Endocannabinoid involvement in reward and impulsivity in addiction

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ISBN: 978-90-5335-470-4

Printed by: Ridderprint BV, Ridderkerk, the Netherlands
Lay out and cover deisgn: Nikki Vermeulen, Ridderprint BV, Ridderkerk, the Netherlands

The studies described in this thesis were performed at the University Medical Center in Utrecht, The Netherlands, and sponsored by TI Pharma.

Printing of this thesis was financially supported by Storz & Bickel

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Endocannabinoïde betrokkenheid bij beloning en impulsiviteit in verslaving

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan,
ingevolge het besluit van het college voor promoties in het openbaar te verdedigen
op maandag 21 november 2011 des ochtends te 10.30 uur

door

Hendrika Heiltje van Hell

geboren op 1 september 1979
te Zwolle

Promotores:	Prof.dr. N.F. Ramsey
	Prof.dr. R.S. Kahn
Co-promotores:	Dr. G. Jager
	Dr. J.M. Jansma

‘Onderzoek alles, behoud het goede.’
1 Tessalonicenzen 5: 21

‘Machtig zijn de werken van de HEER, wie ze liefheeft, onderzoekt ze.’
Psalmen 111: 2

‘Alles wat ik vond is dit: de mens is een eenvoudig schepsel. Zo is hij door God gemaakt,
maar hij heeft talloze gedachtespinsels.’
Prediker 7: 29

Contents

Chapter 1

Introduction

1.1 General introduction

The aim of this thesis is to gain novel insights into the role of the endocannabinoid system in addiction. The studies described in this thesis were performed within the framework of the Pharmacological Imaging of the Cannabinoid System (PhICS) study, an extensive research project aimed at elucidating the role of the endocannabinoid system in symptoms of psychiatric disorders in a multidisciplinary manner. This project was designed in line with the recommendations of the World Health Organization's (WHO) Priority Medicines project [1], that has identified "pharmaceutical gaps" – diseases that pose high burdens to society, but for which 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 consisting of industrial and academic research teams and conducts multidisciplinary research that addresses a large number of diseases mentioned in the WHO's Priority Medicines project. Among these diseases are several psychiatric disorders with a neurobiological basis, including depression, schizophrenia and addiction. An important neurotransmitter system that could be involved in these diseases is the endogenous cannabinoid system. The PhICS study is part of a broader multidisciplinary TI Pharma project addressing the neurophysiological role of the endocannabinoid system. In this project, the endocannabinoid system is studied on a molecular level, on a behavioural level in experimental animal studies, and in clinical studies in humans, using either epidemiological and behavioural studies, or pharmacological challenges in combination with neuropsychological tasks and brain imaging techniques. The PhiCS study has been designed to address the role of the cannabinoid system in cognitive brain function, and this is achieved by investigating neurocognitive performance and brain function in healthy controls and subjects with a psychiatric disorder. In total, six different cognitive domains are covered, namely associative memory, working memory, attention, emotion, impulsivity and reward.

This thesis focuses on the role of the endocannabinoid system in symptoms of addiction, more specifically in altered reward processing and impaired response inhibition. The results of the role of the endocannabinoid system in the other cognitive domains are described in the thesis by Matthijs Bossong, entitled: "Role of the endocannabinoid system in human brain functions relevant for psychiatric disorders".

1.2 Addiction

Addiction is defined as the compulsive physiological and psychological need for a habit-forming substance. Addiction is one of the most disabling diseases of Western society. It often causes health problems and accounts for a substantial proportion of total deaths [2]. Nicotine addiction for instance, is the largest single cause of avoidable death in the European Union. In the United States 28% of the population use tobacco on a regular basis [3], whereas the prevalence of adult nicotine addiction in Europe is around 30%.

These numbers illustrate the extent of the effect of addiction on public health, and the importance of gaining knowledge about processes in the brain related to addiction.
Drugs of abuse are commonly regarded as addictive due to their effects on the brain reward system, and share the property that recreational use of these substances can progress to abuse and subsequently to dependence [4]. Drug addiction is a complex disease, and a common view states that there are three main stages in which drug addiction develops [5]. First, the intoxication stage involves the rewarding effects of drugs, and the association between drugs and cues engaging stimulus-response actions [5,6]. An important brain structure that has been associated with the rewarding effects of drugs is the nucleus accumbens, receiving information regarding reward from the ventral tegmental area. Second, the negative affect stage has been related to the negative emotions experienced during withdrawal, which may cause addicted subjects to proceed into re-taking drugs [5,7]. The amygdala is associated with the negative emotions that represent the negative affect stage. Third, the craving stage may be related to the specific anticipation of drug reward, which is also often associated with relapse [5]. Associative areas such as the hippocampus and also the prefrontal cortex seem to be specifically involved in this stage of addiction.
A common aspect of drug reward is the activation of the mesocorticolimbic dopamine system [8]. Importantly, all drugs of abuse elevate dopamine transmission in the nucleus accumbens [9], an effect that has been associated with the rewarding properties of these drugs. A second neurotransmitter system that has been implicated in addiction is the endocannabinoid system. Ample evidence indicates that the endocannabinoid system may be a promising target for the development of new pharmacological therapies that help relieve symptoms associated with addiction, such as relapse.

1.3 The endocannabinoid system

An important neurotransmitter system that has recently been implicated in addiction is the endocannabinoid system [10-12]. The endocannabinoid system is ubiquitously present in the brain and is involved in many cognitive functions, such as mood, reward, attention and memory. It consists of cannabinoid receptors and endocannabinoids that work on these receptors [13,14]. At least two types of cannabinoid receptors have been identified, being the CB1 and CB2 receptor [15-17]. CB2 receptors are mostly found in peripheral tissue, involved in immune function, while CB1 receptors are more abundant in the central nervous system [16]. CB1 receptors are widely distributed throughout the brain with highest densities in the hippocampus, basal ganglia, and the cerebellum, and more moderate densities in the cerebral cortex and the nucleus accumbens. Low densities of CB1 receptors are found in the hypothalamus and the brain stem [15,18,19]. The two most important endocannabinoids are anandamide and 2-arachidonoylglycerol (2-AG) [12,13,18,19]. They act as retrograde messengers, which means that they are synthesized and released post-synaptically and work on presynaptic receptors, thereby regulating the release of both inhibitory and excitatory

neurotransmitters [12,13]. As such, the endocannabinoid system acts as a 'fine tuning' system that is involved in the control of movement, cognition, learning and memory, emotion and anxiety, reward and pain relief [10,12,20-26].

