-629.
10. Schlaepfer T.E., Pearlson G.D., Wong D.F., Marenco S., Dannals R.F. (1997) PET study of competition between intravenous cocaine and [11C]raclopride at dopamine receptors in human subjects. Am J Psychiatry. 154 (9), 1209-1213.
11. Boileau I., Assaad J.M., Pihl R.O., Benkelfat C., Leyton M., Diksic M., Tremblay R.E., Dagher A. (2003) Alcohol promotes dopamine release in the human nucleus accumbens. Synapse. 49 (4), 226-231.
12. Brody A.L., Olmstead R.E., London E.D., Farahi J., Meyer J.H., Grossman P., Lee G.S., Huang J., Hahn E.L., Mandelkern M.A. (2004) Smoking-induced ventral striatum dopamine release. Am J Psychiatry. 161 (7), 1211-1218.
13. Brody A.L., Mandelkern M.A., Olmstead R.E., Scheibal D., Hahn E., Shiraga S., Zamora-Paja E., Farahi J., Saxena S., London E.D., McCracken J.T. (2006) Gene variants of brain dopamine pathways and smoking-induced dopamine release in the ventral caudate/nucleus accumbens. Arch Gen Psychiatry. 63 (7), 808-816.
14. Gaoni Y., Mechoulam R. (1964) Isolation, structure and partial synthesis of an active constituent of hashish. J Am Chem Soc. 86, 1646-1647.
15. Tanda G., Goldberg S.R. (2003) Cannabinoids: reward, dependence, and underlying neurochemical mechanisms--a review of recent preclinical data. Psychopharmacology. 169 (2), 115-134.
16. Lupica C.R., Riegel A.C., Hoffman A.F. (2004) Marijuana and cannabinoid regulation of brain reward circuits. Br J Pharmacol. 143 (2), 227-234.
17. Gardner E.L. (2005) Endocannabinoid signaling system and brain reward: emphasis on dopamine. Pharmacol Biochem Behav. 81 (2), 263-284.
18. French E.D., Dillon K., Wu X. (1997) Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. Neuroreport. 8 (3), 649-652.
19. French E.D. (1997) Delta9-tetrahydrocannabinol excites rat VTA dopamine neurons through activation of cannabinoid CB1 but not opioid receptors. Neurosci Lett. 226 (3), 159-162.
20. Gessa G.L., Melis M., Muntoni A.L., Diana M. (1998) Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB1 receptors. Eur J Pharmacol. 341 (1), 39-44.
21. Ng Cheong Ton J.M., Gerhardt G.A., Friedemann M., Etgen A.M., Rose G.M., Sharpless N.S., Gardner E.L. (1988) The effects of delta 9-tetrahydrocannabinol on potassium-evoked release of dopamine in the rat caudate nucleus: an in vivo electrochemical and in vivo microdialysis study. Brain Res. 451 (1-2), 59-68.

22. Chen J.P., Paredes W., Li J., Smith D., Lowinson J., Gardner E.L. (1990) Delta 9-tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely-moving rats as measured by intracerebral microdialysis. Psychopharmacology (Berl). 102 (2), 156-162.
23. Tanda G., Pontieri F.E., Di Chiara G. (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. Science. 276 (5321), 2048-2050.
24. Malone D.T., Taylor D.A. (1999) Modulation by fluoxetine of striatal dopamine release following Delta9-tetrahydrocannabinol: a microdialysis study in conscious rats. Br J Pharmacol. 128 (1), 21-26.
25. Fadda P., Scherma M., Spano M.S., Salis P., Melis V., Fattore L., Fratta W. (2006) Cannabinoid self-administration increases dopamine release in the nucleus accumbens. Neuroreport. 17 (15), 1629-1632.
26. Laruelle M., Iyer R.N., Al-Tikriti M.S., Zea-Ponce Y., Malison R., Zoghbi S.S., Baldwin R.M., Kung H.F., Charney D.S., Hoffer P.B., Innis R.B., Bradberry C.W. (1997) Microdialysis and SPECT measurements of amphetamine-induced dopamine release in nonhuman primates. Synapse. 25 (1), 1-14.
27. Derogatis J.R. (1983) SCL-90-R: administration, scoring, and procedures manual - II. Clinical Psychometric Research, Townson.
28. Zuurman L., Roy C., Schoemaker R.C., Hazekamp A., den Hartigh J., Bender J.C., Verpoorte R., Pinquier J.L., Cohen A.F., van Gerven J.M. (2008) Effect of intrapulmonary tetrahydrocannabinol administration in humans. J Psychopharmacol. 22 (7), 707-716.
29. Carson R.E., Breier A., de Bartolomeis A., Saunders R.C., Su T.P., Schmall B., Der M.G., Pickar D., Eckelman W.C. (1997) Quantification of amphetamine-induced changes in [11C]raclopride binding with continuous infusion. J Cereb Blood Flow Metab. 17 (4), 437-447.
30. van der Weerdt A.P., Boellaard R., Knaapen P., Visser C.A., Lammertsma A.A., Visser F.C. (2004) Postinjection transmission scanning in myocardial 18F-FDG PET studies using both filtered backprojection and iterative reconstruction. J Nucl Med. 45 (2), 169-175.
31. Overall J.E., Gorham D.R. (1962) The brief psychiatric rating scale. Psychological Reports. 10, 799-812.
32. Hedlund J.L., Vieweg B.W. (1980) The Brief Psychiatric Rating Scale (BPRS): A comprehensive review. Journal of Operational Psychiatry. 11 (1), 48-65.
33. Bond A., Lader M. (1974) The use of analogue scales in rating subjective feelings. Br J Med Psychol. 47, 211-218.
34. Bowdle T.A., Radant A.D., Cowley D.S., Kharasch E.D., Strassman R.J., Roy-Byrne P.P. (1998) Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. Anesthesiology. 88 (1), 82-88.
35. Schuit R.C., Luurtsema G., Greuter H.N.J.M., Hendrikse N.H., Windhorst A.D., van Berckel B.N., Lammertsma A.A. (2007) Intravenous amphetamine administration does not affect metabolism of [11C] raclopride. Journal of labelled compounds and radiopharmaceuticals. 50, S471.
36. Hulshoff Pol H.E., Schnack H.G., Mandl R.C., van Haren N.E., Koning H., Collins D.L., Evans A.C., Kahn R.S. (2001) Focal gray matter density changes in schizophrenia. Arch Gen Psychiatry. 58 (12), 1118-1125.
37. Mawlawi O., Martinez D., Slifstein M., Broft A., Chatterjee R., Hwang D.R., Huang Y., Simpson N., Ngo K., Van Heertum R., Laruelle M. (2001) Imaging human mesolimbic dopamine transmission with positron emission tomography: I. Accuracy and precision of D(2) receptor parameter measurements in ventral striatum. J Cereb Blood Flow Metab. 21 (9), 1034-1057.
38. Cizek J., Herholz K., Vollmar S., Schrader R., Klein J., Heiss W.D. (2004) Fast and robust registration of PET and MR images of human brain. Neuroimage. 22 (1), 434-442.
39. Innis R.B., Cunningham V.J., Delforge J., Fujita M., Gjedde A., Gunn R.N., Holden J., Houle S., Huang S.C., Ichise M., Iida H., Ito H., Kimura Y., Koeppe R.A., Knudsen G.M., Knuuti J., Lammertsma A.A., Laruelle M., Logan J., Maguire R.P., Mintun M.A., Morris E.D., Parsey R., Price J.C., Slifstein M., Sossi V., Suhara T., Votaw J.R., Wong D.F., Carson R.E. (2007) Consensus nomenclature for in vivo imaging of reversibly binding radioligands. J Cereb Blood Flow Metab. 27 (9), 1533-1539.
40. Lammertsma A.A., Bench C.J., Hume S.P., Osman S., Gunn K., Brooks D.J., Frackowiak R.S. (1996) Comparison of methods for analysis of clinical [11C]raclopride studies. J Cereb Blood Flow Metab. 16 (1), 42-52.
41. D'Souza D.C., Perry E., MacDougall L., Ammerman Y., Cooper T., Wu Y.T., Braley G., Gueorguieva R., Krystal J.H. (2004) The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. Neuropsychopharmacology. 29 (8), 1558-1572.
42. Ilan A.B., Gevins A., Coleman M., ElSohly M.A., De Wit H. (2005) Neurophysiological and subjective profile of marijuana with varying concentrations of cannabinoids. Behav Pharmacol. 16 (5-6), 487-496.

43. Strougo A., Zuurman L., Roy C., Pinquier J.L., van Gerven J.M., Cohen A.F., Schoemaker R.C. (2008) Modelling of the concentration--effect relationship of THC on central nervous system parameters and heart rate -- insight into its mechanisms of action and a tool for clinical research and development of cannabinoids. J Psychopharmacol. 22 (7), 717-726.
44. Huestis M.A., Henningfield J.E., Cone E.J. (1992) Blood cannabinoids. I. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana. J Anal Toxicol. 16 (5), 276-282.
45. Ramaekers J.G., Kauert G., van Ruitenbeek P., Theunissen E.L., Schneider E., Moeller M.R. (2006) High-potency marijuana impairs executive function and inhibitory motor control. Neuropsychopharmacology. 31 (10), 2296-2303.
46. Schlicker E., Kathmann M. (2001) Modulation of transmitter release via presynaptic cannabinoid receptors. Trends Pharmacol Sci. 22 (11), 565-572.
47. Koob G.F., Sanna P.P., Bloom F.E. (1998) Neuroscience of addiction. Neuron. 21 (3), 467-476.
48. Cardenas L., Houle S., Kapur S., Busto U.E. (2004) Oral D-amphetamine causes prolonged displacement of [11C]raclopride as measured by PET. Synapse. 51 (1), 27-31.
49. Laruelle M. (2000) Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review. J Cereb Blood Flow Metab. 20 (3), 423-451.
50. Arseneault L., Cannon M., Witton J., Murray R.M. (2004) Causal association between cannabis and psychosis: examination of the evidence. Br J Psychiatry. 184, 110-117.
51. Moore T.H., Zammit S., Lingford-Hughes A., Barnes T.R., Jones P.B., Burke M., Lewis G. (2007) Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet. 370 (9584), 319-328.
52. Linszen D.H., Dingemans P.M., Lenior M.E. (1994) Cannabis abuse and the course of recent-onset schizophrenic disorders. Arch Gen Psychiatry. 51 (4), 273-279.
53. D'Souza D.C., Abi-Saab W.M., Madonick S., Forselius-Bielen K., Doersch A., Braley G., Gueorguieva R., Cooper T.B., Krystal J.H. (2005) Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. Biol Psychiatry. 57 (6), 594-608.
54. Seeman P., Lee T. (1975) Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurons. Science. 188 (4194), 1217-1219.
55. Angrist B., Van Kammen D.P. (1984) CNS stimulants as tools in the study of schizophrenia. Trends in Neurosciences. 7, 388-390.

3

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, Arterial Spin Labeling 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.

Introduction

The present paper describes the objectives and methods of a large Dutch pharmacological 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) Priority Medicines project[1], 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[2-4]. It consists of cannabinoid receptors and endocannabinoid ligands that work on these receptors. There are at least two different cannabinoid receptors, but in the brain CB_1 receptors are the most important and they are widely distributed throughout the brain (see for extensive reviews on the eCB system Ameri, 1999[5], Wilson and Nicoll, 2002[6] and Piomelli, 2003[7]). 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 postsynaptically and work on presynaptic receptors, thereby regulating the release of both inhibitory and excitatory neurotransmitters[6,7]. 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[2-4].

Modulation of the eCB system by administering exogenous cannabinoids such as Δ9-tetrahydrocannabinol (THC), the main psychoactive constituent of cannabis[8], produces a diverse range of acute effects by activating the CB_1 receptor. Apart from the euphoriant effect or "high"[9-11], THC also induces impairments in working memory[9,10,12], episodic memory[13] (see for a review Ranganathan and D'Souza, 2006[4]), and attention[14-16]. THC also affects impulse control[17,18]. High-dose intoxication with cannabis can result in acute psychosis, usually of a transient nature[19,20]. THC possesses rewarding properties: it is self-administered by monkeys[21] and enhances striatal dopamine levels in both animals[22] and humans[11] (see for a review Lupica et al., 2004[2]). 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 3.1 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[23,24] and worsens its clinical outcome[25,26]. Further, patients with schizophrenia demonstrate both enhanced CB_1 receptor densities in cortical regions[27-29] and increased levels of endogenous cannabinoids in cerebral spinal fluid[30,31] and plasma[32]. Finally, there is a substantial body of evidence from both preclinical 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[33] and Solowij and Michie, 2007[34]).

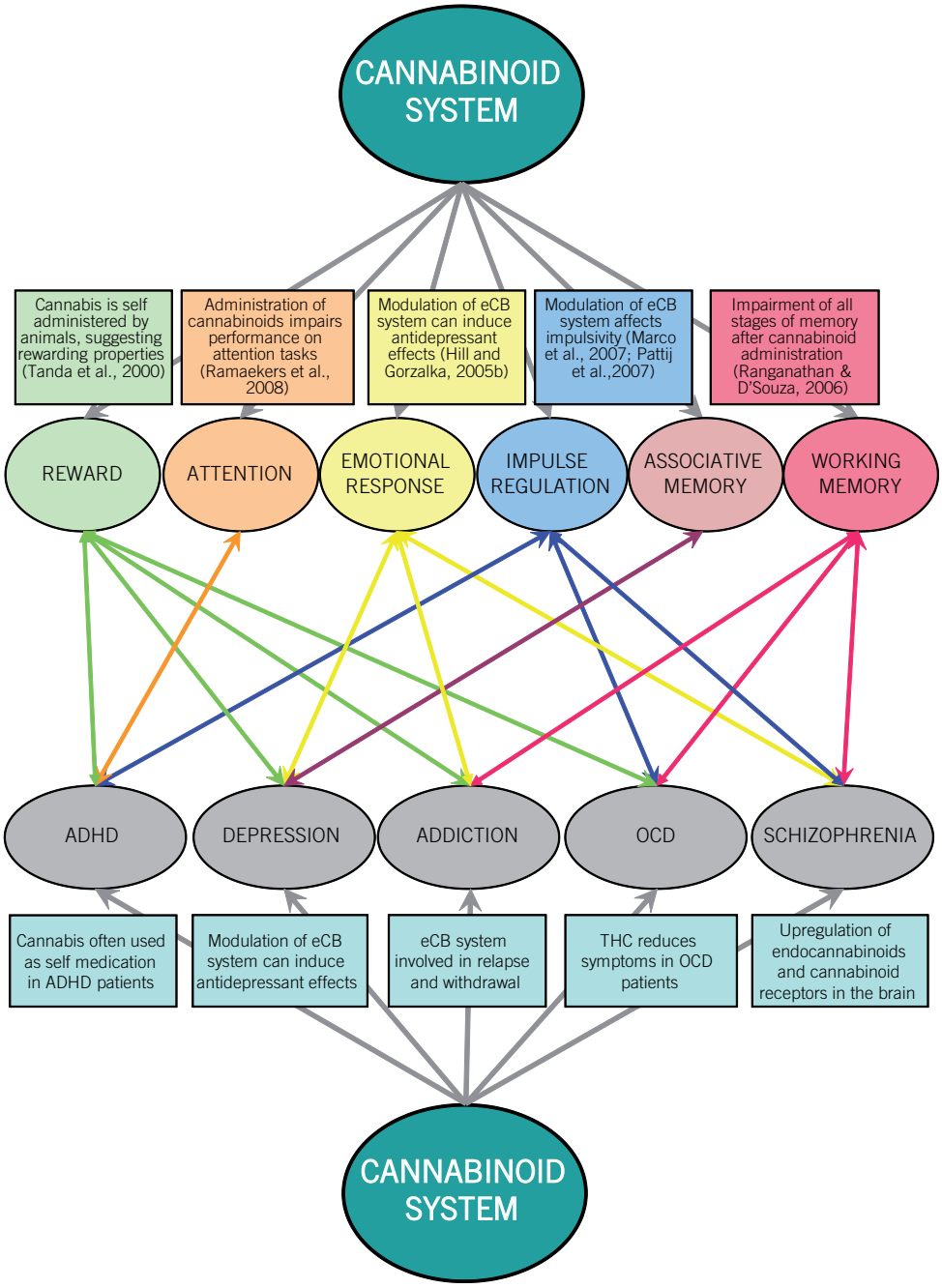

Figure 3.1 A schematic presentation of the rationale behind the PhICS study. There is evidence for involvement of the endocannabinoid (eCB) system in both psychiatric disorders (lower part) and different domains of cognition (upper part). Impairments in cognition are significant symptoms in psychiatric disorders (see also Table 3.2). 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.
ADHD, attention deficit hyperactivity disorder; OCD, obsessive compulsive disorder

The eCB system is involved in different aspects of drug addiction, including reward, withdrawal and relapse (see for reviews De Vries and Schoffelmeer, 2005[35], Maldonado et al., 2006[36] and Fattore et al., 2007[37]). 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 CB_1 receptor knockout mice and after the administration of the CB_1 antagonist rimonabant[36]. Further, CB_1 agonists reinstate drug seeking behavior of drugs of abuse, whereas rimonabant blocks this effect[35,37]. In humans, clinical trials are performed to investigate the effect of rimonabant on the cessation of smoking nicotine[38] and in the reduction of food intake in obesity[39].

Key symptoms of Attention Deficit Hyperactivity Disorder (ADHD) are disturbed impulse regulation and attention[40]. Preclinical studies indicate that the eCB system is involved in impulse regulation, since CB_1 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[41,42]. Impaired performance on attention tasks after administration of cannabinoids to both animals and humans indicates the involvement of the eCB system in attention[16,43]. The cognitive deficits in ADHD may be caused by a dysregulation of dopaminergic frontal-subcortical circuits, also affecting the reward system[44,45].

In depression, the role of the eCB system is less straightforward (see for reviews Witkin et al., 2005[3] and Hill and Gorzalka, 2005[46]). Preclinical studies have demonstrated that both facilitation[47,48] and inhibition[49,50] 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[39].

In Obsessive Compulsive Disorder (OCD) impairments in working memory, attention and impulse regulation are core symptoms[51]. As mentioned before, there are several indications that the eCB system is involved in these symptoms (see for a review Solowij and Michie, 2007[34]). Interestingly, treatment with THC reduces obsessive compulsive symptoms in patients with Gilles de la Tourette-Syndrome[52] and OCD[53].

In summary, various lines of preclinical 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 3.1).

The PhICS study is unique in its multidisciplinarity and the wide array of convergent methods used. Core methodology in PhICS involves measuring brain function in humans with a neuroimaging technique called pharmacological Magnetic Resonance Imaging (phMRI) (see for a review Honey and Bullmore, 2004[54]). PhMRI is a powerful tool to map direct modulation of brain function by psychopharmacological agents, in this case the CB_1 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 3.1). 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 twelve 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[55]. 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 3.1 for all criteria of participation.

Table 3.1 In- 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
• 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 (B.M.I.) <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 3 months before the start of the study
• Claustrophobia
• Metal objects in or around the body (braces, pacemaker, metal fragments)

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 4 hours before their arrival at the hospital. Subjects need to refrain from cannabis for at least 2 weeks before the first study day until study completion and from alcohol for 48 hours before each study day. 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 functional MRI 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 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 3.2 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.

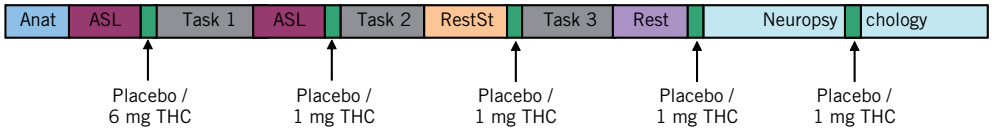

Figure 3.2 Outline of a study day. The scanning session comprises three functional MRI scans during a cognitive challenge. In addition, an anatomical scan, a resting state scan and Arterial Spin Labeling (ASL) scans are acquired. Subjects receive subsequent doses of THC or placebo. The first THC dose 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.

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[56,57]. 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[56,57], 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 GMP-compliant procedures (Farmalyse BV, Zaandam, The Netherlands) and each mg 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 s 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 CNS effects. These doses are based on pharmacokinetic/pharmacodynamic (PK/PD) modeling of the CNS effects induced by THC[58].

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[59]. For OCD patients the Y-BOX questionnaire is used. The ADHD rating scale is used for assessing symptomatology in ADHD patients[60], the Beck Depression Inventory for depressive patients[61], and the Fägerstrom Test for Nicotine Dependence (FTND) for smokers[62]. 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)[63] and the Behavioral Inhibition Scale / Behavioral Activation Scale (BIS/BAS)[64] 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, as well as the resting state scan[65] (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 s; 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 220.8x240x159.6; matrix 368×400×113; voxel size 0.6x0.6x0.6 mm, 266 slices; sagittal orientation).

fMRI data are preprocessed and analyzed using SPM5 (Wellcome Trust Centre for Neuroimaging, London, UK). Preprocessing of data includes realignment 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 (FWHM = 8 mm). Statistical analysis is performed for every fMRI task separately using a General Linear Model 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[40,44], these patients perform fMRI tasks known to activate brain networks underlying these cognitive domains. Table 3.2 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.

Table 3.2 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 5-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 5-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 Task	Paired Associates Learning
	Attention		Simple and 5-choice Reaction Time Task

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

Working memory Working memory is assessed using a modified version of the Sternberg recognition task[66]. 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[67-69].

Reward To activate reward circuitry an adapted version of the monetary incentive delay task as developed by Knutson and colleagues is used[70]. 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)[71] and consists of a continuous stream of four-digit numerals presented every 0.75 sec. 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)[71,72].

Impulse regulation As a measure of impulsivity, brain activity underlying inhibitory motor control is assessed with a stop signal task[73,74]. 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[73-75].

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[76]. 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[76,77].

Associative memory Associative memory is assessed with a pictorial task involving three different task conditions. 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[78,79].

Arterial Spin Labeling

Since functional MRI measures the BOLD (blood oxygen level dependent) signal, THC-induced global changes in cerebral blood flow may affect the fMRI findings. Arterial Spin Labeling (ASL) permits the non-invasive quantification of global and regional brain perfusion

(see for a review Petersen et al., 2006[80]). 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 8 sec. For measurement of the magnetization of arterial blood (M0) 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)[81].

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[82]. 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[83]. Psychedelic effects are assessed using an adapted version of a 13-item visual analogue rating scale, originally described by Bowdle and colleagues[84,85]. The visual analogue scales "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[85]. 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)[85].

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 CB_1-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 3.2).

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[86]. 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[77,87]. However, in these studies THC was administered orally. Since THC plasma concentrations are much higher after intrapulmonary compared to oral administration[11,85], we expect to detect the same degree of THC-induced effects with a sample size of twelve subjects. In addition, groups of twelve subjects provide ample power to demonstrate significant subjective and psychedelic effects of THC[11,85] and to detect differences in cerebral blood flow between patients and healthy controls using ASL[88]. 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 drop-out 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 3.3). First, in functional MRI 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.

Table 3.3 The three steps of functional MRI 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 vs. Off
2.	Effect of THC	THC administration	THC vs. Placebo
3.	Effect on symptom	Psychiatric disorders	Patient vs. Control

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 3.1).
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 central nervous system 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 paragraph describes THC-induced physiological and subjective effects in a group of 13 healthy subjects (all male, age 21.6 ± 2.1 (SD)).

THC plasma concentrations

THC plasma concentration reached a maximum of 58.1 ± 31.3 (standard error of the mean (SEM)) ng/ml 5 min 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 5 min 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 min after the first THC administration. Plasma concentration of 11-OH-THC peaked at 5 min after the first inhalation (2.8 ± 3.0 ng/ml) (see Figure 3.3).

Heart rate

Heart rate was measured at 20 time points during the test days. Per subject, heart rate scores (beats per minute (bpm)) were mean corrected, for placebo and THC sessions separately. Figure 3.4 depicts average mean corrected heart rate curves (± standard error of mean (SEM); N=13) over time during placebo and THC sessions. GLM repeated 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 3.5). 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 functional MRI 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*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.

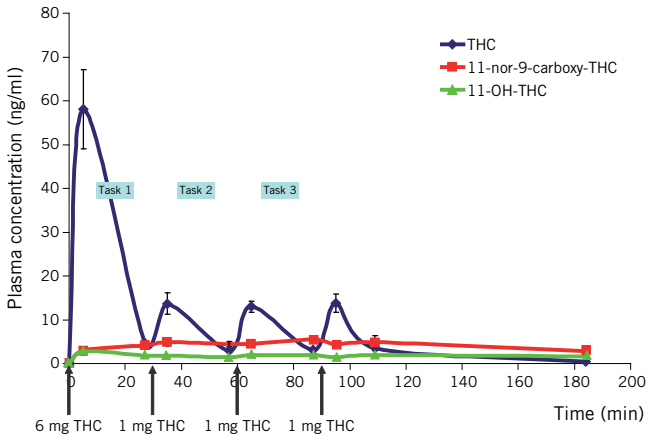

Figure 3.3 Plasma concentrations of Δ9-tetrahydrocannabinol (THC) and its main metabolites 11-OH-THC and 11-nor-9-carboxy-THC after inhalation of 6, 1, 1 and 1 mg THC with 30 minutes intervals (mean ± standard error of mean (SEM); n = 13). At the X-axis vertical arrows indicate the time points of THC administration. The task blocks in the figure indicate time blocks when fMRI tasks were administered in randomized order.

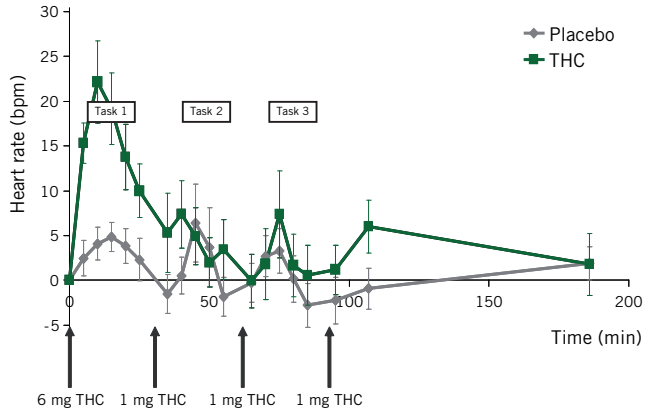

Figure 3.4 Mean corrected heart rate in beats per minute (bpm) over time. Error bars denote standard errors of mean (SEM). At the X-axis vertical arrows indicate the time points of THC administration. The task blocks in the figure indicate time blocks when fMRI tasks were administered in randomized order.

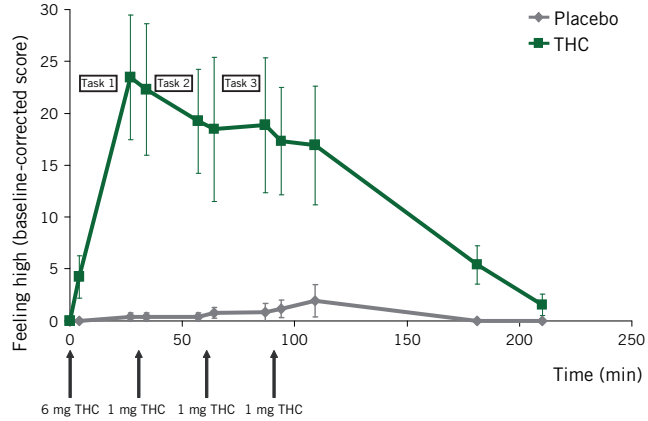

Figure 3.5 Mean corrected VAS scores of Feeling High over time in minutes. Error bars denote standard errors of mean (SEM). At the X-axis vertical arrows indicate the time points of THC administration. The task blocks in the figure indicate time blocks when fMRI tasks were administered in randomized order.

Imaging data

Figure 3.6 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 3.6), 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 3.6) 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 lab using the same task paradigm in different groups of subjects[79,89,90].

For working memory, the network of activated regions shown (middle panel Figure 3.6) 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 7 consonants) with activity during the control condition (memory set of 1 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[68,69,91].

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 3.6, lower part). These regions have been shown to be critically involved in aspects of impulse regulation[73,92].

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 endocannabinoid system. Brain imaging data of the placebo sessions demonstrate that brain activation during specified

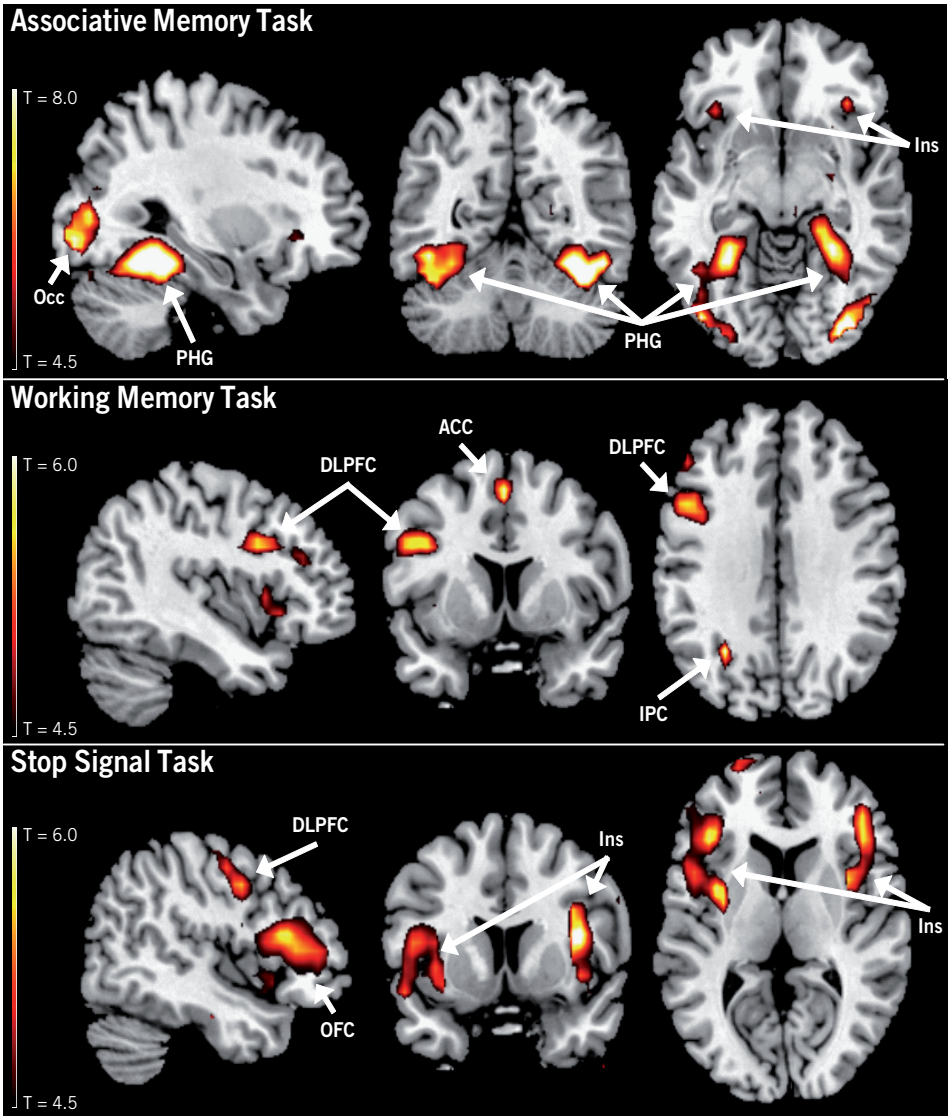

Figure 3.6 Overview of brain regions critically involved in associative memory, working memory and impulse regulation as measured with the task paradigms used in PhICS. All activation maps reflect suprathreshold ($T > 4.5$, $p < 0.05$, corrected for multiple comparisons) group brain activity during placebo 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 7 (memory set of 7 consonants) with load 1. DLPFC = dorsolateral prefrontal cortex, ACC = anterior cingulate cortex, IPG = 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 gyrus.

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 public private partnership 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 preclinical and clinical research and combines technologies ranging from in-vitro approaches to behavioral models matched between animals and humans. It is expected this multidisciplinary 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 functional MRI 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 multidisciplinary study design of PhICS is the measurement of many other physiological 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 helps determining 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 cannabis 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 feedback 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 3.1), with a focus on "intermediate phenotypes"[93]. 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[94,95] 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 malfunctioning endocannabinoid 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 multidisciplinary 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[9]. A similar effect may occur for brain activation. For the patients, a THC-challenge may further aggravate cognitive dysfunction, both at the behavioral and the neurophysiological 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[26,96]. 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 schizophrenia, such as anhedonia, apathy and social withdrawal[97].

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 psychiatric and neurological disorders. When we know how the eCB system is involved, the next step may be development of medication influencing this system to relief these symptoms. Thus, the results from the PhICS study are likely of great interest for research and development departments of pharmaceutical companies. Other future research directions include confirmation of and expanding the findings of the PhICS study via converging methods. For example, future pharmacological study designs could be applied in humans using direct of indirect eCB antagonist. In addition, blocking the degradation of endocannabinoids in humans with a fatty acid amide hydrolase (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 eCB 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.

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References

1. Kaplan W, Laing R. Priority Medicines for Europe and the World. Geneva, Switzerland; 2004 Nov.
2. Lupica C.R., Riegel A.C., Hoffman A.F. (2004) Marijuana and cannabinoid regulation of brain reward circuits. Br J Pharmacol. 143 (2), 227-234.
3. Witkin J.M., Tzavara E.T., Nomikos G.G. (2005) A role for cannabinoid CB1 receptors in mood and anxiety disorders. Behav Pharmacol. 16 (5-6), 315-331.
4. Ranganathan M., D'Souza D.C. (2006) The acute effects of cannabinoids on memory in humans: a review. Psychopharmacology (Berl). 188 (4), 425-444.
5. Ameri A. (1999) The effects of cannabinoids on the brain. Prog Neurobiol. 58 (4), 315-348.
6. Wilson R.I., Nicoll R.A. (2002) Endocannabinoid signaling in the brain. Science. 296 (5568), 678-682.
7. Piomelli D. (2003) The molecular logic of endocannabinoid signalling. Nat Rev Neurosci. 4 (11), 873-884.
8. Gaoni Y., Mechoulam R. (1964) Isolation, structure and partial synthesis of an active constituent of hashish. J Am Chem Soc. 86, 1646-1647.
9. D'Souza D.C., Perry E., MacDougall L., Ammerman Y., Cooper T., Wu Y.T., Braley G., Gueorguieva R., Krystal J.H. (2004) The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. Neuropsychopharmacology. 29 (8), 1558-1572.
10. Ilan A.B., Smith M.E., Gevins A. (2004) Effects of marijuana on neurophysiological signals of working and episodic memory. Psychopharmacology (Berl). 176 (2), 214-222.
11. Bossong M.G., van Berckel B.N., Boellaard R., Zuurman L., Schuit R.C., Windhorst A.D., van Gerven J.M., Ramsey N.F., Lammertsma A.A., Kahn R.S. (2009) Δ9-Tetrahydrocannabinol induces dopamine release in the human striatum. Neuropsychopharmacology. 34 (3), 759-766.
12. Ilan A.B., Gevins A., Coleman M., ElSohly M.A., De Wit H. (2005) Neurophysiological and subjective profile of marijuana with varying concentrations of cannabinoids. Behav Pharmacol. 16 (5-6), 487-496.
13. Curran H.V., Brignell C., Fletcher S., Middleton P., Henry J. (2002) Cognitive and subjective dose-response effects of acute oral Delta 9-tetrahydrocannabinol (THC) in infrequent cannabis users. Psychopharmacology. 164 (1), 61-70.
14. Casswell S., Marks D. (1973) Cannabis induced impairment of performance of a divided attention task. Nature. 241 (5384), 60-61.
15. Marks D.F., MacAvoy M.G. (1989) Divided attention performance in cannabis users and non-users following alcohol and cannabis separately and in combination. Psychopharmacology. 99 (3), 397-401.
16. Ramaekers J.G., Kauert G., Theunissen E.L., Toennes S.W., Moeller M.R. (2009) Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. J Psychopharmacol. 23 (3), 266-277.
17. McDonald J., Schleifer L., Richards J.B., de Wit H. (2003) Effects of THC on behavioral measures of impulsivity in humans. Neuropsychopharmacology. 28 (7), 1356-1365.
18. Ramaekers J.G., Kauert G., van Ruitenbeek P., Theunissen E.L., Schneider E., Moeller M.R. (2006) High-potency marijuana impairs executive function and inhibitory motor control. Neuropsychopharmacology. 31 (10), 2296-2303.
19. Chopra G.S., Smith J.W. (1974) Psychotic reactions following cannabis use in East Indians. Arch Gen Psychiatry. 30 (1), 24-27.
20. Thomas H. (1996) A community survey of adverse effects of cannabis use. Drug Alcohol Depend. 42 (3), 201-207.
21. Tanda G., Munzar P., Goldberg S.R. (2000) Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. Nat Neurosci. 3 (11), 1073-1074.
22. Tanda G., Pontieri F.E., Di C.G. (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. Science. 276 (5321), 2048-2050.
23. Arseneault L., Cannon M., Witton J., Murray R.M. (2004) Causal association between cannabis and psychosis: examination of the evidence. Br J Psychiatry. 184, 110-117.
24. Moore T.H., Zammit S., Lingford-Hughes A., Barnes T.R., Jones P.B., Burke M., Lewis G. (2007) Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet. 370 (9584), 319-328.
25. Linszen D.H., Dingemans P.M., Lenior M.E. (1994) Cannabis abuse and the course of recent-onset schizophrenic disorders. Arch Gen Psychiatry. 51 (4), 273-279.
26. D'Souza D.C., Abi-Saab W.M., Madonick S., Forselius-Bielen K., Doersch A., Braley G., Gueorguieva R., Cooper T.B., Krystal J.H. (2005) Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. Biol Psychiatry. 57 (6), 594-608.

27. Dean B., Sundram S., Bradbury R., Scarr E., Copolov D. (2001) Studies on [3H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. Neuroscience. 103 (1), 9-15.
28. Zavitsanou K., Garrick T., Huang X.F. (2004) Selective antagonist [3H]SR141716A binding to cannabinoid CB1 receptors is increased in the anterior cingulate cortex in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 28 (2), 355-360.
29. Newell K.A., Deng C., Huang X.F. (2006) Increased cannabinoid receptor density in the posterior cingulate cortex in schizophrenia. Exp Brain Res. 172 (4), 556-560.
30. Leweke F.M., Giuffrida A., Wurster U., Emrich H.M., Piomelli D. (1999) Elevated endogenous cannabinoids in schizophrenia. Neuroreport. 10 (8), 1665-1669.
31. Giuffrida A., Leweke F.M., Gerth C.W., Schreiber D., Koethe D., Faulhaber J., Klosterkotter J., Piomelli D. (2004) Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. Neuropsychopharmacology. 29 (11), 2108-2114.
32. De Marchi N., De Petrocellis L., Orlando P., Daniele F., Fezza F., Di Marzo V. (2003) Endocannabinoid signalling in the blood of patients with schizophrenia. Lipids Health Dis. 2, 5.
33. Lichtman A.H., Varvel S.A., Martin B.R. (2002) Endocannabinoids in cognition and dependence. Prostaglandins Leukot Essent Fatty Acids. 66 (2-3), 269-285.
34. Solowij N., Michie P.T. (2007) Cannabis and cognitive dysfunction: parallels with endophenotypes of schizophrenia? J Psychiatry Neurosci. 32 (1), 30-52.
35. De Vries T.J., Schoffelmeer A.N. (2005) Cannabinoid CB1 receptors control conditioned drug seeking. Trends Pharmacol Sci. 26 (8), 420-426.
36. Maldonado R., Valverde O., Berrendero F. (2006) Involvement of the endocannabinoid system in drug addiction. Trends Neurosci. 29 (4), 225-232.
37. Fattore L., Spano M.S., Deiana S., Melis V., Cossu G., Fadda P., Fratta W. (2007) An endocannabinoid mechanism in relapse to drug seeking: a review of animal studies and clinical perspectives. Brain Res Rev. 53 (1), 1-16.
38. Cahill K., Ussher M. (2007) Cannabinoid type 1 receptor antagonists (rimonabant) for smoking cessation. Cochrane Database Syst Rev. (4).
39. Christensen R., Kristensen P.K., Bartels E.M., Bliddal H., Astrup A. (2007) Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. Lancet. 370 (9600), 1706-1713.
40. Biederman J., Faraone S.V. (2005) Attention-deficit hyperactivity disorder. Lancet. 366 (9481), 237-248.
41. Marco E.M., Adriani W., Canese R., Podo F., Viveros M.P., Laviola G. (2007) Enhancement of endocannabinoid signalling during adolescence: Modulation of impulsivity and long-term consequences on metabolic brain parameters in early maternally deprived rats. Pharmacol Biochem Behav. 86 (2), 334-345.
42. Pattij T., Janssen M.C., Schepers I., Gonzalez-Cuevas G., De Vries T.J., Schoffelmeer A.N. (2007) Effects of the cannabinoid CB1 receptor antagonist rimonabant on distinct measures of impulsive behavior in rats. Psychopharmacology (Berl). 193 (1), 85-96.
43. Verrico C.D., Jentsch J.D., Roth R.H., Taylor J.R. (2004) Repeated, intermittent delta(9)-tetrahydrocannabinol administration to rats impairs acquisition and performance of a test of visuospatial divided attention. Neuropsychopharmacology. 29 (3), 522-529.
44. Scheres A., Milham M.P., Knutson B., Castellanos F.X. (2007) Ventral striatal hyporesponsiveness during reward anticipation in attention-deficit/hyperactivity disorder. Biol Psychiatry. 61 (5), 720-724.
45. Plichta M.M., Vasic N., Wolf C., Lesch K.P., Brummer D., Jacob C., Fallgatter A.J., Gron G. (2008) Neural Hyporesponsiveness and Hyperresponsiveness During Immediate and Delayed Reward Processing in Adult Attention-Deficit/Hyperactivity Disorder. Biol Psychiatry.
46. Hill M.N., Gorzalka B.B. (2005) Is there a role for the endocannabinoid system in the etiology and treatment of melancholic depression? Behav Pharmacol. 16 (5-6), 333-352.
47. Hill M.N., Gorzalka B.B. (2005) Pharmacological enhancement of cannabinoid CB1 receptor activity elicits an antidepressant-like response in the rat forced swim test. Eur Neuropsychopharmacol. 15 (6), 593-599.
48. McLaughlin R.J., Hill M.N., Morrish A.C., Gorzalka B.B. (2007) Local enhancement of cannabinoid CB1 receptor signalling in the dorsal hippocampus elicits an antidepressant-like effect. Behav Pharmacol. 18 (5-6), 431-438.
49. Shearman L.P., Rosko K.M., Fleischer R., Wang J., Xu S., Tong X.S., Rocha B.A. (2003) Antidepressant-like and anorectic effects of the cannabinoid CB1 receptor inverse agonist AM251 in mice. Behav Pharmacol. 14 (8), 573-582.
50. Griebel G., Stemmelin J., Scatton B. (2005) Effects of the cannabinoid CB1 receptor antagonist rimonabant in models of emotional reactivity in rodents. Biol Psychiatry. 57 (3), 261-267.