The endocannabinoid system has been implicated in addiction, particularly in the intoxication stage and the craving stage of addiction [11,12]. Rewarding effects of drugs of abuse such as nicotine, ethanol, and opioids appear to be mediated by the endocannabinoid system, as blocking the endocannabinoid system with the antagonist rimonabant blocks the rewarding effects of these drugs [11,27]. In addition, the endocannabinoid system has been associated with craving and relapse to cocaine, nicotine and ethanol [28], as rimonabant decreased relapse, and an endocannabinoid agonist significantly increased relapse. In humans, clinical studies demonstrated efficacy of the cannabinoid antagonist rimonabant in treatment for smoking cessation [29].

1.3.1 Cannabis

The main psycho-active constituent of cannabis, Δ^9-tetrahydrocannabinol (THC), is a partial CB1 receptor agonist, modulating the release of several neurotransmitters, such as glutamate, GABA, and dopamine [13,14]. THC increases dopamine release in the striatum, as has been shown by animals studies [19,30-32], and a Positron Emission Tomography (PET) study in humans [33]. This specific characteristic of cannabis, or specifically THC, indicates the abuse liability of cannabis [34], and as a consequence, cannabis is one of the most widely used drugs of abuse in the world. In the Dutch population, cannabis use has increased in the past decade, and although daily use has become less frequent, a growing group of daily users seeks treatment to be able to deal with their heavy use (for a review see [35]). This phenomenon may be associated with the fact that the potency of cannabis has increased dramatically in recent years [36,37]. The potency of cannabis is mainly determined by its THC content, and in samples of a Dutch variety ('Nederwiet') the concentration of THC increased from 9% in 2000 to 20% in 2004 [36].

1.3.2 Challenging the endocannabinoid system

In the PhICS study, the endocannabinoid system is challenged using the partial CB1 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 endogenous cannabinoids such as anandamide and 2-AG) or withdrawn from the market due to the occurrence of severe side effects (the CB1 antagonist rimonabant). A validated method to administer THC in humans is by using a intrapulmonary route, in which purified THC is dissolved in a small amount of alcohol, and vaporized into a balloon with a Volcano® Vaporizer [38,39]. This produces significant and dose-dependent physiological responses, which allows for the use of this method in clinical studies.

1.4 Addiction and cognitive brain function

1.4.1 Reward

A first important concept related to addiction is reward processing. A reward can be defined as an object or event that elicits approach and is worked for [8], and is associated with a pleasurable impact [40]. As a result of its characteristics, a reward can have a strong influence on behavior, as well as on cognitive processes such as attention, learning, and emotion. Disturbed reward processing is associated with a number of psychiatric disorders in humans, such as depression [41], addiction [12], and attention-deficit hyperactivity disorder (ADHD) [42].

The brain reward system consists of interconnected neurons, linking the ventral tegmental area (VTA) with the nucleus accumbens and the medial prefrontal cortex [43]. The nucleus accumbens is an important brain area that is implicated in reward processing, as has been described in animals [44] and in humans [45,46].

An essential neurotransmitter of the reward system is dopamine [8], but also GABAergic and glutamatergic transmissions play a role in regulating reward-processes [31,47]. There is converging evidence of the involvement of the endocannabinoid system in reward processing [44]. First, brain areas in which endocannabinoid receptors are located, such as the ventral tegmental area, the striatum, and the frontal cortex, are all areas that are also important for reward processes [18,48]. Second, the rewarding effects of many drugs of abuse, such as nicotine, alcohol, and cocaine are thought to be mediated by the cannabinoid system. Third, the endocannabinoids anandamide and 2-AG have rewarding properties, as has been shown in animal studies. In addition, THC has rewarding properties [44,49].

1.4.2 Impulsivity

A second important concept with regard to addiction is impulsivity [50]. Impulsivity has been defined as acting inappropriately to a situation, leading to undesirable consequences [51] or as acting without forethought [52]. Impulsivity is a heterogeneous trait, and can be divided in two main subcategories: first, having difficulty to inhibit a prepotent response, or impaired response inhibition, and second, the inability to postpone reward, or delay aversion [51,52]. This thesis focuses on the role of the endocannabinoid system in response inhibition. Similar to reward, the endocannabinoid system has also been implicated in response inhibition, through animal studies [53,54] and human studies [22,55].

Functional imaging studies have identified several brain areas that are important for response inhibition, such as the inferior frontal gyrus, the anterior cingulate and the striatum [56-59]. The striatum has been identified as an area that is strongly connected to motor areas such as the primary motor cortex and the supplementary motor cortex [59]. Further, the inferior frontal cortex has been shown to be crucial in the process of inhibition [56].

Evidence from pharmacological studies using dopaminergic intervention has indicated a role for dopamine in response inhibition [60]. In addition, serotonine as well as the adrenergic system have been implicated in response inhibition [52].

High dosages of THC have been shown to impair response inhibition in humans [22,55,61]. In animal studies, inhibitory control rather than response inhibition itself showed impairments after administration of cannabinoid agonists [53]. In addition, rimonabant has been shown to improve inhibitory control in animals [53].

1.5 Research questions

The main aim of the PhICS study is to elucidate the role of the endocannabinoid system in cognitive symptoms of psychiatric disorders. The focus of this thesis is on addiction, as it has been shown that the endocannabinoid system may be involved particularly in symptoms of addiction, such as altered reward processing and increased impulsivity.
The main method that is used to address these questions is functional Magnetic Resonance Imaging (fMRI). We use fMRI in combination with either a cognitive challenge (*chapter two*), a pharmacological challenge (*chapter four*), or both (*chapter five, six and seven*).
The key questions addressed in this thesis are as follows::

1) does chronic use of cannabis or nicotine alter reward processing?
2) does THC alter baseline neurophysiological processes?
3) is the endocannabinoid system involved in normal reward processing?
4) is the endocannabinoid system involved in impaired reward processing as is seen in subjects with a nicotine addiction?
5) is the endocannabinoid involved in impulsivity in healthy controls and in nicotine users?

1.6 Functional Imaging

1.6.1 MRI techniques

The studies described here all have used MRI based techniques to measure brain activity. fMRI is a non-invasive measurement of brain activity. It is sensitive to the so-called Blood Oxygenation Level-Dependent (BOLD) signal [62]. The BOLD fMRI signal provides a measure of oxygen use in the brain with a high spatial resolution. It has been shown to have good correspondence with underlying neuronal activity [63,64]. BOLD fMRI is not an absolute measure of brain activity, but can provide a reliable measure of relative change in brain activity between several conditions, if measured in a single session. Brain areas showing significant signal increase during a cognitive task compared to a control task are regarded as involved in that particular task. This pattern of relative signal change can be reproduced between sessions with good reliability if averaged over several subjects [65]. Thus, the signal change between two task conditions measured during a scanning session after the administration of a pharmacological challenge can be compared to the signal change measured during a separate placebo session (pharmacological fMRI).

1.6.2 Pharmacological MRI

Pharmacological fMRI (phMRI) is a relatively new technique, especially used to measure BOLD signal changes in combination with the administration of a pharmacological challenge. Brain activation after administration of a certain drug is compared to brain activation after placebo, hence offering a non-invasive technique to assess neurophysiological processes caused by that specific drug [66]. 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 [67]. This makes this technique highly suitable for use in the PhICS study, as THC is used to challenge the endocannabinoid system to study its role in specific cognitive domains.

1.6.3 Arterial Spin Labelling

A pharmacological challenge such as THC can induce changes in brain activity that are not related to cognitive changes per se. A baseline measurement can show alterations that may be related to neurophysiological changes, such as changes in respiration, heart rate, or brain perfusion. As THC can induce vasodilation, it is likely that THC administration causes baseline changes in perfusion, as has been shown previously with Positron Emission Tomography (PET) studies [68-70]. Recently, an interesting functional MRI technique has been developed which is called arterial spin labeling (ASL). This technique allows us to measure brain perfusion in a similar way as by using PET, but without the need for infusing a radioactive tracer. ASL has been validated as a reliable technique to measure brain perfusion [71]. ASL uses blood as an endogenous tracer by tagging it magnetically [72,73]. Blood is magnetically labelled with a Radio Frequency (RF) pulse in the arteries in the neck, and then imaged when labelled blood has reached the blood vessels in the brain. By using control images in which blood is not labelled, and subtracting these images from labelled images, a quantitative and stable method of imaging perfusion is provided.

1.6.4 Resting-state fMRI

With the application of pharmacological agents during functional MRI studies, an interesting question to be asked is whether baseline brain activity alters with administration of, in this case, THC. However, measuring baseline brain activity is not as straightforward as it seems, especially because fMRI is a technique that adresses relative signal changes, which means that it always needs a baseline to compare signal changes to. Interest is rapidly growing in resting-state fMRI. This technique focuses on measuring the baseline fluctuations of the brain [74-77], and analysis methods are still being developed. We chose to apply a robust measure of signal fluctuations, and to not rely on assumptions in terms of specific networks (independent component analysis (ICA) [74]). A reliable measure of fluctuation amplitude of the BOLD signal over time is the temporal signal-to-noise ratio (tSNR), computed by dividing the mean BOLD signal over a period of time by its standard deviation. This measure of variability of the BOLD signal represents fluctuations in brain activity, and is used here to assess changes following THC administration.

1.6.5 Experimental paradigms

1.6.5.1 Reward processing

Reward processing in humans is often investigated with fMRI using a Monetary Incentive Delay (MID) task [78]. In this task, the processes of anticipation and consummatory phase of reward, are separated. Subjects are presented with a cue, indicating either the appearance of a neutral target or a rewarding target. After a delay (the anticipation phase), a target is presented on which subjects need to press a button as fast as possible. Feedback is presented, indicating whether subjects won or lost, or not (the consummatory phase). For the anticipatory phase of reward processing, the most important brain area is the nucleus accumbens [45,46,78,79]. During feedback of reward, mostly medial and middle frontal brain areas are activated [78,79]. As the study described in *chapter five* did not show clear nucleus accumbens activation, we adapted the paradigm for the study described in *chapter six*, using different levels of reward, and introducing a loss trial. This was done to increase salience of the rewarding trials, which increased brain activation in the nucleus accumbens.

1.6.5.2 Response inhibition

To measure response inhibiton during functional imaging, the stop-signal task is a valid paradigm [80]. This paradigm consists of a simple reaction time task, in which infrequently and unpredictably, after onset of the go-stimulus, a stop-signal appears (either a sound or a visual stimulus). The stop-signal indicates that the go-response needs to be withheld [80]. Performance is measured using the stop-signal reaction time (SSRT), computed by the difference between the mean go reaction time and the mean stop-signal delay, as such providing a measure for one's ability to stop an ongoing response. Brain areas that are often found with fMRI are the inferior frontal cortex, the anterior cingulate cortex and the striatum [56,58,59,81].

1.7 Outline of this thesis

The aim of this thesis is to provide additional insights in the role of the endocannabinoid system in symptoms of addiction. Altered reward processing is an important feature of addiction, as is increased impulsivity.

In *chapter two* we describe an fMRI study on the chronic effects of cannabis use on brain activity during reward processing. We compared a group of long-term cannabis users with a group of healthy controls, and a group of nicotine users. We hypothesized that subjects with an addiction (either cannabis or nicotine) would show a blunted reward response in the nucleus accumbens.

In *chapter three*, the objectives and methods of the Pharmacological Imaging of the Cannabinoid System (PhICS) study are described. The PhICS-study is a comprehensive research project in which the role of the eCB system in cognitive symptoms of psychiatric disorders is assessed.

In *chapter four*, we aimed to clarify the role of the eCB system in neurophysiological processes during rest. Before studying the involvement of the eCB system in cognitive processes, such as memory, emotion, and reward, we should first have insight in the basic processes in the brain. Therefore, we used ASL and resting-state fMRI, comparing THC with placebo, and related behavioral parameters such as heart rate and feeling high to the imaging results. We hypothesized that the areas where CB1 receptors are located, such as limbic areas and the prefrontal cortex, would show differences between THC and placebo, and feeling high would account for a significant part of the variance in these areas.

In *chapter five*, we assessed the role of the eCB system in reward processing. We did this by administering THC to healthy volunteers and measuring brain activity during a reward task. We hypothesized that reward-related brain activity would be altered after THC administration, especially in regions involved in reward processing and densely populated with CB1 receptors, such as the prefrontal cortex and the striatum.