51. de Geus F., Denys D.A., Sitskoorn M.M., Westenberg H.G. (2007) Attention and cognition in patients with obsessive-compulsive disorder. Psychiatry Clin Neurosci. 61 (1), 45-53.
52. Muller-Vahl K.R., Schneider U., Koblenz A., Jobges M., Kolbe H., Daldrup T., Emrich H.M. (2002) Treatment of Tourette's syndrome with Delta 9-tetrahydrocannabinol (THC): a randomized crossover trial. Pharmacopsychiatry. 35 (2), 57-61.
53. Schindler F., Anghelescu I., Regen F., Jockers-Scherubl M. (2008) Improvement in refractory obsessive compulsive disorder with dronabinol. Am J Psychiatry. 165 (4), 536-537.
54. Honey G., Bullmore E. (2004) Human pharmacological MRI. Trends Pharmacol Sci. 25 (7), 366-374.
55. Craft R.M. (2005) Sex differences in behavioral effects of cannabinoids. Life Sci. 77 (20), 2471-2478.
56. Hazekamp A., Ruhaak R., Zuurman L., van Gerven J., Verpoorte R. (2006) Evaluation of a vaporizing device (Volcano) for the pulmonary administration of tetrahydrocannabinol. J Pharm Sci. 95 (6), 1308-1317.
57. Abrams D.I., Vizoso H.P., Shade S.B., Jay C., Kelly M.E., Benowitz N.L. (2007) Vaporization as a smokeless cannabis delivery system: a pilot study. Clin Pharmacol Ther. 82 (5), 572-578.
58. Strougo A., Zuurman L., Roy C., Pinquier J.L., van Gerven J.M., Cohen A.F., Schoemaker R.C. (2008) Modelling of the concentration--effect relationship of THC on central nervous system parameters and heart rate -- insight into its mechanisms of action and a tool for clinical research and development of cannabinoids. J Psychopharmacol. 22 (7), 717-726.
59. Kay S.R., Fiszbein A., Opler L.A. (1987) The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull. 13 (2), 261-276.
60. Kooij S.J.J., Boonstra M.A., Swinkels S.H., Bekker E.M., de Noord I., Buitelaar J.K. (2008) Reliability, validity, and utility of instruments for self-report and informant report concerning symptoms of ADHD in adult patients. J Atten Disord. 11 (4), 445-458.
61. Beck A.T., Ward C.H., Mendelson M., Mock J., Erbaugh J. (1961) An inventory for measuring depression. Arch Gen Psychiatry. 4, 561-571.
62. Heatherton T.F., Kozlowski L.T., Frecker R.C., Fagerstrom K.O. (1991) The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. Br J Addict. 86 (9), 1119-1127.
63. Zuckerman M., Link K. (1968) Construct validity for the sensation-seeking scale. J Consult Clin Psychol. 32 (4), 420-426.
64. Carver C.S., White T.L. (1994) Behavioral inhibition, behavioral activation, and affetive responses to impending reward and punishment: the BIS/BAS scales. Journal of Personality and Social Psychology. 67, 319-333.
65. Neggers S.F., Hermans E.J., Ramsey N.F. (2008) Enhanced sensitivity with fast three-dimensional blood-oxygen-level-dependent functional MRI: comparison of SENSE-PRESTO and 2D-EPI at 3 T. NMR Biomed. 21 (7), 663-676.
66. Sternberg S. (1966) High-speed scanning in human memory. Science. 153 (736), 652-654.
67. Jansma J.M., Ramsey N.F., Slagter H.A., Kahn R.S. (2001) Functional anatomical correlates of controlled and automatic processing. J Cogn Neurosci. 13 (6), 730-743.
68. Ramsey N.F., Jansma J.M., Jager G., van Raalten T., Kahn R.S. (2004) Neurophysiological factors in human information processing capacity. Brain. 127 (Pt 3), 517-525.
69. Jager G., Kahn R.S., van den Brink W., van Ree J.M., Ramsey N.F. (2006) Long-term effects of frequent cannabis use on working memory and attention: an fMRI study. Psychopharmacology. 185 (3), 358-368.
70. Knutson B., Adams C.M., Fong G.W., Hommer D. (2001) Anticipation of increasing monetary reward selectively recruits nucleus accumbens. J Neurosci. 21 (16), RC159.
71. Strakowski S.M., Adler C.M., Holland S.K., Mills N., DelBello M.P. (2004) A preliminary fMRI study of sustained attention in euthymic, unmedicated bipolar disorder. Neuropsychopharmacology. 29 (9), 1734-1740.
72. Adler C.M., Sax K.W., Holland S.K., Schmithorst V., Rosenberg L., Strakowski S.M. (2001) Changes in neuronal activation with increasing attention demand in healthy volunteers: an fMRI study. Synapse. 42 (4), 266-272.
73. Li C.S., Huang C., Constable R.T., Sinha R. (2006) Imaging response inhibition in a stop-signal task: neural correlates independent of signal monitoring and post-response processing. J Neurosci. 26 (1), 186-192.
74. Chevrier A.D., Noseworthy M.D., Schachar R. (2007) Dissociation of response inhibition and performance monitoring in the stop signal task using event-related fMRI. Hum Brain Mapp. 28 (12), 1347-1358.
75. Vink M., Kahn R.S., Raemaekers M., van den Heuvel M., Boersma M., Ramsey N.F. (2005) Function of striatum beyond inhibition and execution of motor responses. Hum Brain Mapp. 25 (3), 336-344.
76. Hariri A.R., Mattay V.S., Tessitore A., Kolachana B., Fera F., Goldman D., Egan M.F., Weinberger D.R.

(2002) Serotonin transporter genetic variation and the response of the human amygdala. Science. 297 (5580), 400-403.
77. Phan K.L., Angstadt M., Golden J., Onyewuenyi I., Popovska A., de Wit H. (2008) Cannabinoid modulation of amygdala reactivity to social signals of threat in humans. J Neurosci. 28 (10), 2313-2319.
78. Henke K., Buck A., Weber B., Wieser H.G. (1997) Human hippocampus establishes associations in memory. Hippocampus. 7 (3), 249-256.
79. Jager G., van Hell H.H., De Win M.M., Kahn R.S., van den Brink W., van Ree J.M., Ramsey N.F. (2007) Effects of frequent cannabis use on hippocampal activity during an associative memory task. Eur Neuropsychopharmacol. 17 (4), 289-297.
80. Petersen E.T., Zimine I., Ho Y.C., Golay X. (2006) Non-invasive measurement of perfusion: a critical review of arterial spin labelling techniques. Br J Radiol. 79 (944), 688-701.
81. van Osch M.J., Teeuwisse W.M., van Walderveen M.A., Hendrikse J., Kies D.A., van Buchem M.A. (2009) Can arterial spin labeling detect white matter perfusion signal? Magn Reson Med. 62 (1), 165-173.
82. Damoiseaux J.S., Rombouts S.A., Barkhof F., Scheltens P., Stam C.J., Smith S.M., Beckmann C.F. (2006) Consistent resting-state networks across healthy subjects. Proc Natl Acad Sci U S A. 103 (37), 13848-13853.
83. Bond A., Lader M. (1974) The use of analogue scales in rating subjective feelings. Br J Med Psychol. 47, 211-218.
84. Bowdle T.A., Radant A.D., Cowley D.S., Kharasch E.D., Strassman R.J., Roy-Byrne P.P. (1998) Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. Anesthesiology. 88 (1), 82-88.
85. Zuurman L., Roy C., Schoemaker R.C., Hazekamp A., den Hartigh J., Bender J.C., Verpoorte R., Pinquier J.L., Cohen A.F., van Gerven J.M. (2008) Effect of intrapulmonary tetrahydrocannabinol administration in humans. J Psychopharmacol. 22 (7), 707-716.
86. Zandbelt B.B., Gladwin T.E., Raemaekers M., van Buuren M., Neggers S.F., Kahn R.S., Ramsey N.F., Vink M. (2008) Within-subject variation in BOLD-fMRI signal changes across repeated measurements: quantification and implications for sample size. Neuroimage. 42 (1), 196-206.
87. Borgwardt S.J., Allen P., Bhattacharyya S., Fusar-Poli P., Crippa J.A., Seal M.L., Fraccaro V., Atakan Z., Martin-Santos R., O'Carroll C., Rubia K., McGuire P.K. (2008) Neural basis of delta-9-tetrahydrocannabinol and cannabidiol: effects during response inhibition. Biol Psychiatry. 64 (11), 966-973.
88. O'Gorman R.L., Mehta M.A., Asherson P., Zelaya F.O., Brookes K.J., Toone B.K., Alsop D.C., Williams S.C. (2008) Increased cerebral perfusion in adult attention deficit hyperactivity disorder is normalised by stimulant treatment: a non-invasive MRI pilot study. Neuroimage. 42 (1), 36-41.
89. Jager G., de Win M.M., Vervaeke H.K., Schilt T., Kahn R.S., van den Brink W., van Ree J.M., Ramsey N.F. (2007) Incidental use of ecstasy: no evidence for harmful effects on cognitive brain function in a prospective fMRI study. Psychopharmacology. 193 (3), 403-414.
90. Jager G., de Win M.M., van der Tweel I., Schilt T., Kahn R.S., van den Brink W., van Ree J.M., Ramsey N.F. (2008) Assessment of cognitive brain function in ecstasy users and contributions of other drugs of abuse: results from an fMRI study. Neuropsychopharmacology. 33 (2), 247-258.
91. Rypma B., D'Esposito M. (1999) The roles of prefrontal brain regions in components of working memory: effects of memory load and individual differences. Proc Natl Acad Sci U S A. 96 (11), 6558-6563.
92. Li C.S., Luo X., Yan P., Bergquist K., Sinha R. (2009) Altered impulse control in alcohol dependence: neural measures of stop signal performance. Alcohol Clin Exp Res. 33 (4), 740-750.
93. Gottesman I.I., Gould T.D. (2003) The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry. 160 (4), 636-645.
94. Whalen P.J., Shin L.M., Somerville L.H., McLean A.A., Kim H. (2002) Functional neuroimaging studies of the amygdala in depression. Semin Clin Neuropsychiatry. 7 (4), 234-242.
95. Fakra E., Salgado-Pineda P., Delaveau P., Hariri A.R., Blin O. (2008) Neural bases of different cognitive strategies for facial affect processing in schizophrenia. Schizophr Res. 100 (1-3), 191-205.
96. Grech A., van Os J., Jones P.B., Lewis S.W., Murray R.M. (2005) Cannabis use and outcome of recent onset psychosis. Eur Psychiatry. 20 (4), 349-353.
97. Compton M.T., Furman A.C., Kaslow N.J. (2004) Lower negative symptom scores among cannabis-dependent patients with schizophrenia-spectrum disorders: preliminary evidence from an African American first-episode sample. Schizophr Res. 71 (1), 61-64.

4

Effects of Δ9-tetrahydrocannabinol (THC) administration on human encoding and recall memory function: a pharmacological fMRI study

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Abstract

Deficits in memory function are an incapacitating aspect of various psychiatric and neurological disorders. Animal studies have recently provided strong evidence for involvement of the endocannabinoid (eCB) system in memory function. Neuropsychological studies in humans have shown less convincing evidence, but suggest that administration of cannabinoid substances affects encoding rather than recall of information. In this study we examined the effects of perturbation of the eCB system on memory function during both encoding and recall. We performed a pharmacological magnetic resonance imaging (MRI) study with a placebo-controlled, cross-over design, investigating effects of Δ9-tetrahydrocannabinol (THC) inhalation on associative memory-related brain function in 13 healthy volunteers. Performance and brain activation during associative memory were assessed using a pictorial memory task, consisting of separate encoding and recall conditions. Administration of THC caused reductions in activity during encoding in right insula, right inferior frontal gyrus and left middle occipital gyrus, and a network-wide increase in activity during recall, which was most prominent in bilateral cuneus and precuneus. THC administration did not affect task performance, but while during placebo recall activity significantly explained variance in performance, this effect disappeared after THC. These findings suggest eCB involvement in encoding of pictorial information. Increased precuneus activity could reflect impaired recall function, but the absence of THC effects on task performance suggests a compensatory mechanism. These results further emphasize the eCB system as a potential novel target for treatment of memory disorders, and a promising target for development of new therapies to reduce memory deficits in humans.

Introduction

Learning and memory are critical in our daily lives. Deficits in memory function are associated with various psychiatric and neurological disorders, such as Alzheimer's disease, schizophrenia and mood disorders, and can be severely incapacitating.

Recently, animal studies have provided strong evidence for the involvement of the endocannabinoid (eCB) system in memory[1-7]. The eCB system, consisting of cannabinoid receptors and accompanying endogenous ligands, is a retrograde messenger system that regulates both excitatory and inhibitory neurotransmission[8,9]. As such, the eCB system may act to 'fine tune' the control of important brain functions, including learning and memory[10]. Modulation of the eCB system by systemic administration of exogenous cannabinoids, such as Δ9-tetrahydrocannabinol (THC), the main psychoactive component in cannabis and partial agonist of the CB_1 receptor, impairs performance on various learning and memory paradigms in animals[1-7]. This suggests that the eCB system may be an important target for the development of novel therapies for memory dysfunction in psychiatric disorders. However, animal findings may not translate directly to humans, and there is a need to study the specific role of the eCB system in humans. In humans, cannabinoids produce a diverse range of acute effects[11], with

increases in heart rate and subjective effects such as 'feeling high' as the strongest and most consistently reported measures[12]. Despite the consistent findings of memory impairments in animals after cannabinoid administration and the robust cannabinoid-induced human subjective and physiological effects, the evidence for impact of cannabinoid intoxication on learning and memory performance is less convincing. A large number of neuropsychological studies have reported no acute effects of cannabinoid administration on learning and memory paradigms[12-19]. Recall of items acquired before cannabis use is also generally not affected[20-22]. Effects of cannabinoids on memory performance have, however, been reported in the free recall of information that is previously learned under the influence of cannabinoids[23-25]. This suggests that cannabinoids influence encoding but not recall of information. Notwithstanding reported effects on memory in humans, the effect size is typically surprisingly small.

Assuming that the eCB system does play an important role in memory in both humans and animals, neuropsychology results may be affected by the ability of the human brain to reduce the effects of perturbations of the eCB system on behavior by functional compensation. A more effective method to measure the role of eCB in memory function in humans can be provided by direct visualization of brain activity during performance of a memory task in a pharmacological functional Magnetic Resonance Imaging (fMRI) study.

In this study we applied this approach, and measured the effect of THC administration on encoding and recall brain function in an fMRI study. Based on neuropsychological findings, we tested the hypothesis that THC administration affects encoding, resulting in reduced encoding-related brain activity in a memory network including (para)hippocampal and prefrontal areas[26,27]. In addition, in line with neuropsychological findings, we did not expect direct effects of THC on recall processes, although compensatory mechanisms for the affected encoding function may lead to increases in activity during recall. These hypotheses were tested in an fMRI study with a double-blind, randomized, placebo-controlled, crossover design, using a pictorial associative memory task, containing separate encoding and recall conditions[26,27].

Materials and methods

This study is part of the Pharmacological Imaging of the Cannabinoid System (PhICS) study, of which design and objectives are provided in a methods paper[28].

Subjects

Fourteen healthy male right-handed subjects were recruited through advertisements on the Internet and in local newspapers. All subjects used cannabis on an incidental basis, defined as having used cannabis at least four times but at most once a week in the year before inclusion in the study. All subjects were in good physical health as assessed by medical history, physical examination, electrocardiogram (ECG), and routine laboratory tests. Subjects were asked to refrain from cannabis for at least two weeks before the first study day until study completion. A maximum use of five cigarettes per day was allowed. Illicit drug use other than cannabis

was restricted to a maximum of five times lifetime and not within six months prior to inclusion. Urine screening for cannabis, cocaine, amphetamine, methamphetamine, 3,4-methylenedioxy-methamphetamine (MDMA), morphine, methadone, tricyclic antidepressants (TCA), barbiturates and benzodiazepines was performed at screening and on both study days. Subjects with a positive drug test were excluded from the study. Subjects were also asked to abstain from alcohol for 48 hours before each study day. Smoking was not allowed from the moment of arrival until the end of a study day. Alcohol and nicotine use was assessed by self-report. Subjects were asked to fast for at least four hours before arrival. On the beginning of each test day, they were served a standard meal. For further details on inclusion and exclusion criteria we refer to Van Hell et al. (2011)[28]. All volunteers gave written informed consent before entry into the study and were compensated for their participation. The study was approved by the Independent Ethics Committee of the University Medical Center Utrecht, the Netherlands. Results are reported on thirteen out of the fourteen included subjects. One subject did not complete the second scanning session due to anxiety. See for subject characteristics Table 4.1.

Design and procedure

In a double-blind, randomized, placebo-controlled, crossover pharmacological MRI study, subjects underwent two scanning sessions after either administration of placebo or THC. Study days were scheduled two weeks apart to allow for complete clearance of drugs. Two weeks before the first study day, participants were familiarized with the scanner environment using a mock scanner. Verbal intelligence was estimated with the Dutch Adult Reading Test (DART), the Dutch version of the National Adult Reading Test[29].

Table 4.1 Subject characteristics (n = 13)

Characteristic	Mean ± SD	Range
Age (years)	21.6 ± 2.1	18 - 27
IQ	104.8 ± 5.6	94 - 111
Height (cm)	185.9 ± 5.3	176 - 196
Weight (kg)	78.7 ± 9.1	64 - 96
BMI (kg/m^2)	22.7 ± 2.3	18.6 – 27.8
Cannabis use (Occasions / year)	17.0 ± 12.4	5 – 52
Tobacco smoking (Cigarettes / week)	2.7 ± 7.7	0 - 28
Alcohol consumption (Units / week)	16.7 ± 8.7	2 - 30
Coffee consumption (Units / week)	11.2 ± 9.9	0 - 28
Illicit drug use (Occasions lifetime)	1.3 ± 1.6	0 - 4

Use of cannabis, tobacco, alcohol and coffee was given for the year before inclusion in the study. Subjects refrained from cannabis for at least two weeks before the first study day until study completion and from alcohol for 48 hours before each study day. Caffeine intake and smoking were not allowed from the moment of arrival until the end of a study day. Illicit drug use other than cannabis was at least more than six months before the first study day. All subjects showed negative urine screening at both study days.

At the beginning of each study day, a catheter was placed percutaneously in the left arm for the withdrawal of blood samples. Subsequently, subjects performed three cognitive paradigms, during which functional MRI scans were obtained. One of these paradigms was the associative memory task. Paradigm sequence was randomized between subjects, but remained unchanged within subjects across sessions. Results of the other two paradigms are reported elsewhere. On study days, subjects received subsequent doses of THC or placebo with 30 minutes intervals. Drugs were administered before each fMRI task using a Volcano® vaporizer (Storz–Bickel GmbH, Tuttlingen, Germany) according to a method described earlier[30-32]. The first THC dose was 6 mg, followed by three doses of 1 mg each to maintain stable levels of CNS effects throughout the scanning procedure. Doses were based on pharmacokinetic/pharmacodynamic (PK/PD) modeling of CNS effects induced by THC[33]. See Van Hell et al. (2011)[28] for detailed study procedures.

Drug levels and behavioral measurements

Venous blood samples were collected to determine plasma concentrations of THC and its two most important metabolites, 11-OH-THC and 11-nor-9-carboxy-THC. Blood samples were processed according to Zuurman et al. (2008)[31]. Subjective and psychedelic effects were determined with two sets of visual analogue scales (VAS)[34,35]. Both rating scales were performed consecutively at baseline and before and after performance of the associative memory task. Visual analogue scales were analyzed as described previously[31]. Correlations between THC peak concentration and behavioral changes (THC versus placebo) were determined using Pearson's correlation coefficient. Heart rate and respiration were monitored continuously during scanning, as described by Van Buuren et al. (2009)[36]. Mean heart rate was calculated by dividing the total number of heart beat trigger signals by the duration of the associative memory task. Data were corrected for baseline values and analyzed with paired t-tests.

Task paradigm

Associative memory was assessed with a pictorial memory task (denoted as PMT) involving three different task conditions (Figure 4.1)[26,27]. First, an encoding condition (EN) was conducted in which subjects were presented with two pictures, one of a person and one of a house. Subjects were asked to decide whether the person might either be an inhabitant or a visitor of the house and to memorize the combination of pictures. There was no correct or incorrect answer. The purpose of the instruction was to engage subjects in a semantic evaluation of the two pictures which was expected to lead to a deep level of encoding of the paired pictures, irrespective of the decision. In the second condition, single item pictures had to be classified (denoted as SC). Two identical pictures were shown and subjects had to indicate whether a house or a person was presented. This condition was chosen as a control task. SC requires identical amount of perceptual processing and motor response as the two experimental conditions, but without a memory component. The third condition was a recall task (RE) which required subjects to recognize specific combinations of pictures previously presented during EN. Half of the stimuli were new combinations and half were combinations previously presented

during EN. For all conditions, subjects were instructed to press one of two buttons according to the instruction in the respective task condition, with emphasis on accuracy without stressing speed of response.

Each task condition was presented in an epoch consisting of an instruction slide of 4000 ms followed by 6 stimuli. Each stimulus contained two pictures on a white background and was presented for 4000 ms, followed by an 850 ms fixation cross. Rest periods of half the epoch duration were also included. Altogether, a fixed order sequence of all task conditions was repeated four times, resulting in total task duration of 9 minutes. The PMT task contained different stimuli on both study days. Performance accuracy was assessed for SC and RE and was calculated as the mean percentage of correctly identified stimuli.

Image Acquisition

Image acquisition was performed on a Philips Achieva 3.0 Tesla scanner (Philips Medical Systems, Best, the Netherlands). Functional images were obtained using a 3D PRESTO-SENSE pulse sequence[37] with shimmed background and the following parameters: TR 22.5 ms; TE 33.2 ms; flip angle = 10°; FOV 224×256×160; matrix 56× 64×40; voxel size 4 mm isotropic; scan time 0.6075 s; 40 slices (sagittal orientation). A total of 900 functional images were acquired. Immediately after the PMT task, one volume with a flip angle of 27° was acquired for image co-registration. A T1-weighted structural image was obtained for anatomical registration with the following parameters: TR 9.5 ms; TE 4.7 ms; flip angle = 8°; FOV 220.8x240x159.6; matrix 368×400×266; voxel size 0.6 mm isotropic, 266 slices (sagittal orientation).

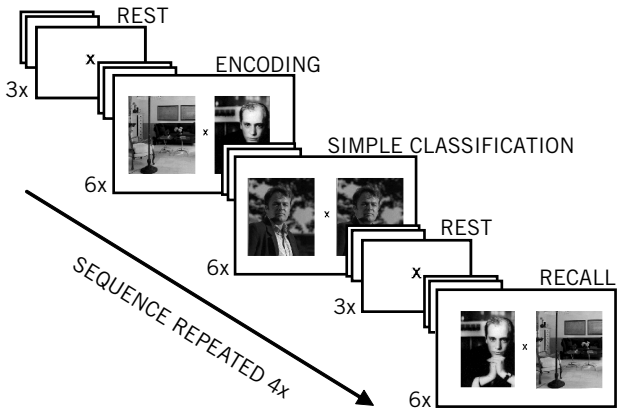


Figure 4.1 Schematic outline of the pictorial memory task (PMT) used to assess associative memory. First, an encoding condition ('ENCODING') was conducted in which subjects were presented with two pictures, one of a person and one of a house. In the second condition, identical pictures had to be classified as a house or a person ('SIMPLE CLASSIFICATION'). This condition was the control condition. The third condition was a recall task ('RECALL') required subjects to recognize specific combinations of pictures previously presented during ENCODING. Half of the stimuli were new combinations and half were combinations previously presented.

Functional MRI analysis

After reconstruction, imaging data were preprocessed and analyzed using SPM5 (Wellcome Trust Centre for Neuroimaging, London, UK). Preprocessing of data included realignment of functional images and co-registration with the anatomical scan using the volume with a flip angle of 27°. Subsequently, functional scans were spatially normalized into MNI-space38 and smoothed (FWHM = 8 mm). For each individual subject, regression coefficients for each voxel (b-values) were obtained from a general linear model regression analysis using a factor matrix that contained factors modeling the EN, SC and RE condition (four blocks each) as well as the instructions that were presented during the task. To correct for drifts in the signal, a high-pass filter with a cut-off frequency of 0.005 Hz was applied to the data.

We chose to perform Region of Interest (ROI) analyses including areas that were involved in the task, as this analysis provides a good balance between power and information and allows for calculation and presentation of effect sizes[39,40]. Group activation maps were created for the contrasts EN-SC and RE-SC, for both the placebo and THC condition. All four maps were thresholded ($t = 4.5$, $p < 0.05$, corrected for multiple comparisons) and placebo and THC maps were pooled, resulting in two group activation maps (EN-SC and RE-SC). For both the EN-SC and RE-SC contrast, clusters of at least ten neighboring voxels were defined as ROIs, thus resulting in two sets of ROIs. Constructing the ROIs based on the highest values in either the THC or the placebo session prevents bias towards either the placebo or THC session[40,41]. Mean signal change for each ROI, each subject and each session (placebo and THC) was based on regression coefficients (b-values) averaged over voxels in each ROI, extracted using Marsbar[42].

To measure THC effects on encoding, a repeated-measures MANOVA was performed on ROIs based on the EN-SC contrast with drug (two levels: THC and placebo), condition (two levels: EN and SC) and ROI (ten levels) as within-subjects factors. Post hoc paired t-test analyses were performed in comparison to SC to further investigate effects in individual ROIs. To measure effects of THC on recall activity, a repeated-measures MANOVA was performed on ROIs based on the RE-SC contrast with drug (two levels), condition (two levels: RE and SC), and ROI (seven levels) as within-subjects factors. Follow-up paired t-test analyses were again performed for every ROI.

To assess relationships between brain activity and performance and to determine whether activity patterns within involved networks predicted performance, regression analyses were conducted with ROIs as independent variables and accuracy as dependent variable. This was done for each set of ROIs (encoding and recall), and for each session (placebo, THC). If the overall GLM model was significant, individual follow-up correlation analyses were performed between performance and ROIs.

Post-hoc paired t-tests were not corrected for multiple comparisons if the main MANOVA effect was significant, as they were considered as a further exploration of an already significant effect. All hypothesis tests were performed using SPSS 17.

Results

Drug levels and behavioral measurements

THC plasma concentration reached a maximum of 58.1 ± 31.3 ng/ml 5 min 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 5 min after each respective dose. Analysis of subjective and psychedelic effects before and after performance of PMT revealed a significant THC-induced increase in VAS score of 'feeling high' ($F(1,12) = 9.98$, $p = 0.008$) and a decrease on 'alertness' ($F(1,12) = 13.95$, $p = 0.003$) compared to placebo. In addition, THC caused a trend towards both increased internal perception (reflecting inner feelings that do not correspond with reality) and external perception (reflecting misperception of external stimuli or changes in the awareness of the environment) ($F(1,12) = 3.79$, $p = 0.075$ and $F(1,12) = 3.46$, $p = 0.087$, respectively). Subjective and psychedelic effects are summarized in Table 4.2. Peak THC concentration was positively correlated with alterations (THC versus placebo) in 'feeling high' ($r = 0.620$; $p = 0.031$) and negatively with changes in 'alertness' ($r = -0.746$; $p = 0.005$). Heart rate increased significantly after THC compared with placebo (8.5 ± 10.2 and 2.1 ± 4.9 bpm increase compared to baseline, respectively; $p = 0.046$). For a more detailed description of drug levels and behavioral measurements following THC see Van Hell et al. (2011)[28].

Task performance

Performance accuracy on the PMT task did not differ between THC and placebo sessions for both SC (99.4 ± 0.4% for both sessions; $p = 1.000$) and RE (91.4 ± 3.3 and 89.4 ± 2.5%, respectively; $p = 0.430$) (Figure 4.2).

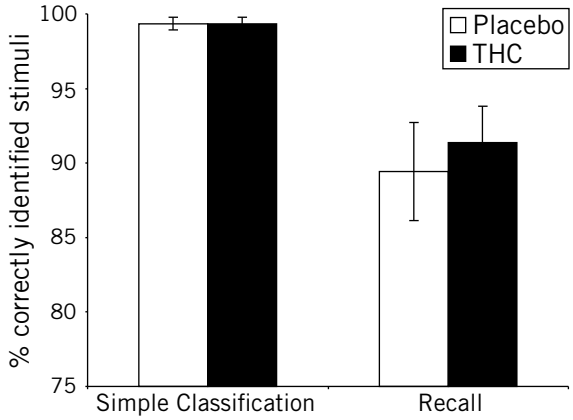


Figure 4.2 Performance accuracy on the PMT task during simple classification (left) and recall (right) in placebo session (white bars) and THC session (black bars). There was no significant difference in performance between sessions (n = 13; mean ± SEM).

Selection of regions of interest

The EN-SC contrast yielded a network of ten brain regions, comprising bilateral fusiform gyrus / parahippocampal gyrus, inferior frontal gyrus, insula and middle occipital gyrus, right putamen and left supplementary motor area (Table 4.3). The RE-SC contrast showed a network of seven regions, comprising bilateral fusiform / parahippocampal gyrus, cuneus / precuneus, middle occipital gyrus and left superior parietal gyrus (Table 4.4).

Effects of THC on encoding activity

For the ten encoding ROIs, a significant interaction effect was found between condition, drug and ROI ($F(9,108) = 2.20$; $p = 0.028$). This indicates that THC induced a change in the pattern of activity during encoding. There was a trend towards a significant effect of drug ($F(1,12) = 4.15$; $p = 0.064$), but no significant difference in the effect of THC on conditions (drug * condition, $F(1,12) = 2.47$; $p = 0.142$). To elucidate which ROI(s) were involved in the significant interaction, post hoc analyses (not corrected for multiple comparisons) were performed on each ROI. These demonstrated significantly reduced brain activity after THC administration (relative to placebo) in the right insula (from 0.53 ± 0.07 to 0.33 ± 0.06, $p = 0.019$), right inferior frontal gyrus (from 0.54 ± 0.09 to 0.22 ± 0.17, $p = 0.031$) and left middle occipital gyrus (from 0.54 ± 0.06 to 0.39 ± 0.07, $p = 0.033$). The mean b-values are shown in Figure 4.3.

Effects of THC on recall activity

Repeated measures analysis showed no significant effect of drug ($F(1,12) = 1.14$; $p = 0.306$) in the seven recall ROIs, but THC affected the RE and SC conditions significantly different (drug * condition, $F(1,12) = 5.92$; $p = 0.032$). A significant interaction effect between condition, drug and ROI ($F(6,72) = 3.02$; $p = 0.011$) indicated that these drug by condition effects differed between ROIs. Post hoc analysis (not corrected for multiple comparisons) showed a significant THC-induced increase in brain activity relative to placebo in the left (from 0.37 ± 0.11 to 0.76 ± 0.09, $p = 0.014$) and right precuneus (from 0.33 ± 0.09 to 0.78 ± 0.10, $p = 0.004$) (mean b-values, see Figure 4.4).

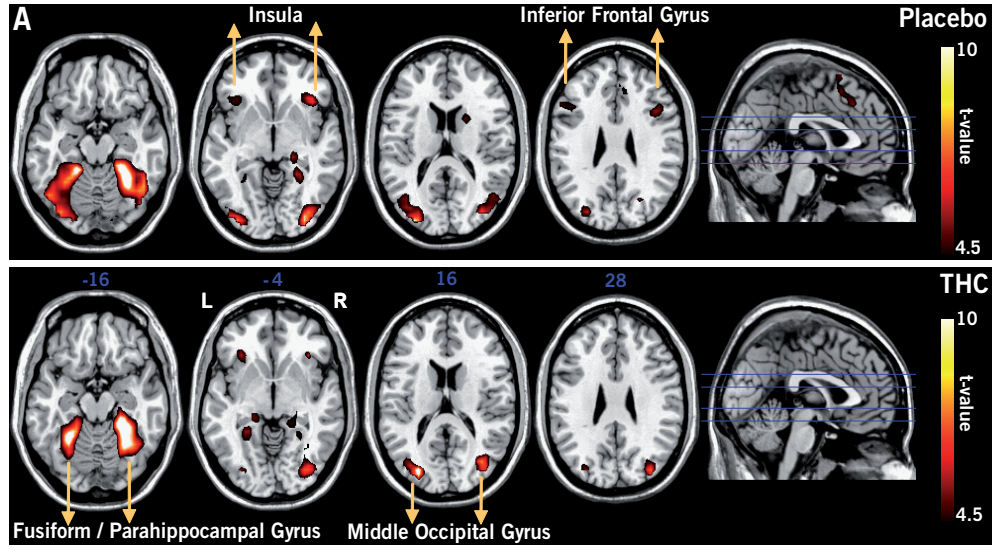
Table 4.2 Subjective and psychedelic effects of Δ9-tetrahydrocannabinol (THC) (n = 13)

Assessment	Drug effect	Mean placebo score (± SD)	Mean THC score (± SD)
VAS Feeling High	$F(1, 12) = 9.98$, $p = 0.008$*	0.38 ± 1.39	17.31 ± 19.16
VAS Internal Perception	$F(1, 12) = 3.79$, $p = 0.075$	-0.35 ± 1.41	1.69 ± 3.78
VAS External Perception	$F(1, 12) = 3.46$, $p = 0.087$	0.35 ± 0.72	6.76 ± 12.43
VAS Alertness	$F(1, 12) = 13.95$, $p = 0.003$*	-2.09 ± 7.00	-13.57 ± 9.38
VAS Contentedness	$F(1, 12) = 1.09$, $p = 0.318$	-2.77 ± 3.64	-4.85 ± 6.69
VAS Calmness	$F(1, 12) = 2.44$, $p = 0.144$	3.56 ± 8.97	-2.21 ± 11.12

Statistical analysis was performed with baseline corrected values using repeated measures ANOVA with drug and time as factors. * Significant difference between placebo and THC. VAS, Visual Analogue Scale.

Brain activity versus performance

Overall THC administration did not affect performance. To assess whether activity patterns within involved networks predicted performance, regression analyses were conducted with ROIs as independent variables and accuracy as dependent variable for each set of ROIs (encoding and recall), and for each session (placebo, THC). This revealed that during the placebo session a significant part of the variance in performance was explained by recall activity ($F = 17.37$; $p = 0.003$), but not during the THC session ($F = 0.65$; $p = 0.71$). Encoding activity patterns contained no predictive value for performance during placebo ($F = 0.54$; $p = 0.79$) or THC ($F = 0.90$; $p = 0.63$).



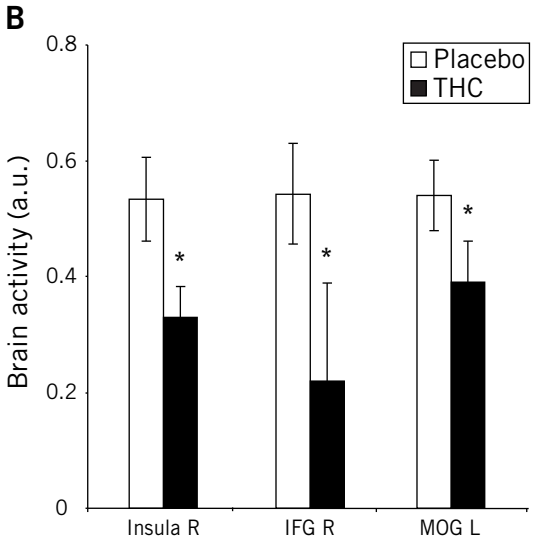


Figure 4.3 Brain activity during encoding (EN-SC). **A**, group activation maps after (top) placebo and (bottom) THC administration (n = 13; T > 4.5, $p <$ 0.05, corrected for multiple comparisons, clusters ≥ 10 voxels). L = left, R = right. **B**, effect of THC administration on brain activity in the right insula, right inferior frontal gyrus and left middle occipital gyrus (mean ± SEM). (* $p < 0.05$; a.u. = arbitrary units).

A closer look at individual recall ROIs for the placebo session indicated that 3 of 7 were negatively correlated with performance: left fusiform / parahippocampal gyrus (r = -0.83, $p < 0.001$) and left and right middle occipital gyrus (r = -0.63, $p = 0.02$ and r = -0.82, $p = 0.001$, respectively) (Figure 4.5). This shows that under normal circumstances good performance is associated with low activity in these regions during recall, while this association disappears after THC.

Table 4.3 Significantly activated brain regions during encoding (n = 13)

Encoding – Simple Classification						
Activated brain regions	**Brodmann area**	**Number of voxels**	**X**	**Y**	**Z**	**Maximum t-value**
Fusiform / Parahippocampal gyrus L	37	291	-28	-52	-12	13.10
Fusiform / Parahippocampal gyrus R	37	330	36	-60	-20	15.44
Inferior frontal gyrus L	44	13	-56	24	28	5.30
Inferior frontal gyrus R	48	19	40	16	28	5.97
Insula L	47	28	-32	28	-4	6.25
Insula R	47	22	40	24	-4	7.45
Middle occipital gyrus L	19	271	-28	-80	16	11.96
Middle occipital gyrus R	39	244	40	-80	24	9.82
Putamen R	48	17	20	8	16	5.57
Supplementary motor area L	6	37	4	16	52	6.25

Group activation maps for placebo and THC were thresholded at t = 4.5, $p < 0.05$, corrected for multiple comparisons, cluster size ≥ 10 voxels. X, Y and Z are MNI coordinates and represent the highest t-value in a cluster. Brodmann areas are obtained from the location in the AAL atlas indicated by the MNI coordinates. L, left; R, right.

Table 4.4 Significantly activated brain regions during recall (n = 13)

Recall – Simple Classification						
Activated brain regions	**Brodmann area**	**Number of voxels**	**X**	**Y**	**Z**	**Maximum t-value**
Cuneus / Precuneus L	19	182	-12	-72	40	11.21
Cuneus / Precuneus R	23	167	12	-68	24	8.27
Fusiform / Parahippocampal gyrus L	37	180	-32	-44	-12	10.83
Fusiform / Parahippocampal gyrus R	37	210	40	-56	-20	8.54
Middle occipital gyrus L	19	14	-28	-84	24	6.12
Middle occipital gyrus R	19	94	32	-72	20	7.90
Superior parietal gyrus L	19	179	-12	-72	40	11.21

Group activation maps for placebo and THC were thresholded at t = 4.5, $p < 0.05$, corrected for multiple comparisons, cluster size ≥ 10 voxels. X, Y and Z are MNI coordinates and represent the highest t-value in a cluster. Brodmann areas are obtained from the location in the AAL atlas indicated by the MNI coordinates. L, left; R, right.

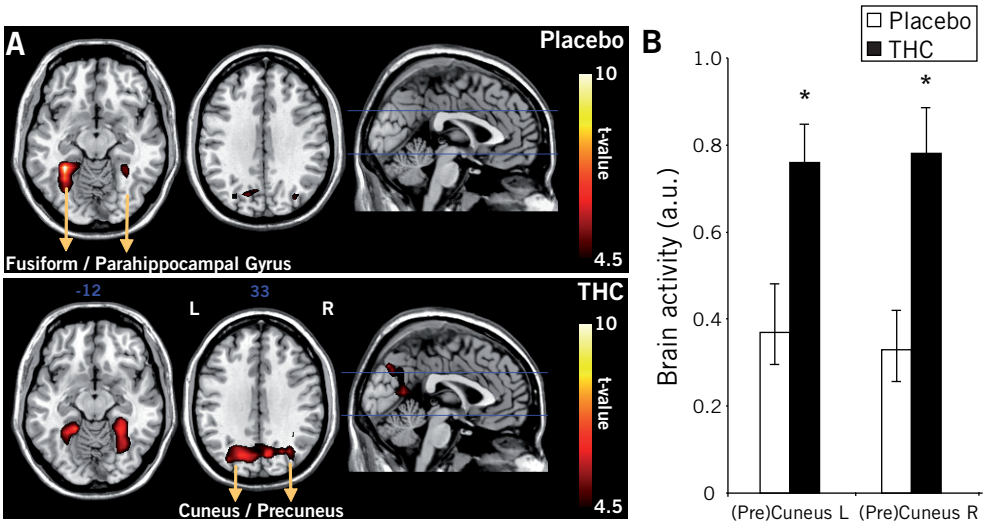


Figure 4.4 Brain activity during recall (RE-SC). **A**, group activation maps after (top) placebo and (bottom) THC administration (n = 13; T > 4.5, $p < 0.05$, corrected for multiple comparisons, cluster size ≥ 10 voxels). L = left, R = right. **B**, effect of THC administration on brain activity in the bilateral cuneus / precuneus (mean ± SEM). (* $p < 0.05$; a.u. = arbitrary units).

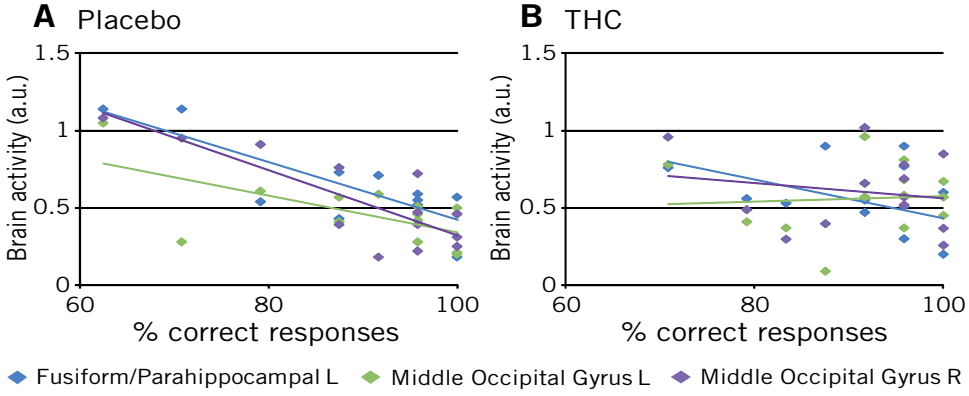


Figure 4.5 Correlation between recall brain activity and performance accuracy during placebo (A) and THC (B). The left fusiform / parahippocampal and bilateral middle occipital gyrus showed a significant inverse correlation with performance in the placebo session ($p < 0.05$), while there was no significant correlation with performance in the THC session, nor in any of the other ROIs.

Discussion

This study tested the hypothesis that a cannabinoid challenge affects associative memory processes in humans. Activity in the network of regions involved in encoding of paired pictorial stimuli was significantly affected by THC administration, with reduced levels of activity in the right insula, right inferior frontal gyrus and left middle occipital gyrus. During recall, THC administration was associated with a network wide increase in activity, which was strongest in a bilateral region comprising cuneus and precuneus. Recall performance was not affected by THC administration. However, during the placebo session recall activity significantly explained variance in performance, with a strong inverse correlation in fusiform / parahippocampal and middle occipital gyrus, indicating that good performance was associated with low activity. This association disappeared after THC. Our interpretation is that under normal circumstances some subjects were able to use a very efficient recognition strategy for recall, if information was sufficiently deep encoded. After THC, these subjects were most affected, as they could not apply this efficient recall strategy anymore. Hence, the inverse correlation between performance and recall activity disappeared after THC, although average performance itself was not reduced.

Although the design of the study does not provide conclusive evidence concerning the stage of memory processing that is most affected by THC, several arguments can be made for encoding as being more directly affected by THC, while the changes during recall are more likely to reflect a form of compensation for the affected encoding. First, THC induced opposite activity changes in encoding and recall. The interpretation that THC reduced encoding depth, indicated by less activity, while subjects could compensate during recall, at the expense of more activity, fits these differential effects. Second, behavioral studies in humans have indicated impairments in the free recall of information that is previously learned under the influence of cannabinoids[23-25], but recall of items acquired before cannabis use is generally not affected[20-22], which indicates that cannabinoids influence encoding but not recall of information. Third, as task performance did not reach a ceiling during placebo, it was optimally sensitive to detect any changes in performance. Still, no performance effects were found, which suggests that subjects were able to compensate for the effects of THC. An alternative explanation could be that THC did also directly affect the recall process, for instance by disturbing the retrieval process of previously encoded information. However, this interpretation would be in contrast with the mentioned previous findings that have indicated that THC does not affect recall of material encoded before drug intake.

In the absence of effects of THC on associative memory performance, it could be argued that the reported effects of THC may not be related to associative memory, but are rather caused by non-specific effects of THC intoxication. There are however several reasons to argue that the effects are indeed related to associative memory. First, as mentioned earlier, the opposite effect of THC on encoding and recall activity suggests differential effects of THC that are specific for each process, and not task-independent. Second, the reduced correlation between performance and recall activity after THC indicates a direct effect of THC on the association

between brain activity and task performance. Third, the reported effects of THC on brain activity reflect differences between the control and experimental task. These differences lie predominantly in the addition of an associative memory component in the experimental task. Thus, the effects of THC on brain activity are most likely associated with processes that directly or indirectly affect associative memory. Intoxicating, task-independent effects of THC can be expected to be present in both the control and experimental task.

Several fMRI studies have suggested important roles for some of the brain regions implicated in memory encoding in the current study[43-45]. The insula has been implicated in the process of selecting relevant item information, whereas the inferior frontal gyrus has been implicated in the organization of multiple pieces of information, possibly by building associations among items[46-49]. The middle occipital gyrus may not only be involved in the visual processing of to-be-remembered stimuli[50], but also in maintenance and imagery of visual information[51]. As all these functions include attentional processes, and the right insula and inferior frontal gyrus are part of the ventral attention network[52,53], the decrease in activity in these brain areas after THC administration may be related to disturbed attentional processes, which is in line with the reported THC-induced reduction in alertness. A possible alternative interpretation for the reduced encoding activity after THC would be that encoding was performed more efficiently under the influence of THC. However, both animal and human behavioral studies argue against this, as previous studies have not indicated increased efficiency of encoding after THC, only impairments[1-7,23-25]. A potential mechanism underlying the THC-induced decreases in brain activity may be found in the regulatory role of the eCB system in neurotransmitter release. As shown in electrophysiological animal studies, activation of cannabinoid receptors reduces both GABA and glutamate release from presynaptic terminals[9,54]. This eCB-mediated inhibition of synaptic transmission is critically involved in learning and memory processes[54], and has been demonstrated in the prefrontal cortex[55], among other brain regions.

In our study we found an increase in activity in bilateral precuneus after THC during recall. Previous imaging studies have suggested a pivotal role for the bilateral cuneus and precuneus in recall memory[56-64]. It is suggested that the (pre)cuneus is particularly involved in recall of context-rich memories, such as memories entailing specific contextual associations[58-60,62,63]. Increased involvement of the precuneus has been demonstrated when subjects claimed to recognize items based on conscious recollection of contextual details rather than on feelings of familiarity[60,64,65]. More specifically, it may signal whether context information should be used to recognize an item correctly[66]. The enhanced precuneus activity found in the current study after THC administration thus could be related to a change in retrieval strategy, with increased utilization of contextual associations to accurately recall information. One mechanism would be that after THC administration recall relies more on processing of individual features of to-be-remembered items, such as the color of a person's shirt, than on the recognition of the complete composition of the picture, which can be expected to be more efficient. Importantly, increases in precuneus activity during recall memory have also been associated with compensatory mechanisms in individuals with and at risk for mild cognitive impairment or Alzheimer's disease[67-69].

To date, only one other functional MRI study has been published that investigated the acute effects of THC administration on learning and memory[70]. A normal linear decrease in activity in the parahippocampal gyrus present over repeated encoding blocks was no longer evident after oral THC administration. As in the current study, task performance was unaffected. Because Bhattacharyya and colleagues presented the same stimuli during four blocks of encoding, thereby investigating the effect of THC on learning activity, only the imaging results for the first presentation of stimuli are comparable to our study. These findings are in line with our results in that a THC-induced reduction in encoding activity was found. However, differences in recall activity in the first session were not reported[70].

This study has several limitations. First, the sample size was relatively small. We therefore cannot exclude the possibility that subtle effects of THC on brain activity have been missed. Second, inclusion of incidental cannabis users, as opposed to non-users, may have hampered interpretation of the results as previous cannabis use may have affected the endocannabinoid system. The choice for incidental cannabis users was based on ethical grounds[28]. Third, absence of significant differences between placebo and THC in performance accuracy may suggest that the memory task used in this study was not an appropriate task to assess memory function. However, we have previously shown that performance on this task correlates inversely with the amount of cannabis used in the year prior to testing, in heavy cannabis users[27], indicating that the task is sensitive to impairment. Finally, non-specific THC-induced changes on cerebral blood flow may have confounded our results[71]. However, we have designed our study to minimize the influence of this effect by comparing brain activity between task-specific conditions and a closely matched control condition, as the non-specific effect of THC on blood flow can be expected to be present in all conditions. Further, as we found both significant decreases and increases in activity after THC administration, it is highly unlikely that our findings can be explained by such non-specific effects.

In conclusion, findings reported in this paper contribute to the growing body of evidence that suggests the involvement of the eCB system in learning and memory processes. Our results further emphasize the eCB system as a potential novel target for treatment of memory disorders, encouraging further research into novel, eCB-targeting compounds.

Disclosure Statement

The authors report no biomedical financial interests or potential conflict of interest.

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References

1. Hampson R.E., Deadwyler S.A. (2000) Cannabinoids reveal the necessity of hippocampal neural encoding for short-term memory in rats. J Neurosci. 20 (23), 8932-8942.
2. Lichtman A.H., Dimen K.R., Martin B.R. (1995) Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. Psychopharmacology. 119 (3), 282-290.
3. Lichtman A.H., Martin B.R. (1996) Delta 9-tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. Psychopharmacology. 126 (2), 125-131.
4. Mallet P.E., Beninger R.J. (1998) The cannabinoid CB1 receptor antagonist SR141716A attenuates the memory impairment produced by delta9-tetrahydrocannabinol or anandamide. Psychopharmacology. 140 (1), 11-19.
5. Wegener N., Kuhnert S., Thuns A., Roese R., Koch M. (2008) Effects of acute systemic and intra-cerebral stimulation of cannabinoid receptors on sensorimotor gating, locomotion and spatial memory in rats. Psychopharmacology. 198 (3), 375-385.
6. Wise L.E., Thorpe A.J., Lichtman A.H. (2009) Hippocampal CB(1) receptors mediate the memory impairing effects of Delta(9)-tetrahydrocannabinol. Neuropsychopharmacology. 34 (9), 2072-2080.
7. Yim T.T., Hong N.S., Ejaredar M., McKenna J.E., McDonald R.J. (2008) Post-training CB1 cannabinoid receptor agonist activation disrupts long-term consolidation of spatial memories in the hippocampus. Neuroscience. 151 (4), 929-936.
8. Piomelli D. (2003) The molecular logic of endocannabinoid signalling. Nat Rev Neurosci. 4 (11), 873-884.
9. Wilson R.I., Nicoll R.A. (2002) Endocannabinoid signaling in the brain. Science. 296 (5568), 678-682.
10. Ranganathan M., D'Souza D.C. (2006) The acute effects of cannabinoids on memory in humans: a review. Psychopharmacology. 188 (4), 425-444.
11. Hall W., Solowij N. (1998) Adverse effects of cannabis. Lancet. 352 (9140), 1611-1616.
12. Zuurman L., Ippel A.E., Moin E., van Gerven J.M. (2009) Biomarkers for the effects of cannabis and THC in healthy volunteers. Br J Clin Pharmacol. 67 (1), 5-21.
13. Block R.I., Wittenborn J.R. (1984) Marijuana effects on semantic memory: verification of common and uncommon category members. Psychol Rep. 55 (2), 503-512.
14. Chait L.D., Perry J.L. (1994) Acute and residual effects of alcohol and marijuana, alone and in combination, on mood and performance. Psychopharmacology. 115 (3), 340-349.
15. Darley C.F., Tinklenberg J.R., Roth W.T., Vernon S., Kopell B.S. (1977) Marijuana effects on long-term memory assessment and retrieval. Psychopharmacology. 52 (3), 239-241.
16. Hart C.L., van Gorp W., Haney M., Foltin R.W., Fischman M.W. (2001) Effects of acute smoked marijuana on complex cognitive performance. Neuropsychopharmacology. 25 (5), 757-765.
17. Hart C.L., Ward A.S., Haney M., Comer S.D., Foltin R.W., Fischman M.W. (2002) Comparison of smoked marijuana and oral Delta(9)-tetrahydrocannabinol in humans. Psychopharmacology. 164 (4), 407-415.
18. Hart C.L., Ilan A.B., Gevins A., Gunderson E.W., Role K., Colley J., Foltin R.W. (2010) Neurophysiological and cognitive effects of smoked marijuana in frequent users. Pharmacol Biochem Behav. 96 (3), 333-341.
19. McDonald J., Schleifer L., Richards J.B., de Wit H. (2003) Effects of THC on behavioral measures of impulsivity in humans. Neuropsychopharmacology. 28 (7), 1356-1365.
20. Abel E.L. (1971) Marihuana and memory: acquisition or retrieval? Science. 173 (4001), 1038-1040.
21. Darley C.F., Tinklenberg J.R., Roth W.T., Hollister L.E., Atkinson R.C. (1973) Influence of marijuana on storage and retrieval processes in memory. Memory & Cognition. 1, 196-200.
22. Dornbush R.L. (1974) Marijuana and memory: effects of smoking on storage. Trans N Y Acad Sci. 36 (1), 94-100.
23. Curran H.V., Brignell C., Fletcher S., Middleton P., Henry J. (2002) Cognitive and subjective dose-response effects of acute oral Delta 9-tetrahydrocannabinol (THC) in infrequent cannabis users. Psychopharmacology. 164 (1), 61-70.
24. D'Souza D.C., Perry E., MacDougall L., Ammerman Y., Cooper T., Wu Y.T., Braley G., Gueorguieva R., Krystal J.H. (2004) The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. Neuropsychopharmacology. 29 (8), 1558-1572.
25. Miller L.L., Cornett T.L. (1978) Marijuana: dose effects on pulse rate, subjective estimates of intoxication, free recall and recognition memory. Pharmacol Biochem Behav. 9 (5), 573-577.
26. Henke K., Buck A., Weber B., Wieser H.G. (1997) Human hippocampus establishes associations in memory. Hippocampus. 7 (3), 249-256.

27. Jager G., van Hell H.H., De Win M.M., Kahn R.S., van den Brink W., van Ree J.M., Ramsey N.F. (2007) Effects of frequent cannabis use on hippocampal activity during an associative memory task. Eur Neuropsychopharmacol. 17 (4), 289-297.
28. van Hell H.H., Bossong M.G., Jager G., Kahn R.S., Ramsey N.F. (2011) Pharmacological Imaging of the Cannabinoid System (PhICS): towards understanding the role of the brain endocannabinoid system in human cognition. Int J Methods Psychiatr Res. 20, 10-27.
29. Schmand B., Bakker D., Saan R., Louman J. (1991) [The Dutch Reading Test for Adults: a measure of premorbid intelligence level]. Tijdschr Gerontol Geriatr. 22 (1), 15-19.
30. Hazekamp A., Ruhaak R., Zuurman L., van Gerven J., Verpoorte R. (2006) Evaluation of a vaporizing device (Volcano) for the pulmonary administration of tetrahydrocannabinol. J Pharm Sci. 95 (6), 1308-1317.
31. Zuurman L., Roy C., Schoemaker R.C., Hazekamp A., den Hartigh J., Bender J.C., Verpoorte R., Pinquier J.L., Cohen A.F., van Gerven J.M. (2008) Effect of intrapulmonary tetrahydrocannabinol administration in humans. J Psychopharmacol. 22 (7), 707-716.
32. Bossong M.G., van Berckel B.N., Boellaard R., Zuurman L., Schuit R.C., Windhorst A.D., van Gerven J.M., Ramsey N.F., Lammertsma A.A., Kahn R.S. (2009) Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. Neuropsychopharmacology. 34 (3), 759-766.
33. Strougo A., Zuurman L., Roy C., Pinquier J.L., van Gerven J.M., Cohen A.F., Schoemaker R.C. (2008) Modelling of the concentration--effect relationship of THC on central nervous system parameters and heart rate -- insight into its mechanisms of action and a tool for clinical research and development of cannabinoids. J Psychopharmacol. 22 (7), 717-726.
34. Bond A., Lader M. (1974) The use of analogue scales in rating subjective feelings. Br J Med Psychol. 47, 211-218.
35. Bowdle T.A., Radant A.D., Cowley D.S., Kharasch E.D., Strassman R.J., Roy-Byrne P.P. (1998) Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. Anesthesiology. 88 (1), 82-88.
36. van Buuren M., Gladwin T.E., Zandbelt B.B., van den Heuvel M., Ramsey N.F., Kahn R.S., Vink M. (2009) Cardiorespiratory effects on default-mode network activity as measured with fMRI. Hum Brain Mapp. 30 (9), 3031-3042.
37. Neggers S.F., Hermans E.J., Ramsey N.F. (2008) Enhanced sensitivity with fast three-dimensional blood-oxygen-level-dependent functional MRI: comparison of SENSE-PRESTO and 2D-EPI at 3 T. NMR Biomed. 21 (7), 663-676.
38. Collins D.L., Neelin P., Peters T.M., Evans A.C. (1994) Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. J Comput Assist Tomogr. 18 (2), 192-205.
39. Poldrack R.A. (2007) Region of interest analysis for fMRI. Soc Cogn Affect Neurosci. 2 (1), 67-70.
40. Kriegeskorte N., Simmons W.K., Bellgowan P.S., Baker C.I. (2009) Circular analysis in systems neuroscience: the dangers of double dipping. Nat Neurosci. 12 (5), 535-540.
41. Mehta M.A., O'Daly O.G. (2011) Pharmacological application of fMRI. Methods Mol Biol. 711, 551-565.
42. Brett M., Anton J.-L., Valabregue R., Poline J.-B. (2002) Region of interest analysis using an SPM toolbox. Neuroimage. 497, Abstract 497.
43. Dobbins I.G., Foley H., Schacter D.L., Wagner A.D. (2002) Executive control during episodic retrieval: multiple prefrontal processes subserve source memory. Neuron. 35 (5), 989-996.
44. Kelley W.M., Miezin F.M., McDermott K.B., Buckner R.L., Raichle M.E., Cohen N.J., Ollinger J.M., Akbudak E., Conturo T.E., Snyder A.Z., Petersen S.E. (1998) Hemispheric specialization in human dorsal frontal cortex and medial temporal lobe for verbal and nonverbal memory encoding. Neuron. 20 (5), 927-936.
45. Wagner A.D., Poldrack R.A., Eldridge L.L., Desmond J.E., Glover G.H., Gabrieli J.D. (1998) Material-specific lateralization of prefrontal activation during episodic encoding and retrieval. Neuroreport. 9 (16), 3711-3717.
46. Blumenfeld R.S., Ranganath C. (2007) Prefrontal cortex and long-term memory encoding: an integrative review of findings from neuropsychology and neuroimaging. Neuroscientist. 13 (3), 280-291.
47. Simons J.S., Spiers H.J. (2003) Prefrontal and medial temporal lobe interactions in long-term memory. Nat Rev Neurosci. 4 (8), 637-648.
48. Staresina B.P., Davachi L. (2006) Differential encoding mechanisms for subsequent associative recognition and free recall. J Neurosci. 26 (36), 9162-9172.
49. Summerfield C., Greene M., Wager T., Egner T., Hirsch J., Mangels J. (2006) Neocortical connectivity during episodic memory formation. PLoS Biol. 4 (5), e128.
50. Ishai A., Ungerleider L.G., Martin A., Haxby J.V. (2000) The representation of objects in the human occipital and temporal cortex. J Cogn Neurosci. 12 Suppl 2, 35-51.

51. Johnson M.R., Mitchell K.J., Raye C.L., D'Esposito M., Johnson M.K. (2007) A brief thought can modulate activity in extrastriate visual areas: Top-down effects of refreshing just-seen visual stimuli. Neuroimage. 37 (1), 290-299.
52. Corbetta M., Shulman G.L. (2002) Control of goal-directed and stimulus-driven attention in the brain. Nat Rev Neurosci. 3 (3), 201-215.
53. Corbetta M., Patel G., Shulman G.L. (2008) The reorienting system of the human brain: from environment to theory of mind. Neuron. 58 (3), 306-324.
54. Heifets B.D., Castillo P.E. (2009) Endocannabinoid signaling and long-term synaptic plasticity. Annu Rev Physiol. 71, 283-306.
55. Lafourcade M., Elezgarai I., Mato S., Bakiri Y., Grandes P., Manzoni O.J. (2007) Molecular components and functions of the endocannabinoid system in mouse prefrontal cortex. PLoS One. 2 (1), e709.
56. Burgess N., Maguire E.A., Spiers H.J., O'Keefe J. (2001) A temporoparietal and prefrontal network for retrieving the spatial context of lifelike events. Neuroimage. 14 (2), 439-453.
57. Fletcher P.C., Frith C.D., Baker S.C., Shallice T., Frackowiak R.S., Dolan R.J. (1995) The mind's eye--precuneus activation in memory-related imagery. Neuroimage. 2 (3), 195-200.
58. Gardini S., Cornoldi C., De Beni R., Venneri A. (2006) Left mediotemporal structures mediate the retrieval of episodic autobiographical mental images. Neuroimage. 30 (2), 645-655.
59. Gilboa A., Winocur G., Grady C.L., Hevenor S.J., Moscovitch M. (2004) Remembering our past: functional neuroanatomy of recollection of recent and very remote personal events. Cereb Cortex. 14 (11), 1214-1225.
60. Henson R.N., Rugg M.D., Shallice T., Josephs O., Dolan R.J. (1999) Recollection and familiarity in recognition memory: an event-related functional magnetic resonance imaging study. J Neurosci. 19 (10), 3962-3972.
61. Krause B.J., Schmidt D., Mottaghy F.M., Taylor J., Halsband U., Herzog H., Tellmann L., Muller-Gartner H.W. (1999) Episodic retrieval activates the precuneus irrespective of the imagery content of word pair associates. A PET study. Brain. 122 (Pt 2), 255-263.
62. Lundstrom B.N., Petersson K.M., Andersson J., Johansson M., Fransson P., Ingvar M. (2003) Isolating the retrieval of imagined pictures during episodic memory: activation of the left precuneus and left prefrontal cortex. Neuroimage. 20 (4), 1934-1943.
63. Lundstrom B.N., Ingvar M., Petersson K.M. (2005) The role of precuneus and left inferior frontal cortex during source memory episodic retrieval. Neuroimage. 27 (4), 824-834.
64. Wiesmann M., Ishai A. (2008) Recollection- and familiarity-based decisions reflect memory strength. Front Syst Neurosci. 2, 1.
65. Wheeler M.E., Buckner R.L. (2004) Functional-anatomic correlates of remembering and knowing. Neuroimage. 21 (4), 1337-1349.
66. Dörfel D., Werner A., Schaefer M., von Kummer R., Karl A. (2009) Distinct brain networks in recognition memory share a defined region in the precuneus. Eur J Neurosci. 30 (10), 1947-1959.
67. Schwindt G.C., Black S.E. (2009) Functional imaging studies of episodic memory in Alzheimer's disease: a quantitative meta-analysis. Neuroimage. 45 (1), 181-190.
68. Seidenberg M., Guidotti L., Nielson K.A., Woodard J.L., Durgerian S., Antuono P., Zhang Q., Rao S.M. (2009) Semantic memory activation in individuals at risk for developing Alzheimer disease. Neurology. 73 (8), 612-620.
69. Woodard J.L., Seidenberg M., Nielson K.A., Antuono P., Guidotti L., Durgerian S., Zhang Q., Lancaster M., Hantke N., Butts A., Rao S.M. (2009) Semantic memory activation in amnestic mild cognitive impairment. Brain. 132 (Pt 8), 2068-2078.
70. Bhattacharyya S., Fusar-Poli P., Borgwardt S., Martin-Santos R., Nosarti C., O'Carroll C., Allen P., Seal M.L., Fletcher P.C., Crippa J.A., Giampietro V., Mechelli A., Atakan Z., McGuire P. (2009) Modulation of mediotemporal and ventrostriatal function in humans by Delta9-tetrahydrocannabinol: a neural basis for the effects of Cannabis sativa on learning and psychosis. Arch Gen Psychiatry. 66 (4), 442-451.
71. Iannetti G.D., Wise R.G. (2007) BOLD functional MRI in disease and pharmacological studies: room for improvement? Magn Reson Imaging. 25 (6), 978-988.

5

Effects of Δ9-tetrahydrocannabinol (THC) on human working memory efficiency

Under revision

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Abstract

Background

Schizophrenia patients often exhibit reduced load sensitivity in working memory (WM) brain activity, together with reduced task performance at high loads, commonly referred to as inefficient WM function. Furthermore, evidence indicates involvement of the endocannabinoid (eCB) system in both pathophysiology of schizophrenia and WM function. In the present study, we examined eCB involvement in schizophrenia WM deficits by testing if perturbation of the eCB system induces WM inefficiency in healthy subjects, resembling that of schizophrenia patients.

Methods

A pharmacological functional magnetic resonance imaging (fMRI) study was conducted with a placebo-controlled, cross-over design, investigating effects of the eCB agonist Δ9-tetrahydrocannabinol (THC) on WM function in 17 healthy volunteers. Performance and brain activity during WM were assessed using a parametric Sternberg item-recognition paradigm (SIRP) with five difficulty levels.

Results

Performance accuracy was significantly reduced after THC. In the placebo condition, brain activity increased linearly with rising WM load. THC administration enhanced activity for low WM loads, and reduced the linear relationship between WM load and activity in the WM system as a whole, and in left dorsolateral prefrontal cortex, inferior temporal gyrus, inferior parietal gyrus and cerebellum in particular.

Conclusions

THC affected the response to increasing WM load in terms of task performance and brain activity in the WM system. This profile of performance and brain activity corresponds with current concepts of WM inefficiency, and resembles that of schizophrenia patients. These results provide compelling support for eCB involvement in WM function and indirect evidence for involvement in cognitive deficits in schizophrenia.

Introduction

Impairment of cognitive function is currently considered a core feature of schizophrenia[1]. Working memory (WM), the ability for short term storage and manipulation of information, is a central component of cognitive function. Current information provided by functional imaging studies has indicated that patients with schizophrenia often exhibit a reduced load sensitivity of brain activity with increasing WM load, which is also often combined with enhanced prefrontal brain activity for tasks with low WM load[2-6]. This has led to the theoretical notion

that impaired cognitive function in schizophrenia is related to neurophysiologically inefficient WM function[7,8]. According to this WM inefficiency hypothesis, both brain activity and performance level that are normally related to a higher WM load will already occur at a lower load (see Figure 5.1). Inefficiency refers to the disproportionate magnitude of brain activity in relation to workload, and compromised performance as a result. Although the notion of inefficient WM in schizophrenia is equivocal and ambiguous results have been reported[9-11], it has unique value in its explanatory power of load-dependent results found in schizophrenia. Evidence is accumulating for involvement of the endocannabinoid (eCB) system in the pathophysiology of schizophrenia. For instance, schizophrenia patients exhibit enhanced levels of endogenous cannabinoids in cerebrospinal fluid[12], and altered post mortem cannabinoid CB1 receptor densities in the brain[13,14]. Epidemiological studies indicate that the use of cannabis increases the risk for developing schizophrenia[15]. Also, in patients, cannabis use has been associated with higher relapse rates, poor treatment outcome and increased severity of symptoms[16], as well as accelerated loss of grey matter volume[17]. In healthy subjects, administration of exogenous cannabinoids such as Δ9-tetrahydrocannabinol (THC), the main psychoactive component in cannabis and a partial agonist of the CB_1 receptor, impairs performance on various WM paradigms[18-20], indicating that the eCB system may play a role in WM.

With these considerations, the goal of this study was to examine the possibility that the eCB system is involved in WM inefficiency as observed in schizophrenia patients. If our study results support this hypothesis, it could be cause to target the eCB system for medical treatment of cognitive impairment in the illness. Direct testing of the hypothesis would require administration of an eCB antagonist to schizophrenia patients, but that is currently not possible due to the fact that the only antagonist available for human use (rimonabant) has been withdrawn from the market as there is reason to believe it may increase the risk of suicide[21]. As an alternative approach, we investigated the effect of the partial eCB agonist THC on WM performance and brain function in healthy subjects, and assessed whether perturbation of the eCB system would induce inefficient WM function similar to what has been reported for schizophrenia.

The hypothesis was tested in an fMRI study with a placebo-controlled, cross-over design, using a parametric Sternberg item-recognition paradigm (SIRP)[3,22,23] with five difficulty levels to establish a reliable activity and performance profile. This paradigm is well suited to test the hypothesis, as it has been shown to induce a gradual WM load increase with increasing memory size, while keeping subjects engaged in the task even at high WM load[22,24,25]. For a SIRP, it has been shown that brain activity increases linearly with increasing WM load, tapering off until a maximum is reached (Figure 5.1a)[24-27]. In addition, performance is high until task load causes a gradual increase in errors (Figure 5.1b). Following the WM inefficiency hypothesis, two important characteristics that can be expected after THC administration are a reduced load-dependent increase in brain activity, as well as increased activity at low loads. In addition, it is hypothesized that the decline in performance starts at a lower level (Figure 5.1).

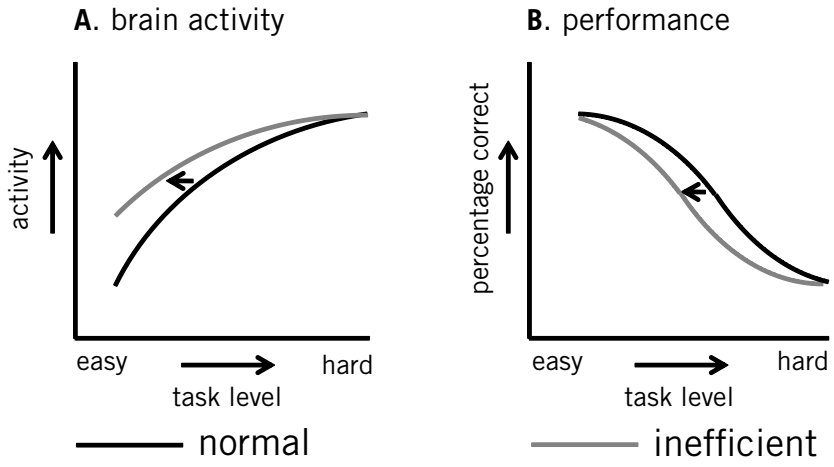

Figure 5.1 Effect of WM inefficiency on profile of brain activity and performance in a parametric design. **A**, A shift of the load-response curve to the left will effectively reduce the load-dependent increase in brain activity, while increasing activity for easy tasks. **B**, A shift of the performance curve to the left will effectively cause a drop off in performance at a lower load.

Methods and materials

This study is part of the Pharmacological Imaging of the Cannabinoid System (PhICS) study, of which design and objectives are provided in a methods paper[28].

Subjects

Twenty-five healthy male right-handed subjects were recruited through flyers, posters and internet advertisements. All subjects were incidental cannabis users, defined as having used cannabis at least four times but at most once a week in the year before inclusion in the study. All subjects were in good physical health as assessed by medical history, physical examination, electrocardiogram (ECG), and routine laboratory tests. A maximum use of five cigarettes per day was allowed. Illicit drug use other than cannabis was restricted to a maximum of five times lifetime and not within six months prior to inclusion. Subjects were asked to refrain from cannabis for at least two weeks before the first study day until study completion. Compliance was tested by means of a urine sample at the beginning of each test day. In- and exclusion criteria are described in further detail in van Hell et al. (2011)[28]. After complete description of the study to the subjects, written informed content was obtained. The study was approved by the Independent Ethics Committee of the University Medical Center Utrecht, the Netherlands. Four subjects did not complete the study procedure due to a strong disruptive response to THC. They experienced a brief period of elevated anxiety which vanished rapidly. Four other subjects were excluded because of technical malfunction or movement-related errors. Results are reported on seventeen out of the twenty-five included subjects. See Table 5.1 for subject characteristics.

Table 5.1 Subject characteristics (n = 17).

Characteristic	Mean ± SD	Range
Age (years)	21.4 ± 2.1	18 - 27
IQ	105.4 ± 5.4	94 - 113
Height (cm)	183.6 ± 4.5	176 - 191
Weight (kg)	75.6 ± 7.5	64 - 91
BMI (kg/m^2)	22.4 ± 2.0	18.6 - 27.8
Cannabis use (Occasions / year)	18.1 ± 12.2	4 - 52
Tobacco smoking (Cigarettes / week)	2.5 ± 6.9	0 - 28
Alcohol consumption (Units / week)	14.9 ± 8.8	2 - 30
Coffee consumption (Units / week)	11.2 ± 10.2	0 - 28
Illicit drug use (Occasions lifetime)	1.5 ± 2.0	0 - 5

Use of cannabis, tobacco, alcohol and coffee was given for the year before inclusion in the study. Subjects refrained from cannabis for at least two weeks before the first study day until study completion and from alcohol for 48 hours before each study day. Caffeine intake and smoking were not allowed from the moment of arrival until the end of a study day. Illicit drug use other than cannabis was at least more than six months before the first study day. All subjects showed negative urine screening at both study days.

Design and procedure

In a double-blind, randomized, placebo-controlled, crossover pharmacological MRI study, subjects underwent scanning sessions after administration of placebo and of THC. Study days were scheduled two weeks apart to allow for complete clearance of drugs. On each study day, subjects performed three cognitive paradigms, during which functional MRI scans were obtained. One of these paradigms was the SIRP. Paradigm sequence was randomized between subjects, but remained unchanged within subjects across sessions. Results of other assessments are reported elsewhere[28,29]. On study days, subjects received subsequent doses of THC or placebo with 30 minutes intervals. Drugs were administered before each functional MRI task using a Volcano® vaporizer (Storz–Bickel GmbH, Tuttlingen, Germany) according to a method described earlier[30,31]. The first THC dose was 6 mg, followed by three doses of 1 mg each to maintain stable levels of CNS effects. See Van Hell et al. (2011)[28] for detailed study procedures.

Drug levels and behavioral measurements

Venous blood samples were collected to determine plasma concentrations of THC and its two main metabolites, 11-OH-THC and 11-nor-9-carboxy-THC. Blood samples were processed according to Zuurman et al. (2008)[31]. Subjective effects were determined with two sets of visual analogue scales[32,33]. Both rating scales were performed consecutively at baseline and before and after WM task performance, and were analyzed as described previously[30,31]. Correlations between THC peak concentration and behavioral changes (THC versus placebo) were determined using Pearson's correlation coefficient. Heart rate and respiration were monitored continuously during scanning[34]. Data were corrected for baseline values and analyzed with paired t-tests.

Task paradigm

WM function was assessed with a Sternberg item-recognition paradigm[22] (denoted SIRP) (Figure 5.2). Participants were instructed to memorize alternating sets of one, three, five, seven or nine uppercase consonants, respectively denoted as load1, load3, load5, load7, load9. Each letter set was presented for 6600 ms followed by a fixation cross of 600 ms. After presentation of this memory set, eight single consonants were displayed in sequence for 1500 ms each, separated by a fixation cross of 900 ms. Subjects were instructed to press a button as quickly as possible if the probe was present in the preceding memory set ('target'). No action was required if the probe was not part of the memory set ('non-target'). Four blocks for each level were presented, resulting in a total of 20 task blocks, together with four rest blocks. The order of blocks was counterbalanced. The number of targets per block varied from three to seven, with an average of five targets per block. Total task duration was 11 minutes. Memory sets differed for both study days for all subjects.

Outcome measures included accuracy and reaction time (RT), for each load condition. Accuracy was calculated as the mean percentage of correctly identified targets (% hits) and correctly rejected non-targets (100% - % false alarms). Accuracy and reaction time were tested for an effect of THC through a repeated measures ANOVA with factors drug (placebo, THC) and load (five levels, load1 to load9).

Image Acquisition

Image acquisition was performed on a Philips Achieva 3.0 Tesla scanner (Philips Medical Systems, Best, the Netherlands). Functional images were obtained using a PRESTO-SENSE pulse sequence[35] (parameters: TR 22.5 ms; TE 33.2 ms; flip angle = 10°; FOV 224×256×160; matrix 56× 64×40; voxel size 4 mm isotropic; scan time 0.6075 s; 40 slices (sagittal orientation); 1055 volumes). A high contrast volume with a flip angle of 27° (FA27) was scanned for registration purposes. A T1-weighted structural image was obtained for anatomical registration (parameters: TR 9.5 ms; TE 4.7 ms; flip angle = 8°; FOV 220.8x240x159.6; matrix 368×400×266; voxel size 0.6 mm isotropic, 266 slices (sagittal orientation)).

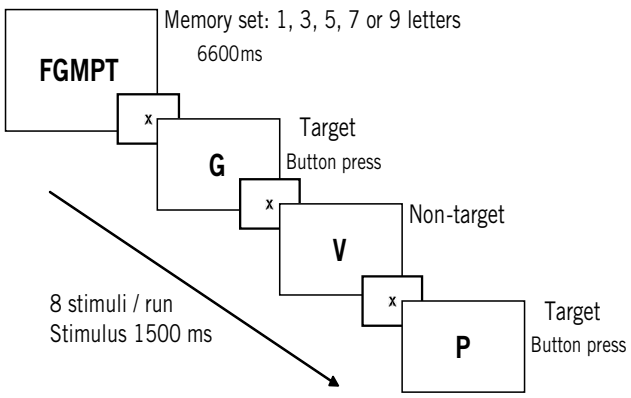

Figure 5.2 Schematic outline of the Sternberg item-recognition paradigm (SIRP) used to assess WM. Each task block starts with the presentation of a memory set consisting of 1, 3, 5, 7 or 9 consonants and is followed by eight stimuli, each separated by a fixation cross of 900 ms. Subjects have to press a button as quickly as possible if the stimulus belongs to the target set. See for detailed information the Methods and Materials section.

Functional MRI analysis

Functional MRI data were preprocessed and analyzed using SPM5 (Wellcome Trust Centre for Neuroimaging, London, UK). Preprocessing included realignment of functional images, and co-registration with the anatomical volume using the FA27 volume. Anatomical volumes were used to calculate parameters for spatially normalization of the functional scans into standard MNI space. After spatial normalization, functional volumes were spatially smoothed (FWHM = 8 mm) to reduce the effect of between-subject spatial variability in activation.

First level single subject analysis included a general linear model regression analysis that contained factors modeling the response period for each level, the memory set presentation period, as well as factors to correct for slow drifts in the signal up to 0.007 Hz. Load1 was used as a baseline condition constituting minimal WM load to control for any global effects of THC on task activity that were not related to WM. Individual as well as group activity maps were generated for the contrasts load3 – load1, load5 – load1, load7 – load1 and load9 – load1, both for placebo and THC.

Regions of Interest (ROIs) were determined using an independent sample of 46 healthy controls from a separate study[36] to avoid bias in ROI definition[37]. ROIs were based on clusters of neighboring voxels (cluster size ≥ 10 voxels) showing a significant signal increase in the load5 (the highest level measured in that study) compared to load1 (threshold: t = 4.5, $p < 0.05$, corrected for multiple comparisons) resulting in twelve ROIs (Table 5.2 and Figure 5.3). Regression coefficients (b-values) for the response period were averaged per ROI for all contrasts for both the placebo and THC session, thus resulting in eight b-values per ROI.

Effects of THC were determined using a repeated measures ANOVA with linear contrast for load (SPSS) over all twelve ROIs (factors: drug (two levels, THC or placebo), load (four levels, load3-load1 to load9-load1) and ROI (12 levels, all included regions), as well as for each separate ROI with factors drug (placebo, THC) and load (four levels). Post-hoc paired t-tests were performed for the lowest (load3-load1) and highest (load9-load1) contrast to test for occurrence of hypo- or hyperactivity.

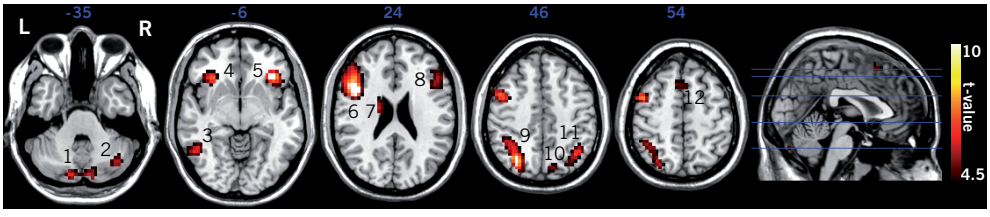

Figure 5.3 Regions of interest (ROIs) used to assess effects of THC administration on brain activity. ROIs were determined using an independent sample of 46 healthy controls and included regions showing activity in a group activity map calculated for the contrast load5 – load1 (thresholded at T = 4.5, $p < 0.05$, corrected for multiple comparisons, cluster size ≥ 10 voxels). Numbers above slices indicate MNI z coordinates. ROI numbers correspond to those shown in Table 5.2 and Table 5.4.

Table 5.2 Regions of interest: significantly activated brain regions for load5 – load1 in an independent sample of 46 subjects.

ROI number	Activated brain regions	Brodmann area	Number of voxels	X	Y	Z
1	Cerebellum L	-	76	-16	-84	-36
2	Cerebellum R	-	21	40	-68	-36
3	Inferior temporal gyrus L	37	88	-48	-52	-8
4	Insula / Inferior frontal gyrus L	47	64	-32	28	-8
5	Insula / Inferior frontal gyrus R	47	94	36	28	-4
6	Dorsolateral prefrontal cortex L	9 / 46	441	-44	12	24
7	Caudate L	-	29	-16	-8	20
8	Dorsolateral prefrontal cortex R	9 / 46	107	44	20	28
9	Inferior parietal gyrus L	7	179	-28	-68	44
10	Precuneus R	7	15	12	-76	44
11	Inferior parietal gyrus R	7	49	36	-60	44
12	Anterior cingulate cortex	8	19	-8	20	56

Group activity maps were thresholded at t = 4.5, $p < 0.05$, corrected for multiple comparisons, cluster size ≥ 10 voxels. X, Y and Z are MNI coordinates and represent the highest t-value in a cluster. ROI numbers correspond to those shown in Figure 5.3. ROI, region of interest; L, left; R, right

Table 5.3 . Subjective and psychotropic effects of Δ9-tetrahydrocannabinol (THC) (n = 17).