Chapter six describes a study concerning the role of the eCB system in reward processing in addiction. Despite ample knowledge of the association between addiction and the eCB system in animal studies, in humans the mechanisms behind this association have never been investigated. THC was administered to a group of healthy volunteers and to a group of subjects with a nicotine addiction. As the nucleus accumbens is an important brain area in reward processing, addiction, and the eCB system, we performed a Region-of-Interest (ROI) analysis to assess differences between controls and nicotine users in reward processing in the nucleus accumbens after THC administration. We hypothesized that nicotine users would show attenuated reward activity compared to controls, and that THC would increase this attenuation.

In *chapter seven*, we describe a study into the role of the eCB system in impulsivity in addiction. Impulsivity is measured using a stop-signal task, that assesses response inhibition. Subjects completed the stop-signal task both in the scanner during which fMRI scans were performed, and in a neuropsychological test battery outside the scanner (CANTAB). We focussed on the striatum, as this brain structure is specifically involved in response inhibition. Response inhibition was expected to decline after THC administration, and brain activity in the striatum was hypothesized to decrease. Nicotine users were expected to show increased impulsivity compared to healthy controls, and THC would increase this impairment.

In *chapter eight*, we summarize and discuss the findings, merits and limitations of the presented studies.

Chapter 2

Chronic effects of cannabis use on the human reward system: an fMRI study

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European Neuropsychopharmacology 20(3): 153-163

Abstract

Cannabis is one of the most used drugs of abuse. It affects the brain reward system in animals, and has proven rewarding and addictive potential in humans. We used functional MRI to measure brain activity during reward anticipation in a monetary reward task. Long-term cannabis users were compared to healthy controls. An additional control group consisting of nicotine users was included. Cannabis users showed attenuated brain activity during reward anticipation in the nucleus accumbens compared to non-smoking controls, but not compared to smoking controls. Cannabis users showed decreased reward anticipation activity in the caudate nucleus, compared to both non-smoking and smoking controls. These data suggest that nicotine may be responsible for attenuated reward anticipation activity in the accumbens, but that differences in the caudate are associated with the use of cannabis. Our findings imply that chronic cannabis use as well as nicotine, may cause an altered brain response to rewarding stimuli.

Introduction

Cannabis is one of the most widely used drugs since ancient times. Nowadays, it is commonly accepted that the drug has addictive potential. In the Dutch population cannabis use has increased in the past decade, and although daily use has become less frequent, a growing group of (daily) users seeks treatment to be able to deal with their heavy use (for a review see [35]). This may be associated with the fact that the potency of cannabis has increased significantly in recent years [36, 37].

Cannabis has an effect on the reward system, as is clearly shown in animal studies [9, 19, 31]. The rewarding effects of cannabis might be responsible for its addictive properties. Like most other drugs of abuse, prolonged cannabis exposure decreases baseline sensitivity of reward systems in animal studies [19, 30, 82], implying that in frequent cannabis users more reward is needed to reach the same subjective feeling of reward than in non-users. Indeed, chronic cannabis use, as well as use of other drugs, is associated with anhedonia [83, 84], i.e. an inability to experience pleasure from normally pleasurable life events. Anhedonia is an important symptom in several psychiatric disorders, such as depression, addiction, and schizophrenia [85], and is correlated with craving and relapse in addiction [84, 86]. It is associated with a dysfunctional reward system [85, 87], indicating that anhedonia in chronic cannabis use may be due to impaired reward processing. However, there is no direct evidence for an altered response in the brain reward system in humans after chronic cannabis use.

An endocannabinoid system has been uncovered, with both cannabinoid receptors and endocannabinoid ligands (for a review see [13]), that has a neuromodulatory role in the central nervous system [88]. There are at least two types of cannabinoid receptors, but in the brain mainly CB1 receptors are found. High densities of CB1 receptors are found in the basal ganglia, the cerebellum, and the hippocampus [18, 30]. CB1 receptors modulate GABAergic, glutamatergic and dopaminergic neurotransmission. The endocannabinoid system has been associated with several neurological and psychiatric disorders, and therefore, there is much interest in this system regarding its potential role in therapy [12].

The main psycho-active constituent of cannabis is Δ^9-tetrahydrocannabinol (THC), which works directly on the endocannabinoid system [13]. From animal studies it is known that the acute effect of THC in the brain includes an elevation of dopaminergic transmission in the nucleus accumbens [19, 30], most likely through CB1 receptor activation on GABAergic neurons in the ventral tegmental area and the nucleus accumbens [48]. A recent positron emission tomography (PET) study has confirmed that also in humans, dopamine release occurs in the striatum after acute THC administration [33]. Long-term effects include a decrease in dopamine neurotransmission in areas of the reward system [19]. The mechanism for this is not yet fully elucidated, but both down-regulation and desensitization of CB1 receptors after chronic cannabis use have been reported [89, 90]. It has been postulated that subjects prone to drug abuse suffer from a hypodopaminergic reward system. According to this reward deficiency hypothesis [91], drugs of abuse are the only way to effectively normalize reward functioning.

Although many animal studies have been performed to elucidate the influence of cannabis on reward systems in the brain, little is known about the effects of cannabis on the brain reward system in humans. The aim of this study is to investigate the long-term effects of cannabis on the human reward system. To assess effects of chronic cannabis use on reward, we used monetary reward as a substitute of natural rewards to induce brain responses. Hence, a monetary incentive delay (MID) task is used, that has proven to effectively activate areas associated with reward [45, 46]. Brain activity during the MID task is measured with functional magnetic resonance imaging (fMRI). We focussed specifically on the nucleus accumbens, since this area is an important region of the reward system as has been shown in animal studies [18, 30] and humans [46]. In the MID task, brain activity during the appetitive, or anticipatory phase of reward is separated from activity during reward outcome. Areas important for anticipation of reward are the nucleus accumbens, the caudate nucleus, putamen, and thalamus. During reward outcome, focus lies on more frontal areas, such as the orbitofrontal cortex and the mesial prefrontal cortex [78].

Given that cannabis is often consumed together with nicotine and that these substances interact in animals [92, 93] and in humans [94], it is important to assess the effects of nicotine. Nicotine may alter the reward system also, given that it is an addictive drug [95]. Therefore, non-using controls are needed as a reference of a normal functioning reward system. A control group consisting of regular smokers was included, as well as a control group that does not use cannabis or nicotine. Due to the cross-sectional nature of the study, we cannot exclude that other factors than chronic cannabis use may contribute to the results (e.g. pre-existing characteristics). For the sake of readability, we applied the term 'chronic use' throughout the paper.