Assessment	Drug effect	Mean placebo score (± SD)	Mean THC score (± SD)
VAS Feeling High	F(1, 15) = 16.95, $p = 0.001$*	0.88 ± 2.15	15.88 ± 14.14
VAS Internal Perception	F(1, 15) = 1.40, $p = 0.254$	-0.12 ± 1.23	0.71 ± 1.24
VAS External Perception	F(1, 15) = 8.16, $p = 0.012$*	0.74 ± 1.26	3.75 ± 3.62
VAS Alertness	F(1, 16) = 10.29, $p = 0.005$*	-2.79 ± 5.65	-11.39 ± 8.89
VAS Contentedness	F(1, 16) = 4.81, $p = 0.043$*	-2.09 ± 4.43	-5.97 ± 6.89
VAS Calmness	F(1, 16) = 0.14, $p = 0.718$	-3.46 ± 6.04	-2.35 ± 10.96

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

Results

Drug levels and behavioral measurements

Plasma concentrations of THC and its main metabolites were 70.0 ± 40.6 ng/ml (THC), 2.5 ± 1.6 ng/ml (11-nor-9-carboxy-THC) and 2.6 ± 2.5 ng/ml (11-OH-THC), 5 min after inhalation of 6 mg THC. Analysis of subjective effects revealed a significant THC-induced increase in VAS score of 'feeling high' (F(1,15) = 16.95, $p = 0.001$) and external perception (reflecting misperception of external stimuli or changes in the awareness of the environment) (F(1,15) = 8.16, $p = 0.012$) compared to placebo. In addition, THC significantly reduced 'alertness'

(F(1,16) = 10.29, $p = 0.005$) and 'contentedness' (F(1,16) = 4.81, $p = 0.043$). Subjective effects are summarized in Table 5.3. Peak THC concentration was negatively correlated with changes in 'alertness' (r = -0.699; $p = 0.003$). Heart rate increased significantly after THC compared with placebo (15.6 ± 16.4 and 2.9 ± 8.1 bpm increase compared to baseline, respectively; $p = 0.005$). For a more detailed description of drug levels and behavioral measurements following THC see Van Hell et al. (2011)[28].

Task performance

Reaction times increased with rising WM load (F(1,64) = 342.9, $p < 0.001$) and were significantly longer after THC administration compared to placebo (F(1,16) = 17.48, $p = 0.001$). There was no significant linear drug by load interaction (F = 0.56, $p = 0.47$) (Figure 5.4a).
Accuracy decreased with rising WM load (F(1,16) = 54.35, $p < 0.001$) and was significantly reduced by THC (F(1,16) = 17.91, $p = 0.001$). There was a trend for an interaction between load and drug for accuracy (F = 3.14, $p = 0.09$) (Figure 5.4b).

Brain activity

Group activity maps yielded a commonly reported WM network of activated brain regions (see for illustration Figure 5.5). The linear interaction effect between drug and load was assessed directly, to test the hypothesis. Analysis of activity in the WM network as a whole revealed a linear difference in load response between placebo and THC, as indicated by a significant linear interaction effect between drug and load (F(1,16) = 9.39; $p = 0.007$). As Figure 5.6a shows, this reflects that the load-dependent increase in activity in the placebo condition was reduced after THC administration. Activity in the WM network showed a significant THC-induced increase for the load3 – load1 condition specifically (from 0.32 ± 0.09 to 0.57 ± 0.08; $p = 0.047$) (Table 5.4 and Figure 5.6a).

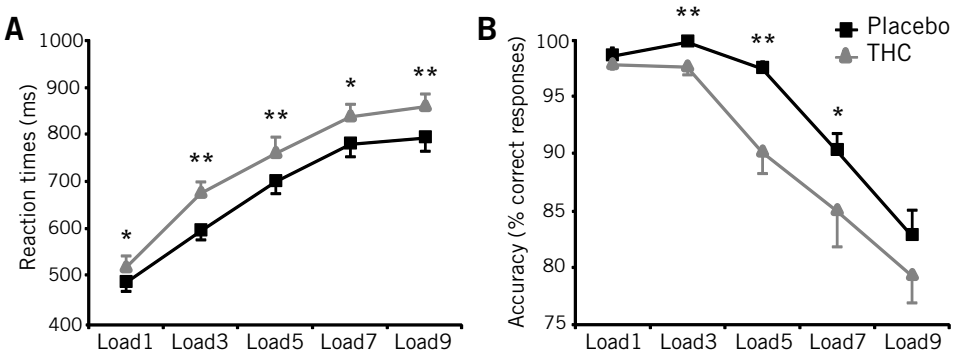

Figure 5.4 SIRP task performance. **A**, Reaction times of correct responses after placebo and THC administration. **B**, Performance accuracy as percentage of correct responses after placebo and THC administration (n = 17; mean ± SEM). ** Significant difference ($p < 0.05$) and * trend towards significant difference ($p < 0.10$) between THC and placebo.

Analysis of individual ROIs revealed a significant linear difference in load response between placebo and THC in the left dorsolateral prefrontal cortex ($F(1,16) = 9.65$; $p = 0.007$), left inferior temporal gyrus ($F(1,16) = 5.27$; $p = 0.036$), left inferior parietal gyrus ($F(1,16) = 4.98$; $p = 0.040$) and cerebellum ($F(1,16) = 4.84$; $p = 0.043$). This indicates that also in these specific ROIs, THC attenuated the load-dependent rise in activity that was present after placebo administration. In these ROIs, THC administration significantly increased activity for the load3 – load1 condition in the left inferior parietal gyrus (from 0.23 ± 0.12 to 0.72 ± 0.13; $p = 0.012$) and left inferior temporal gyrus (from 0.15 ± 0.07 to 0.44 ± 0.11; $p = 0.028$). ROI results are summarized in Table 5.4 and Figure 5.6b.

Table 5.4 Effects of Δ9-tetrahydrocannabinol (THC) on activity in regions of interest (n = 17).

ROI	Activated brain region	Linear effects (F(1,16))			Load effects	
		Drug	Load	Drug * load	Load3-1	Load9-1
	WM network	0.025, $p = 0.88$	**17.31, $p = 0.001$***	**9.39, $p = 0.007$ ***	**$p = 0.047$ ***	$p = 0.913$
1	Cerebellum L	0.05, $p = 0.85$	0.051, $p = 0.82$	**4.84, $p = 0.043$ ***	$p = 0.238$	$p = 0.617$
2	Cerebellum R	1.16, $p = 0.30$	1.93 $p = 0.18$	2.39, $p = 0.142$	$p = 0.629$	$p = 0.228$
3	Inferior temporal gyrus L	2.35. $p = 0.14$	0.47 $p = 0.50$	**5.27, $p = 0.036$ ***	**$p = 0.028$ ***	$p = 0.305$
4	Insula / Inferior frontal gyrus L	0.07, $p = 0.80$	0.12, $p = 0.74$	3.84, $p = 0.068$	$p = 0.344$	$p = 0.512$
5	Insula / Inferior frontal gyrus R	0.95, $p = 0.34$	0.36, $p = 0.56$	1.02, $p = 0.328$	$p = 0.109$	$p = 0.367$
6	Dorsolateral prefrontal cortex L	0.22, $p = 0.65$	**4.86, $p = 0.04$***	**9.65, $p = 0.007$ ***	$p = 0.329$	$p = 0.201$
7	Caudate L	1.98, $p = 0.18$	2.35, $p = 0.15$	2.76, $p = 0.116$	$p = 0.128$	$p = 0.662$
8	Dorsolateral prefrontal cortex R	0.002, $p = 0.97$	1.09, $p = 0.31$	0.19, $p = 0.671$	$p = 0.672$	$p = 0.949$
9	Inferior parietal gyrus L	**5.05, $p = 0.04$**	3.23, $p = 0.91$	**4.98, $p = 0.040$ ***	**$p = 0.012$ ***	$p = 0.154$
10	Precuneus R	**4.55, $p = 0.049$**	0.45, $p = 0.51$	3.18, $p = 0.094$	**$p = 0.030$ ***	$p = 0.161$
11	Inferior parietal gyrus R	**4.58, p =0.048**	1.82, $p = 0.20$	3.40, $p = 0.084$	**$p = 0.028$ ***	$p = 0.221$
12	Anterior cingulate cortex	0.051, $p = 0.82$	**14.02, $p = 0.002$***	1.58, $p = 0.226$	$p = 0.519$	$p = 0.867$

Linear effects were determined with repeated measures linear contrast ANOVA, with drug and load as factors. Load effects were assessed with paired t-tests. ROI numbers correspond to those shown in Figure 5.3. See Table 5.2 for details on ROIs. * Significant effect ($p < 0.05$); ROI = region of interest; WM = working memory; L = left; R = right

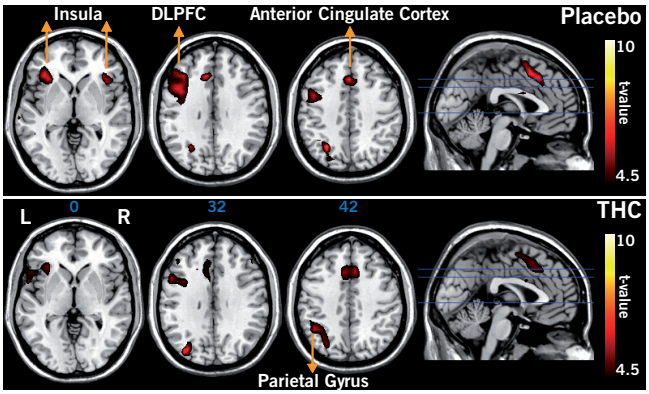

Figure 5.5 Group activity maps for load9 – load1 after (top) placebo and (bottom) THC administration (n = 17; $T > 4.5$, $p < 0.05$, corrected for multiple comparisons, cluster size ≥ 10 voxels). Numbers above slices indicate MNI z coordinates. DLPFC, dorsolateral prefrontal cortex; L, left; R, right.

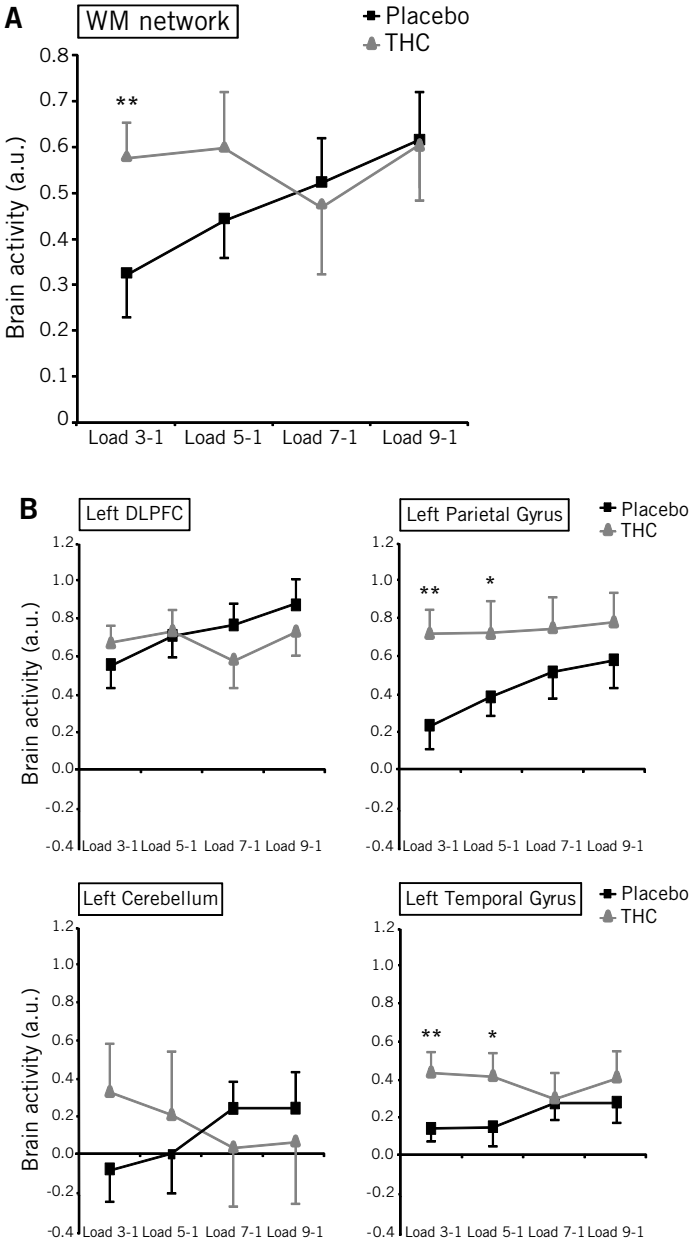

Figure 5.6 SIRP brain activity for increasing WM loads after placebo and THC administration in **A**, the entire WM network, and **B**, ROIs showing a significant linear interaction effect between drug and load ($p < 0.05$) (n = 17, mean ± SEM). ** Significant difference ($p < 0.05$) and * trend towards significant difference ($p < 0.10$) between THC and placebo. For detailed information on statistical results see Table 5.4. a.u., arbitrary units; DLPFC, dorsolateral prefrontal cortex.

Discussion

An fMRI study with a THC challenge was performed in healthy volunteers to test the hypothesis that perturbation of the eCB system can induce neurophysiological inefficiency in WM function in healthy subjects, similar to that observed in schizophrenia. In the placebo condition, brain activity in the WM network increased linearly with rising WM load, in accordance with previous findings[24-27] and confirming that the parametric manipulation of WM load was effective. THC administration reduced this linear relationship between WM load and WM network activity, and induced hyperactivity at a low WM load. In addition, performance started to decline at a lower load after THC administration. This effect of THC administration on WM brain function and performance is similar to the WM deficits reported in schizophrenia[2-6] and sheds some light on the concept of neurophysiological WM inefficiency[7,8].

Our results indicate involvement of the eCB system in WM function. To our knowledge, no other functional imaging studies have examined the effect of THC on WM function so far. The present findings are in line with several previously published electroencephalography (EEG) studies that assessed the effects of cannabis on WM function. A decline in EEG theta power has been reported after acute cannabis intake during WM task performance[18,19]. Another study demonstrated that the cannabis-induced decrease in resting state theta power after performing a WM task was correlated with decline of WM accuracy on the task[20]. Given that EEG theta power is associated with the maintenance of multiple WM items[38], these findings are consistent with those in the current study, indicating WM deficits after administration of cannabinoids.

Functional imaging studies have also demonstrated reduced activity of the prefrontal cortex in schizophrenia patients during cognitive task performance[2,9-11], which suggests that cognitive problems of schizophrenia patients may be related to hypofrontality[39]. Although the hypothesis of WM efficiency predominantly predicts reduced sensitivity for WM load, as well as an increase in activity for low loads, it does not exclude hypofrontality. It is possible that in addition to lower load sensitivity, the highest level of activity may be reduced in schizophrenia. However, hypofrontality may not always be a reflection of brain function, but may also be an artefact of task design. For instance, n-back tasks are essentially all-or-nothing tasks, where performance drops rapidly with loads that exceed capacity, promoting disengagement of the participant. Task disengagement will result in a strong decline in brain activity. The SIRP differs in that subjects can still perform moderately well with memory sets that exceed their capacity, allowing for continued engagement, and a more linear degradation in performance. Indeed, in the current study there was no indication of reduction of activity for high load compared to intermediate load either after placebo or THC, as has been hypothesized previously[7,8]. Both reaction time and accuracy results did not indicate disengagement for the THC session.

A mechanism that may be involved in both the increase in activity at low levels of WM load and reduced accuracy as a result of eCB perturbation is automatization of task performance. Typically, brain activity is reduced and accuracy is improved if a WM task, or parts of a WM task, can be automated[23,40,41]. For the Sternberg paradigm, it has been shown that particularly maintenance of a fixed memory set, which contributes largely to its activation pattern, is

sensitive for automatization at very short time periods[41]. Thus, our results could be explained by a reduced capacity to automate within each block, as these had fixed memory sets. Interestingly, this notion corresponds with a recent study with medication-naïve schizophrenia patients, who were shown to benefit less from automatization of a Sternberg WM task in terms of brain activity reduction[42].

Alternatively, inefficient WM function after THC administration may be related to a THC-induced reduction in alertness, as reported by the subjects in the current study. Subjects may have been impaired in directing attention to task-specific stimuli, which is a key process of WM[43]. The effects of THC may have lead to an increase in effort to keep task performance on par, which may be associated with the hyperactivity in the WM network for lower WM loads. Importantly, impaired attention is considered a fundamental cognitive deficit of schizophrenia patients[44].

This study has several limitations. First, inclusion of incidental cannabis users, as opposed to non-users, may hamper interpretation of the results, as previous cannabis use may have affected the eCB system. The choice for incidental cannabis users was based on ethical grounds[28]. Second, non-specific THC-induced changes on cerebral blood flow may have confounded our results[45]. However, we have designed our study to minimize the influence of this effect by comparing brain activity between task-specific conditions and a closely matched control condition (load1), as the non-specific effects of THC on blood flow can be expected to be present in all conditions. Third, it may be possible that the effects of THC may be specific for either maintenance of the memory set or response selection processes. The blocked fMRI design we used in the current study however does not allow for investigation of function-specific changes in brain activity.

In conclusion, this study shows that THC administration induces changes in the relationship between WM load, brain activity and task performance. These changes are strikingly similar to the relationships reported in schizophrenia, reflecting diminished neurophysiological efficiency of WM function. The findings provide indirect but compelling support for the notion that endocannabinoid brain systems play a role in working memory deficits in schizophrenia.

Acknowledgments

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on Neuropsychopharmacology for Young Scientists; Nice, France; March 2010; the Summer Meeting of the British Association for Psychopharmacology; Harrogate, United Kingdom; July 2010; and the 23rd Annual Congress of the European College of Neuropsychopharmacology; Amsterdam, the Netherlands; September 2010.

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References

1. Green M.F. (1996) What are the functional consequences of neurocognitive deficits in schizophrenia? Am J Psychiatry. 153 (3), 321-330.
2. Jansma J.M., Ramsey N.F., van der Wee N.J., Kahn R.S. (2004) Working memory capacity in schizophrenia: a parametric fMRI study. Schizophr Res. 68 (2-3), 159-171.
3. Manoach D.S., Press D.Z., Thangaraj V., Searl M.M., Goff D.C., Halpern E., Saper C.B., Warach S. (1999) Schizophrenic subjects activate dorsolateral prefrontal cortex during a working memory task, as measured by fMRI. Biol Psychiatry. 45 (9), 1128-1137.
4. Callicott J.H., Bertolino A., Mattay V.S., Langheim F.J., Duyn J., Coppola R., Goldberg T.E., Weinberger D.R. (2000) Physiological dysfunction of the dorsolateral prefrontal cortex in schizophrenia revisited. Cereb Cortex. 10 (11), 1078-1092.
5. Ramsey N.F., Koning H.A., Welles P., Cahn W., van der Linden J.A., Kahn R.S. (2002) Excessive recruitment of neural systems subserving logical reasoning in schizophrenia. Brain. 125 (Pt 8), 1793-1807.
6. Johnson M.R., Morris N.A., Astur R.S., Calhoun V.D., Mathalon D.H., Kiehl K.A., Pearlson G.D. (2006) A functional magnetic resonance imaging study of working memory abnormalities in schizophrenia. Biol Psychiatry. 60 (1), 11-21.
7. Manoach D.S. (2003) Prefrontal cortex dysfunction during working memory performance in schizophrenia: reconciling discrepant findings. Schizophr Res. 60 (2-3), 285-298.
8. Callicott J.H., Mattay V.S., Verchinski B.A., Marenco S., Egan M.F., Weinberger D.R. (2003) Complexity of prefrontal cortical dysfunction in schizophrenia: more than up or down. Am J Psychiatry. 160 (12), 2209-2215.
9. Carter C.S., Perlstein W., Ganguli R., Brar J., Mintun M., Cohen J.D. (1998) Functional hypofrontality and working memory dysfunction in schizophrenia. Am J Psychiatry. 155 (9), 1285-1287.
10. Driesen N.R., Leung H.C., Calhoun V.D., Constable R.T., Gueorguieva R., Hoffman R., Skudlarski P., Goldman-Rakic P.S., Krystal J.H. (2008) Impairment of working memory maintenance and response in schizophrenia: functional magnetic resonance imaging evidence. Biol Psychiatry. 64 (12), 1026-1034.
11. Koch K., Wagner G., Nenadic I., Schachtzabel C., Schultz C., Roebel M., Reichenbach J.R., Sauer H., Schlosser R.G. (2008) Fronto-striatal hypoactivation during correct information retrieval in patients with schizophrenia: an fMRI study. Neuroscience. 153 (1), 54-62.
12. Leweke F.M., Giuffrida A., Wurster U., Emrich H.M., Piomelli D. (1999) Elevated endogenous cannabinoids in schizophrenia. Neuroreport. 10 (8), 1665-1669.
13. Dean B., Sundram S., Bradbury R., Scarr E., Copolov D. (2001) Studies on [3H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. Neuroscience. 103 (1), 9-15.
14. Dalton V.S., Long L.E., Weickert C.S., Zavitsanou K. (2011) Paranoid Schizophrenia is Characterized by Increased CB(1) Receptor Binding in the Dorsolateral Prefrontal Cortex. Neuropsychopharmacology. 36 (8), 1620-1630.
15. Moore T.H., Zammit S., Lingford-Hughes A., Barnes T.R., Jones P.B., Burke M., Lewis G. (2007) Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet. 370 (9584), 319-328.
16. Linszen D.H., Dingemans P.M., Lenior M.E. (1994) Cannabis abuse and the course of recent-onset schizophrenic disorders. Arch Gen Psychiatry. 51 (4), 273-279.
17. Rais M., Cahn W., Van H.N., Schnack H., Caspers E., Hulshoff P.H., Kahn R. (2008) Excessive brain volume loss over time in cannabis-using first-episode schizophrenia patients. Am J Psychiatry. 165 (4), 490-496.
18. Ilan A.B., Smith M.E., Gevins A. (2004) Effects of marijuana on neurophysiological signals of working and episodic memory. Psychopharmacology (Berl). 176 (2), 214-222.
19. Ilan A.B., Gevins A., Coleman M., ElSohly M.A., De Wit H. (2005) Neurophysiological and subjective profile of marijuana with varying concentrations of cannabinoids. Behav Pharmacol. 16 (5-6), 487-496.
20. Bocker K.B., Hunault C.C., Gerritsen J., Kruidenier M., Mensinga T.T., Kenemans J.L. (2010) Cannabinoid modulations of resting state EEG theta power and working memory are correlated in humans. J Cogn Neurosci. 22 (9), 1906-1916.
21. Christensen R., Kristensen P.K., Bartels E.M., Bliddal H., Astrup A. (2007) Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. Lancet. 370 (9600), 1706-1713.
22. Sternberg S. (1966) High-speed scanning in human memory. Science. 153 (736), 652-654.

23. Jansma J.M., Ramsey N.F., Slagter H.A., Kahn R.S. (2001) Functional anatomical correlates of controlled and automatic processing. J Cogn Neurosci. 13 (6), 730-743.
24. Jansma J.M., Ramsey N.F., de Zwart J.A., van Gelderen P., Duyn J.H. (2007) fMRI study of effort and information processing in a working memory task. Hum Brain Mapp. 28 (5), 431-440.
25. Altamura M., Elvevag B., Blasi G., Bertolino A., Callicott J.H., Weinberger D.R., Mattay V.S., Goldberg T.E. (2007) Dissociating the effects of Sternberg working memory demands in prefrontal cortex. Psychiatry Res. 154 (2), 103-114.
26. Callicott J.H., Mattay V.S., Bertolino A., Finn K., Coppola R., Frank J.A., Goldberg T.E., Weinberger D.R. (1999) Physiological characteristics of capacity constraints in working memory as revealed by functional MRI. Cereb Cortex. 9 (1), 20-26.
27. Braver T.S., Cohen J.D., Nystrom L.E., Jonides J., Smith E.E., Noll D.C. (1997) A parametric study of prefrontal cortex involvement in human working memory. Neuroimage. 5 (1), 49-62.
28. van Hell H.H., Bossong M.G., Jager G., Kahn R.S., Ramsey N.F. (2011) Pharmacological Imaging of the Cannabinoid System (PhICS): towards understanding the role of the brain endocannabinoid system in human cognition. Int J Methods Psychiatr Res. 20, 10-27.
29. van Hell H.H., Bossong M.G., Jager G., Kristo G., van Osch M.J., Zelaya F., Kahn R.S., Ramsey N.F. (2011) Evidence for involvement of the insula in the psychotropic effects of THC in humans: a double-blind, randomized pharmacological MRI study. Int J Neuropsychopharmacol., 1-12.
30. Bossong M.G., van Berckel B.N., Boellaard R., Zuurman L., Schuit R.C., Windhorst A.D., van Gerven J.M., Ramsey N.F., Lammertsma A.A., Kahn R.S. (2009) Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. Neuropsychopharmacology. 34 (3), 759-766.
31. Zuurman L., Roy C., Schoemaker R.C., Hazekamp A., den Hartigh J., Bender J.C., Verpoorte R., Pinquier J.L., Cohen A.F., van Gerven J.M. (2008) Effect of intrapulmonary tetrahydrocannabinol administration in humans. J Psychopharmacol. 22 (7), 707-716.
32. Bond A., Lader M. (1974) The use of analogue scales in rating subjective feelings. Br J Med Psychol. 47, 211-218.
33. Bowdle T.A., Radant A.D., Cowley D.S., Kharasch E.D., Strassman R.J., Roy-Byrne P.P. (1998) Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. Anesthesiology. 88 (1), 82-88.
34. van Buuren M., Gladwin T.E., Zandbelt B.B., van den Heuvel M., Ramsey N.F., Kahn R.S., Vink M. (2009) Cardiorespiratory effects on default-mode network activity as measured with fMRI. Hum Brain Mapp. 30 (9), 3031-3042.
35. Neggers S.F., Hermans E.J., Ramsey N.F. (2008) Enhanced sensitivity with fast three-dimensional blood-oxygen-level-dependent functional MRI: comparison of SENSE-PRESTO and 2D-EPI at 3 T. NMR Biomed. 21 (7), 663-676.
36. van Raalten T.R., Callicott J.H., Sust S., Brooke J., Kahn R.S., Ramsey N.F. (2009) Practice and the dynamic nature of working memory. In: van Raalten T.R. (Editor), The automatic brain; Studies on practice and brain function in healthy subjects and patients with schizophrenia.
37. Kriegeskorte N., Simmons W.K., Bellgowan P.S., Baker C.I. (2009) Circular analysis in systems neuroscience: the dangers of double dipping. Nat Neurosci. 12 (5), 535-540.
38. Jensen O. (2006) Maintenance of multiple working memory items by temporal segmentation. Neuroscience. 139 (1), 237-249.
39. Weinberger D.R., Berman K.F., Zec R.F. (1986) Physiologic dysfunction of dorsolateral prefrontal cortex in schizophrenia. I. Regional cerebral blood flow evidence. Arch Gen Psychiatry. 43 (2), 114-124.
40. Ramsey N.F., Jansma J.M., Jager G., Van Raalten T., Kahn R.S. (2004) Neurophysiological factors in human information processing capacity. Brain. 127 (Pt 3), 517-525.
41. van Raalten T.R., Ramsey N.F., Duyn J., Jansma J.M. (2008) Practice induces function-specific changes in brain activity. PLoS One. 3 (10), e3270.
42. van Veelen N.M., Vink M., Ramsey N.F., Kahn R.S. (2010) Left dorsolateral prefrontal cortex dysfunction in medication-naive schizophrenia. Schizophr Res. 123 (1), 22-29.
43. Corbetta M., Shulman G.L. (2002) Control of goal-directed and stimulus-driven attention in the brain. Nat Rev Neurosci. 3 (3), 201-215.
44. Cornblatt B.A., Keilp J.G. (1994) Impaired attention, genetics, and the pathophysiology of schizophrenia. Schizophr Bull. 20 (1), 31-46.
45. Iannetti G.D., Wise R.G. (2007) BOLD functional MRI in disease and pharmacological studies: room for improvement? Magn Reson Imaging. 25 (6), 978-988.

6

Default mode network is implicated in the effects of Δ9-tetrahydrocannabinol (THC) on human executive function

Submitted

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Abstract

There is increasing evidence for involvement of the endocannabinoid (eCB) system in psychiatric disorders. Impaired executive function is an important symptom among a broad range of neurological and psychiatric disorders, suggesting the possibility of a common underlying brain system that may have been affected. Behavioral evidence indicates that perturbation of the eCB system can impair performance on executive function paradigms. The default mode network (DMN) is associated with goal-oriented behavior independent of the specific task. In order to examine a possible role of DMN in executive function deficits related to the eCB system, a placebo-controlled, crossover functional MRI experiment with Δ9-tetrahydrocannabinol (THC) administration was performed. Effects of THC on brain function and task performance were assessed in twenty healthy volunteers, using a continuous performance task with identical pairs (CPT-IP). Task performance was impaired after THC administration, reflected in both an increase in false alarms and a reduction in detected targets. This was associated with elevated activity in a set of brain regions that has been linked to the DMN, including posterior cingulate cortex and angular gyrus. Level of deactivation in DMN was anti-correlated with performance after THC administration. Regions that were activated by the CPT-IP, notably bilateral prefrontal and parietal cortex, did not show effects of THC. These results suggest that the eCB system may play an important role in executive function, through modulation of the DMN. These results indicate possible involvement of both the DMN and the eCB system in impaired executive function in psychiatric and neurological disorders.

Introduction

The endocannabinoid (eCB) system, consisting of cannabinoid receptors and accompanying endogenous ligands, is a retrograde messenger system that regulates both excitatory and inhibitory neurotransmission[1-3]. Recently, the eCB system has been emerging as a potential candidate for pharmacological targeting of psychiatric syndromes. Animal studies have shown that blocking cannabinoid CB_1 receptors with the eCB antagonist rimonabant prevents self-administration of several drugs of abuse[4,5], while relapse to cocaine, nicotine and ethanol is reduced in abstinent animals pre-treated with rimonabant[6,7]. In humans, the eCB system has been studied by administration of Δ9-tetrahydrocannabinol (THC), the main psychoactive component in cannabis and a partial agonist of the CB_1 receptor, as well as rimonabant and the cannabinoid agent cannabidiol (CBD). CBD has been shown to block psychotic effects induced by THC administration in healthy volunteers[8], while preliminary results indicate that CBD may decrease psychotic symptoms in patients with schizophrenia with few side effects[9]. In addition, results from clinical trials suggest that rimonabant facilitates smoking cessation[10]. Modulation of the eCB system by administration of THC has been shown to impair performance on various executive function paradigms which target high level cognitive functions that are essential for goal-directed behavior[11-19].

A vast body of research papers has shown that goal-oriented behavior is associated with reduced activity in the default mode network (DMN)[20-22]. Moreover, failure to reduce DMN activity is related to errors in goal-oriented behavior[23-28]. Given the previous considerations, the DMN emerges as a promising candidate brain system to be involved in executive function deficits. Goal of this study is to examine involvement of the DMN in executive function deficits related to eCB perturbation after administration of THC.

A pharmacological fMRI challenge study was performed with THC, using a placebo-controlled, cross-over design and a continuous performance task paradigm with identical pairs (CPT-IP)[29] in healthy subjects. The CPT-IP requires processing of a continuously changing stream of data, and is characterized by a heavy reliance on executive function while short-term memory load is relatively small[30,31]. Previous imaging studies using CPT-IP paradigms have shown activation of an executive system predominantly consisting of frontal and parietal regions[32,33]. We compared performance on the CPT-IP task after placebo and after THC administration, and assessed the role of the DMN and the executive system in the effect of THC.

Materials and Methods

This study is part of the Pharmacological Imaging of the Cannabinoid System (PhICS) project, a comprehensive research project on the role of the eCB system in the regulation of cognitive brain function in healthy volunteers and patients with psychiatric disorders. Methods of the entire study are reported in detail in a methodological paper[34]. This study is registered in both the EudraCT database (2007-004247-30) and the Dutch Trial Register (NTR1787).

Subjects

Twenty-three healthy male right-handed subjects were recruited through flyers, posters and internet advertisements. All subjects used cannabis on an incidental basis, defined as having used cannabis at least four times but at most once a week in the year before inclusion in the study. All subjects were in good physical health as assessed by medical history and physical examination, and were screened for axis I psychiatric disorders using the Dutch version of the Mini International Neuropsychiatric Interview for DSM-IV clinical disorders[35]. Subjects were asked to refrain from cannabis for at least two weeks before the first study day until study completion. Illicit drug use other than cannabis was not within six months prior to inclusion. Urine screening for cannabis, cocaine, amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), morphine, methadone, tricyclic antidepressants (TCA), barbiturates and benzodiazepines was performed at screening and on both study days. Subjects with a positive drug test were excluded from the study. Subjects were also asked to abstain from alcohol for 48 hours before each study day. Smoking was not allowed from the moment of arrival until the end of a study day. Alcohol and nicotine use was assessed by self-report. Subjects were asked to fast for at least four hours before arrival. On the beginning of each test day, they were served a standard meal. For further details on

inclusion and exclusion criteria we refer to Van Hell et al. (2011)[34]. All volunteers gave written informed consent before entry into the study and were compensated for their participation. The study was approved by the Independent Ethics Committee of the University Medical Center Utrecht, the Netherlands, in accordance to the Declaration of Helsinki 2008.
Results are reported on twenty out of the twenty-three included subjects. One subject did not complete the study procedure due to the exceeding of acceptable blood pressure levels. Two other subjects were excluded because of an absence of detectable THC plasma levels and technical malfunction during scanning, respectively. Subject characteristics are summarized in Table 6.1. All subjects showed negative urine screening at both study days.

Design and procedure

Subjects underwent two scanning sessions of which one with administration of placebo and one with THC in random sequence. Study days were scheduled two weeks apart to allow for complete clearance of drugs. Two weeks before the first study day, participants were familiarized with the scanner environment using a mock scanner. Verbal intelligence was estimated with the Dutch Adult Reading Test (DART), the Dutch version of the National Adult Reading Test[36].
At the beginning of each study day, a catheter was placed percutaneously in the left arm for the withdrawal of blood samples. Subsequently, subjects performed three cognitive paradigms, during which functional MRI scans were obtained. One of these paradigms was the CPT-IP. Paradigm sequence was randomized between subjects, but remained unchanged within subjects across sessions. Results of other assessments are reported elsewhere[34,37].

Table 6.1 Subject characteristics (n = 20).

Characteristic	Mean ± SD	Range
Age (years)	22.9 ± 4.9	18 - 40
IQ	105.6 ± 5.6	97 - 114
Height (cm)	185.9 ± 7.9	175 - 201
Weight (kg)	77.0 ± 11.3	60 - 110
BMI (kg/m^2)	22.2 ± 2.1	18.5 - 27.2
Cannabis use (Occasions / year)	22.5 ± 15.2	4 - 52
Tobacco smoking (Cigarettes / week)	57.6 ± 60.8	0 - 140
Alcohol consumption (Units / week)	12.5 ± 7.8	2 - 30
Coffee consumption (Units / week)	17.4 ± 12.4	0 - 40
Illicit drug use (Occasions lifetime)	2.0 ± 4.0	0 - 17

Use of cannabis, tobacco, alcohol and coffee was given for the year before inclusion in the study. Subjects refrained from cannabis for at least two weeks before the first study day until study completion and from alcohol for 48 hours before each study day. Caffeine intake and smoking were not allowed from the moment of arrival until the end of a study day. Illicit drug use other than cannabis was at least more than six months before the first study day. All subjects showed negative urine screening at both study days.

On study days, subjects received subsequent doses of THC or placebo with 30 minutes intervals. Drugs were administered before each fMRI task using a Volcano® vaporizer (Storz–Bickel GmbH, Tuttlingen, Germany) according to a method described earlier[38,39]. The first THC dose was 6 mg, followed by three doses of 1 mg each to maintain stable levels of CNS effects. Doses were based on pharmacokinetic/pharmacodynamic (PK/PD) modeling of CNS effects induced by THC[40]. See Van Hell et al. (2011) for detailed study procedures[34].

Drug levels and behavioral measurements

Venous blood samples were collected to determine plasma concentrations of THC and its two most important metabolites, 11-OH-THC and 11-nor-9-carboxy-THC. Blood samples were processed according to Zuurman et al. (2008)[39]. Subjective effects were determined with two sets of visual analogue scales[41,42]. Both rating scales were performed consecutively at baseline and before and after performance of the CPT-IP. Visual analogue scales were analyzed as described previously[38,39]. Heart rate and respiration were monitored continuously during scanning, as described by van Buuren et al. (2009)[43]. Mean heart rate was computed by dividing the total number of heart beat trigger signals by the duration of the CPT-IP. Data were corrected for baseline values and analyzed with paired t-tests.

Task paradigm

Executive function was assessed with a CPT with identical pairs (CPT-IP) consisting of two different task conditions (Figure 6.1)[29,32,33]. In the experimental condition (CPT-IP), participants were presented with a series of four-digit numbers, and were instructed to press a button as quickly as possible when two consecutive numbers were identical. In a control task (CT), subjects were always presented with the same stimulus ('1234'), and were instructed to watch the stimuli, but not to respond. This task was designed to control for the simple visual components of watching flashing numbers. The CPT-IP and CT tasks were given in alternating blocks of 30 s each. Six blocks of each task were presented, together with six rest blocks. The order of blocks was counterbalanced. A total of 40 numbers per block was presented. Every number appeared for 700 ms, followed by a fixation cross of 50 ms. The number of targets per block varied from seven to nine, with an average of eight targets per block.

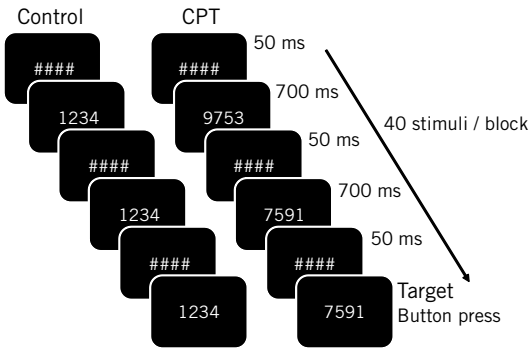

Figure 6.1 Schematic outline of the task used to assess executive function. The task consists of a control (CT, left) and an experimental condition (CPT-IP, right), during which four-digit numbers are presented in sequence. In the experimental condition, subjects have to press a button as quickly as possible when two consecutive numbers are identical. No response is required for the control condition. See for detailed information the Materials and Methods section.

In addition, each block contained eight distracters, defined as numbers consisting of similar digits as the preceding number, but presented in another order. Total task duration was 11 minutes. Numbers differed for both study days for all subjects.
Outcome measures included reaction time for hits (RT), the mean percentage of correctly identified targets (% hits), and the mean percentage of incorrectly identified targets (% false alarms). The ability to discriminate targets from non-targets (d') was calculated as described by Rutschmann et al. (1977)[44]. Group differences in RT and performance accuracy between placebo and THC were analyzed with paired t-tests.

Image Acquisition

Image acquisition was performed on a Philips Achieva 3.0 Tesla scanner (Philips Medical Systems, Best, the Netherlands). Functional images were obtained using a 3D PRESTO-SENSE pulse sequence[45] (parameters: TR 22.5 ms; TE 33.2 ms; flip angle = 10°; FOV 224×256×160; matrix 56× 64×40; voxel size 4 mm isotropic; scan time 0.6075 s; 40 slices (sagittal orientation)). A total of 1105 functional images were acquired. One functional volume with a flip angle of 27° (FA27) was acquired for image co-registration. A T1-weighted structural image was obtained for anatomical registration (parameters: TR 9.5 ms; TE 4.7 ms; flip angle = 8°; FOV 220.8x240x159.6; matrix 368×400×266; voxel size 0.6 mm isotropic, 266 slices (sagittal orientation)).

Functional MRI analysis

After reconstruction, scan volumes were preprocessed and analyzed using SPM5 (Wellcome Trust Centre for Neuroimaging, London, UK). The FA27 functional was co-registered to the high resolution anatomical scan. Subsequently, the anatomical scan was normalized to standard MNI space[46], to calculate parameters for spatial normalization. Functional data were realigned to the FA27 volume, followed by spatial normalization into MNI space, and spatial smoothing (FWHM = 8 mm). First level single subject analysis included a general linear model regression analysis using a factor matrix with factors for the CPT-IP and CT condition, as well as the instructions that were presented during the task. To correct for drifts in the signal, cosine-based regressors were added to the model, corresponding to a high-pass filter with cut-off frequency of 0.004 Hz. Group activity maps were created for both the placebo and THC session for the CPT-IP minus CT contrast. In order to increase power of hypothesis testing in regard to a whole brain voxel wise analysis, we preselected 'task' voxels that showed significant signal changes associated with the experimental paradigm (thresholded at $t > |4.6|$, $p < 0.0001$). To prevent session bias in voxel selection, voxels were included if they were either active either in the placebo or in the THC session. ROIs were identified by clustering groups of at least ten neighboring active voxels. According to the direction of the signal change, ROIs were grouped in two networks: ROIs showing task-related increases are from here on referred to as task-induced activation ('TIA') ROIs. ROIs based on voxels showing signal decrease are from here on referred to as task-induced deactivation ('TID') ROIs.

For hypothesis testing, mean regression coefficients (b-values) for the CPT-IP condition were extracted from each ROI and for both the placebo and THC condition, using the Marsbar SPM tool. Effects of THC on signal changes in the two networks of ROIs were determined in SPSS using repeated measures MANOVA with drug and ROI as within-subjects factors. Effects of THC on activity in ROIs were assessed with paired t-tests, with Bonferroni correction for multiple comparisons when THC did not induce a significant effect of drug in the network analysis.

To directly compare effects of THC in TIA and TID networks, mean regression coefficients for the CPT-IP condition were averaged over all included voxels for either network, for both the placebo and THC condition. Effects of THC were determined in SPSS using repeated measures MANOVA with drug and network as factors. For further understanding of the involvement of eCB in executive function, we correlated TIA and TID network activity with task performance (percentage of correct responses) after placebo and after THC (Pearson's r).