We hypothesized that the responsiveness of the reward system is affected in long-term cannabis users compared to non-users. If the reward deficiency hypothesis [91] were true, a possible effect of cannabis would be an attenuation of neural responses in the nucleus accumbens, as this area is a key structure in the brain reward system which may show decreased sensitivity after long-term cannabis use.

Experimental procedures

Subjects

14 cannabis users (13 males), 14 smokers (11 males) and 13 non-users (11 males) participated in this study. Subjects were recruited through advertisement and word of mouth. Subjects were not seeking treatment for either nicotine or cannabis use and did not use hard drugs for more than 7 occasions lifetime. Groups were matched on age, alcohol use, and years of education (see table 2.1). All subjects were right-handed and were screened for mental disorders using the Mini International Neuropsychiatric Interview for DSM-IV clinical disorders (M.I.N.I: Translated Dutch Version 5.0.0 [96]) and for drug use (self-report questionnaire). A week before testing, subjects abstained from alcohol and cannabis. Normal smoking pattern was maintained, but subjects were not

allowed to smoke after arrival in the hospital, i.e. approximately 2 hours before testing. A urine sample was collected and tested for drugs (i.e. cannabis, methadone, opiates, cocaine, amphetamines, benzodiazepines, and barbiturates) and alcohol use, and smokers and non-users were excluded when tested positive on either. As THC levels can remain elevated even after one week abstinence, cannabis users were excluded when tested positive for alcohol or any drugs other than THC. All participants gave written informed consent. The Ethical Committee of the University Medical Centre of Utrecht approved this study in accordance with the Declaration of Helsinki 2004.

	Cannabis users (N=14)	**Smokers (N=14)**	**Non-users (N=13)**	**F**	**p**
Age (mean)	24 (± 4.4)	25 (± 4.5)	24 (± 2.7)	0.3	0.75
Years of education (mean)	14 (± 0.8)	14 (± 1.2)	15 (± 1.2)	2.4	0.11
Alcohol use last year (mean no. of units)	468 (± 388.8) range 50 - 1500	547 (± 274.3) range 75 – 938	348 (± 361.1) range 30 - 1000	1.9	0.15
Nicotine use per day (mean no. of cigarettes)	6.9 (± 5.6) range 0 – 20	13.2 (± 6.4) range 5 – 20	-	7.7	0.01*
Cannabis use life time (mean no. of joints)	3841 (± 2645.3) range1500 – 9700	-	-	-	-
Cannabis use last year (mean no. of joints)	614 (± 411.6) range 150 – 1400	-	-	-	-
Hard drugs life time (mean no. of occasions)	1.1 (± 2.1) range 0 – 7	0	0	2.8	0.07
Mean RT reward trial (ms)	316.6 (± 42.8)	317.2 (± 25.5)	333.6 (± 46.3)	n.a.	n.a.
Mean RT neutral trial (ms)	339.8 (± 40.3)	343.6 (± 29.5)	369.1 (± 58.4)	n.a.	n.a.

Table 2.1 Demographic characteristics, estimations of amount of substance use, and performance results of the MID task (* significant difference between cannabis users and smokers)

Reward Task

The monetary reward task was based on the monetary incentive delay (MID) task [78] (see figure 2.1). The task consisted of 72 trials, each lasting six seconds on average (range 3-10s). At the beginning of each trial a cue was presented signalling a potentially rewarding (a circle) or non-rewarding (a square) trial. Following this cue, a target was presented to which subjects had to respond. Finally, feedback on performance was given (either a reward or no reward). Subjects were instructed to respond as fast as possible to the target (by pressing a button) irrespective of cue type. Prior to the experiment, ten practice trials were presented so that subjects could familiarize themselves with the task. From these practice data, the shortest reaction time to the target was used to determine the individual time limit

allowed for responses to the target during the task. That is, in case of a reward cue subjects could win two euro when they responded within the time limit. In addition, subjects were rewarded in only fifty percent of the reward trials. This was achieved by increasing the time limit by 400 ms in half of the rewarding trials to make sure subjects would be fast enough to win the trial, and decreasing the time limit by 400 ms in the other half of the rewarding trials to make sure subjects would miss the reward. Hence, all subjects earned the same amount (36 euro).

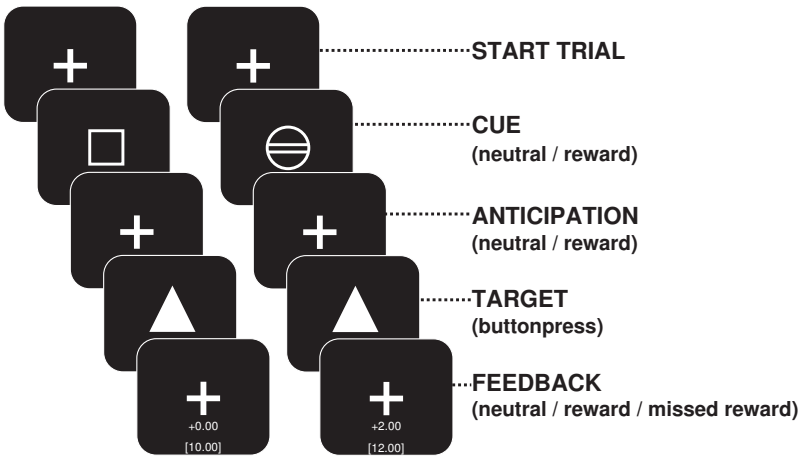

Figure 2.1. Schematical presentation of the task

The task was designed in such a way that the BOLD signal in response to anticipation of reward (i.e. time between cue and target) could be modelled independently from that of actual reward (i.e. feedback). This way, we could focus specifically on anticipation of reward, which induces the highest BOLD response in the reward network [78]. To reduce collinearity between the factors coding for anticipation of reward and actual reward, anticipation time and intertrial interval were varied (3-10 sec; mean 6 sec, and 0-30 sec; mean 4.2 sec, respectively). Only one level of reward was used, and no loss trials were included, to reach maximum power in a relative short time period.

Image acquisition

Brain imaging data were collected on a 1.5T Philips ACS-NT scanner (Philips Medical Systems, Best, The Netherlands) with fast gradients (PT6000). The head was held in place with a strap and padding. Structural and functional images were acquired in transverse orientation from the same section of the brain. For functional scans, a navigated 3D-PRESTO pulse sequence [97] was used with the following parameters: echo time 29.23 ms; repetition time 19.23 ms, flip angle 9°; matrix 40 × 64, 20 slices, field of view 160 × 256 × 80 mm; voxel size 4 mm isotropic; scan duration 1 second per 20-slice volume. Immediately

after functional scans, an additional PRESTO scan of the same volume of brain tissue was acquired with a high (30°) flip angle (FA30) for the image coregistration routine [97]. Finally, a T1-weighted structural image was acquired for anatomical registration purposes. A total of 1008 functional images were acquired for each subject.