Results

Drug levels and behavioral measurements

Plasma concentrations of THC and its main metabolites were 78.4 ± 27.0 ng/ml (THC), 3.9 ± 4.6 ng/ml (11-nor-9-carboxy-THC) and 2.5 ± 2.0 ng/ml (11-OH-THC), 5 min after inhalation of 6 mg THC. Analysis of subjective effects before and after performance of CPT-IP revealed a significant THC-induced increase in VAS score of 'feeling high' ($F(1,19) = 19.10$, $p < 0.001$) and 'external perception' (reflecting misperception of external stimuli or changes in the awareness of the environment) ($F(1,19) = 11.03$, $p = 0.004$) compared to placebo. In addition, THC significantly reduced 'alertness' ($F(1,19) = 9.24$, $p = 0.007$), 'contentedness' ($F(1,19) = 10.03$, $p = 0.005$), and 'calmness' ($F(1,19) = 10.10$, $p = 0.005$). THC caused a trend towards increased 'internal perception' (reflecting inner feelings that do not correspond with reality) ($F(1,19) = 3.42$, $p = 0.080$). Subjective effects are summarized in Table 6.2. Heart rate increased significantly after THC compared with placebo (22.2 ± 14.5 and -1.5 ± 7.8 bpm increase compared to baseline, respectively; $p < 0.001$). For a more detailed description of drug levels and behavioral measurements following THC see Van Hell et al. (2011)[34].

Task performance

THC administration significantly decreased the percentage of correctly identified targets (from 83.7 ± 13.3% to 74.7 ± 19.5%, $p = 0.016$) and enhanced the percentage of false alarms (from 3.5 ± 3.4% to 5.7 ± 4.3%, $p = 0.001$) compared to placebo. The ability to discriminate targets from non-targets as indexed by d' was reduced after THC administration (from 3.1 ± 0.9 to 2.5 ± 1.0, $p = 0.002$). Reaction times on the CPT-IP did not differ between placebo and THC sessions (538.5 ± 32.7 and 552.0 ± 50.3 ms, respectively; $p = 0.296$) (see Figure 6.2).

Selection of regions of interest

Task activity was measured in a set of regions showing task-induced deactivation (TID) and a set of regions showing task-induced activation (TIA). TID showed a network of four regions, comprising posterior cingulate cortex, left inferior temporal gyrus, right cerebellum and left angular gyrus (Table 6.3 and Figure 6.3A). TIA yielded a network of 15 brain regions, comprising bilateral prefrontal cortex, parietal cortex, precentral gyrus, visual cortex, and thalamus, as well as anterior cingulate cortex, mid cingulate gyrus, vermis, and right middle temporal cortex (Table 6.4 and Figure 6.3B).

Effects of THC on task-induced deactivation

Activity in TID regions was significantly increased after THC administration compared to placebo ($F = 13.20$; $p = 0.002$). There was no significant difference in the effect of THC on TID ROIs (drug * ROI interaction, $F = 0.06$, $p = 0.98$) (Table 6.5 and Figure 6.4).

Table 6.2 Subjective effects of Δ9-tetrahydrocannabinol (THC) (n = 20).

VAS Assessment	Drug effect (F(1,19))	Mean placebo score (± SD)	Mean THC score (± SD)
Feeling High	19.10, $p < 0.001$*	2.63 ± 6.41	27.00 ± 25.99
Internal Perception	3.42, $p = 0.080$	0.15 ± 0.63	3.15 ± 7.06
External Perception	11.03, $p = 0.004$*	0.98 ± 2.24	9.15 ± 10.29
Alertness	9.24, $p = 0.007$*	-7.44 ± 7.68	-17.03 ± 12.72
Contentedness	10.03, $p = 0.005$*	-3.60 ± 8.05	-11.68 ± 9.73
Calmness	10.10, $p = 0.005$*	4.94 ± 12.82	-9.63 ± 18.20

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

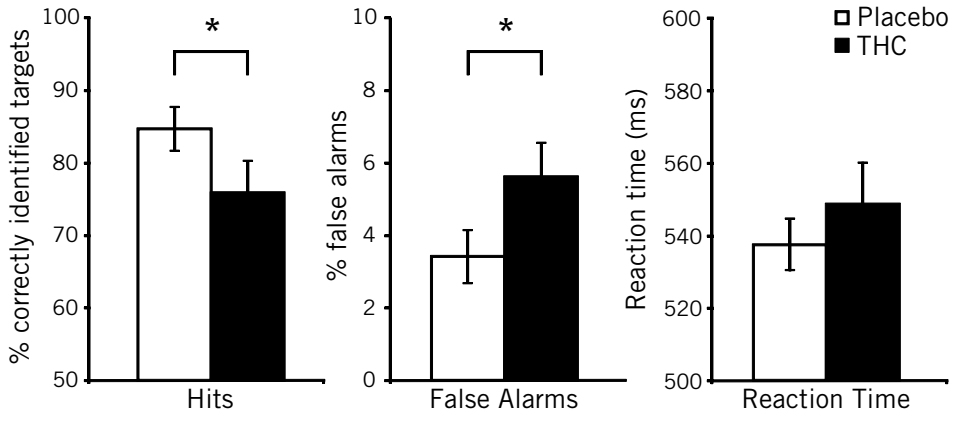

Figure 6.2 Task performance, with (left) the mean percentage of correctly identified targets, (middle) the mean percentage of false alarms, and (right) reaction times of correct responses after placebo and THC administration (n = 20; mean ± SEM). * Significant difference between THC and placebo ($p < 0.05$).

Analysis of individual ROIs revealed a significant THC-induced increase in activity in three out of four ROIs, namely the posterior cingulate gyrus (t = -2.84; $p = 0.01$), left inferior temporal cortex (t = -2.25; $p = 0.04$) and left angular gyrus (t = -2.21; $p = 0.04$) (see Table 6.3, Figure 6.5 and Figure 6.6).

Effects of THC on task-induced activation

Brain activity in TIA regions was not affected by THC administration compared to placebo (F = 0.02; $p = 0.90$), indicating that THC did not induce a change in the pattern of TIA activity during CPT-IP. There was no significant difference in the effect of THC on TIA ROIs (drug * ROI interaction, F = 0.72, $p = 0.71$) (Table 6.5 and Figure 6.4). Follow u*p* analysis in individual TIA ROIs showed that THC administration increased activity in one region (mid cingulate gyrus, t = -2.18; $p = 0.04$). This effect did not survive correction for multiple comparisons for number of TIA ROIs (see Table 6.4, Figure 6.6 and Figure 6.7).

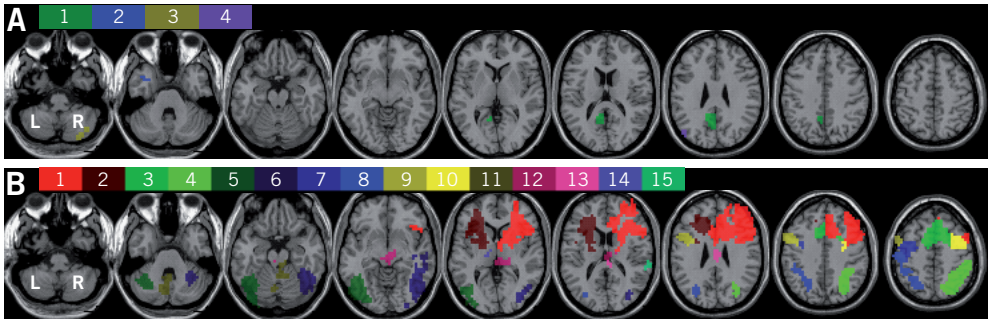

Figure 6.3 Regions of interest (ROIs) used to assess effects of THC administration on **A**, task-induced deactivation (TID), and **B**, task-induced activation (TIA). ROIs are defined in CPT-IP minus CT group activity maps, pooled over placebo and THC (n = 20; t > l4.6l, $p < 0.0001$ uncorrected, clusters ≥ 10 voxels). ROI numbers correspond to those shown in Table 6.3 and Table 6.4, respectively. L, left; R, right.

Table 6.3 Effect of Δ9-tetrahydrocannabinol (THC) on TID ROIs (n = 20).

ROI	Activated brain region	Abbreviation	Cluster size (mm^3)	t value	*p* value
1	Posterior cingulate cortex	PPC	7616	-2.84	0.01 *
2	Inferior temporal cortex L	lITC	1024	-2.25	0.04 *
3	Cerebellum R	rCB	768	-1.47	0.16
4	Angular gyrus L	lAG	640	-2.21	0.04 *

Group activity maps for placebo and THC were thresholded at t < -4.6, $p < 0.0001$ uncorrected, cluster size ≥ 10 voxels. ROI numbers correspond to those shown in Figure 6.3A. Statistical analysis was performed with paired t-tests. * Significant difference between placebo and THC ($p < 0.05$); TID, task-induced deactivation; ROI, region of interest; L, left; R, right.

Task-induced activation vs task-induced deactivation

A direct comparison of the network effect of THC on the absolute value of change in TIA and TID activity, using the network average of all ROIs in TID and TIA, revealed a significant interaction effect between drug and network ($F = 6.97$; $p = 0.02$), reflecting that the TID network was more sensitive to the effects of THC then the TIA network (see Table 6.5 and Figure 6.4). A spatial illustration of the effect of THC administration on TID and TIA activity is shown in Figure 6.8. The upper graph demonstrates the consistent reduction of deactivation in the TID network after THC administration, while the lower graph shows that activity in TIA ROIs is virtually unchanged after THC.

Table 6.4 Effect of Δ9-tetrahydrocannabinol (THC) on TIA ROIs (n = 20).

ROI	Activated brain region	Abbreviation	Cluster size (mm^3)	t value	p value
1	Prefrontal cortex R	rPFC	95616	-0.75	0.46
2	Prefrontal cortex L	lPFC	34048	-0.27	0.79
3	Anterior cingulate cortex	ACC	30528	0.02	0.99
4	Parietal cortex R	rPC	27520	0.34	0.74
5	Visual cortex L	lVC	31040	1.27	0.22
6	Visual cortex R	rVC	31424	1.89	0.07
7	Precentral gyrus L	lPrCG1	10560	-1.54	0.14
8	Parietal cortex L	lPC	17856	0.19	0.85
9	Precentral gyrus L	lPrCG2	8768	1.25	0.23
10	Precentral gyrus R	rPrCG	11584	-1.62	0.12
11	Vermis	VM	9088	0.72	0.48
12	Thalamus R	rTHAL	9344	-0.56	0.58
13	Mid cingulate gyrus	MCG	2560	-2.18	0.04 *
14	Thalamus L	lTHAL	1408	-1.26	0.22
15	Middle temporal cortex R	rMTC	896	0.41	0.69

Group activity maps for placebo and THC were thresholded at $t > 4.6$, $p < 0.0001$ uncorrected, cluster size ≥ 10 voxels. ROI numbers correspond to those shown in Figure 6.3B. Statistical analysis was performed with paired t-tests. * Significant difference between placebo and THC ($p < 0.05$). TIA, task-induced activation; ROI, region of interest; L, left; R, right.

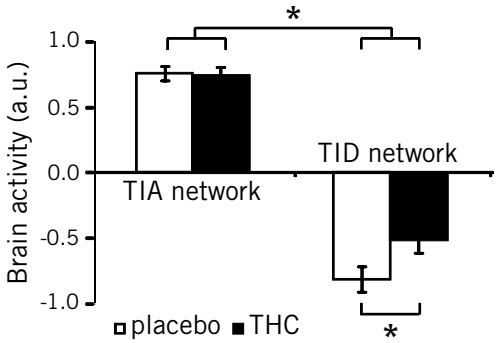

Figure 6.4 Brain activity in the TIA (left) and TID network (right, all voxels combined), after administration of placebo (white) and THC (black) (n = 20; mean ± SEM). A significant interaction effect between drug and network indicates that THC had a different effect on activity in the TID than in the TIA network. See also Table 6.5. * Significant effect ($p < 0.05$). TIA, task-induced activation; TID, task-induced deactivation; a.u., arbitrary units.

Table 6.5 Effect of Δ9-tetrahydrocannabinol (THC) on TIA and TID set of regions (n = 20).

Effect	F value	*p* value
drug * network (TIA, TID) [a]	6.97	**0.02** *
drug (TIA) [b]	0.02	0.90
drug * region (TIA) [b]	0.72	0.71
drug (TID) [b]	13.20	**0.002** **
drug * region (TID) [b]	0.06	0.98

Statistical analysis was performed using repeated measures ANOVA with [a] drug and network and [b] drug and ROI as factors. See also Figure 6.4. * Significant at $p < 0.05$; ** Significant at $p < 0.01$. TIA, task-induced activation; TID, task-induced deactivation.

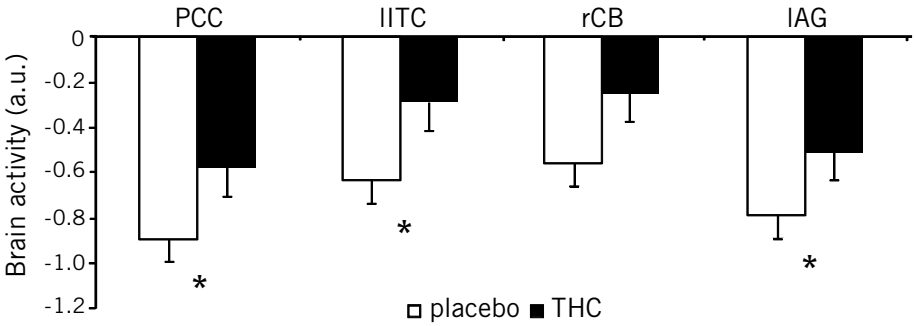

Figure 6.5 Brain activity in TID regions, after administration of placebo (white) and THC (black) (n = 20; mean ± SEM). Three regions showed a significantly reduced inhibition after THC administration, if not corrected for multiple comparisons. * Significant effect ($p < 0.05$). Abbreviations are given in Table 6.3. a.u., arbitrary units.

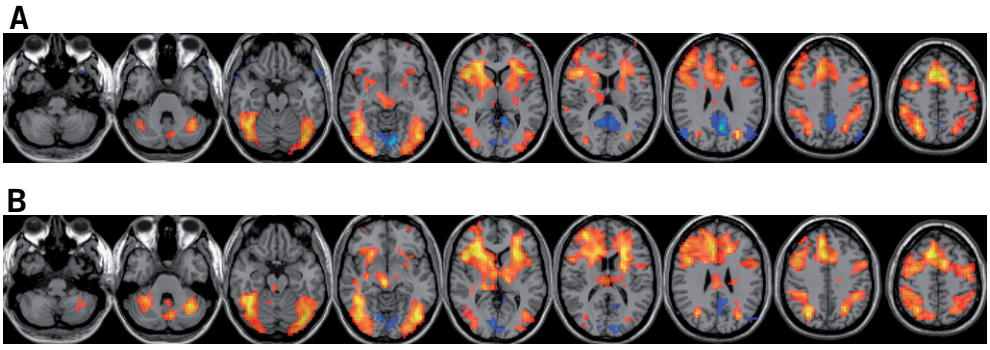

Figure 6.6 Activity patterns during performance of CPT-IP (baseline: rest) after administration of **A**, placebo, and **B**, THC (n = 20; t > |4.6|, $p < 0.0001$ uncorrected, clusters ≥ 10 voxels).

Correlations between performance and task-induced deactivation

In a follow-up analysis we examined if activity in the TID network was related to performance across subjects (percent correct responses). Activity in TID after THC showed a significant negative correlation with performance (r = -0.43, $p = 0.03$) (Figure 6.9A). Follow up analysis in the four TID ROIs indicated a significant negative correlation in posterior cingulate cortex (r = -0.38, $p = 0.049$), right cerebellum (r = -0.44, $p = 0.026$) and left angular gyrus (r = -0.53, $p = 0.008$). No significant correlation was found between performance and TID after placebo (r = -0.04; $p = 0.85$) (see Figure 6.9).

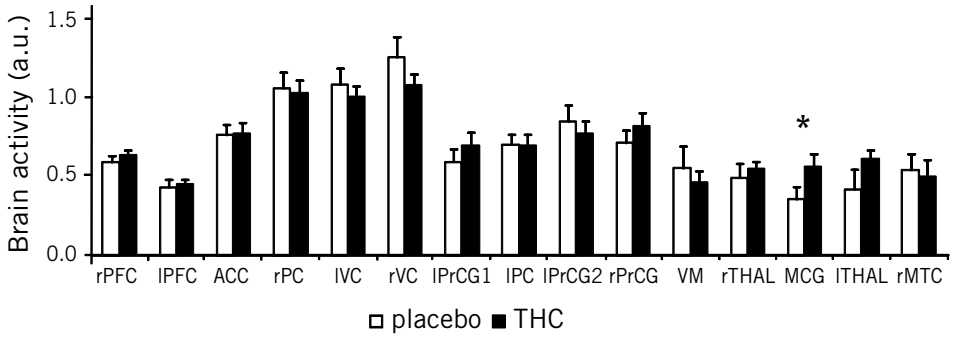

Figure 6.7 Brain activity in TIA regions, after administration of placebo (white) and THC (black) (n = 20; mean ± SEM). One region (mid cingulate cortex) showed a significant increase after THC administration, if not corrected for multiple comparisons. * Significant effect ($p < 0.05$). Abbreviations are given in Table 6.4. a.u., arbitrary units.

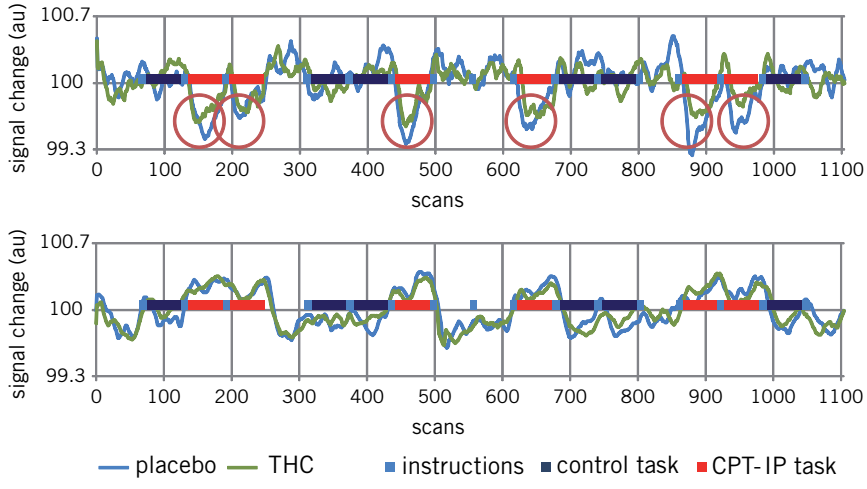

Figure 6.8 Activity over time in the TID (upper graph) and TIA network (lower graph) during performance of the CPT-IP after placebo (blue) and THC (green) administration (n = 20; mean ± SEM). TIA, task-induced activation; TID, task-induced deactivation; au, arbitrary units.

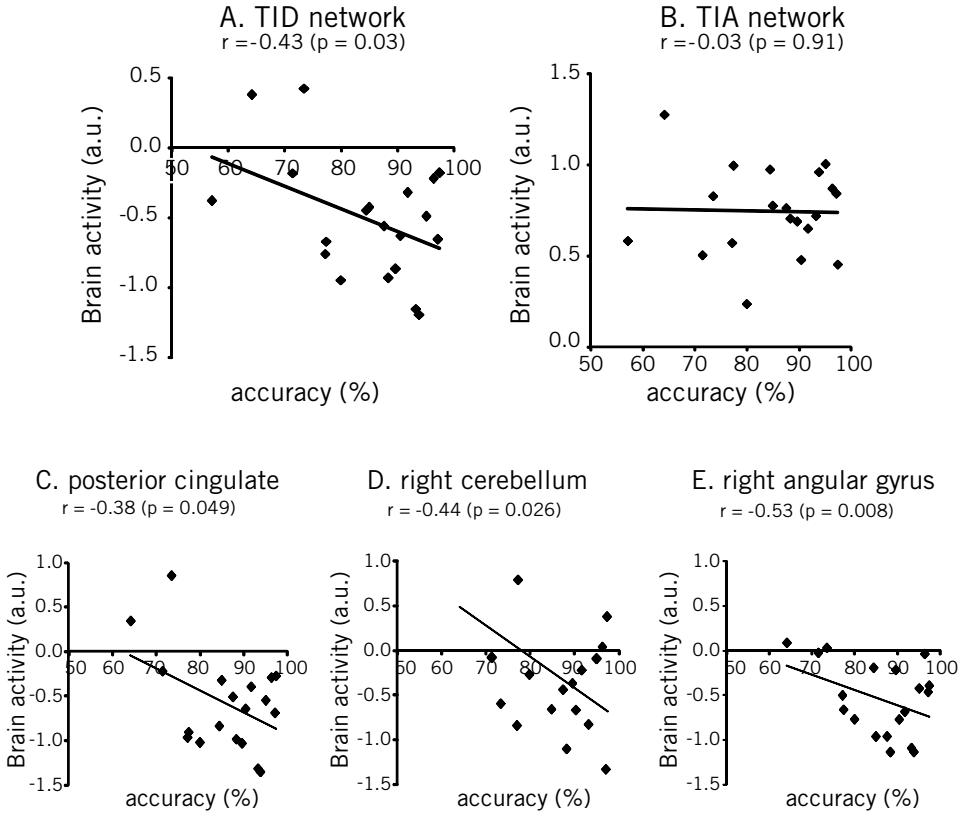

Figure 6.9 Graphs illustrating correlations between performance (percentage correct responses) and activity in **A**, the TID network, **B**, the TIA network, **C**, posterior cingulate cortex, **D**, right cerebellum, and **E**, right angular gyrus. TIA, task-induced activation; TID, task-induced deactivation; a.u., arbitrary units.

Discussion

The role of the eCB system in executive function was studied in an fMRI study with a THC challenge, focusing on DMN. After administration of THC, subjects showed impaired task performance, reflected in both an increase in false alarms and a reduction in detected targets, indicating a small but consistent deficit in executive function. The set of regions that were deactivated during the task showed less deactivation after THC administration than after placebo. In addition, it was found that after THC administration, task performance was negatively correlated with activity in the deactivated brain regions. There was no correlation between performance and deactivation after placebo. In contrast, the set of regions that were positively activated by the task did not change activity after THC administration. Together, these results indicate that the involvement of the eCB system in executive function is linked to the DMN. In our study, effects of THC on DMN activity were predominantly found in the posterior cingulate cortex (PCC) and angular gyrus. While both regions are routinely identified

as part of the DMN[21], specifically the PCC has been recognized as a pivotal integrating node in the DMN[47-50].

A possible role of DMN in executive function is addressed by the default mode interference hypothesis as proposed by Sonuga-Barke and Castellanos (2007)[51]. The interference theory states that functions that are performed by the DMN interfere with successful goal-oriented performance. In the context of a normally functioning brain, the DMN component is attenuated during goal-directed action, and the level of attenuation is independent of task content. Neuroimaging studies support this notion, as several studies have linked reduced activity in DMN with successful task execution[23-28]. In addition, is has been shown that the level of reduction in DMN activity reflects the relative resources that need to be allocated to task execution[52-54]. How exactly interference occurs is largely unknown, but a possibility is that DMN functions use similar resources as needed for goal-oriented behavior. Possible functions of the DMN include conscious processes that occur in the absence of goal- oriented behavior, such as self-referential mental processing[55-57], mind-wandering[58], and mental explorations and simulations[47].

The THC-induced effects on DMN activity as demonstrated in the current study suggest a role for the eCB system in regulation of default mode activity. A potential neurobiological explanation for the changes in DMN activity after THC administration may be found in the modulating role of the eCB system in neurotransmitter release. The eCB system is a retrograde messenger system that regulates both GABA and glutamate neurotransmission according to an 'on-demand' principle: endocannabinoids are released when and where they are needed[1-3]. This eCB-mediated regulation of synaptic transmission is a widespread phenomenon in the brain, and is thought to play an important role in higher cognitive functions[2,3]. It has been shown that THC administration can disrupt this function of the eCB system[59,60]. Recent studies indicate that negative BOLD responses are tightly coupled to reductions in neuronal activity[61,62], most likely mediated by increased GABA transmission in the DMN[63]. Importantly, increasing cognitive load was associated with more DMN deactivation and higher GABA concentrations[63]. This suggests that THC administration may affect DMN activity through disruption of eCB-mediated GABA neurotransmission.

Our results may have implications for understanding impairment in executive function related to psychiatric and neurological disorders. Executive function impairments are associated with various psychiatric and neurological disorders, including schizophrenia, attention-deficit hyperactivity disorder (ADHD), obsessive-compulsive disorder, depression, Alzheimer's disease and Tourette's syndrome[64-67]. The presence of a similar symptom in such a wide range of disorders suggests the possibility of a common underlying mechanism. Evidence is accumulating for a role of DMN in executive function impairment in patients with schizophrenia, as several studies have shown reduced deactivation in DMN during various tasks in patients with schizophrenia[68-71]. In addition, reduced connectivity of PCC with other DMN nodes was demonstrated in schizophrenia patients during task execution[72]. Reduced deactivation in DMN during task performance has also been identified in other patient groups such as youth with ADHD[73,74] and patients with Alzheimer's disease[75,76]. Our results suggest the possibility that

abnormal involvement of the eCB system may be a factor in the abnormal DMN activity associated with aforementioned disorders. As such, the eCB system could be involved in cognitive deficits in these disorders.

The current study demonstrated, as expected, an extensive set of regions that were positively activated by the task, both after placebo and after THC administration. Previous imaging studies using executive function paradigms have shown activation of a similar network of brain areas, predominantly consisting of (right) frontal and parietal regions[32,33,77,78]. Activation of this network, also referred to as the Central Executive System (CES)[79,80], has been associated with several functions necessary for successful executive function, such as selection of sensory stimuli and the subsequent linking of stimuli to appropriate motor responses[81,82], detecting of visual stimuli[83-86], preparation of a specific response[87], and linking of relevant stimuli to responses, as it is modulated when people change their motor plan according to a stimulus[88]. In the present study, THC did not affect activity in the CES, in spite of clear effects on performance. Previous studies have reported reduced activity in the CES in psychiatric disorders such as ADHD[89-91] and schizophrenia[92-97], an effect that is likely related to impaired task performance[95,98]. One explanation for this apparent discrepancy could be that performance deficits as shown in our study after THC administration are moderate compared to those of psychiatric patients. For example, decreased CES activity in schizophrenia patients in the study of Salgado-Pineda et al. (2004) was associated with a 33% reduction in the mean percentage of correctly identified targets[93]. This view is further supported by studies in which CES activity of schizophrenia patients was not reduced during adequate performance of moderately difficult central executive tasks[99-103].

To our knowledge, there are no previous imaging studies addressing the role of the eCB system in executive function. However, one related PET study with a dichotic listening task showed that smoking of cannabis caused decreased blood flow in visual and auditory cortices[104]. These changes appeared to be task-independent, and thus were interpreted to reflect direct effects of cannabis on the brain. The study did not show effects of cannabis on task-related deactivation, which may be related to the absence of any task performance effects after smoking of cannabis.

An increasing number of imaging studies use a pharmacological challenge to study effects on cognition. For instance, the dopamine transporter inhibitor modafinil has been shown to increase DMN deactivation during a simple visuomotor task. The modafinil effect in the ventromedial prefrontal cortex was significantly correlated with reaction time[105]. Treatment with methylphenidate has been shown to normalize DMN activity in off-methylphenidate patients with ADHD who showed attenuated DMN activity during low incentive conditions[106]. These studies provide converging evidence for an important role of DMN in cognitive performance. Several limitations have to be taken into account in interpreting the results of this study. First, inclusion of incidental cannabis users, as opposed to non-users, may have affected interpretation of the results as previous cannabis use may have affected the eCB system. The choice for incidental cannabis users was based on ethical grounds[34]. Second, non-specific THC-induced changes on cerebral blood flow may have confounded our results[107].

However, the fact that the reduced deactivation in DMN after THC is correlated with performance indicates that the effect is specifically related to task execution. Finally, although the study was designed to be double-blind, THC induced behavioral effects that were identified by most subjects, possibly causing expectancy effects across sessions. The influence of expectancy was minimized by using a randomized crossover design, thus balancing the effects of expectancy across study days. Still, it cannot be excluded that expectancy effects may have affected our results to some extent.

In conclusion, this study shows specific reduction of DMN activity related to THC administration, which was associated with reduced task performance. These results suggest an important role for the eCB system in both DMN modulation and executive function. The association of the eCB system with DMN modulation may be relevant for psychiatric disorders associated with executive function deficits, such as schizophrenia and ADHD, as well as for neurological disorders such as Alzheimer's disease.

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References

1. Piomelli D. (2003) The molecular logic of endocannabinoid signalling. Nat Rev Neurosci. 4 (11), 873-884.
2. Heifets B.D., Castillo P.E. (2009) Endocannabinoid signaling and long-term synaptic plasticity. Annu Rev Physiol. 71, 283-306.
3. Kano M., Ohno-Shosaku T., Hashimotodani Y., Uchigashima M., Watanabe M. (2009) Endocannabinoid-mediated control of synaptic transmission. Physiol Rev. 89 (1), 309-380.
4. Cohen C., Perrault G., Voltz C., Steinberg R., Soubrie P. (2002) SR141716, a central cannabinoid (CB(1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. Behav Pharmacol. 13 (5-6), 451-463.
5. Maldonado R., Valverde O., Berrendero F. (2006) Involvement of the endocannabinoid system in drug addiction. Trends Neurosci. 29 (4), 225-232.
6. De Vries T.J., Schoffelmeer A.N. (2005) Cannabinoid CB1 receptors control conditioned drug seeking. Trends Pharmacol Sci. 26 (8), 420-426.
7. De Vries T.J., Shaham Y., Homberg J.R., Crombag H., Schuurman K., Dieben J., Vanderschuren L.J., Schoffelmeer A.N. (2001) A cannabinoid mechanism in relapse to cocaine seeking. Nat Med. 7 (10), 1151-1154.
8. Bhattacharyya S., Morrison P.D., Fusar-Poli P., Martin-Santos R., Borgwardt S., Winton-Brown T., Nosarti C., O' Carroll C.M., Seal M., Allen P., Mehta M.A., Stone J.M., Tunstall N., Giampietro V., Kapur S., Murray R.M., Zuardi A.W., Crippa J.A., Atakan Z., McGuire P.K. (2010) Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. Neuropsychopharmacology. 35 (3), 764-774.
9. Leweke F.M., Koethe D., Gerth C.W., Nolden B.M., Schreiber D., Gross S., Schultze-Lutter F., Juelicher A., Hellmich M., Klosterkotter J. (2005) The Endocannabinoid Modulator Cannabidiol as an Antipsychotic. Results from the First Controlled Randomized Clinical Trial in Acute Schizophrenia. Biological Psychiatry. 57, 135S.
10. Cahill K., Ussher M.H. (2011) Cannabinoid type 1 receptor antagonists for smoking cessation. Cochrane Database Syst Rev. (3), CD005353.
11. Carlin A.S., Bakker C.B., Halpern L., Post R.D. (1972) Social facilitation of marijuana intoxication: impact of social set and pharmacological activity. J Abnorm Psychol. 80 (2), 132-140.
12. Klonoff H. (1974) Marijuana and driving in real-life situations. Science. 186 (4161), 317-324.
13. Casswell S. (1975) Cannabis intoxication: effects of monetary incentive on performance, a controlled investigation of behavioural tolerance in moderate users of cannabis. Percept Mot Skills. 41 (2), 423-434.
14. Hooker W.D., Jones R.T. (1987) Increased susceptibility to memory intrusions and the Stroop interference effect during acute marijuana intoxication. Psychopharmacology (Berl). 91 (1), 20-24.
15. Ramaekers J.G., Kauert G., van Ruitenbeek P., Theunissen E.L., Schneider E., Moeller M.R. (2006) High-potency marijuana impairs executive function and inhibitory motor control. Neuropsychopharmacology. 31 (10), 2296-2303.
16. Ramaekers J.G., Robbe H.W., O'Hanlon J.F. (2000) Marijuana, alcohol and actual driving performance. Hum Psychopharmacol. 15 (7), 551-558.
17. Hart C.L., Haney M., Vosburg S.K., Comer S.D., Foltin R.W. (2005) Reinforcing effects of oral Delta9-THC in male marijuana smokers in a laboratory choice procedure. Psychopharmacology (Berl). 181 (2), 237-243.
18. Morrison P.D., Zois V., McKeown D.A., Lee T.D., Holt D.W., Powell J.F., Kapur S., Murray R.M. (2009) The acute effects of synthetic intravenous Delta9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. Psychol Med., 1-10.
19. Zuurman L., Ippel A.E., Moin E., van Gerven J.M. (2009) Biomarkers for the effects of cannabis and THC in healthy volunteers. Br J Clin Pharmacol. 67 (1), 5-21.
20. Shulman G.L., Fiez J.A., Corbetta M., Buckner R.L., Miezin F.M., Raichle M.E., Petersen S.E. (1997) Common blood flow changes across visual tasks: II. Decreases in cerebral cortex. J Cogn Neurosci. 9, 648-663.
21. Raichle M.E., MacLeod A.M., Snyder A.Z., Powers W.J., Gusnard D.A., Shulman G.L. (2001) A default mode of brain function. Proc Natl Acad Sci U S A. 98 (2), 676-682.
22. Mazoyer B., Zago L., Mellet E., Bricogne S., Etard O., Houde O., Crivello F., Joliot M., Petit L., Tzourio-Mazoyer N. (2001) Cortical networks for working memory and executive functions sustain the conscious resting state in man. Brain Res Bull. 54 (3), 287-298.
23. Otten L.J., Rugg M.D. (2001) When more means less: neural activity related to unsuccessful memory encoding. Curr Biol. 11 (19), 1528-1530.

24. Drummond S.P., Bischoff-Grethe A., Dinges D.F., Ayalon L., Mednick S.C., Meloy M.J. (2005) The neural basis of the psychomotor vigilance task. Sleep. 28 (9), 1059-1068.
25. Polli F.E., Barton J.J., Cain M.S., Thakkar K.N., Rauch S.L., Manoach D.S. (2005) Rostral and dorsal anterior cingulate cortex make dissociable contributions during antisaccade error commission. Proc Natl Acad Sci U S A. 102 (43), 15700-15705.
26. Weissman D.H., Roberts K.C., Visscher K.M., Woldorff M.G. (2006) The neural bases of momentary lapses in attention. Nat Neurosci. 9 (7), 971-978.
27. Shulman G.L., Astafiev S.V., McAvoy M.P., d'Avossa G., Corbetta M. (2007) Right TPJ deactivation during visual search: functional significance and support for a filter hypothesis. Cereb Cortex. 17 (11), 2625-2633.
28. Eichele T., Debener S., Calhoun V.D., Specht K., Engel A.K., Hugdahl K., von Cramon D.Y., Ullsperger M. (2008) Prediction of human errors by maladaptive changes in event-related brain networks. Proc Natl Acad Sci U S A. 105 (16), 6173-6178.
29. Cornblatt B.A., Risch N.J., Faris G., Friedman D., Erlenmeyer-Kimling L. (1988) The Continuous Performance Test, identical pairs version (CPT-IP): I. New findings about sustained attention in normal families. Psychiatry Res. 26 (2), 223-238.
30. Baddeley A. (Editor) (1986) Working memory. Claredon Press, Oxford.
31. Norman D.A., Shallice T. (1986) Attention and action: willed and automatic control of behavior. In: Davidson R.J., Schwarts G.E., Shapiro D. (Eds), Consciousness and self-regulation. Plenum, New York, p. 1-18.
32. Adler C.M., Sax K.W., Holland S.K., Schmithorst V., Rosenberg L., Strakowski S.M. (2001) Changes in neuronal activation with increasing attention demand in healthy volunteers: an fMRI study. Synapse. 42 (4), 266-272.
33. Strakowski S.M., Adler C.M., Holland S.K., Mills N., DelBello M.P. (2004) A preliminary FMRI study of sustained attention in euthymic, unmedicated bipolar disorder. Neuropsychopharmacology. 29 (9), 1734-1740.
34. van Hell H.H., Bossong M.G., Jager G., Kahn R.S., Ramsey N.F. (2011) Pharmacological Imaging of the Cannabinoid System (PhICS): towards understanding the role of the brain endocannabinoid system in human cognition. Int J Methods Psychiatr Res. 20, 10-27.
35. Sheehan D.V., Lecrubier Y., Sheehan K.H., Amorim P., Janavs J., Weiller E., Hergueta T., Baker R., Dunbar G.C. (1998) The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry. 59 Suppl 20, 22-33.
36. Schmand B., Bakker D., Saan R., Louman J. (1991) [The Dutch Reading Test for Adults: a measure of premorbid intelligence level]. Tijdschr Gerontol Geriatr. 22 (1), 15-19.
37. van Hell H.H., Jager G., Bossong M.G., Brouwer A., Jansma J.M., Zuurman L., van Gerven J.M.A., Kahn R.S., Ramsey N.F. (2011) Involvement of the endocannabinoid system in reward processing in the human brain. Psychopharmacology (Berl). 2011 Aug 6 [Epub ahead of print].
38. Bossong M.G., van Berckel B.N., Boellaard R., Zuurman L., Schuit R.C., Windhorst A.D., van Gerven J.M., Ramsey N.F., Lammertsma A.A., Kahn R.S. (2009) Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. Neuropsychopharmacology. 34 (3), 759-766.
39. Zuurman L., Roy C., Schoemaker R.C., Hazekamp A., den Hartigh J., Bender J.C., Verpoorte R., Pinquier J.L., Cohen A.F., van Gerven J.M. (2008) Effect of intrapulmonary tetrahydrocannabinol administration in humans. J Psychopharmacol. 22 (7), 707-716.
40. Strougo A., Zuurman L., Roy C., Pinquier J.L., van Gerven J.M., Cohen A.F., Schoemaker R.C. (2008) Modelling of the concentration--effect relationship of THC on central nervous system parameters and heart rate -- insight into its mechanisms of action and a tool for clinical research and development of cannabinoids. J Psychopharmacol. 22 (7), 717-726.
41. Bond A., Lader M. (1974) The use of analogue scales in rating subjective feelings. Br J Med Psychol. 47, 211-218.
42. Bowdle T.A., Radant A.D., Cowley D.S., Kharasch E.D., Strassman R.J., Roy-Byrne P.P. (1998) Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. Anesthesiology. 88 (1), 82-88.
43. van Buuren M., Gladwin T.E., Zandbelt B.B., van den Heuvel M., Ramsey N.F., Kahn R.S., Vink M. (2009) Cardiorespiratory effects on default-mode network activity as measured with fMRI. Hum Brain Mapp. 30 (9), 3031-3042.
44. Rutschmann J., Cornblatt B., Erlenmeyer-Kimling L. (1977) Sustained attention in children at risk for schizophrenia. Report on a continuous performance test. Arch Gen Psychiatry. 34 (5), 571-575.

45. Neggers S.F., Hermans E.J., Ramsey N.F. (2008) Enhanced sensitivity with fast three-dimensional blood-oxygen-level-dependent functional MRI: comparison of SENSE-PRESTO and 2D-EPI at 3 T. NMR Biomed. 21 (7), 663-676.
46. Collins D.L., Neelin P., Peters T.M., Evans A.C. (1994) Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. J Comput Assist Tomogr. 18 (2), 192-205.
47. Buckner R.L., Andrews-Hanna J.R., Schacter D.L. (2008) The brain's default network: anatomy, function, and relevance to disease. Ann N Y Acad Sci. 1124, 1-38.
48. Fransson P., Marrelec G. (2008) The precuneus/posterior cingulate cortex plays a pivotal role in the default mode network: Evidence from a partial correlation network analysis. Neuroimage. 42 (3), 1178-1184.
49. Song M., Liu Y., Zhou Y., Wang K., Yu C., Jiang T. (2009) Default network and intelligence difference. Conf Proc IEEE Eng Med Biol Soc. 2009, 2212-2215.
50. Leech R., Kamourieh S., Beckmann C.F., Sharp D.J. (2011) Fractionating the default mode network: distinct contributions of the ventral and dorsal posterior cingulate cortex to cognitive control. J Neurosci. 31 (9), 3217-3224.
51. Sonuga-Barke E.J., Castellanos F.X. (2007) Spontaneous attentional fluctuations in impaired states and pathological conditions: a neurobiological hypothesis. Neurosci Biobehav Rev. 31 (7), 977-986.
52. McKiernan K.A., Kaufman J.N., Kucera-Thompson J., Binder J.R. (2003) A parametric manipulation of factors affecting task-induced deactivation in functional neuroimaging. J Cogn Neurosci. 15 (3), 394-408.
53. McKiernan K.A., D'Angelo B.R., Kaufman J.N., Binder J.R. (2006) Interrupting the "stream of consciousness": an fMRI investigation. Neuroimage. 29 (4), 1185-1191.
54. Jansma J.M., Ramsey N.F., de Zwart J.A., van Gelderen P., Duyn J.H. (2007) fMRI study of effort and information processing in a working memory task. Hum Brain Mapp. 28 (5), 431-440.
55. Gusnard D.A., Raichle M.E. (2001) Searching for a baseline: functional imaging and the resting human brain. Nat Rev Neurosci. 2 (10), 685-694.
56. Gusnard D.A., Akbudak E., Shulman G.L., Raichle M.E. (2001) Medial prefrontal cortex and self-referential mental activity: relation to a default mode of brain function. Proc Natl Acad Sci U S A. 98 (7), 4259-4264.
57. Johnson S.C., Baxter L.C., Wilder L.S., Pipe J.G., Heiserman J.E., Prigatano G.P. (2002) Neural correlates of self-reflection. Brain. 125 (Pt 8), 1808-1814.
58. Mason M.F., Norton M.I., van Horn J.D., Wegner D.M., Grafton S.T., Macrae C.N. (2007) Wandering minds: the default network and stimulus-independent thought. Science. 315 (5810), 393-395.
59. Mato S., Chevaleyre V., Robbe D., Pazos A., Castillo P.E., Manzoni O.J. (2004) A single in-vivo exposure to Delta 9THC blocks endocannabinoid-mediated synaptic plasticity. Nat Neurosci. 7 (6), 585-586.
60. Hoffman A.F., Oz M., Yang R., Lichtman A.H., Lupica C.R. (2007) Opposing actions of chronic Delta9-tetrahydrocannabinol and cannabinoid antagonists on hippocampal long-term potentiation. Learn Mem. 14 (1-2), 63-74.
61. Shmuel A., Augath M., Oeltermann A., Logothetis N.K. (2006) Negative functional MRI response correlates with decreases in neuronal activity in monkey visual area V1. Nat Neurosci. 9 (4), 569-577.
62. Devor A., Tian P., Nishimura N., Teng I.C., Hillman E.M., Narayanan S.N., Ulbert I., Boas D.A., Kleinfeld D., Dale A.M. (2007) Suppressed neuronal activity and concurrent arteriolar vasoconstriction may explain negative blood oxygenation level-dependent signal. J Neurosci. 27 (16), 4452-4459.
63. Northoff G., Walter M., Schulte R.F., Beck J., Dydak U., Henning A., Boeker H., Grimm S., Boesiger P. (2007) GABA concentrations in the human anterior cingulate cortex predict negative BOLD responses in fMRI. Nat Neurosci. 10 (12), 1515-1517.
64. Nuechterlein K.H., Dawson M.E. (1984) Information processing and attentional functioning in the developmental course of schizophrenic disorders. Schizophr Bull. 10 (2), 160-203.
65. Cornblatt B.A., Keilp J.G. (1994) Impaired attention, genetics, and the pathophysiology of schizophrenia. Schizophr Bull. 20 (1), 31-46.
66. Perry R.J., Hodges J.R. (1999) Attention and executive deficits in Alzheimer's disease. A critical review. Brain. 122 (Pt 3), 383-404.
67. Willcutt E.G., Doyle A.E., Nigg J.T., Faraone S.V., Pennington B.F. (2005) Validity of the executive function theory of attention-deficit/hyperactivity disorder: a meta-analytic review. Biol Psychiatry. 57 (11), 1336-1346.
68. Pomarol-Clotet E., Salvador R., Sarro S., Gomar J., Vila F., Martinez A., Guerrero A., Ortiz-Gil J., Sans-Sansa B., Capdevila A., Cebamanos J.M., McKenna P.J. (2008) Failure to deactivate in the prefrontal cortex in schizophrenia: dysfunction of the default mode network? Psychol Med. 38 (8), 1185-1193.