Data analysis

Preprocessing and analysis of individual fMRI timeseries data was performed with SPM2 (Wellcome Department of Imaging Neuroscience, London, UK). For each individual subject, regression-coefficients for each voxel were obtained from a general linear model regression analysis using a factor matrix that contained factors representing event-related changes time-locked to the anticipation of neutral (36 events) and reward (36 events) trials. The third factor modelled heamodynamic responses during responding to the target (72 events). Three factors described the brain response during neutral feedback (36 events), feedback of rewarded targets (18 events), and feedback of missed targets (18 events). To correct for drifts in the signal, a high-pass filter with a cut-off frequency of 0.006 Hz was applied to the data. The task was designed such that the overlap between factors of interest was minimized (variance inflation factor (VIF) of neutral anticipation = 1.24; VIF of reward anticipation = 1.21, VIF of feedback of rewarded targets: 1.60; VIF of feedback of missed targets: 1.70). Factors for all six conditions (anticipation of neutral trials, anticipation of rewarding trials, response to the target, neutral feedback, feedback of rewarded targets, and feedback of missed targets) were convolved with a canonical heamodynamic response function. Group activation maps were generated for each factor using MULTISTAT [98]. This constitutes a mixed-effects analysis. Group results were tested for significance ($p<0.05$, corrected for multiple comparisons) resulting in a critical t value of 4.5 for every voxel.

Results

Three cannabis users were positive on cannabis metabolites in their urine (122 ng/ml, 138 ng/ml, and 144 ng/ml). All analyses were done with and without these subjects. No significant differences were found between these analyses.

Behavioral data analysis

A repeated-measures general linear model analysis with task condition (2 levels: reward and neutral) as within- and group (3 levels: non-users, users, and smokers) as between-subject factors revealed a main effect of condition ($F(1,38)=81.74$, $p<0.01$), indicating that all three groups responded faster to the target during reward trials compared to neutral trials (see table 2.1). The group by condition interaction was not significant ($F(2,38)=1.34$, $p=0.27$), indicating that reaction times were equal for all groups in both task conditions. All subjects won an equal amount of money (36 euros).

Imaging data analysis

For each group a whole-brain analysis was performed to obtain reward anticipation activity by contrasting activity during anticipation of reward versus anticipation of no-reward. Non-users showed reward-related activity in the nucleus accumbens, the caudate, putamen, thalamus, and several frontal areas, indicating that the task activated reward-related brain areas. Cannabis users and smokers showed similar activation patterns, albeit less pronounced than in non-users. In addition, cannabis users showed activation in the fusiform gyrus, whereas smokers showed activation in the left insula and the parahippocampal gyrus (see figure 2.2A).

A whole-brain two-sample t-test was performed to compare anticipation activity between non-users and cannabis users (see figure 2.2B). Cannabis users showed reduced reward anticipation activity compared to non-smoking controls in the left and right nucleus accumbens and caudate nucleus, the left putamen and the right inferior and medial frontal gyrus, the superior frontal gyrus bilaterally and the left cingulate gyrus ($p<0.05$, corrected for multiple comparisons). Areas in which cannabis users showed enhanced reward anticipation activity compared to non-smoking controls were the middle temporal gyrus bilaterally, the right cuneus, and the right parahippocampal gyrus (see figure 2.2B and table 2.2). In these areas, anticipation activity in healthy controls was negative compared to baseline.

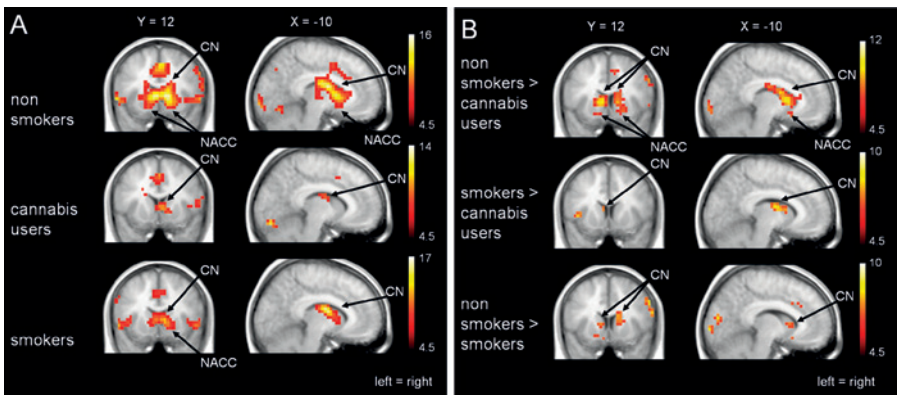

Figure 2.2 Group activation maps for reward activity at a threshold of $p < 0.05$, corrected for multiple comparisons. A: non-users, cannabis users and smokers. B: non-users versus cannabis users, smokers versus cannabis users, and non-users versus smokers. CN = caudate nucleus, NACC = nucleus accumbens

To explore differences between smoking tobacco and using cannabis, we checked whether smokers and cannabis users also differed in brain activity in areas that were differentially activated between non-users and cannabis users. In these areas, cannabis users also showed reduced anticipation activity compared to smoking controls, with the exception of the nucleus accumbens bilaterally, the right medial frontal gyrus, and the

left cingulate gyrus. Cannabis users showed enhanced anticipation activity compared to smoking controls in the left middle temporal gyrus (see figure 2.2B and table 2.3). This indicates that in the nucleus accumbens, the medial frontal gyrus, the left cingulate gyrus, and the temporal gyrus, changes in anticipation activity may not be due to cannabis use alone, but that nicotine may also be involved.