69. Whitfield-Gabrieli S., Thermenos H.W., Milanovic S., Tsuang M.T., Faraone S.V., McCarley R.W., Shenton M.E., Green A.I., Nieto-Castanon A., LaViolette P., Wojcik J., Gabrieli J.D., Seidman L.J. (2009) Hyperactivity and hyperconnectivity of the default network in schizophrenia and in first-degree relatives of persons with schizophrenia. Proc Natl Acad Sci U S A. 106 (4), 1279-1284.
70. Hasenkamp W., James G.A., Boshoven W., Duncan E. (2011) Altered engagement of attention and default networks during target detection in schizophrenia. Schizophr Res. 125 (2-3), 169-173.
71. Schneider F.C., Royer A., Grosselin A., Pellet J., Barral F.G., Laurent B., Brouillet D., Lang F. (2011) Modulation of the default mode network is task-dependant in chronic schizophrenia patients. Schizophr Res. 125 (2-3), 110-117.
72. Sambataro F., Blasi G., Fazio L., Caforio G., Taurisano P., Romano R., Di G.A., Gelao B., Lo B.L., Papazacharias A., Popolizio T., Nardini M., Bertolino A. (2010) Treatment with olanzapine is associated with modulation of the default mode network in patients with Schizophrenia. Neuropsychopharmacology. 35 (4), 904-912.
73. Fassbender C., Zhang H., Buzy W.M., Cortes C.R., Mizuiri D., Beckett L., Schweitzer J.B. (2009) A lack of default network suppression is linked to increased distractibility in ADHD. Brain Res. 1273, 114-128.
74. Peterson B.S., Potenza M.N., Wang Z., Zhu H., Martin A., Marsh R., Plessen K.J., Yu S. (2009) An FMRI study of the effects of psychostimulants on default-mode processing during Stroop task performance in youths with ADHD. Am J Psychiatry. 166 (11), 1286-1294.
75. Lustig C., Snyder A.Z., Bhakta M., O'Brien K.C., McAvoy M., Raichle M.E., Morris J.C., Buckner R.L. (2003) Functional deactivations: change with age and dementia of the Alzheimer type. Proc Natl Acad Sci U S A. 100 (24), 14504-14509.
76. Pihlajamaki M., Sperling R.A. (2009) Functional MRI assessment of task-induced deactivation of the default mode network in Alzheimer's disease and at-risk older individuals. Behav Neurol. 21 (1), 77-91.
77. Fink G.R., Halligan P.W., Marshall J.C., Frith C.D., Frackowiak R.S., Dolan R.J. (1997) Neural mechanisms involved in the processing of global and local aspects of hierarchically organized visual stimuli. Brain. 120 (Pt 10), 1779-1791.
78. Hager F., Volz H.P., Gaser C., Mentzel H.J., Kaiser W.A., Sauer H. (1998) Challenging the anterior attentional system with a continuous performance task: a functional magnetic resonance imaging approach. Eur Arch Psychiatry Clin Neurosci. 248 (4), 161-170.
79. D'Esposito M., Detre J.A., Alsop D.C., Shin R.K., Atlas S., Grossman M. (1995) The neural basis of the central executive system of working memory. Nature. 378 (6554), 279-281.
80. Baddeley A. (2003) Working memory: looking back and looking forward. Nat Rev Neurosci. 4 (10), 829-839.
81. Corbetta M., Patel G., Shulman G.L. (2008) The reorienting system of the human brain: from environment to theory of mind. Neuron. 58 (3), 306-324.
82. Corbetta M., Shulman G.L. (2002) Control of goal-directed and stimulus-driven attention in the brain. Nat Rev Neurosci. 3 (3), 201-215.
83. Corbetta M., Miezin F.M., Shulman G.L., Petersen S.E. (1993) A PET study of visuospatial attention. J Neurosci. 13 (3), 1202-1226.
84. Gitelman D.R., Nobre A.C., Parrish T.B., LaBar K.S., Kim Y.H., Meyer J.R., Mesulam M. (1999) A large-scale distributed network for covert spatial attention: further anatomical delineation based on stringent behavioural and cognitive controls. Brain. 122 (Pt 6), 1093-1106.
85. Nobre A.C., Sebestyen G.N., Gitelman D.R., Mesulam M.M., Frackowiak R.S., Frith C.D. (1997) Functional localization of the system for visuospatial attention using positron emission tomography. Brain. 120 (Pt 3), 515-533.
86. Coull J.T., Frackowiak R.S., Frith C.D. (1998) Monitoring for target objects: activation of right frontal and parietal cortices with increasing time on task. Neuropsychologia. 36 (12), 1325-1334.
87. Connolly J.D., Goodale M.A., Menon R.S., Munoz D.P. (2002) Human fMRI evidence for the neural correlates of preparatory set. Nat Neurosci. 5 (12), 1345-1352.
88. Rushworth M.F., Paus T., Sipila P.K. (2001) Attention systems and the organization of the human parietal cortex. J Neurosci. 21 (14), 5262-5271.
89. Tamm L., Menon V., Reiss A.L. (2006) Parietal attentional system aberrations during target detection in adolescents with attention deficit hyperactivity disorder: event-related fMRI evidence. Am J Psychiatry. 163 (6), 1033-1043.
90. Stevens M.C., Pearlson G.D., Kiehl K.A. (2007) An FMRI auditory oddball study of combined-subtype attention deficit hyperactivity disorder. Am J Psychiatry. 164 (11), 1737-1749.

91. Rubia K., Smith A.B., Halari R., Matsukura F., Mohammad M., Taylor E., Brammer M.J. (2009) Disorder-specific dissociation of orbitofrontal dysfunction in boys with pure conduct disorder during reward and ventrolateral prefrontal dysfunction in boys with pure ADHD during sustained attention. Am J Psychiatry. 166 (1), 83-94.
92. Volz H., Gaser C., Hager F., Rzanny R., Ponisch J., Mentzel H., Kaiser W.A., Sauer H. (1999) Decreased frontal activation in schizophrenics during stimulation with the continuous performance test--a functional magnetic resonance imaging study. Eur Psychiatry. 14 (1), 17-24.
93. Salgado-Pineda P., Junque C., Vendrell P., Baeza I., Bargallo N., Falcon C., Bernardo M. (2004) Decreased cerebral activation during CPT performance: structural and functional deficits in schizophrenic patients. Neuroimage. 21 (3), 840-847.
94. Kiehl K.A., Stevens M.C., Celone K., Kurtz M., Krystal J.H. (2005) Abnormal hemodynamics in schizophrenia during an auditory oddball task. Biol Psychiatry. 57 (9), 1029-1040.
95. Gur R.E., Turetsky B.I., Loughead J., Snyder W., Kohler C., Elliott M., Pratiwadi R., Ragland J.D., Bilker W.B., Siegel S.J., Kanes S.J., Arnold S.E., Gur R.C. (2007) Visual attention circuitry in schizophrenia investigated with oddball event-related functional magnetic resonance imaging. Am J Psychiatry. 164 (3), 442-449.
96. Weiss E.M., Siedentopf C., Golaszewski S., Mottaghy F.M., Hofer A., Kremser C., Felber S., Fleischhacker W.W. (2007) Brain activation patterns during a selective attention test--a functional MRI study in healthy volunteers and unmedicated patients during an acute episode of schizophrenia. Psychiatry Res. 154 (1), 31-40.
97. Hong L.E., Schroeder M., Ross T.J., Buchholz B., Salmeron B.J., Wonodi I., Thaker G.K., Stein E.A. (2011) Nicotine enhances but does not normalize visual sustained attention and the associated brain network in schizophrenia. Schizophr Bull. 37 (2), 416-425.
98. Lawrence N.S., Ross T.J., Hoffmann R., Garavan H., Stein E.A. (2003) Multiple neuronal networks mediate sustained attention. J Cogn Neurosci. 15 (7), 1028-1038.
99. Callicott J.H., Bertolino A., Mattay V.S., Langheim F.J., Duyn J., Coppola R., Goldberg T.E., Weinberger D.R. (2000) Physiological dysfunction of the dorsolateral prefrontal cortex in schizophrenia revisited. Cereb Cortex. 10 (11), 1078-1092.
100. Manoach D.S., Gollub R.L., Benson E.S., Searl M.M., Goff D.C., Halpern E., Saper C.B., Rauch S.L. (2000) Schizophrenic subjects show aberrant fMRI activation of dorsolateral prefrontal cortex and basal ganglia during working memory performance. Biol Psychiatry. 48 (2), 99-109.
101. Ramsey N.F., Koning H.A., Welles P., Cahn W., van der Linden J.A., Kahn R.S. (2002) Excessive recruitment of neural systems subserving logical reasoning in schizophrenia. Brain. 125 (Pt 8), 1793-1807.
102. Jansma J.M., Ramsey N.F., van der Wee N.J., Kahn R.S. (2004) Working memory capacity in schizophrenia: a parametric fMRI study. Schizophr Res. 68 (2-3), 159-171.
103. Potkin S.G., Turner J.A., Brown G.G., McCarthy G., Greve D.N., Glover G.H., Manoach D.S., Belger A., Diaz M., Wible C.G., Ford J.M., Mathalon D.H., Gollub R., Lauriello J., O'Leary D., van Erp T.G., Toga A.W., Preda A., Lim K.O. (2009) Working memory and DLPFC inefficiency in schizophrenia: the FBIRN study. Schizophr Bull. 35 (1), 19-31.
104. O'Leary D.S., Block R.I., Koeppel J.A., Schultz S.K., Magnotta V.A., Ponto L.B., Watkins G.L., Hichwa R.D. (2007) Effects of smoking marijuana on focal attention and brain blood flow. Hum Psychopharmacol. 22 (3), 135-148.
105. Minzenberg M.J., Yoon J.H., Carter C.S. (2011) Modafinil modulation of the default mode network. Psychopharmacology (Berl). 215 (1), 23-31.
106. Liddle E.B., Hollis C., Batty M.J., Groom M.J., Totman J.J., Liotti M., Scerif G., Liddle P.F. (2011) Task-related default mode network modulation and inhibitory control in ADHD: effects of motivation and methylphenidate. J Child Psychol Psychiatry. 52 (7), 761-771.
107. Iannetti G.D., Wise R.G. (2007) BOLD functional MRI in disease and pharmacological studies: room for improvement? Magn Reson Imaging. 25 (6), 978-988.

7

Role of the endocannabinoid system in human brain function related to emotional processing

In preparation

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Abstract

Introduction

Processing of emotions is affected in psychiatric disorders such as major depression. Behavioral evidence suggests that modulation of the endocannabinoid (eCB) system with administration of cannabinoid substances changes emotional responses. This may be mediated by functional alterations in a network of brain regions associated with emotional processing. In the present study we examined eCB involvement in human brain function related to processing of positive and negative emotions.

Methods

A pharmacological magnetic resonance imaging (MRI) study was conducted with a placebo-controlled, cross-over design, investigating effects of the eCB agonist Δ9-tetrahydrocannabinol (THC) on brain function related to emotional processing in 11 healthy volunteers. Performance and brain activity during emotional processing were assessed using an emotional faces task consisting of stimuli with either negative or positive emotional content.

Results

After THC administration, performance accuracy was decreased for matching stimuli with negative but not positive emotional content. Processing of emotions activated a network of brain regions mainly consisting of amygdala, orbital frontal gyrus, hippocampus, superior parietal gyrus, prefrontal cortex, and regions in the occipital cortex. THC had distinct effects on brain activity in this network, in that it reduced activity for negative emotions, while activity related to positive emotions was unaffected.

Conclusion

THC administration reduced both task performance and brain activity for negative emotions, without effects on processing of positive emotions. This indicates that THC administration changed emotional bias in healthy subjects, mainly reflected in decreased reactivity towards negative stimuli. These results provide compelling support for involvement of the eCB system in emotional processing.

Introduction

Abnormalities in emotional processing are among the most important characteristics of psychiatric disorders such as major depression[1], bipolar disorder[2] and schizophrenia[3-5], with significant consequences for social functioning and subjective well-being of patients.

Evidence is accumulating for involvement of the endocannabinoid (eCB) system in emotional processing[6-8]. The eCB system, consisting of cannabinoid receptors and accompanying endogenous ligands, is a retrograde messenger system that regulates both excitatory and

inhibitory neurotransmission[9-11]. For example, the most common reason for the recreational use of cannabis is that it produces a euphoriant effect. This 'high' includes a feeling of intoxication, with decreased anxiety, alertness, depression and tension[12-14]. In addition, animal studies show that disruption of eCB-mediated synaptic regulation through genetic deletion or pharmacological blockade of cannabinoid receptors produces anxiety- or depressive-like states[15-20]. Administration of low doses of cannabinoid agonists or drugs that enhance levels of endogenous cannabinoids reduces anxiety-like behavior[17,21-27]. Converging models posit that distinct neural systems are involved in different aspects of emotional processing[2,32-34]. For instance, occipital and temporal lobes, including the fusiform gyrus, have been implicated in the perceptual processing of emotional stimuli such as facial expressions, while the amygdala and orbital frontal cortex are involved in emotion recognition and generation of (automatic) emotional reactions in response to a stimulus. The anterior cingulate and prefrontal cortex are associated with the (voluntary) regulation of emotional reactions[2,32-34]. Cannabinoid receptors are highly expressed in many of these key regions for emotional processing[35-37]. So far, the role of the eCB system in human emotional processing has been investigated in two functional neuroimaging studies with administration of THC[38,39]. Both studies have focused on brain activity related to processing of emotional faces, but came to different conclusions. Only examining the effects of THC in the amygdala region, Phan et al. (2008) found reduced amygdala reactivity for processing of both fearful and happy faces[38]. Fusar-Poli et al. (2009) noticed THC-induced decreases in activity in frontal and parietal brain regions when subjects viewed fearful faces, but showed no significant effects of THC administration on the amygdala response[39]. The purpose of the present study was to further elucidate involvement of the eCB system in human emotional processing. This was examined in a functional MRI (fMRI) study with healthy volunteers, measuring the effects of THC administration on brain function related to stimuli with either negative or positive emotional content. Brain activity was assessed in the network of regions involved in emotional processing, providing the opportunity to restrict analysis to brain areas of interest, and to calculate and present effect sizes. Based on previous neuroimaging studies with emotional stimuli, we expected that processing of facial expressions would activate a network of brain regions consisting of amygdala, orbital frontal gyrus, hippocampus, prefrontal cortex, and regions in the parietal and occipital cortex. Based on the studies of Phan et al. (2008)[38] and Fusar-Poli et al. (2009)[39], it was hypothesized that inhalation of THC would induce a decrease in brain activity underlying processing of negative emotions. THC administration may enhance brain activity for positive emotions, as part of the reported reductions in anxiety- or depressive-like responses in both humans and animals[12-14,17,21-27].

Materials and Methods

This study is part of the Pharmacological Imaging of the Cannabinoid System (PhICS) project, a comprehensive research project on the role of the eCB system in the regulation of cognitive brain function in healthy volunteers and patients with psychiatric disorders. Methods of the

entire study are reported in detail in a methodological paper[40]. This study is registered in both the EudraCT database (2007-004247-30) and the Dutch Trial Register (NTR1787).

Subjects

Fourteen healthy male right-handed subjects were recruited through flyers, posters and internet advertisements. All subjects used cannabis on an incidental basis, defined as having used cannabis at least four times but at most once a week in the year before inclusion in the study. All subjects were in good physical health as assessed by medical history and physical examination, and were screened for axis I psychiatric disorders using the Dutch version of the Mini International Neuropsychiatric Interview for DSM-IV clinical disorders[41]. Subjects were asked to refrain from cannabis for at least two weeks before the first study day until study completion. Illicit drug use other than cannabis was not within six months prior to inclusion. Urine screening for cannabis, cocaine, amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), morphine, methadone, tricyclic antidepressants (TCA), barbiturates and benzodiazepines was performed at screening and on both study days. Subjects with a positive drug test were excluded from the study. Subjects were also asked to abstain from alcohol for 48 hours before each study day. Smoking was not allowed from the moment of arrival until the end of a study day. Alcohol and nicotine use was assessed by self-report. Subjects were asked to fast for at least four hours before arrival. On the beginning of each test day, they were served a standard meal. For further details on inclusion and exclusion criteria we refer to Van Hell et al. (2011)[40]. All volunteers gave written informed consent before entry into the study and were compensated for their participation. The study was approved by the Independent Ethics Committee of the University Medical Center Utrecht, the Nether-

Table 7.1 Subject characteristics (n = 11).

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

Use of cannabis, tobacco, alcohol and coffee was given for the year before inclusion in the study. Subjects refrained from cannabis for at least two weeks before the first study day until study completion and from alcohol for 48 hours before each study day. Caffeine intake and smoking were not allowed from the moment of arrival until the end of a study day. Illicit drug use other than cannabis was at least more than six months before the first study day. All subjects showed negative urine screening at both study days.

lands, in accordance to the Declaration of Helsinki 2008. Results are reported on eleven out of the fourteen included subjects. One subject did not complete the study procedure due to strong feelings of anxiety during one of the scanning sessions. Two other subjects were excluded because of an absence of detectable THC plasma levels and movement-related errors during scanning, respectively. Subject characteristics are summarized in Table 7.1. All subjects showed negative urine screening at both study days.

Design and procedure

In a double-blind, randomized, placebo-controlled, crossover pharmacological MRI study, subjects underwent two scanning sessions after administration of placebo and of THC. Study days were two weeks apart to allow for complete clearance of drugs. Two weeks before the first study day, participants were familiarized with the scanner environment using a mock scanner. On the beginning of each study day, a catheter was placed percutaneously in the left arm for the withdrawal of blood samples. Subsequently, subjects performed three cognitive paradigms, during which functional MRI scans were obtained. Paradigm sequence was randomized between subjects, but remained unchanged within subjects across sessions. Here we report on the results of the emotional faces task. Results of other assessments are reported elsewhere[40,42,43]. On study days, subjects received subsequent doses of THC or placebo with 30 minutes intervals. Drugs were administered before each fMRI task using a Volcano® vaporizer (Storz–Bickel GmbH, Tuttlingen, Germany) according to a method described earlier[44-46]. The first THC dose was 6 mg, followed by three doses of 1 mg each to maintain stable levels of CNS effects. Doses were based on pharmacokinetic/pharmacodynamic (PK/PD) modeling of CNS effects induced by THC[47]. See Van Hell et al. (2011) for detailed study procedures[40].

Drug levels and behavioral measurements

Venous blood samples were collected to determine plasma concentrations of THC and its two most important metabolites, 11-OH-THC and 11-nor-9-carboxy-THC. Blood samples were processed according to Zuurman et al. (2008)[45]. Subjective effects were determined with two sets of visual analogue scales[48,49]. Both rating scales were performed consecutively at baseline and before and after performance of the emotional faces task. Visual analogue scales were analyzed as described previously[45,46]. Heart rate and respiration were monitored continuously during scanning, as described by van Buuren et al. (2009)[50]. Mean heart rate was computed by dividing the total number of heart beat trigger signals by the duration of the task. Data were corrected for baseline values and analyzed with paired t-tests.

Task paradigm

Emotional processing was assessed with an emotional faces task consisting of two conditions involving processing of facial expressions of emotion (fearful ('FF') and happy faces ('HF'), respectively) and a sensorimotor control condition ('CT') (Figure 7.1)[38,51]. During FF and HF, subjects viewed a trio of unfamiliar faces and selected one of the two bottom faces that

expressed the same facial emotion as the target face on top. The target and congruent probe face displayed either a fearful or happy expression, while the incongruent probe face displayed a neutral expression. The identity of all three faces was always different. FF and HF were interspersed with a sensorimotor control condition in which subjects viewed a trio of simple geometric shapes (circles, vertical and horizontal ellipses) and selected one of the two bottom shapes identical to the target shape on top. Subjects responded by pressing one of two buttons with their right thumb.

The emotional faces task consisted of 17 experimental blocks of 24 s: four each for FF and HF, interleaved with 9 control blocks, for a total task length of 7 min. The order of blocks was counterbalanced. All blocks were preceded by a 4 s instruction (in Dutch): "Match Faces" or "Match Shapes", followed by four different trios of images presented sequentially for 5 s each, randomized for all conditions. Trios of faces were balanced for gender. All facial images were derived from a standard set of pictures of facial affect[52].

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

Image Acquisition

Image acquisition was performed on a Philips Achieva 3.0 Tesla scanner (Philips Medical Systems, Best, the Netherlands). Functional images were obtained using a 3D PRESTO-SENSE pulse sequence[53] with the following parameters: TR 22.5 ms; TE 33.2 ms; flip angle = 10°; FOV 224×256×160; matrix 56× 64×40; voxel size 4 mm isotropic; scan time 0.6075 s; 40

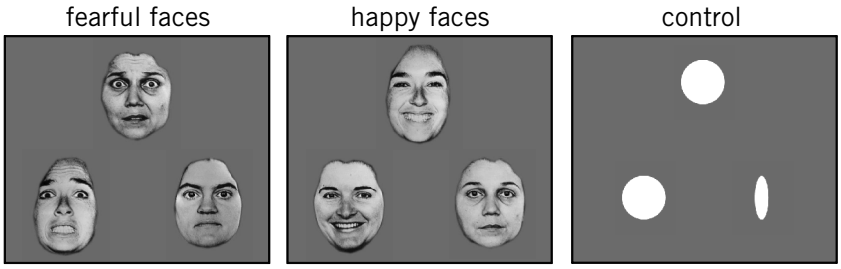

Figure 7.1 Schematic outline of the task used to assess effects of THC on processing of facial expressions of emotion. The task consists of two experimental conditions (fearful faces (left) and happy faces (middle), respectively), during which subjects viewed a trio of unfamiliar faces and selected one of the two bottom faces that expressed the same facial emotion as the target face on top. Experimental conditions were interspersed with a sensorimotor control condition (right), during which subjects viewed a trio of simple geometric shapes (circles, vertical and horizontal ellipses) and selected one of the two bottom shapes identical to the target shape on top. Each block consisted of four different trios of images presented sequentially for 5 s each. See for detailed information the Materials and methods section.

slices (sagittal orientation). A total of 700 functional images were acquired. Immediately after the emotional faces task, one volume with a flip angle of 27° was acquired for image co-registration. A T1-weighted structural image was obtained for anatomical registration with the following parameters: TR 9.5 ms; TE 4.7 ms; flip angle = 8°; FOV 220.8x240x159.6; matrix 368×400×266; voxel size 0.6 mm isotropic, 266 slices (sagittal orientation).

Functional MRI analysis

After reconstruction, scan volumes were preprocessed and analyzed using SPM5 (Wellcome Trust Centre for Neuroimaging, London, UK). Preprocessing of data included realignment of functional images to the first image and co-registration with the anatomical scan using the volume with a flip angle of 27°. Subsequently, the anatomical scan was normalized to standard MNI space[54], and the transformation parameters were used to normalize the functional scans. After normalization, functional scans were smoothed (FWHM = 8 mm).

For a first level single subject analysis, regression coefficients for each voxel (b-values) were obtained from a general linear model regression analysis using a factor matrix that contained factors modeling FF and HF (four blocks each) as well as the instructions that were presented before each block. To correct for drifts in the signal, cosine-based regressors were added to the model, corresponding to a cut-off frequency of 0.006 Hz. Group activity maps were created for the contrasts FF-CT and HF-CT, for both the placebo and THC condition. Voxels were selected that reached an activity threshold ($t = 4.1$, $p < 0.001$, uncorrected for multiple comparisons) in at least one of these four maps, thereby including voxels without bias for effects of interest. Clusters with a minimum of ten neighboring voxels were defined as regions of interest (ROIs). Mean regression coefficients (b-values) for each ROI were extracted using Marsbar[55], for both contrasts and for both the placebo and THC session, thus resulting in four b-values per ROI per subject.

Effects of THC on activity in the network of ROIs were determined using repeated measures ANOVA (Huynh-Feldt correction) with drug (two levels), condition (two levels: FF-CT and HF-CT) and ROI (twelve levels) as within-subjects factors. Post hoc paired t-test analyses were performed to further investigate effects of THC on individual task conditions.

Effects of THC on activity in individual ROIs were assessed using repeated measures ANOVA with drug and condition as within-subjects factors. All hypothesis tests were performed using SPSS 17.

Results

Drug levels and behavioral measurements

Plasma concentrations of THC and its main metabolites were 82.3 ± 45.9 ng/ml (THC), 4.4 ± 5.5 ng/ml (11-nor-9-carboxy-THC) and 2.6 ± 1.3 ng/ml (11-OH-THC), 5 min after inhalation of 6 mg THC. Analysis of subjective effects before and after performance of the emotional faces task revealed a significant THC-induced increase in VAS score of 'feeling high' ($F(1,10)$

= 11.06, $p = 0.008$), 'internal perception' (reflecting inner feelings that do not correspond with reality) ($F(1,10) = 6.21$, $p = 0.032$), and 'external perception' (reflecting misperception of external stimuli or changes in the awareness of the environment) ($F(1,10) = 11.97$, $p = 0.006$) compared to placebo. In addition, THC significantly reduced 'alertness' ($F(1,10) = 8.19$, $p = 0.017$), 'contentedness' ($F(1,10) = 6.96$, $p = 0.025$), and 'calmness' ($F(1,10) = 7.72$, $p = 0.020$). THC caused a trend towards a significant increase in VAS score of 'anxiety' ($F(1,10) = 3.60$, $p = 0.087$). Subjective effects are summarized in Table 7.2. Heart rate increased significantly after THC compared with placebo (16.0 ± 13.9 and -1.5 ± 9.9 bpm increase compared to baseline, respectively; $p < 0.001$). For a more detailed description of drug levels and behavioral measurements following THC see Van Hell et al. (2011)[40].

Task performance

The effect of THC administration on performance accuracy was significantly different between the two experimental conditions (drug * condition, $F(1,10) = 7.11$; $p = 0.024$), with a THC-induced decrease in the mean percentage of correctly identified emotions for FF only (from 99.4 ± 1.9% to 93.8 ± 7.4%, $p = 0.024$). Reaction times differed significantly between conditions (condition, $F(1,10) = 17.53$; $p = 0.002$), with the longest response time for FF, but showed no effects of THC administration (drug, $F(1,10) = 2.59$; $p = 0.139$) (see Figure 7.2).

Selection of regions of interest

Processing of facial expressions of emotion (pooled FF-CT and HF-CT group activity maps) yielded a network of twelve brain regions, comprising vermis, bilateral prefrontal cortex, hippocampus and occipital cortex, and right amygdala / parahippocampal gyrus, inferior orbital frontal gyrus, supplementary motor area, superior parietal gyrus and middle frontal gyrus (Table 7.3 and Figure 7.3).

Table 7.2 Subjective effects of Δ9-tetrahydrocannabinol (THC) (n = 11).

Assessment	Drug effect (F(1,10))	Mean placebo score (± SD)	Mean THC score (± SD)
VAS Feeling High	11.06, $p = 0.008$ **	1.14 ± 4.79	34.77 ± 32.76
VAS Internal Perception	6.21, $p = 0.032$ **	-0.32 ± 1.06	5.14 ± 6.86
VAS External Perception	11.97, $p = 0.006$ **	0.68 ± 2.25	10.23 ± 7.93
VAS Alertness	8.19, $p = 0.017$ **	-5.63 ± 3.80	-18.86 ± 14.30
VAS Contentedness	6.96, $p = 0.025$ **	-2.36 ± 6.19	-9.73 ± 10.38
VAS Calmness	7.72, $p = 0.020$ **	5.11 ± 10.90	-11.25 ± 20.23
VAS Anxiety	3.60, $p = 0.087$ *	-1.59 ± 3.92	7.50 ± 13.69

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

Brain activity

Brain activity in the network of ROIs showed a significant interaction effect between drug and condition ($F(1,10)= 6.66$; $p = 0.027$), indicating that THC administration had a different effect on the processing of FF and HF. There was no significant effect of drug ($F(1,10) = 0.14$, $p = 0.718$), and no difference in the effect of THC between ROIs (drug * condition * ROI interaction,

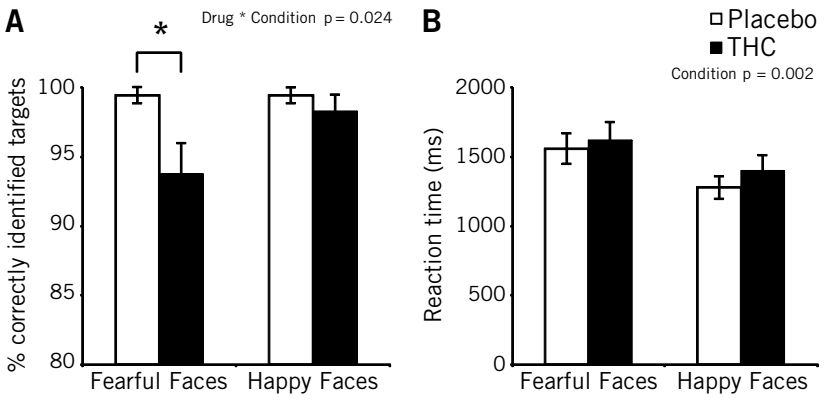

Figure 7.2 Task performance. **A**, Performance accuracy as mean percentage of correctly identified targets after placebo and THC administration. **B**, Reaction times of correct responses after placebo and THC administration (n = 11; mean ± SEM). * Significant difference between placebo and THC ($p < 0.05$). ms, milliseconds.

Table 7.3 Effects of Δ9-tetrahydrocannabinol (THC) on brain activity involved in matching of facial expressions of emotion (n = 11; mean ± SEM).

ROI number	Activated brain region	Cluster size	Drug effect (F(1,10))	Drug * condition effect (F(1,10))
	Network	**2433**	**0.14, $p = 0.718$**	**6.66, $p = 0.027$ ***
1	Vermis	13	0.40, $p = 0.544$	**13.70, $p = 0.004$ ***
2	Occipital cortex L	923	0.56, $p = 0.472$	**10.12, $p = 0.010$ ***
3	Occipital cortex R	1164	1.14, $p = 0.312$	**5.79, $p = 0.037$ ***
4	Amygdala / Parahippocampal gyrus R	14	0.84, $p = 0.382$	0.14, $p = 0.716$
5	Inferior orbital frontal gyrus R	46	2.93, $p = 0.118$	3.18, $p = 0.105$
6	Hippocampus L	27	0.16, $p = 0.698$	**8.06, $p = 0.018$ ***
7	Hippocampus R	34	3.46, $p = 0.092$	2.87, $p = 0.121$
8	Prefrontal cortex L	24	0.55, $p = 0.477$	4.64, $p = 0.057$
9	Prefrontal cortex R	131	0.01, $p = 0.932$	**5.26, $p = 0.045$ ***
10	Superior parietal gyrus R	22	2.47, $p = 0.147$	**7.94, $p = 0.018$ ***
11	Middle frontal gyrus R	15	2.56, $p = 0.141$	2.69, $p = 0.132$
12	Supplementary motor area R	20	4.09, $p = 0.071$	**6.52, p = 0.029 ***

Group activity maps for placebo and THC were thresholded at $t = 4.1$, $p < 0.001$, uncorrected for multiple comparisons, cluster size ≥ 10 voxels. ROI numbers correspond to those shown in Figure 7.3. Statistical analysis was performed with repeated measures ANOVA (Huynh-Feldt corrected). * Significant effect ($p < 0.05$). ROI, region of interest; L, left; R, right.

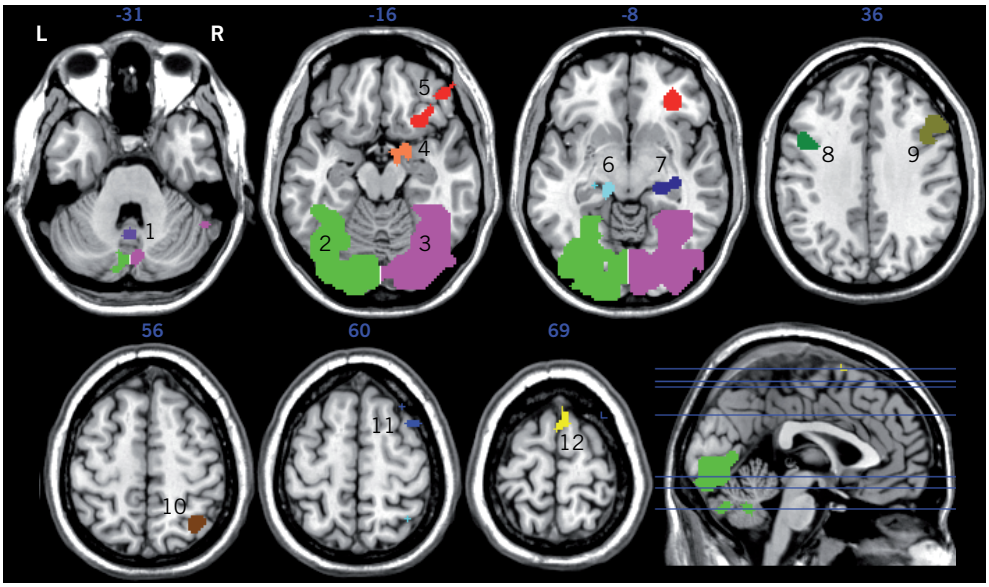

Figure 7.3 Regions of interest (ROIs) used to assess effects of THC administration on brain activity. ROIs are defined in group activity maps that were pooled for the placebo and THC condition of both the contrasts FF-CT and HF-CT ($n = 11$; $t > 4.1$, $p < 0.001$, uncorrected for multiple comparisons, clusters ≥ 10 voxels). Numbers above slices indicate MNI z coordinates. ROI numbers correspond to those shown in Table 7.3. L, left; R, right.

$F(6,64) = 1.69$, $p = 0.133$). Post hoc analysis revealed a significant THC-induced decrease in FF activity ($p = 0.017$), while HF activity was unaffected ($p = 0.285$). This suggests that the significant interaction effect between drug and condition is mainly reflected in decreased processing of FF (Table 7.3 and Figure 7.4).

Analysis of individual ROIs showed a significant interaction effect between drug and condition in the vermis ($F(1,10) = 13.70$; $p = 0.004$), left occipital cortex ($F(1,10) = 10.12$; $p = 0.010$), right occipital cortex ($F(1,10) = 5.79$; $p = 0.037$), left hippocampus ($F(1,10) = 8.06$; $p = 0.018$), right prefrontal cortex ($F(1,10) = 5.26$; $p = 0.045$), right superior parietal gyrus ($F(1,10) = 7.94$; $p = 0.018$), and right supplementary motor area ($F(1,10) = 6.52$; $p = 0.029$) (not corrected for multiple comparisons). No significant effects of drug were demonstrated in individual ROIs. ROI results are summarized in Table 7.3 and Figure 7.5.

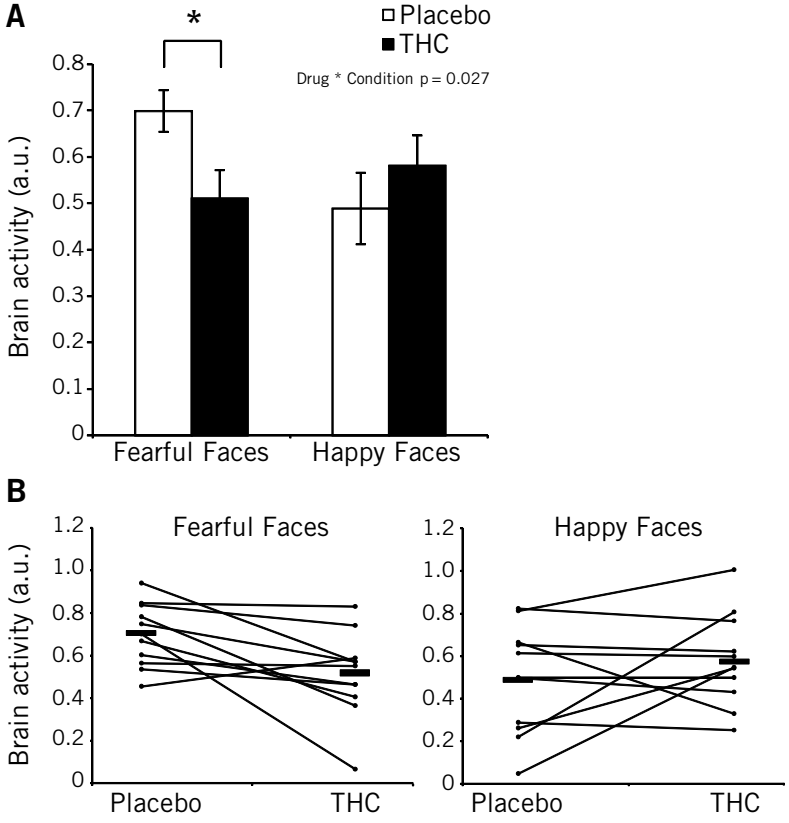

Figure 7.4 Activity in the network of ROIs during matching of negative and positive emotions (n = 11). **A**, Mean network activity after placebo and THC administration (mean ± SEM). **B**, Network activity after placebo and THC administration presented for individual subjects. * Significant difference between placebo and THC ($p < 0.05$). a.u., arbitrary units.

Discussion

An fMRI study with a THC challenge was performed in healthy volunteers to study involvement of the eCB system in emotional processing. After THC administration, performance accuracy was decreased for matching stimuli with negative but not positive emotional content. THC had distinct effects on brain activity related to positive versus negative emotions in a network of brain regions mainly consisting of amygdala, orbital frontal gyrus, hippocampus, superior parietal gyrus, prefrontal cortex, and regions in the occipital cortex, in that THC administration reduced activity for negative emotions, whereas activity related to positive emotions was unaffected.

THC administration reduced both task performance and brain activity for negative emotions, without effects on processing of positive emotions. These results indicate that THC administration changed emotional bias in healthy subjects, mainly reflected in decreased reactivity towards negative stimuli. This view is supported by the commonly reported acute

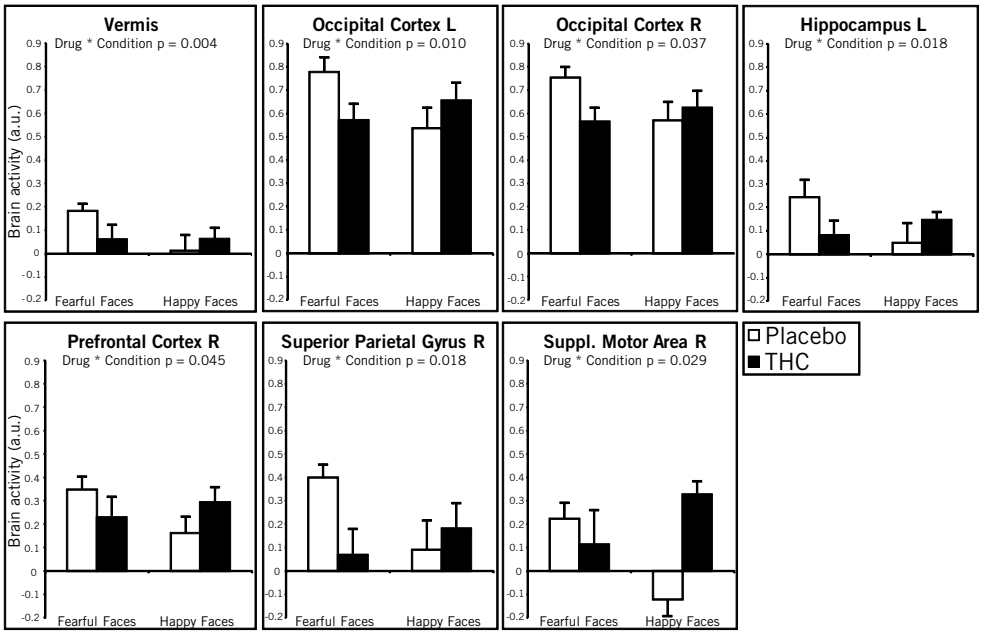

Figure 7.5 Brain activity during matching of negative and positive emotions in ROIs that demonstrated a significant interaction effect between drug and condition (n = 11; mean ± SEM). a.u., arbitrary units; L, left; R, right.

behavioral effects of cannabis. The main feature of the recreational use of cannabis is that it produces a euphoriant effect. This 'high' includes a feeling of intoxication, with decreased anxiety, alertness, depression and tension[12]. Importantly, these effects of cannabis are the most common reasons for using the drug[13,14]. This is also consistent with animal studies that demonstrated reduced anxiety-like behavior after administration of either low doses of exogenous cannabinoid agonists including THC or drugs that enhance levels of endogenous cannabinoids[17,21-27].