Region		Max T value	Talairach coordinates of max T value		
			x	y	z
non users > cannabis users					
Nucleus accumbens	L	5.8	-16	14	-10
Nucleus accumbens	R	5.5	16	13	-6
Caudate Head	L	9.3	-12	8	3
Caudate Head	R	8.1	12	8	3
Putamen	L	7.2	-28	0	-3
Inferior Frontal Gyrus, BA 9/47	R	8.8	32	23	-11
Medial Frontal Gyrus, BA 6	R	6.6	4	37	35
Superior Frontal Gyrus, BA 10	R	8.0	20	59	15
Superior Frontal Gyrus, BA 9	L	7.3	-28	52	27
Cingulate Gyrus, BA 24	L	7.9	-4	2	33
Inferior Occipital Gyrus, BA 17	L	11.8	-20	-94	-5
Middle Occipital Gyrus, BA 18	L	8.6	-24	-93	5
cannabis users > non users					
Middle Temporal gyrus, BA 39	L	7.6	-48	-68	20
Middle Temporal gyrus, BA 39	R	5.2	40	-64	20
Cuneus, BA 18	R	5.7	12	-76	24
Parahippocampal gyrus, BA 35	R	9.1	24	-16	-24

Table 2.2: Maximum t-value and Talairach coordinates of brain regions that show significantly altered reward activity in cannabis users compared to non-users.

Reward outcome activity was defined as brain activity during feedback of rewarded targets contrasted with brain activity during feedback of missed targets (see figure 2.3). In non-using controls, areas that are involved in the outcome of reward are the middle and superior temporal gyrus, middle frontal gyrus, fusiform gyrus, lingual gyrus, cingulate gyrus, as well as the inferior occipital gyrus, the pre- and postcentral gyrus and the inferior parietal lobule. The anterior cingulate was activated, as well as the left parahippocampal gyrus and the insula bilaterally. No significant activity was found in the nucleus accumbens. In cannabis users, mostly the same areas were involved in reward outcome, but also the left putamen and the right thalamus were activated. In smokers, frontal areas were found, as well as the insula, the parahippocampal and fusiform gyrus.

Region		Max T value	Talairach coordinates of max T value		
			x	y	z
smokers > cannabis users					
Caudate Body	L	6.6	-8	8	8
Caudate Body	R	7.4	16	-4	20
Putamen	L	6.9	-24	0	-8
Thalamus	L	6.9	-16	-8	16
Inferior Frontal Gyrus, BA 47	R	5.4	48	20	-4
Superior Frontal Gyrus	R	5.8	20	60	12
Superior Frontal Gyrus, BA 9	L	4.6	-24	56	28
cannabis users > smokers					
Middle Temporal Gyrus, BA 39	L	7.6	-48	-68	20

Table 2.3: Maximum t-value and Talairach coordinates of brain regions that show significantly altered reward activity in cannabis users compared to smokers

A whole-brain two-sample t-test revealed significantly altered outcome activity in several brain areas in non-users compared to cannabis users (see table 2.4 and figure 2.3B). Importantly, cannabis users showed more outcome activtiy in the right caudate nucleus and the putamen bilaterally. No significant differences were found in the nucleus accumbens. In areas that were significantly different between non-users and cannabis users, we checked whether differences in outcome activity between non users and cannabis users may have been due to nicotine use, by comparing smokers with cannabis users, and smokers with non-using controls. Areas that were differentially activated between smokers and cannabis users are shown in table 2.5. The putamen was more activated in cannabis users than in non-using and smoking controls, indicating that changes in this area were due to cannabis use, and not to smoking.

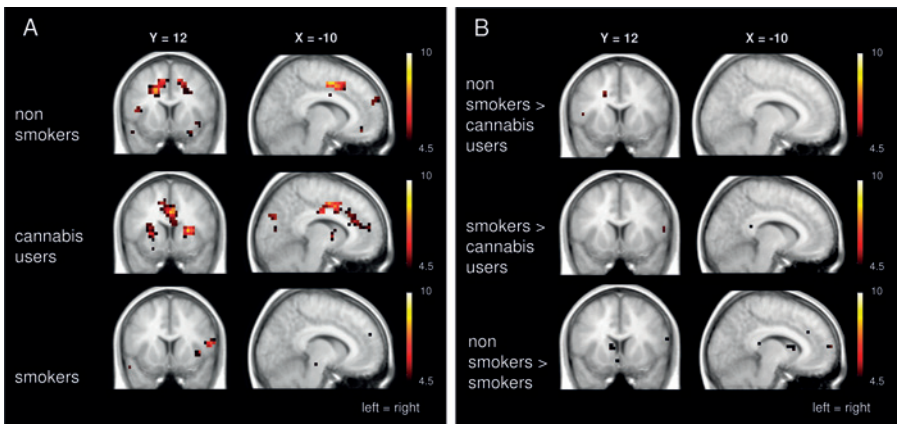

Figure 2.3 Group activation maps for reward outcome activity at a threshold of $p < 0.05$, corrected for multiple comparisons. A: non-users, cannabis users and smokers. B: non-users versus cannabis users, smokers versus cannabis users, and non-users versus smokers.

Region		Max T value	Talairach coordinates of max T value		
			x	y	z
non users > cannabis users					
Fusiform Gyrus. BA 20	L	5.8	-44	-28	-12
Middle Temporal Gyrus. BA 19	L	5.7	-36	-62	10
Lingual Gyrus. BA 18	L	5.2	-12	-68	3
Posterior Cingulate. BA 30	R	5.0	1	-62	10
Cingulate Gyrus. BA 32	R	5.6	20	17	30
Middle Frontal Gyrus. BA 8	R	5.7	28	33	40
Inferior Occipital Gyrus. BA 18	R	5.0	33	-83	-6
Claustrum	R	5.6	36	0	-9
Middle Frontal Gyrus. BA 11	R	4.9	43	34	-14
Middle Temporal Gyrus. BA 21	R	4.9	53	-1	-13
Postcentral Gyrus. BA 3	R	4.7	55	-18	22
Inferior Parietal Lobule. BA 40	R	5.6	63	-30	31
Superior Temporal Gyrus. BA 22	R	5.2	62	-43	14
cannabis users > non users					
Middle Temporal Gyrus. BA 39	L	7.5	-51	-65	22
Postcentral Gyrus. BA 40	L	7.2	-55	-26	20
Parahippocampal Gyrus. BA 36	L	5.5	-27	-39	-8
Inferior Frontal Gyrus. BA 47	L	5.0	-38	27	-4
Putamen	L	8.4	-20	4	8
Precuneus. BA 31	L	7.4	-8	-65	26
Cingulate Gyrus. BA 31	L	4.8	-14	-25	38
Medial Frontal Gyrus. BA 10	R	8.4	6	58	1
Cingulate Gyrus. BA 32	R	7.9	8	32	24
Cingulate Gyrus. BA 23	R	5.3	4	-18	29
Caudate Body	R	8.0	12	4	7
Putamen	R	6.7	19	8	-11
Precuneus. BA 19	R	7.7	24	-72	30
Cingulate Gyrus. BA 24	R	7.1	22	-10	35
Precentral Gyrus. BA 6	R	7.1	48	-6	34
Inferior Frontal Gyrus. BA 9	R	4.9	48	10	32
Middle Occipital Gyrus. BA 19	R	7.4	43	78	15