A potential mechanism underlying the effects of THC administration on brain activity may be found in the regulatory role of the eCB system in neurotransmitter release. The eCB system is a retrograde messenger system that regulates both excitatory and inhibitory neurotransmission according to an 'on-demand' principle: endocannabinoids are released when and where they are needed[9-11]. This eCB-mediated regulation of synaptic transmission is a widespread phenomenon in the brain, and is thought to play an important role in higher brain functions, including emotional processing[10,56]. In line with our result that administration of the eCB agonist THC reduces reactivity towards negative stimuli, it has been demonstrated that elimination of eCB-mediated synaptic transmission through genetic deletion or pharmacological blockade of cannabinoid receptors produces anxiety- or depressive-like states in animals[6,15-20,57]. This is also consistent with human clinical trials testing the eCB antagonist rimonabant and inverse agonist taranabant for treatment of obesity, which showed depressed mood and anxiety as the most common adverse events[58-61].

THC plasma concentrations and reported subjective effects in our study indicate that a moderate high dose of THC was used[62,63]. In line with behavioral animal studies that used high doses of THC[17,22,24-26], subjective ratings in the present study are more in the direction of anxiety-like effects, with a trend towards a significant THC-induced increase in the VAS score of 'anxiety', and significantly reduced measures of 'contentedness' and 'calmness'. These behavioral findings seem to contradict brain activity effects of THC. One possibility may be that a temporary change in self-reported subjective states is not reflected in brain activity related to emotional processing. The circumstances of the experiment may have interacted with effects of THC to increase feelings of anxiety after THC, while processing of emotional stimuli was only affected by THC itself.

In addition, the impact of subjective effects of THC on brain activity related to emotional processing may differ between regions. Possibly, the subjective anxiety-like effects of THC administration may have specifically masked THC-induced effects on the response of the amygdala, as it has been shown that particularly amygdala activity may be involved in the subjective response to pharmacologically induced anxiety[64]. This view is supported by results of two previous fMRI studies that investigated eCB involvement in emotional processing with administration of THC[38,39]. Fusar-Poli et al. (2009) showed THC-induced decreases in activity in frontal and parietal brain regions when subjects viewed fearful faces, but no significant effects of THC administration on the amygdala response[39]. These brain activity findings were accompanied by strong subjective anxiety-like effects of THC[39]. On the other hand, only studying effects of THC in the amygdala region, Phan et al. (2008) found reduced amygdala reactivity for processing of both fearful and happy faces[38]. In this study, no THC-induced changes in subjective ratings were reported[38]. Therefore, anxiety-like subjective effects as reported in the current study after THC administration may have affected amygdala activity related to emotional processing.

In the present study, THC administration reduced both task performance and brain activity for negative emotions, whereas processing of positive emotions was unaffected. The absence of effects on processing of positive emotional stimuli was not in line with our hypothesis, as we expected a THC-induced increase in activity for positive emotions as part of the reported reductions in anxiety- or depressive-like responses in both humans and animals[12-14,17,21-27]. However, effects of THC on human reactivity towards positive facial emotions may be more comparable to results of animal studies that investigate eCB involvement in social behavior[65,66]. These studies showed that administration of a direct cannabinoid agonist (WIN55,212-2) reduced social interaction, whereas some (URB597, VDM11) but not all (AM404) compounds that augment levels of endogenous cannabinoids enhanced this behavior[65,66]. This complex role of the eCB system in social interaction may explain the absence of effects of THC on reactivity towards positive emotions.

Findings in the current study indicate potential for eCB-mediated medication in the treatment of symptoms of depression. Individuals diagnosed with major depressive disorder exhibit an attentional bias towards negative emotional cues and a bias away from positive emotional cues[67,68], together with increased neural activity in response to negative emotions and

diminished neural activity in response to positive emotions in brain structures related to emotional processing[69-71]. Administration of antidepressant medication reduces this bias in patients[69,70,72], and appears to induce a comparable shift in emotional bias in healthy volunteers as did THC in the current study[73,74].

Some limitations have to be taken into account in interpreting the results of this study. First, the sample size was relatively small. We therefore cannot exclude the possibility that subtle effects of THC on brain activity have been missed. Second, inclusion of incidental cannabis users, as opposed to non-users, may affect interpretation of the results, as previous cannabis use may have influenced the eCB system. The choice for incidental cannabis users was based on ethical grounds[40].

In conclusion, this study shows that THC induces a shift in emotional bias, which is mainly reflected in decreased processing of negative emotions. Our results further emphasize the eCB system as a potential novel target for treatment of symptoms of depression.

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References

1. Leppanen J.M. (2006) Emotional information processing in mood disorders: a review of behavioral and neuroimaging findings. Curr Opin Psychiatry. 19 (1), 34-39.
2. Phillips M.L., Ladouceur C.D., Drevets W.C. (2008) A neural model of voluntary and automatic emotion regulation: implications for understanding the pathophysiology and neurodevelopment of bipolar disorder. Mol Psychiatry. 13 (9), 829, 833-829, 857.
3. Pinkham A.E., Penn D.L., Perkins D.O., Lieberman J. (2003) Implications for the neural basis of social cognition for the study of schizophrenia. Am J Psychiatry. 160 (5), 815-824.
4. Phillips M.L., Drevets W.C., Rauch S.L., Lane R. (2003) Neurobiology of emotion perception II: Implications for major psychiatric disorders. Biol Psychiatry. 54 (5), 515-528.
5. Aleman A., Kahn R.S. (2005) Strange feelings: do amygdala abnormalities dysregulate the emotional brain in schizophrenia? Prog Neurobiol. 77 (5), 283-298.
6. Viveros M.P., Marco E.M., File S.E. (2005) Endocannabinoid system and stress and anxiety responses. Pharmacol Biochem Behav. 81 (2), 331-342.
7. Hill M.N., Hillard C.J., Bambico F.R., Patel S., Gorzalka B.B., Gobbi G. (2009) The therapeutic potential of the endocannabinoid system for the development of a novel class of antidepressants. Trends Pharmacol Sci. 30 (9), 484-493.
8. Gaetani S., Cuomo V., Piomelli D. (2003) Anandamide hydrolysis: a new target for anti-anxiety drugs? Trends Mol Med. 9 (11), 474-478.
9. Piomelli D. (2003) The molecular logic of endocannabinoid signalling. Nat Rev Neurosci. 4 (11), 873-884.
10. Heifets B.D., Castillo P.E. (2009) Endocannabinoid signaling and long-term synaptic plasticity. Annu Rev Physiol. 71, 283-306.
11. Kano M., Ohno-Shosaku T., Hashimotodani Y., Uchigashima M., Watanabe M. (2009) Endocannabinoid-mediated control of synaptic transmission. Physiol Rev. 89 (1), 309-380.
12. Ashton C.H. (2001) Pharmacology and effects of cannabis: a brief review. Br J Psychiatry. 178, 101-106.
13. Green B., Kavanagh D.J., Young R.M. (2004) Reasons for cannabis use in men with and without psychosis. Drug Alcohol Rev. 23 (4), 445-453.
14. Hathaway A.D. (2003) Cannabis effects and dependency concerns in long-term frequent users: a missing piece of the public health puzzle. Addiction Research and Theory. 11 (6), 441-458.
15. Navarro M., Hernandez E., Munoz R.M., Del A., I, Villanua M.A., Carrera M.R., Rodriguez de F.F. (1997) Acute administration of the CB1 cannabinoid receptor antagonist SR 141716A induces anxiety-like responses in the rat. Neuroreport. 8 (2), 491-496.
16. Martin M., Ledent C., Parmentier M., Maldonado R., Valverde O. (2002) Involvement of CB1 cannabinoid receptors in emotional behaviour. Psychopharmacology (Berl). 159 (4), 379-387.
17. Haller J., Varga B., Ledent C., Freund T.F. (2004) CB1 cannabinoid receptors mediate anxiolytic effects: convergent genetic and pharmacological evidence with CB1-specific agents. Behav Pharmacol. 15 (4), 299-304.
18. Haller J., Bakos N., Szirmay M., Ledent C., Freund T.F. (2002) The effects of genetic and pharmacological blockade of the CB1 cannabinoid receptor on anxiety. Eur J Neurosci. 16 (7), 1395-1398.
19. Uriguen L., Perez-Rial S., Ledent C., Palomo T., Manzanares J. (2004) Impaired action of anxiolytic drugs in mice deficient in cannabinoid CB1 receptors. Neuropharmacology. 46 (7), 966-973.
20. Griebel G., Stemmelin J., Scatton B. (2005) Effects of the cannabinoid CB1 receptor antagonist rimonabant in models of emotional reactivity in rodents. Biol Psychiatry. 57 (3), 261-267.
21. Berrendero F., Maldonado R. (2002) Involvement of the opioid system in the anxiolytic-like effects induced by Delta(9)-tetrahydrocannabinol. Psychopharmacology (Berl). 163 (1), 111-117.
22. Valjent E., Mitchell J.M., Besson M.J., Caboche J., Maldonado R. (2002) Behavioural and biochemical evidence for interactions between Delta 9-tetrahydrocannabinol and nicotine. Br J Pharmacol. 135 (2), 564-578.
23. Kathuria S., Gaetani S., Fegley D., Valino F., Duranti A., Tontini A., Mor M., Tarzia G., La Rana G., Calignano A., Giustino A., Tattoli M., Palmery M., Cuomo V., Piomelli D. (2003) Modulation of anxiety through blockade of anandamide hydrolysis. Nat Med. 9 (1), 76-81.
24. Hill M.N., Gorzalka B.B. (2004) Enhancement of anxiety-like responsiveness to the cannabinoid CB(1) receptor agonist HU-210 following chronic stress. Eur J Pharmacol. 499 (3), 291-295.
25. Marco E.M., Perez-Alvarez L., Borcel E., Rubio M., Guaza C., Ambrosio E., File S.E., Viveros M.P. (2004) Involvement of 5-HT1A receptors in behavioural effects of the cannabinoid receptor agonist CP 55,940 in male rats. Behav Pharmacol. 15 (1), 21-27.

26. Patel S., Hillard C.J. (2006) Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: further evidence for an anxiolytic role for endogenous cannabinoid signaling. J Pharmacol Exp Ther. 318 (1), 304-311.
27. Rubino T., Sala M., Vigano D., Braida D., Castiglioni C., Limonta V., Guidali C., Realini N., Parolaro D. (2007) Cellular mechanisms underlying the anxiolytic effect of low doses of peripheral Delta9-tetrahydrocannabinol in rats. Neuropsychopharmacology. 32 (9), 2036-2045.
28. Sergerie K., Chochol C., Armony J.L. (2008) The role of the amygdala in emotional processing: a quantitative meta-analysis of functional neuroimaging studies. Neurosci Biobehav Rev. 32 (4), 811-830.
29. Stein M.B., Goldin P.R., Sareen J., Zorrilla L.T., Brown G.G. (2002) Increased amygdala activation to angry and contemptuous faces in generalized social phobia. Arch Gen Psychiatry. 59 (11), 1027-1034.
30. Phan K.L., Fitzgerald D.A., Nathan P.J., Tancer M.E. (2006) Association between amygdala hyperactivity to harsh faces and severity of social anxiety in generalized social phobia. Biol Psychiatry. 59 (5), 424-429.
31. Etkin A., Wager T.D. (2007) Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. Am J Psychiatry. 164 (10), 1476-1488.
32. Haxby J.V., Hoffman E.A., Gobbini M.I. (2000) The distributed human neural system for face perception. Trends Cogn Sci. 4 (6), 223-233.
33. Adolphs R. (2002) Recognizing emotion from facial expressions: psychological and neurological mechanisms. Behav Cogn Neurosci Rev. 1 (1), 21-62.
34. Ochsner K.N., Gross J.J. (2005) The cognitive control of emotion. Trends Cogn Sci. 9 (5), 242-249.
35. Herkenham M., Lynn A.B., Johnson M.R., Melvin L.S., De Costa B.R., Rice K.C. (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci. 11 (2), 563-583.
36. Glass M., Dragunow M., Faull R.L. (1997) Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neuroscience. 77 (2), 299-318.
37. Katona I., Rancz E.A., Acsady L., Ledent C., Mackie K., Hajos N., Freund T.F. (2001) Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. J Neurosci. 21 (23), 9506-9518.
38. Phan K.L., Angstadt M., Golden J., Onyewuenyi I., Popovska A., De Wit H. (2008) Cannabinoid modulation of amygdala reactivity to social signals of threat in humans. J Neurosci. 28 (10), 2313-2319.
39. Fusar-Poli P., Crippa J.A., Bhattacharyya S., Borgwardt S.J., Allen P., Martin-Santos R., Seal M., Surguladze S.A., O'Carrol C., Atakan Z., Zuardi A.W., McGuire P.K. (2009) Distinct effects of delta9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. Arch Gen Psychiatry. 66 (1), 95-105.
40. van Hell H.H., Bossong M.G., Jager G., Kahn R.S., Ramsey N.F. (2011) Pharmacological Imaging of the Cannabinoid System (PhICS): towards understanding the role of the brain endocannabinoid system in human cognition. Int J Methods Psychiatr Res. 20, 10-27.
41. Sheehan D.V., Lecrubier Y., Sheehan K.H., Amorim P., Janavs J., Weiller E., Hergueta T., Baker R., Dunbar G.C. (1998) The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry. 59 Suppl 20, 22-33.
42. van Hell H.H., Jager G., Bossong M.G., Brouwer A., Jansma J.M., Zuurman L., van Gerven J.M.A., Kahn R.S., Ramsey N.F. (2011) Involvement of the endocannabinoid system in reward processing in the human brain. Psychopharmacology (Berl). 2011 Aug 6 [Epub ahead of print].
43. van Hell H.H., Bossong M.G., Jager G., Kristo G., van Osch M.J., Zelaya F., Kahn R.S., Ramsey N.F. (2011) Evidence for involvement of the insula in the psychotropic effects of THC in humans: a double-blind, randomized pharmacological MRI study. Int J Neuropsychopharmacol. 2011 Apr 14 [Epub ahead of print].
44. Hazekamp A., Ruhaak R., Zuurman L., van Gerven J., Verpoorte R. (2006) Evaluation of a vaporizing device (Volcano) for the pulmonary administration of tetrahydrocannabinol. J Pharm Sci. 95 (6), 1308-1317.
45. Zuurman L., Roy C., Schoemaker R.C., Hazekamp A., den Hartigh J., Bender J.C., Verpoorte R., Pinquier J.L., Cohen A.F., van Gerven J.M. (2008) Effect of intrapulmonary tetrahydrocannabinol administration in humans. J Psychopharmacol. 22 (7), 707-716.
46. Bossong M.G., van Berckel B.N., Boellaard R., Zuurman L., Schuit R.C., Windhorst A.D., van Gerven J.M., Ramsey N.F., Lammertsma A.A., Kahn R.S. (2009) Δ9-tetrahydrocannabinol induces dopamine release in the human striatum. Neuropsychopharmacology. 34 (3), 759-766.
47. Strougo A., Zuurman L., Roy C., Pinquier J.L., van Gerven J.M., Cohen A.F., Schoemaker R.C. (2008) Modelling of the concentration--effect relationship of THC on central nervous system parameters and

heart rate -- insight into its mechanisms of action and a tool for clinical research and development of cannabinoids. J Psychopharmacol. 22 (7), 717-726.
48. Bond A., Lader M. (1974) The use of analogue scales in rating subjective feelings. Br J Med Psychol. 47, 211-218.
49. Bowdle T.A., Radant A.D., Cowley D.S., Kharasch E.D., Strassman R.J., Roy-Byrne P.P. (1998) Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. Anesthesiology. 88 (1), 82-88.
50. van Buuren M., Gladwin T.E., Zandbelt B.B., van den Heuvel M., Ramsey N.F., Kahn R.S., Vink M. (2009) Cardiorespiratory effects on default-mode network activity as measured with fMRI. Hum Brain Mapp. 30 (9), 3031-3042.
51. Hariri A.R., Mattay V.S., Tessitore A., Kolachana B., Fera F., Goldman D., Egan M.F., Weinberger D.R. (2002) Serotonin transporter genetic variation and the response of the human amygdala. Science. 297 (5580), 400-403.
52. Ekman P., Friesen W.V. (Eds) (1976) Pictures of facial affect. Consulting Psychologists Press, Palo Alto.
53. Neggers S.F., Hermans E.J., Ramsey N.F. (2008) Enhanced sensitivity with fast three-dimensional blood-oxygen-level-dependent functional MRI: comparison of SENSE-PRESTO and 2D-EPI at 3 T. NMR Biomed. 21 (7), 663-676.
54. Collins D.L., Neelin P., Peters T.M., Evans A.C. (1994) Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. J Comput Assist Tomogr. 18 (2), 192-205.
55. Brett M., Anton J.-L., Valabregue R., Poline J.-B. (2002) Region of interest analysis using an SPM toolbox. Neuroimage. 497, Abstract 497.
56. Marsicano G., Wotjak C.T., Azad S.C., Bisogno T., Rammes G., Cascio M.G., Hermann H., Tang J., Hofmann C., Zieglgansberger W., di Marzo V, Lutz B. (2002) The endogenous cannabinoid system controls extinction of aversive memories. Nature. 418 (6897), 530-534.
57. Lafenetre P., Chaouloff F., Marsicano G. (2007) The endocannabinoid system in the processing of anxiety and fear and how CB1 receptors may modulate fear extinction. Pharmacol Res. 56 (5), 367-381.
58. Christensen R., Kristensen P.K., Bartels E.M., Bliddal H., Astrup A. (2007) Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. Lancet. 370 (9600), 1706-1713.
59. Addy C., Rothenberg P., Li S., Majumdar A., Agrawal N., Li H., Zhong L., Yuan J., Maes A., Dunbar S., Cote J., Rosko K., van Dyck K., de Lepeleire I., de Hoon J., van Hecken A., Depre M., Knops A., Gottesdiener K., Stoch A., Wagner J. (2008) Multiple-dose pharmacokinetics, pharmacodynamics, and safety of taranabant, a novel selective cannabinoid-1 receptor inverse agonist, in healthy male volunteers. J Clin Pharmacol. 48 (6), 734-744.
60. Nissen S.E., Nicholls S.J., Wolski K., Rodes-Cabau J., Cannon C.P., Deanfield J.E., Despres J.P., Kastelein J.J., Steinhubl S.R., Kapadia S., Yasin M., Ruzyllo W., Gaudin C., Job B., Hu B., Bhatt D.L., Lincoff A.M., Tuzcu E.M. (2008) Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. JAMA. 299 (13), 1547-1560.
61. Nathan P.J., O'Neill B.V., Napolitano A., Bullmore E.T. (2010) Neuropsychiatric Adverse Effects of Centrally Acting Antiobesity Drugs. CNS Neurosci Ther.
62. Huestis M.A., Henningfield J.E., Cone E.J. (1992) Blood cannabinoids. I. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana. J Anal Toxicol. 16 (5), 276-282.
63. Ramaekers J.G., Kauert G., van Ruitenbeek P., Theunissen E.L., Schneider E., Moeller M.R. (2006) High-potency marijuana impairs executive function and inhibitory motor control. Neuropsychopharmacology. 31 (10), 2296-2303.
64. Eser D., Leicht G., Lutz J., Wenninger S., Kirsch V., Schule C., Karch S., Baghai T., Pogarell O., Born C., Rupprecht R., Mulert C. (2009) Functional neuroanatomy of CCK-4-induced panic attacks in healthy volunteers. Hum Brain Mapp. 30 (2), 511-522.
65. Trezza V., Vanderschuren L.J. (2008) Bidirectional cannabinoid modulation of social behavior in adolescent rats. Psychopharmacology (Berl). 197 (2), 217-227.
66. Trezza V., Vanderschuren L.J. (2009) Divergent effects of anandamide transporter inhibitors with different target selectivity on social play behavior in adolescent rats. J Pharmacol Exp Ther. 328 (1), 343-350.
67. Gur R.C., Erwin R.J., Gur R.E., Zwil A.S., Heimberg C., Kraemer H.C. (1992) Facial emotion discrimination: II. Behavioral findings in depression. Psychiatry Res. 42 (3), 241-251.
68. Surguladze S.A., Young A.W., Senior C., Brebion G., Travis M.J., Phillips M.L. (2004) Recognition accuracy and response bias to happy and sad facial expressions in patients with major depression. Neuropsychology. 18 (2), 212-218.

69. Fu C.H., Williams S.C., Brammer M.J., Suckling J., Kim J., Cleare A.J., Walsh N.D., Mitterschiffthaler M.T., Andrew C.M., Pich E.M., Bullmore E.T. (2007) Neural responses to happy facial expressions in major depression following antidepressant treatment. Am J Psychiatry. 164 (4), 599-607.
70. Fu C.H., Williams S.C., Cleare A.J., Brammer M.J., Walsh N.D., Kim J., Andrew C.M., Pich E.M., Williams P.M., Reed L.J., Mitterschiffthaler M.T., Suckling J., Bullmore E.T. (2004) Attenuation of the neural response to sad faces in major depression by antidepressant treatment: a prospective, event-related functional magnetic resonance imaging study. Arch Gen Psychiatry. 61 (9), 877-889.
71. Surguladze S., Brammer M.J., Keedwell P., Giampietro V., Young A.W., Travis M.J., Williams S.C., Phillips M.L. (2005) A differential pattern of neural response toward sad versus happy facial expressions in major depressive disorder. Biol Psychiatry. 57 (3), 201-209.
72. Harmer C.J., O'Sullivan U., Favaron E., Massey-Chase R., Ayres R., Reinecke A., Goodwin G.M., Cowen P.J. (2009) Effect of acute antidepressant administration on negative affective bias in depressed patients. Am J Psychiatry. 166 (10), 1178-1184.
73. Harmer C.J., Mackay C.E., Reid C.B., Cowen P.J., Goodwin G.M. (2006) Antidepressant drug treatment modifies the neural processing of nonconscious threat cues. Biol Psychiatry. 59 (9), 816-820.
74. Murphy S.E., Norbury R., O'Sullivan U., Cowen P.J., Harmer C.J. (2009) Effect of a single dose of citalopram on amygdala response to emotional faces. Br J Psychiatry. 194 (6), 535-540.

8

General discussion

The focus of this thesis is the role of the endocannabinoid system in a number of cognitive domains that are typically affected in psychiatric disorders, including memory encoding and recall, working memory, executive function and emotional processing. In addition, endocannabinoid involvement in regulation of dopamine release in the striatum is addressed, as this is a robust pathophysiological feature of psychiatric disorders such as schizophrenia and addiction. This is all studied with the use of neuroimaging techniques after challenging the endocannabinoid system of healthy volunteers with administration of THC, a partial agonist of the CB_1 receptor.
This final chapter provides a general discussion of the findings presented in this thesis. In addition, some limitations and directions for future research are discussed.

General discussion

The endocannabinoid system and higher cognitive functions

Chapter 4 - 6 describe results of fMRI studies on the role of the endocannabinoid system in higher cognitive functions such as encoding and recall memory processes, working memory and executive function. Both animal studies and human neuropsychological studies with administration of cannabinoid substances have already provided ample evidence for involvement of the endocannabinoid system in cognition. However, administration of THC in combination with neuroimaging techniques provides the unique opportunity to demonstrate how the endocannabinoid system is involved in human brain function. For example, our studies show that the effects of THC on brain activity related to memory processes are stronger than previous neuropsychological studies would suggest. In the absence of changes in memory task performance, more delicate effects of THC on the brain are present, which suggest that the human brain can compensate for (small) disturbances in memory (**Chapter 4**). In addition, after THC administration, a profile of task performance, working memory load and brain activity is demonstrated that suggests working memory inefficiency (**Chapter 5**). Another example of the added value of neuroimaging studies is the association between a THC-induced decrease in performance on an executive function paradigm and reduced deactivation of the default mode network (**Chapter 6**). This provides a strong indication of how the endocannabinoid system is involved in executive function.
Domains described in **Chapter 4 - 6** show great overlap in the cognitive processes involved. For example, performance of a memory task comprises processes of executive function, memory and attention[1]. A comparison of the results presented in these chapters may provide a further explanation of the role of the endocannabinoid system in human cognition. Most likely, the endocannabinoid system is not involved in modulation of the central executive system, as suggested by findings shown in **Chapter 6**. This would imply that the effects of THC on associative and working memory (**Chapter 4** and **Chapter 5**) are related with memory processes rather than impairments in executive function. Our findings also show that impaired executive task performance after THC administration is associated with reduced deactivation

of the default mode network (**Chapter 6**). As the level of deactivation of this network may reflect the relative resources that need to be allocated to task performance[2-4], this suggests that subjects may have been impaired in directing attention to task-specific stimuli after THC administration. This is further supported by the significant THC-induced decrease in the subjective measure of alertness, which is present in all fMRI studies described in this thesis. Reduced alertness may also be associated with brain activity changes related to encoding and recall memory processes and working memory. This is suggested by reductions in activity in encoding-related brain regions, as affected regions have been implicated in attentional processes[5,6] (**Chapter 4**). In addition, decreases in alertness after THC may have led to an increase in effort to keep task performance on par, which may be associated with the hyperactivity for lower working memory loads (**Chapter 5**).

Human neuropsychological studies with continuous performance paradigms can shed more light on the role of the endocannabinoid system in memory and attentional processes. These paradigms typically require central executive function, which is the constant reorganization or updating of information, maintenance, the ability to temporarily keep information in mind[7,8], and the allocation of attention to a continuous stream of data, demonstrated by response to specific target stimuli[9]. A number of neuropsychological studies have reported no effects of smoking of cannabis or administration of THC on continuous performance paradigms[10-15]. Importantly, tasks used in these studies are easy cognitive tasks with a high memory component, mostly relying on maintenance of information. Most likely, subjects are able to compensate for effects of THC in simple cognitive tasks with increased recruitment of neural resources, as has been suggested in **Chapter 4**. Decreased performance after acute administration of cannabinoids has, however, been reported on more challenging continuous performance paradigms[16,17]. Tasks used in these studies are difficult cognitive tasks with a low memory component, typically relying on fast processing of information. In these difficult tasks, compensational neural mechanisms may not be able to overcome the effects of THC intoxication on performance accuracy. As demonstrated in **Chapter 6**, reduced performance on these tasks after THC administration may be associated with reduced deactivation of the default mode network rather than effects on activity in the central executive system. This may be due to a THC-induced decrease in attention that is directed towards task performance. Taken together, as higher cognitive demands induce stronger deactivation of the default mode system activity[2-4], this suggests that the acute effects of THC only interfere with deactivation of the default mode system when a certain level of deactivation is required, possibly resulting in impaired task performance.

As explained in **Chapter 1**, the overall role of the endocannabinoid system is to maintain normal homeostasis of neurotransmission. The endocannabinoid system is a retrograde messenger system that controls both inhibitory and excitatory neurotransmission according to an 'on-demand' principle: it is activated when and where it is needed (see Figure 1.1)[18-21]. This endocannabinoid-mediated modulation of synaptic transmission is a widespread phenomenon in the brain, and is thought to play an important role in higher cognitive functions[20,21]. It has been shown that THC administration can disrupt this function of the

endocannabinoid system[22,23]. Neurophysiological inefficient working memory function after THC administration as shown in **Chapter 5** may therefore well be a reflection of the synaptic inefficiency that is induced by disruption of endocannabinoid-mediated neurotransmission. Effects of THC administration on deactivation of the default mode network as demonstrated in **Chapter 6** also suggest a role for the endocannabinoid system in regulation of default mode activity. Recent studies indicate that negative BOLD responses are tightly coupled to reductions in neuronal activity[24,25], most likely mediated by increased GABA transmission in the default mode network[26]. Importantly, increasing cognitive load was associated with more deactivation of the default mode network and higher GABA concentrations[26]. This suggests that THC administration may affect default mode activity through disruption of endocannabinoid-mediated GABA neurotransmission.

In all studies described in this thesis, THC administration induced strong subjective and physiological effects. Based on these findings, one might have expected more robust THC-induced effects on brain function. However, results of the fMRI studies described in **Chapter 4 - 7** revealed that strong intoxicating effects are not necessarily reflected in cognitive brain function. Overall, a pattern of subtle THC effects on task performance was demonstrated associated with task-specific alterations in brain activity.

One explanation may be that intoxicating effects of THC are predominantly task-independent. As for all fMRI paradigms brain activity was compared between task-specific conditions and a closely matched control condition, reported effects of THC on brain activity reflect processes that directly or indirectly affect cognition. Task-independent effects of THC can be expected to be present in all conditions, and therefore should not show up as effects in the fMRI studies. An alternative explanation could be that the human brain possesses the capability to compensate for functional disturbances as induced by THC administration, in order to maintain normal levels of task performance. This is suggested by the results presented in **Chapter 4**.

The endocannabinoid system and striatal dopamine release

Chapter 2 showed results of a PET study in which the effects of THC administration on striatal dopamine release were investigated. THC reduced [^{11}C]raclopride binding in the ventral striatum and precommissural dorsal putamen, which is consistent with an increase in dopamine levels in these regions. These results suggest endocannabinoid control over striatal dopamine release, which indicates an important role for the endocannabinoid system in psychiatric disorders such as schizophrenia and addiction.

Recent studies have shed new light on our finding of increased dopamine levels in the human striatum after THC administration. Whereas we showed a moderate but significant THC-induced increase in dopamine transmission in subregions of the striatum, two other neuroimaging studies did not demonstrate effects of THC administration on striatal dopamine concentrations[27,28]. Although at first sight results of these three studies may appear contradictory, animal and human findings on striatal dopamine levels after THC administration seem to point into the same direction: THC induces strong behavioral and physiological effects[27-29], which are not associated with a large magnitude of dopamine release in the striatum[27-33]. This suggests

that it is unlikely that the robust behavioral effects of THC are exclusively mediated by the striatal dopamine system. Possibly, these effects of THC may be partially mediated via direct activation of the endocannabinoid system. Notwithstanding our finding of a modest degree of THC-induced striatal dopamine release, this would even further emphasize the endocannabinoid system as a novel target in the treatment of psychiatric disorders such as schizophrenia and addiction.

Implications for psychiatric disorders

Chapter 7 described effects of THC administration on processing of negative and positive emotions. THC administration reduced both task performance and brain activity for negative emotions, whereas processing of positive emotions was unaffected. This suggests that THC administration altered emotional bias in healthy subjects, mainly reflected in decreased reactivity towards negative stimuli. THC administration to healthy volunteers induces a comparable change in emotional bias as shown with antidepressant medication in both healthy subjects[34,35] and patients with major depression[36,37]. This suggests involvement of the endocannabinoid system in impaired emotional processing of these patients. Recent animal studies also strongly suggest endocannabinoid involvement in depression, as pharmacological augmentation of levels of endogenous cannabinoids has been shown to unequivocally reduce anxiety- or depressive-like behavior[38-40]. Hence, it seems that the endocannabinoid system may be a fruitful target for new treatment, but it will be a challenge to develop agonistic compounds that are devoid of dependence-creating properties.

Results described in this thesis suggest that the endocannabinoid system is involved in both the release of dopamine in the human striatum (**Chapter 2**) as well as in working memory efficiency (**Chapter 5**). Importantly, deficits in both domains have been implicated in schizophrenia[41-43]. In addition, strong similarities are shown in the profile of performance and brain activity in the working memory system between healthy subjects after THC administration and schizophrenia patients[44-48] (**Chapter 5**). Altogether, these findings contribute to the growing body of evidence that suggests the involvement of the endocannabinoid system in schizophrenia[49-59], and further emphasize this system as a potential novel target for treatment of schizophrenia symptoms.

Limitations

Several limitations have to be taken into account in interpreting the results of the studies described in this thesis. First, although this thesis focused on the role of the endocannabinoid system in domains that are relevant for psychiatric disorders, no studies with psychiatric patients have been performed. As an alternative approach, we investigated the role of the endocannabinoid system in symptoms of psychiatric disorders by assessing the effects of the partial CB_1 agonist THC on brain function in healthy volunteers. Similarities in brain function between healthy volunteers after THC administration and psychiatric patients provide indirect

evidence for endocannabinoid involvement in symptoms of these patients. Second, all subjects who participated in these studies were incidental cannabis users. Previous cannabis use may have caused *a priori* adaptations in the functioning of the endocannabinoid system, and thus may have affected interpretation of the results. The choice for incidental cannabis users was based on ethical grounds, as THC administration to cannabis naïve subjects was considered an eminent risk for developing drug dependence. Third, although the study was designed to be double-blind, THC induced behavioral effects that were identified by most subjects, possibly causing expectancy effects across sessions. The influence of expectancy was minimized by using a randomized crossover design, thus balancing the effects of expectancy across study days. Still, it cannot be excluded that expectancy effects may have affected our results to some extent. Fourth, task-independent effects of THC administration on baseline brain activity or cerebral blood flow may have affected our results[60,61]. However, there are several reasons to argue that it is highly unlikely that our findings can be explained by these effects. All studies were designed to minimize the influence of task-independent effects of THC by comparing brain activity between task-specific conditions and a closely matched control condition, as the non-specific effects of THC can be expected to be present in all conditions. In addition, changes in correlations between brain activity and task performance after THC administration indicate task-specific effects of THC. Finally, we found both significant decreases and increases in activity after THC administration, which also suggest that effects of THC are specific for particular cognitive processes.

Future perspectives

The growing indication that the endocannabinoid system plays a role in brain functions that are implicated in psychiatric disorders encourages research with psychiatric patients. As discussed, the endocannabinoid system is in particular a promising target in the treatment of symptoms of schizophrenia and depression.

With regard to schizophrenia, results presented in **Chapter 2** and **Chapter 5** are of specific interest, as the two most robust pathophysiological features of schizophrenia are increased dopamine function in the striatum[42,43] and altered functioning of the prefrontal cortex during performance of complex cognitive tasks[62,63]. We have demonstrated that these features of schizophrenia are under control of the endocannabinoid system, as administration of THC to healthy volunteers increases striatal dopamine levels (**Chapter 2**) and alters prefrontal brain activity patterns similar to what has been shown in schizophrenia patients (**Chapter 5**). This suggests that administration of a cannabinoid antagonist to schizophrenia patients may normalize these functions. At present, however, such cannabinoid compounds are not yet available for this type of studies. The cannabinoid antagonist rimonabant had been withdrawn from the market as there was reason to believe it may induce depression and suicide[64-66]. These severe adverse events are also reported in clinical trials testing the cannabinoid inverse agonist taranabant for treatment of obesity[66,67]. Currently, the most promising cannabinoid

compound for development as an antipsychotic drug is the plant-derived cannabinoid agent cannabidiol (CBD). Although the mode of action of CBD is not fully understood, there are indications that it acts as a cannabinoid CB_1/CB_2 receptor inverse agonist[68], and that it inhibits the uptake and metabolism of anandamide[69], thereby enhancing levels of endogenous cannabinoids. Studies with administration of CBD to healthy volunteers do not show any significant increases on measures of psychotic symptoms, sedation, negative schizophrenia-like symptoms or intoxication[70-72]. However, CBD has been shown to block THC-induced psychotic symptoms in healthy volunteers[73] and to inhibit L-DOPA-elicited psychosis in Parkinson's disease[74]. Interestingly, studies in cannabis users show that smoking of cannabis with a high CBD content is associated with fewer psychotic-like symptoms[75,76]. A preliminary report of a controlled clinical trial suggests that CBD decreases psychotic symptoms in schizophrenia to a similar extent as the conventional antipsychotic amisulpride, but with significantly fewer side effects[77]. Therefore, one future neuroimaging study could be to compare the effects of CBD administration between schizophrenia patients and healthy controls. This could either be a single dose of CDB or a multi day treatment. Effects could be examined on behavioral symptoms, striatal dopamine function (PET) or prefrontal brain activity patterns (fMRI). Alternatively, people at ultra high risk for developing of schizophrenia could be included to investigate the potential of CBD to reduce prodromal schizophrenia symptoms. Assessment of all parameters in the same subjects provides the opportunity to correlate findings and to enhance interpretation of results.

With regard to depression, results presented in **Chapter 7** are of particular interest. Individuals diagnosed with major depressive disorder exhibit an attentional bias towards negative emotional cues and a bias away from positive emotional cues[78,79], together with increased neural activity in response to negative emotions and diminished neural activity in response to positive emotions in brain structures related to emotional processing[36,80,81]. As demonstrated in **Chapter 7**, administration of THC induces a comparable change in emotional bias as shown with antidepressant medication in both healthy subjects[34,35] and patients with major depression[36,37]. This suggests that targeting the endocannabinoid system of depressive patients with a cannabinoid agonist may reduce their symptoms. However, apart from the possible addictive properties of cannabinoid agonists (**Chapter 2**), results from studies on the effects of THC-like compounds on anxiety are not consistent, showing both anxiolytic as well as anxiogenic effects[39,72,82]. A possible explanation could be that direct stimulation of the CB_1 receptor by systemic administration of an exogenous cannabinoid agonist does not implicitly mimic the physiological function of the endocannabinoid system. Obviously, this approach lacks the 'on-demand' requirement that characterizes endocannabinoid functioning. Drugs that can enhance levels of endogenous cannabinoids by inhibiting their reuptake or degradation (indirect agonists) do exhibit this feature, and would therefore be a promising alternative to investigate functioning of the endocannabinoid system in patients with major depression. This view is supported by the decrease in anxiety- or depressive-like behavior of animals after administration of cannabinoid inverse agonists such as AM404 and URB597[38-40]. However, these compounds are not yet available for human research. From this human clinical

perspective, the cannabinoid compound CBD may also be a potential treatment in the domain of depression, possibly through its indirect agonistic properties[69]. In healthy volunteers, administration of CBD has been shown to decrease measures of anxiety[83], both the amygdala response and skin conductance fluctuation associated with processing of negative emotions[72], and THC-induced elevations in anxiety scores[84,85]. Furthermore, CBD reduced anxiety caused by simulated public speaking in social phobia patients[86], and subjective anxiety of patients with generalized social anxiety disorder[87]. Thus, another future neuroimaging study could be to compare the effects of CBD administration between depression patients and healthy controls on brain function related to the processing of emotions.

Besides setting up new studies to investigate endocannabinoid involvement in psychiatric patients, another possibility is to further explore the role of the endocannabinoid system in healthy volunteers in the cognitive domains described in this thesis. First, performance of connectivity analyses provides the opportunity to study the effects of THC on the relationship between brain areas. Basically, the fMRI data analysis package SPM has two options: psycho-physiological interaction (PPI) and dynamic causal modeling (DCM). With PPI, a seed region is defined and connectivity between this region and all voxels in the brain is tested. Possible seed regions could be the prefrontal cortex in the context of working memory (**Chapter 5**) or the posterior cingulate cortex during performance of a central executive task (**Chapter 6**). DCM tests the connectivity in a pre-defined network, which could be networks of 'functionally defined ROIs' for the different fMRI paradigms. Second, as all subjects that participated in the PhICS project have performed three fMRI tasks, activity patterns for separate cognitive paradigms could be correlated. For example, it would be interesting to assess relationships between effects of THC on network-wide activity during recall memory, working memory and central executive functions.

Conclusion

In this thesis, we have investigated the effect of THC on brain function in several domains implicated in psychiatric disorders. Together, these results provide support for endocannabinoid involvement in the control of different cognitive functions as well as dopamine release in the striatum. Findings also provide indirect evidence for possible involvement of the endocannabinoid system in psychiatric disorders. With these results, the endocannabinoid system becomes a promising candidate for novel therapies to target symptoms in psychiatric disorders such as schizophrenia or depression.

References

1. Baddeley A. (2003) Working memory: looking back and looking forward. Nat Rev Neurosci. 4 (10), 829-839.
2. McKiernan K.A., Kaufman J.N., Kucera-Thompson J., Binder J.R. (2003) A parametric manipulation of factors affecting task-induced deactivation in functional neuroimaging. J Cogn Neurosci. 15 (3), 394-408.
3. McKiernan K.A., D'Angelo B.R., Kaufman J.N., Binder J.R. (2006) Interrupting the "stream of consciousness": an fMRI investigation. Neuroimage. 29 (4), 1185-1191.
4. Jansma J.M., Ramsey N.F., de Zwart J.A., van Gelderen P., Duyn J.H. (2007) fMRI study of effort and information processing in a working memory task. Hum Brain Mapp. 28 (5), 431-440.
5. Corbetta M., Patel G., Shulman G.L. (2008) The reorienting system of the human brain: from environment to theory of mind. Neuron. 58 (3), 306-324.
6. Corbetta M., Shulman G.L. (2002) Control of goal-directed and stimulus-driven attention in the brain. Nat Rev Neurosci. 3 (3), 201-215.
7. Baddeley A. (Editor) (1986) Working memory. Claredon Press, Oxford.
8. Norman D.A., Shallice T. (1986) Attention and action: willed and automatic control of behavior. In: Davidson R.J., Schwarts G.E., Shapiro D. (Eds), Consciousness and self-regulation. Plenum, New York, p. 1-18.
9. Cornblatt B.A., Risch N.J., Faris G., Friedman D., Erlenmeyer-Kimling L. (1988) The Continuous Performance Test, identical pairs version (CPT-IP): I. New findings about sustained attention in normal families. Psychiatry Res. 26 (2), 223-238.
10. Weil A.T., Zinberg N.E., Nelsen J.M. (1968) Clinical and psychological effects of marihuana in man. Science. 162 (859), 1234-1242.
11. Vachon L., Sulkowski A., Rich E. (1974) Marihuana effects on learning, attention and time estimation. Psychopharmacologia. 39 (1), 1-11.
12. Casswell S. (1975) Cannabis intoxication: effects of monetary incentive on performance, a controlled investigation of behavioural tolerance in moderate users of cannabis. Percept Mot Skills. 41 (2), 423-434.
13. Wilson W.H., Ellinwood E.H., Mathew R.J., Johnson K. (1994) Effects of marijuana on performance of a computerized cognitive-neuromotor test battery. Psychiatry Res. 51 (2), 115-125.
14. Curran H.V., Brignell C., Fletcher S., Middleton P., Henry J. (2002) Cognitive and subjective dose-response effects of acute oral Delta 9-tetrahydrocannabinol (THC) in infrequent cannabis users. Psychopharmacology (Berl). 164 (1), 61-70.
15. D'Souza D.C., Perry E., MacDougall L., Ammerman Y., Cooper T., Wu Y.T., Braley G., Gueorguieva R., Krystal J.H. (2004) The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. Neuropsychopharmacology. 29 (8), 1558-1572.
16. Sharma S., Moskowitz H. (1974) Effects of two levels of attention demand on vigilance performance under marihuana. Percept Mot Skills. 38 (3), 967-970.
17. Hunault C.C., Mensinga T.T., Bocker K.B., Schipper C.M., Kruidenier M., Leenders M.E., de Vries I., Meulenbelt J. (2009) Cognitive and psychomotor effects in males after smoking a combination of tobacco and cannabis containing up to 69 mg delta-9-tetrahydrocannabinol (THC). Psychopharmacology (Berl). 204 (1), 85-94.
18. Wilson R.I., Nicoll R.A. (2002) Endocannabinoid signaling in the brain. Science. 296 (5568), 678-682.
19. Piomelli D. (2003) The molecular logic of endocannabinoid signalling. Nat Rev Neurosci. 4 (11), 873-884.
20. Heifets B.D., Castillo P.E. (2009) Endocannabinoid signaling and long-term synaptic plasticity. Annu Rev Physiol. 71, 283-306.
21. Kano M., Ohno-Shosaku T., Hashimotodani Y., Uchigashima M., Watanabe M. (2009) Endocannabinoid-mediated control of synaptic transmission. Physiol Rev. 89 (1), 309-380.
22. Mato S., Chevaleyre V., Robbe D., Pazos A., Castillo P.E., Manzoni O.J. (2004) A single in-vivo exposure to Delta 9THC blocks endocannabinoid-mediated synaptic plasticity. Nat Neurosci. 7 (6), 585-586.
23. Hoffman A.F., Oz M., Yang R., Lichtman A.H., Lupica C.R. (2007) Opposing actions of chronic Delta9-tetrahydrocannabinol and cannabinoid antagonists on hippocampal long-term potentiation. Learn Mem. 14 (1-2), 63-74.
24. Shmuel A., Augath M., Oeltermann A., Logothetis N.K. (2006) Negative functional MRI response correlates with decreases in neuronal activity in monkey visual area V1. Nat Neurosci. 9 (4), 569-577.

25. Devor A., Tian P., Nishimura N., Teng I.C., Hillman E.M., Narayanan S.N., Ulbert I., Boas D.A., Kleinfeld D., Dale A.M. (2007) Suppressed neuronal activity and concurrent arteriolar vasoconstriction may explain negative blood oxygenation level-dependent signal. J Neurosci. 27 (16), 4452-4459.
26. Northoff G., Walter M., Schulte R.F., Beck J., Dydak U., Henning A., Boeker H., Grimm S., Boesiger P. (2007) GABA concentrations in the human anterior cingulate cortex predict negative BOLD responses in fMRI. Nat Neurosci. 10 (12), 1515-1517.
27. Stokes P.R., Mehta M.A., Curran H.V., Breen G., Grasby P.M. (2009) Can recreational doses of THC produce significant dopamine release in the human striatum? Neuroimage. 48 (1), 186-190.
28. Barkus E., Morrison P.D., Vuletic D., Dickson J., Ell P.J., Pilowsky L.S., Brenneisen R., Holt D.W., Powell J., Kapur S., Murray R.M. (2010) Does intravenous Δ9-tetrahydrocannabinol increase dopamine release? A SPET study. J Psychopharmacol.
29. Bossong M.G., van Berckel B.N., Boellaard R., Zuurman L., Schuit R.C., Windhorst A.D., van Gerven J.M., Ramsey N.F., Lammertsma A.A., Kahn R.S. (2009) Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. Neuropsychopharmacology. 34 (3), 759-766.
30. Ng Cheong Ton J.M., Gerhardt G.A., Friedemann M., Etgen A.M., Rose G.M., Sharpless N.S., Gardner E.L. (1988) The effects of delta 9-tetrahydrocannabinol on potassium-evoked release of dopamine in the rat caudate nucleus: an in vivo electrochemical and in vivo microdialysis study. Brain Res. 451 (1-2), 59-68.
31. Chen J.P., Paredes W., Li J., Smith D., Lowinson J., Gardner E.L. (1990) Delta 9-tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely-moving rats as measured by intracerebral microdialysis. Psychopharmacology (Berl). 102 (2), 156-162.
32. Tanda G., Pontieri F.E., Di Chiara G. (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. Science. 276 (5321), 2048-2050.
33. Malone D.T., Taylor D.A. (1999) Modulation by fluoxetine of striatal dopamine release following Delta9-tetrahydrocannabinol: a microdialysis study in conscious rats. Br J Pharmacol. 128 (1), 21-26.
34. Harmer C.J., Mackay C.E., Reid C.B., Cowen P.J., Goodwin G.M. (2006) Antidepressant drug treatment modifies the neural processing of nonconscious threat cues. Biol Psychiatry. 59 (9), 816-820.
35. Murphy S.E., Norbury R., O'Sullivan U., Cowen P.J., Harmer C.J. (2009) Effect of a single dose of citalopram on amygdala response to emotional faces. Br J Psychiatry. 194 (6), 535-540.
36. Fu C.H., Williams S.C., Cleare A.J., Brammer M.J., Walsh N.D., Kim J., Andrew C.M., Pich E.M., Williams P.M., Reed L.J., Mitterschiffthaler M.T., Suckling J., Bullmore E.T. (2004) Attenuation of the neural response to sad faces in major depression by antidepressant treatment: a prospective, event-related functional magnetic resonance imaging study. Arch Gen Psychiatry. 61 (9), 877-889.
37. Harmer C.J., O'Sullivan U., Favaron E., Massey-Chase R., Ayres R., Reinecke A., Goodwin G.M., Cowen P.J. (2009) Effect of acute antidepressant administration on negative affective bias in depressed patients. Am J Psychiatry. 166 (10), 1178-1184.
38. Kathuria S., Gaetani S., Fegley D., Valino F., Duranti A., Tontini A., Mor M., Tarzia G., La Rana G., Calignano A., Giustino A., Tattoli M., Palmery M., Cuomo V., Piomelli D. (2003) Modulation of anxiety through blockade of anandamide hydrolysis. Nat Med. 9 (1), 76-81.
39. Patel S., Hillard C.J. (2006) Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: further evidence for an anxiolytic role for endogenous cannabinoid signaling. J Pharmacol Exp Ther. 318 (1), 304-311.
40. Hill M.N., Hillard C.J., Bambico F.R., Patel S., Gorzalka B.B., Gobbi G. (2009) The therapeutic potential of the endocannabinoid system for the development of a novel class of antidepressants. Trends Pharmacol Sci. 30 (9), 484-493.
41. Green M.F. (1996) What are the functional consequences of neurocognitive deficits in schizophrenia? Am J Psychiatry. 153 (3), 321-330.
42. Laruelle M., Abi-Dargham A. (1999) Dopamine as the wind of the psychotic fire: new evidence from brain imaging studies. J Psychopharmacol. 13 (4), 358-371.
43. Howes O.D., Montgomery A.J., Asselin M.C., Murray R.M., Grasby P.M., McGuire P.K. (2007) Molecular imaging studies of the striatal dopaminergic system in psychosis and predictions for the prodromal phase of psychosis. Br J Psychiatry Suppl. 51, s13-s18.
44. Manoach D.S., Gollub R.L., Benson E.S., Searl M.M., Goff D.C., Halpern E., Saper C.B., Rauch S.L. (2000) Schizophrenic subjects show aberrant fMRI activation of dorsolateral prefrontal cortex and basal ganglia during working memory performance. Biol Psychiatry. 48 (2), 99-109.
45. Manoach D.S., Press D.Z., Thangaraj V., Searl M.M., Goff D.C., Halpern E., Saper C.B., Warach S. (1999) Schizophrenic subjects activate dorsolateral prefrontal cortex during a working memory task, as measured by fMRI. Biol Psychiatry. 45 (9), 1128-1137.

46. Callicott J.H., Bertolino A., Mattay V.S., Langheim F.J., Duyn J., Coppola R., Goldberg T.E., Weinberger D.R. (2000) Physiological dysfunction of the dorsolateral prefrontal cortex in schizophrenia revisited. Cereb Cortex. 10 (11), 1078-1092.
47. Jansma J.M., Ramsey N.F., van der Wee N.J., Kahn R.S. (2004) Working memory capacity in schizophrenia: a parametric fMRI study. Schizophr Res. 68 (2-3), 159-171.
48. Potkin S.G., Turner J.A., Brown G.G., McCarthy G., Greve D.N., Glover G.H., Manoach D.S., Belger A., Diaz M., Wible C.G., Ford J.M., Mathalon D.H., Gollub R., Lauriello J., O'Leary D., van Erp T.G., Toga A.W., Preda A., Lim K.O. (2009) Working memory and DLPFC inefficiency in schizophrenia: the FBIRN study. Schizophr Bull. 35 (1), 19-31.
49. Linszen D.H., Dingemans P.M., Lenior M.E. (1994) Cannabis abuse and the course of recent-onset schizophrenic disorders. Arch Gen Psychiatry. 51 (4), 273-279.
50. Leweke F.M., Giuffrida A., Wurster U., Emrich H.M., Piomelli D. (1999) Elevated endogenous cannabinoids in schizophrenia. Neuroreport. 10 (8), 1665-1669.
51. Arseneault L., Cannon M., Witton J., Murray R.M. (2004) Causal association between cannabis and psychosis: examination of the evidence. Br J Psychiatry. 184, 110-117.
52. Giuffrida A., Leweke F.M., Gerth C.W., Schreiber D., Koethe D., Faulhaber J., Klosterkotter J., Piomelli D. (2004) Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. Neuropsychopharmacology. 29 (11), 2108-2114.
53. D'Souza D.C., Abi-Saab W.M., Madonick S., Forselius-Bielen K., Doersch A., Braley G., Gueorguieva R., Cooper T.B., Krystal J.H. (2005) Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. Biol Psychiatry. 57 (6), 594-608.
54. Grech A., van Os J., Jones P.B., Lewis S.W., Murray R.M. (2005) Cannabis use and outcome of recent onset psychosis. Eur Psychiatry. 20 (4), 349-353.
55. Newell K.A., Deng C., Huang X.F. (2006) Increased cannabinoid receptor density in the posterior cingulate cortex in schizophrenia. Exp Brain Res. 172 (4), 556-560.
56. Moore T.H., Zammit S., Lingford-Hughes A., Barnes T.R., Jones P.B., Burke M., Lewis G. (2007) Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet. 370 (9584), 319-328.
57. Eggan S.M., Hashimoto T., Lewis D.A. (2008) Reduced cortical cannabinoid 1 receptor messenger RNA and protein expression in schizophrenia. Arch Gen Psychiatry. 65 (7), 772-784.
58. Rais M., Cahn W., Van H.N., Schnack H., Caspers E., Hulshoff P.H., Kahn R. (2008) Excessive brain volume loss over time in cannabis-using first-episode schizophrenia patients. Am J Psychiatry. 165 (4), 490-496.
59. Dalton V.S., Long L.E., Weickert C.S., Zavitsanou K. (2011) Paranoid Schizophrenia is Characterized by Increased CB(1) Receptor Binding in the Dorsolateral Prefrontal Cortex. Neuropsychopharmacology. 36 (8), 1620-1630.
60. Iannetti G.D., Wise R.G. (2007) BOLD functional MRI in disease and pharmacological studies: room for improvement? Magn Reson Imaging. 25 (6), 978-988.
61. van Hell H.H., Bossong M.G., Jager G., Kristo G., van Osch M.J., Zelaya F., Kahn R.S., Ramsey N.F. (2011) Evidence for involvement of the insula in the psychotropic effects of THC in humans: a double-blind, randomized pharmacological MRI study. Int J Neuropsychopharmacol., 1-12.
62. Callicott J.H., Mattay V.S., Verchinski B.A., Marenco S., Egan M.F., Weinberger D.R. (2003) Complexity of prefrontal cortical dysfunction in schizophrenia: more than up or down. Am J Psychiatry. 160 (12), 2209-2215.
63. Manoach D.S. (2003) Prefrontal cortex dysfunction during working memory performance in schizophrenia: reconciling discrepant findings. Schizophr Res. 60 (2-3), 285-298.
64. Christensen R., Kristensen P.K., Bartels E.M., Bliddal H., Astrup A. (2007) Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. Lancet. 370 (9600), 1706-1713.
65. Nissen S.E., Nicholls S.J., Wolski K., Rodes-Cabau J., Cannon C.P., Deanfield J.E., Despres J.P., Kastelein J.J., Steinhubl S.R., Kapadia S., Yasin M., Ruzyllo W., Gaudin C., Job B., Hu B., Bhatt D.L., Lincoff A.M., Tuzcu E.M. (2008) Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. JAMA. 299 (13), 1547-1560.
66. Nathan P.J., O'Neill B.V., Napolitano A., Bullmore E.T. (2010) Neuropsychiatric Adverse Effects of Centrally Acting Antiobesity Drugs. CNS Neurosci Ther. 2010 Jul 7. [Epub ahead of print].
67. Addy C., Rothenberg P., Li S., Majumdar A., Agrawal N., Li H., Zhong L., Yuan J., Maes A., Dunbar S., Cote J., Rosko K., van Dyck K., de Lepeleire I., de Hoon J., van Hecken A., Depre M., Knops A., Gottesdiener K., Stoch A., Wagner J. (2008) Multiple-dose pharmacokinetics, pharmacodynamics, and

safety of taranabant, a novel selective cannabinoid-1 receptor inverse agonist, in healthy male volunteers. J Clin Pharmacol. 48 (6), 734-744.
68. Pertwee R.G. (2008) The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. Br J Pharmacol. 153 (2), 199-215.
69. Bisogno T., Hanus L., De Petrocellis L., Tchilibon S., Ponde D.E., Brandi I., Moriello A.S., Davis J.B., Mechoulam R., Di Marzo V. (2001) Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br J Pharmacol. 134 (4), 845-852.
70. Borgwardt S.J., Allen P., Bhattacharyya S., Fusar-Poli P., Crippa J.A., Seal M.L., Fraccaro V., Atakan Z., Martin-Santos R., O'Carroll C., Rubia K., McGuire P.K. (2008) Neural basis of delta-9-tetrahydrocannabinol and cannabidiol: effects during response inhibition. Biol Psychiatry. 64 (11), 966-973.
71. Bhattacharyya S., Fusar-Poli P., Borgwardt S., Martin-Santos R., Nosarti C., O'Carroll C., Allen P., Seal M.L., Fletcher P.C., Crippa J.A., Giampietro V., Mechelli A., Atakan Z., McGuire P. (2009) Modulation of mediotemporal and ventrostriatal function in humans by Delta9-tetrahydrocannabinol: a neural basis for the effects of Cannabis sativa on learning and psychosis. Arch Gen Psychiatry. 66 (4), 442-451.
72. Fusar-Poli P., Crippa J.A., Bhattacharyya S., Borgwardt S.J., Allen P., Martin-Santos R., Seal M., Surguladze S.A., O'Carrol C., Atakan Z., Zuardi A.W., McGuire P.K. (2009) Distinct effects of Δ9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. Arch Gen Psychiatry. 66 (1), 95-105.
73. Bhattacharyya S., Morrison P.D., Fusar-Poli P., Martin-Santos R., Borgwardt S., Winton-Brown T., Nosarti C., O' Carroll C.M., Seal M., Allen P., Mehta M.A., Stone J.M., Tunstall N., Giampietro V., Kapur S., Murray R.M., Zuardi A.W., Crippa J.A., Atakan Z., McGuire P.K. (2010) Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. Neuropsychopharmacology. 35 (3), 764-774.
74. Zuardi A.W., Crippa J.A., Hallak J.E., Pinto J.P., Chagas M.H., Rodrigues G.G., Dursun S.M., Tumas V. (2009) Cannabidiol for the treatment of psychosis in Parkinson's disease. J Psychopharmacol. 23 (8), 979-983.
75. Morgan C.J., Curran H.V. (2008) Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. Br J Psychiatry. 192 (4), 306-307.
76. Schubart C.D., Sommer I.E., van Gastel W.A., Goetgebuer R.L., Kahn R.S., Boks M.P. (2011) Cannabis with high cannabidiol content is associated with fewer psychotic experiences. Schizophr Res. 130 (1-3), 216-221.
77. Leweke F.M., Koethe D., Gerth C.W., Nolden B.M., Schreiber D., Gross S., Schultze-Lutter F., Juelicher A., Hellmich M., Klosterkotter J. (2005) The Endocannabinoid Modulator Cannabidiol as an Antipsychotic. Results from the First Controlled Randomized Clinical Trial in Acute Schizophrenia. Biological Psychiatry. 57, 135S.
78. Gur R.C., Erwin R.J., Gur R.E., Zwil A.S., Heimberg C., Kraemer H.C. (1992) Facial emotion discrimination: II. Behavioral findings in depression. Psychiatry Res. 42 (3), 241-251.
79. Surguladze S.A., Young A.W., Senior C., Brebion G., Travis M.J., Phillips M.L. (2004) Recognition accuracy and response bias to happy and sad facial expressions in patients with major depression. Neuropsychology. 18 (2), 212-218.
80. Fu C.H., Williams S.C., Brammer M.J., Suckling J., Kim J., Cleare A.J., Walsh N.D., Mitterschiffthaler M.T., Andrew C.M., Pich E.M., Bullmore E.T. (2007) Neural responses to happy facial expressions in major depression following antidepressant treatment. Am J Psychiatry. 164 (4), 599-607.
81. Surguladze S., Brammer M.J., Keedwell P., Giampietro V., Young A.W., Travis M.J., Williams S.C., Phillips M.L. (2005) A differential pattern of neural response toward sad versus happy facial expressions in major depressive disorder. Biol Psychiatry. 57 (3), 201-209.
82. Viveros M.P., Marco E.M., File S.E. (2005) Endocannabinoid system and stress and anxiety responses. Pharmacol Biochem Behav. 81 (2), 331-342.
83. Crippa J.A., Zuardi A.W., Garrido G.E., Wichert-Ana L., Guarnieri R., Ferrari L., Azevedo-Marques P.M., Hallak J.E., McGuire P.K., Filho B.G. (2004) Effects of cannabidiol (CBD) on regional cerebral blood flow. Neuropsychopharmacology. 29 (2), 417-426.
84. Karniol I.G., Shirakawa I., Kasinski N., Pfeferman A., Carlini E.A. (1974) Cannabidiol interferes with the effects of delta 9 - tetrahydrocannabinol in man. Eur J Pharmacol. 28 (1), 172-177.
85. Zuardi A.W., Shirakawa I., Finkelfarb E., Karniol I.G. (1982) Action of cannabidiol on the anxiety and other effects produced by delta 9-THC in normal subjects. Psychopharmacology (Berl). 76 (3), 245-250.

86. Bergamaschi M.M., Queiroz R.H., Chagas M.H., de O., De Martinis B.S., Kapczinski F., Quevedo J., Roesler R., Schroder N., Nardi A.E., Martin-Santos R., Hallak J.E., Zuardi A.W., Crippa J.A. (2011) Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naive social phobia patients. Neuropsychopharmacology. 36 (6), 1219-1226.
87. Crippa J.A., Derenusson G.N., Ferrari T.B., Wichert-Ana L., Duran F.L., Martin-Santos R., Simoes M.V., Bhattacharyya S., Fusar-Poli P., Atakan Z., Santos F.A., Freitas-Ferrari M.C., McGuire P.K., Zuardi A.W., Busatto G.F., Hallak J.E. (2011) Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. J Psychopharmacol. 25 (1), 121-130.

List of abbreviations

2-AG	2-arachidonoylglycerol
AC-PC	anterior commissure-posterior commissure
ADHD	attention-deficit hyperactivity disorder
ANOVA	analysis of variance
ASL	arterial spin labeling
a.u.	arbitrary units
BA	brodmann area
BAS	behavioral activation scale
BIS	behavioral inhibition scale
BMI	body mass index
BOLD	blood oxygenated level-dependent
bpm	beats per minute
BPRS	brief psychiatric rating scale
BP_{ND}	non-displaceable binding potential
CB	cannabinoid
CBD	cannabidiol
CBF	cerebral blood flow
CES	central executive system
CNS	central nervous system
CPT	continuous performance task
CPT-IP	continuous performance task with identical pairs
CT	control task
DART	Dutch adult reading test
DCM	dynamic causal modeling
DMN	default mode network
DSM-IV	diagnostic statistic manual IV
DVR	distribution volume ratio
eCB	endocannabinoid
ECG	electrocardiogram
EEG	electroencephalography
EN	encoding
FA	flip angle
FAAH	fatty acid amide hydrolase
FF	fearful faces
fMRI	functional magnetic resonance imaging
FTND	Fägerstrom test for nicotine dependence
FOV	field of view
FWHM	full width at half maximum
GABA	ɣ-aminobutyric acid
GLM	general linear model

GMP	good manufacturing practice
HF	happy faces
HR	high resolution
Hz	hertz
IQ	intelligence quotient
L	left
Kbol	bolus to infusion ratio
MANOVA	multivariate analysis of variance
MBq	megabecquerel
MDMA	3,4-methylenedioxymethamphetamine
MNI	Montréal neurological institute
MRI	magnetic resonance imaging
mg	milligram
ms	milliseconds
n.a.	not applicable
OCD	obsessive compulsive disorder
PANSS	positive and negative syndrome scale
PCC	posterior cingulate cortex
PET	positron emission tomography
PhICS	pharmacological imaging of the cannabinoid system
phMRI	pharmacological magnetic resonance imaging
PMT	pictorial memory task
PK/PD	pharmacokinetic/pharmacodynamic
PPI	psycho-physiological interaction
PPP	public private partnership
PRESTO	principle of echo shifting with a train of observations
R	right
RE	recall
RF	radio frequency
ROI	region of interest
RT	reaction time
SC	single classification
SCL-90	symptom checklist 90
SD	standard deviation
s / sec	seconds
SEM	standard error of the mean
SENSE	sensitivity encoding
SIRP	Sternberg item-recognition paradigm
SPM	statistical parametric mapping
SSS	sensation seeking scale

TCA	tricyclic antidepressants
TE	echo time
THC	Δ9-tetrahydrocannabinol
TI	top institute
TIA	task-induced activation
TID	task-induced deactivation
TR	repetition time
VAS	visual analogue scale
VINCI	volume imaging in neurological research
WHO	world health organization
WM	working memory

Nederlandse samenvatting

Inleiding

Het roken van cannabis veroorzaakt een breed scala aan acute effecten. Het meest bekende effect en de voornaamste reden om cannabis te gebruiken is het gevoel van euforie, beter bekend als je 'high' of 'stoned' voelen. Verder kunnen mensen hun omgeving anders waarnemen of zogenaamde eet- of lachkicks ervaren. Cannabisgebruik kan ook leiden tot acute angstgevoelens, geheugenstoornissen, milde hallucinaties en verminderde impulscontrole. Dit alles wordt voornamelijk veroorzaakt door Δ9-tetrahydrocannabinol (THC), het belangrijkste psychoactieve bestanddeel van cannabis. THC oefent zijn effecten uit door aan te grijpen op cannabisreceptoren in de hersenen. Echter, de rol van deze receptoren is uiteraard niet het veroorzaken van acute effecten van een lichaamsvreemde stof als THC. Cannabisreceptoren hebben een belangrijke biologische functie in het binden van cannabisachtige stoffen die onze hersenen zelf aanmaken.

Sommige acute effecten van cannabis zijn vergelijkbaar met symptomen van patiënten met een psychiatrische aandoening. Patiënten met schizofrenie hebben bijvoorbeeld vaak geheugenstoornissen, hallucinaties, angstgevoelens en een veranderde impulscontrole. Ook symptomen van depressie, ADHD of verslaving vertonen gelijkenissen met sommige acute effecten van cannabis. Dit suggereert dat de cannabisreceptoren en de cannabisachtige stoffen in de hersenen, gezamenlijk aangeduid als het lichaamseigen cannabissysteem, een rol kunnen spelen bij de symptomen van psychiatrische stoornissen. Het onderzoeken van deze rol van het lichaamseigen cannabissysteem kan een eerste stap zijn in de ontwikkeling van medicatie die werkt via beïnvloeding van dit systeem.

Doel van het proefschrift

Het doel van dit proefschrift is om nieuwe inzichten te verkrijgen in de rol van het lichaamseigen cannabissysteem in verschillende humane hersenfuncties, waaronder leren, geheugen, informatieverwerking en het verwerken van emoties. Dit zijn cognitieve functies die vaak ook aangedaan zijn bij psychiatrische ziektebeelden zoals schizofrenie, depressie of ADHD. Verder is de rol van het lichaamseigen cannabissysteem bij de regulatie van dopamine-afgifte in het striatum onderzocht, omdat een verstoring van deze functie een belangrijk pathofysiologisch kenmerk is van zowel schizofrenie als verslaving.

De studies die in dit proefschrift beschreven worden maken gebruik van hersenscantechnieken om hersenfuncties te meten en in beeld te brengen. Dit gebeurt in combinatie met toediening van THC aan gezonde vrijwilligers. THC bindt aan cannabisreceptoren in de hersenen, en kan zo de normale functie van het lichaamseigen cannabissysteem verstoren. Een overeenkomst in hersenfunctie tussen gezonde vrijwilligers na THC toediening en patiënten met een psychiatrische aandoening is een indicatie dat het lichaamseigen cannabissysteem mogelijk betrokken is bij symptomen van deze patiënten.

Hersenscantechnieken

De hersenscantechnieken die gebruikt zijn om hersenfunctie te meten zijn Positron Emissie Tomografie (PET) en functionele Magnetische Resonantie Imaging (fMRI). PET is een techniek waarmee fysiologische processen in het lichaam zichtbaar gemaakt kunnen worden door gebruik te maken van radioactieve tracers. In **Hoofdstuk 2** is bijvoorbeeld de hoeveelheid dopamine in het striatum gemeten met behulp van PET en de radioactieve tracer [^{11}C]raclopride. Onderzocht is of THC toediening de hoeveelheid dopamine verhoogt. Omdat zowel dopamine als [^{11}C]raclopride binden aan dopamine receptoren in het striatum, zal verhoging van de hoeveelheid dopamine in het striatum leiden tot een verlaging van [^{11}C]raclopride: dopamine verdringt [^{11}C]raclopride van de dopamine receptoren. De verlaagde hoeveelheid radioactiviteit die nu gemeten wordt door de PET scanner is dus een indicatie voor een verhoogde hoeveelheid dopamine in het striatum (zie Figuur 1.2).
Functionele MRI is een techniek die met behulp van een sterke magneet hersenactiviteit zichtbaar maakt door indirect het zuurstofverbruik in de hersenen te meten. Proefpersonen maken in de scanner een cognitieve taak (bijvoorbeeld een geheugen- of een aandachtstaak), waardoor onderzocht kan worden welke hersengebieden betrokken zijn bij het uitvoeren van een specifieke taak. De rol van het lichaamseigen cannabissysteem in verschillende cognitieve hersenfuncties is onderzocht door hersenactiviteit twee keer te meten: één keer na toediening van THC en één keer na toediening van placebo, een niet-werkend middel. Het vergelijken van patronen van hersenactiviteit tussen beide sessies geeft een indicatie hoe het lichaamseigen cannabissysteem betrokken is bij het aansturen van humane cognitieve hersenfuncties. In de studies beschreven in **Hoofdstuk 3 - 7** is gebruik gemaakt van fMRI om hersenactiviteit te meten.

Samenvatting

In **Hoofdstuk 2** is met behulp van PET en de radioactieve tracer [^{11}C]raclopride onderzocht of toediening van THC leidt tot verhoging van afgifte van de neurotransmitter dopamine in het striatum. Dopamine-afgifte in dit hersengebied speelt een belangrijke rol in het ervaren van genot, zoals dat kan voorkomen bij lekker eten of seks, maar ook na gebruik van verslavende stoffen als nicotine of cocaïne. Tevens is een verhoogd functioneren van het dopaminesysteem in het striatum een belangrijk pathofysiologisch kenmerk van schizofrenie. Resultaten in dit hoofdstuk laten zien dat THC toediening leidt tot een verlaagde binding van [^{11}C]raclopride in bepaalde subgebieden van het striatum. Dit betekent dat de hoeveelheid dopamine in deze gebieden verhoogd is. Het humane lichaamseigen cannabissysteem lijkt dus betrokken te zijn bij de regulatie van dopamine-afgifte in het striatum. Dit suggereert een rol voor dit systeem in psychiatrische aandoeningen als verslaving en schizofrenie.

De studies die in dit proefschrift beschreven worden maken deel uit van het groter opgezette PhICS project ('Pharmacological Imaging of the Cannabinoid System'). In **Hoofdstuk 3** worden de doelstellingen en de onderzoeksmethoden van PhICS beschreven. PhICS behelst een omvangrijk onderzoek naar de rol van het lichaamseigen cannabissysteem in de regulatie van cognitieve hersenfuncties van gezonde vrijwilligers en patiënten met een psychiatrische aandoening. Dit is onderzocht met functionele MRI in combinatie met THC toediening, voor zes verschillende cognitieve hersenfuncties: associatief geheugen, werkgeheugen, aandacht, verwerking van emoties, verwerking van beloning en respons inhibitie. Dit hoofdstuk laat de effecten van THC toediening zien op gedragsmatige, subjectieve en fysiologische parameters zoals THC plasmaconcentraties, hartslag en het gevoel van 'high'. Verder zijn de fMRI taken gevalideerd, en wordt het PhICS project in een breder kader besproken.

In **Hoofdstuk 4** is de rol van het lichaamseigen cannabissysteem onderzocht in geheugenprocessen van gezonde vrijwilligers. Dit is gedaan met behulp van een geheugentaak bestaande uit afzonderlijke condities van geheugen: leren en terughalen van informatie (zie Figuur 4.1). Toediening van THC veroorzaakt een vermindering van hersenactiviteit tijdens het leren van informatie in de rechter insula, rechter inferieure frontale cortex en linker occipitale cortex. Dit zijn gebieden die eerder in verband zijn gebracht met processen als aandacht en het selecteren van relevante informatie. Hersenactiviteit neemt juist toe bij het terughalen van informatie, voornamelijk in de bilaterale cuneus en precuneus. Deze gebieden lijken een belangrijke rol te spelen in het inpassen van context en associaties in geheugenprocessen. THC toediening heeft geen invloed op de taakprestatie. Deze bevindingen wijzen op betrokkenheid van het lichaamseigen cannabissysteem bij het leren van informatie. De verhoogde activiteit in de precuneus suggereert dat proefpersonen na THC toediening mogelijk een andere strategie gebruiken om de taak goed uit te voeren. Ze maken bijvoorbeeld meer gebruik van typische kenmerken of associaties (de man met het rode shirt hoort bij het interieur met de staande lamp).

Hoofdstuk 5 laat de resultaten zien van een onderzoek naar de rol van het lichaamseigen cannabissysteem in werkgeheugen. Proefpersonen hebben een taak gemaakt waarbij ze lettersets van toenemende lengte moesten onthouden (1, 3, 5, 7 of 9 letters, zie Figuur 5.2). De taakprestatie neemt af naarmate de taak moeilijker wordt, maar na THC toediening verslechtert de prestatie eerder (bij het onthouden van 5 letters) dan na placebo (7 letters). Hersenactiviteit neemt lineair toe met de stijgende moeilijkheidsgraad van de taak wanneer placebo wordt toegediend. THC toediening verhoogt de activiteit voor de makkelijke taken, en vermindert de lineaire relatie tussen moeilijkheidsgraad en hersenactiviteit. Dit gebeurt zowel in het netwerk van hersengebieden dat betrokken is bij werkgeheugen als in een aantal belangrijke afzonderlijke gebieden zoals de dorsolaterale prefrontale cortex en de inferieure pariëtale gyrus. Dit profiel van moeilijkheidsgraad van de taak, taakprestatie en hersenactiviteit laat zien dat het werkgeheugen inefficiënt functioneert na THC toediening. Immers, om de makkelijke taken goed uit te voeren is er na THC meer activiteit nodig dan na placebo (net zoals een inefficiënte auto meer brandstof verbruikt). Bovendien lijkt het werkgeheugenprofiel na THC toediening sterk op het profiel dat is aangetoond in patiënten met schizofrenie. Deze

resultaten laten zien dat het lichaamseigen cannabissysteem betrokken is bij werkgeheugen. Ook vormen ze een aanwijzing voor een rol van dit systeem in de cognitieve symptomen van patiënten met schizofrenie.

In **Hoofdstuk 6** is gekeken naar de betrokkenheid van het lichaamseigen cannabissysteem bij het verwerken van informatie. Hiervoor hebben proefpersonen een taak uitgevoerd waarbij ze in een hoog tempo getallen moesten verwerken (zie Figuur 6.1). THC toediening verslechtert de prestatie op deze taak. Dit gaat gepaard met een verhoogde activiteit in hersengebieden die deel uitmaken van het zogenaamde default mode netwerk. Activiteit in dit netwerk wordt tijdens het maken van moeilijke taken normaal gesproken onderdrukt, omdat het waarschijnlijk betrokken is bij hersenfuncties die voor een goede taakprestatie 'uitgeschakeld' moeten worden (zoals dagdromen). Een verminderde onderdrukking van hersenactiviteit na THC toediening hangt inderdaad samen met een slechtere taakprestatie. Hersengebieden die specifiek betrokken zijn bij de uitvoering van de taak laten geen effecten van THC zien. Deze resultaten suggereren dat het lichaamseigen cannabissysteem betrokken is bij informatieverwerking door beïnvloeding van het default mode netwerk. Omdat een afname in informatieverwerking een belangrijk symptoom is van veel psychiatrische en neurologische aandoeningen kunnen deze resultaten wijzen op een mogelijke rol van zowel het default mode netwerk als het lichaamseigen cannabissysteem in de cognitieve symptomen van bijvoorbeeld schizofrenie, ADHD of de ziekte van Alzheimer.

Hoofdstuk 7 laat de resultaten zien van een onderzoek naar de rol van het lichaamseigen cannabissysteem in het verwerken van emoties. Dit is gedaan met behulp van een taak waarbij proefpersonen gezichten met positieve en negatieve emoties moesten verwerken (zie Figuur 7.1). THC toediening verslechtert de taakprestatie voor het vergelijken van negatieve, maar niet van positieve emoties. Verwerking van emoties activeert een netwerk van hersengebieden, waaronder de amygdala, orbitale frontale gyrus, hippocampus en prefrontale cortex. THC toediening verlaagt de activiteit in dit netwerk van hersengebieden tijdens het verwerken van negatieve emoties, maar heeft geen effect op de activiteit voor positieve emoties. Dit geeft aan dat onder invloed van THC het belang van emoties verandert, wat vooral tot uiting komt in een verlaagde respons op negatieve stimuli. Deze resultaten laten zien dat het lichaamseigen cannabissysteem een rol speelt in het verwerken van emoties. Een overeenkomst in hersenfunctie na toediening van THC aan gezonde vrijwilligers en antidepressiva aan patiënten met depressie wijst bovendien op mogelijkheden voor beïnvloeding van het lichaamseigen cannabissysteem in de behandeling van symptomen van depressie.

Conclusie

In dit proefschrift zijn onderzoeken beschreven naar de effecten van THC toediening op hersenfuncties die aangedaan zijn bij psychiatrische ziektebeelden. De resultaten laten zien dat het lichaamseigen cannabissysteem een rol speelt in het aansturen van zowel verschillende cognitieve functies als de afgifte van dopamine in het striatum. De bevindingen leveren

bovendien indirect bewijs voor mogelijke betrokkenheid van het lichaamseigen cannabissysteem bij psychiatrische aandoeningen. Dit systeem is daarom een veelbelovend aangrijpingspunt voor nieuwe medicatie ter behandeling van symptomen van psychiatrische ziektebeelden zoals schizofrenie of depressie.

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Cees, de twee dingen die ik aan jou het meest waardeer: je kritische kijk op de (wetenschappelijke) wereld en je gebrek aan nuance. Tezamen zorgt dat nogal eens voor heerlijk botte uitspraken, maar ik weet wie het zegt. Bedankt voor de relativerende gesprekken onder het genot van een al dan niet zelf gebrouwen glas bier.

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List of publications

Bossong M.G., Jansma J.M., Van Hell H.H., Jager G., Kahn R.S., Ramsey N.F.
Role of the endocannabinoid system in human brain function related to emotional processing.
In preparation.

Bossong M.G., Jansma J.M., Van Hell H.H., Jager G., Kahn R.S., Ramsey N.F.
Default mode network is implicated in the effects of Δ9-tetrahydrocannabinol (THC) on human executive function.
Submitted.

Bossong M.G., Jansma J.M., Van Hell H.H., Jager G., Oudman E., Saliasi E., Kahn R.S., Ramsey N.F.
Effects of Δ9-tetrahydrocannabinol (THC) on human working memory efficiency.
Under revision.

Bossong M.G., Jager G., Van Hell H.H., Zuurman L., Jansma J.M., Mehta M.A., Van Gerven J.M.A., Kahn R.S., Ramsey N.F. (2011)
Effects of Δ9-tetrahydrocannabinol (THC) administration on human encoding and recall memory function: a pharmacological fMRI study.
Journal of Cognitive Neuroscience 2011 Nov 8 [Epub ahead of print]

Van Hell H.H., Jager G., **Bossong M.G.**, Brouwer A., Jansma J.M., Zuurman L., Van Gerven J.M.A., Kahn R.S., Ramsey N.F. (2011)
Involvement of the endocannabinoid system in reward processing in the human brain.
Psychopharmacology 2011 Aug 6 [Epub ahead of print].

Van Hell H.H., **Bossong M.G.**, Jager G., Kristo G., Van Osch M.P.J., Zelaya F., Kahn R.S., Ramsey N.F. (2011)
Evidence for involvement of the insula in the psychotropic effects of THC in humans: a double-blind, randomized pharmacological MRI study.
International Journal of Neuropsychopharmacology 14 (10), 1377 - 1388.

Van Hell H.H.*, **Bossong M.G.***, Jager G., Kahn R.S., Ramsey N.F. (2011) Methods of the Pharmacological Imaging of the Cannabinoid System (PhICS) study: towards understanding the role of the brain endocannabinoid system in human cognition.
International Journal of Methods in Psychiatric Research 20 (1), 10 - 27.
* Authors contributed equally.

Bossong M.G., Niesink R.J. (2010)
Adolescent brain maturation, the endogenous cannabinoid system and cannabis-induced schizophrenia.
Progress in Neurobiology 92 (3), 370 - 385.

Bossong M.G., Brunt T.M., Van Dijk J.P., Rigter S.M., Hoek J., Goldschmidt H.M.J., Niesink R.J. (2010)
mCPP: an undesired addition to the ecstasy market.
Journal of Psychopharmacology 24 (9), 1395 - 1401.

Bossong M.G., Van Berckel B.N.M., Boellaard R., Zuurman L., Schuit R.C., Windhorst A.D., Van Gerven J.M.A., Ramsey N.F., Lammertsma A.A., Kahn R.S. (2009)
Δ9-Tetrahydrocannabinol induces dopamine release in the human striatum.
Neuropsychopharmacology 34 (3), 759 - 766.

Van Berckel B.N.M., **Bossong M.G.**, Van Haaren N., Boellaard R., Comans E.F.I., Kloet R., Schuitemaker A., Caspers E., Luurtsema G., Windhorst A.D., Cahn W., Lammertsma A.A., Kahn R.S. (2008)
Microglia activation in recent onset schizophrenia: a quantitative (R)-[^{11}C]PK11195 PET study.
Biological Psychiatry 64 (9), 820 - 822.

Keijsers L., **Bossong M.G.**, Waarlo A.J. (2008)
Participatory evaluation of a Dutch warning campaign for substance-users.
Health, Risk and Society 10 (3), 283 – 295.

Bossong M.G., Keijsers L., Waarlo A.J. (2007)
Delphi: een stimulerend middel om consensus te bereiken; procesevaluatie van een waarschuwingscampagne voor verontreinigde cocaïne
Kwalon 35, 25 - 31.

Bossong M.G., Van Dijk J.P., Niesink R.J. (2005)
Methylone and mCPP, two new drugs of abuse?
Addiction Biology 10 (4), 321 - 323.

Curriculum vitae

Matthijs Bossong was born on April 1st, 1980 in Vught. He graduated from secondary school (Maurick College, Vught) in 1998. In that same year he moved to Utrecht to study Biomedical Sciences at Utrecht University. This is where he became interested in the research field of neuropharmacology. He performed his first internship at the Department of Medical Pharmacology, University Medical Center Utrecht, and his second internship at the Trimbos Institute, the Netherlands Institute of Mental Health and Addiction. In August 2003 he obtained his Master's degree in Biomedical Sciences. After graduation, Matthijs started working as junior investigator at the Drug Information and Monitoring System of the Trimbos Institute, where he contributed to the monitoring of markets of recreational drugs in the Netherlands. In January 2006, he started as research assistant at the Department of Psychiatry, UMC Utrecht, coordinating two PET neuroimaging projects on the effects of cannabis on dopamine release in healthy volunteers and microglia activation in schizophrenia patients, respectively. Matthijs started his PhD in April 2007 at the Department of Neurology and Neurosurgery, UMC Utrecht, under supervision of Prof. Nick Ramsey and Prof. René Kahn. The results of his research are described in this thesis. Since September 2011, Matthijs has been working as postdoctoral research fellow in the group of Prof. Philip McGuire at the Institute of Psychiatry, King's College London, investigating the neurobiological factors underlying the onset of psychosis.