Table 2.4: Maximum t-value and Talairach coordinates of brain regions that show significantly altered reward outcome activity in non users compared to cannabis users

Region		Max T value	Talairach coordinates of max T value		
			x	y	z
smokers > cannabis users					
Fusiform Gyrus. BA 37	L	4.6	-44	-40	-8
Posterior Cingulate. BA 23	R	7.6	4	-60	14
Middle Frontal Gyrus. BA 10	R	4.6	28	49	-2
Middle Temporal Gyrus. BA 21	R	5.5	55	-6	-16
cannabis users > smokers					
Superior Temporal Gyrus. BA 41	L	5.8	-55	-25	9
Putamen	L	6.1	-24	14	8
Medial Frontal Gyrus. BA 10	R	8.2	0	59	15
Cingulate Gyrus. BA 24	R	6.8	1	2	34
Putamen	R	7.6	25	0	18

Table 2.5: Maximum t-value and Talairach coordinates of brain regions that show significantly altered reward outcome activity in smokers compared to cannabis users

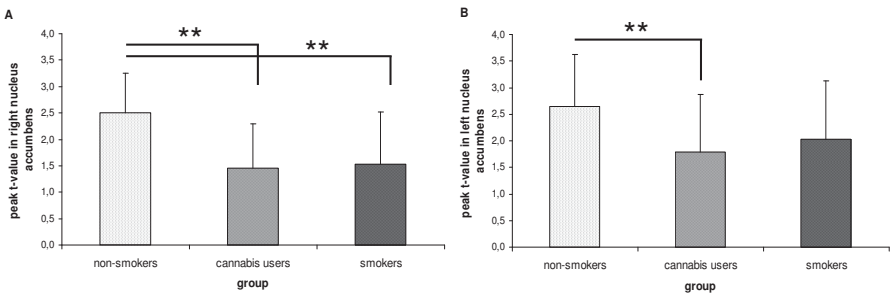

Figure 2.4. Peak t-values of reward activity in the right (A) and left (B) nucleus accumbens in the different groups. *: groups differ significantly ($p < 0.05$); **: groups differ significantly ($p < 0.01$). Error bars denote SD. Note that these t-values represent those of individual subjects, whereas t-values in Table 2.2 - Table 2.5 represent values computed over subjects.

Finally, we performed a Region of Interest (ROI) analysis of the fMRI activity levels in the left and right nucleus accumbens specifically (see figure 2.4 and 2.5). The nucleus accumbens was anatomically defined as a sphere with a diameter of 7 mm, and the center was at Talairach coordinates (x,y,z) 14,14,-8 (right) and -14,14,-8 (left). In the right nucleus accumbens, both cannabis users and smokers showed attenuated anticipation activity compared to non-smoking controls ($p<0.05$). In the left nucleus accumbens, cannabis users and smokers showed a trend towards reduced anticipation activity compared to non-smoking controls ($p<0.1$) (see figure 2.4). No significant differences were found when outcome activity in the nucleus accumbens was compared between groups (see figure 2.5). Since cannabis users smoked less nicotine cigarettes than the smoking control group, additional analyses were performed to ascertain that the observed effects were not due

to a difference in nicotine smoking. First, no correlations were found between number of cigarettes and brain activity in the left and right nucleus accumbens. Second, the effects did not change after adding smoking as a covariate. Third, comparing the ten least smokers (mean 10.5 ± 5.5 cigarettes per day, range 5 – 20) with the ten cannabis users who smoked most (mean 9.5 ± 4.4 cigarettes per day, range 5 – 20), no differences were found between number of cigarettes ($p = 0.66$), and the ROI analysis showed the same effects (see figure 2.4 and 2.5).

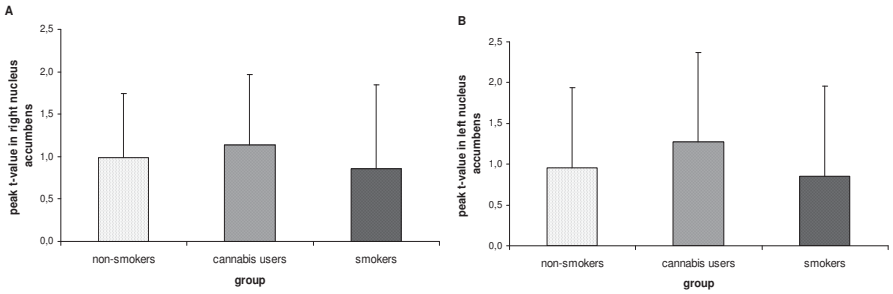

Figure 2.5. Peak t-values of reward outcome activity in the right (A) and left (B) nucleus accumbens in the different groups. There were no significant differences between groups. Error bars denote SD. Note that these t-values represent those of individual subjects, whereas t-values in Table 2.2 – Table 2.5 represent values computed over subjects.

Discussion

Brain responses on a monetary reward task were compared between long-term cannabis users, nicotine users, and healthy controls to investigate the long-term effect of cannabis use. Compared to healthy controls, cannabis users showed attenuated reward anticipation activity in the nucleus accumbens and caudate nucleus bilaterally, the left putamen, thalamus and several frontal areas, areas that are involved in (preparation of) motor processing and normal voluntary movement [99]. These areas are also reported [45, 46, 78] as being important in anticipation of reward. Cannabis users showed enhanced reward anticipation activity in the temporal gyrus, cuneus, and the parahippocampal gyrus, an area that is typically involved in associative memory encoding [100, 101]. In the areas that differed between cannabis users and non-smoking controls, cannabis users were also compared to smokers, and a specific effect was found in the caudate, putamen, thalamus, and frontal regions, as well as the parahippocampal gyrus.

The nucleus accumbens did not exhibit activation during reward outcome, showing the same as in Knutson's study [46], and no differences were found in the nucleus accumbens in reward outcome activity between groups. The putamen was more activated in cannabis users than in non-using and smoking controls, and the caudate showed more outcome activity in both cannabis users and smokers than in non-smokers. The putamen and caudate nucleus are involved in reward-related learning. The putamen is more involved

in associating a certain action in response to a simulus with reward, while the caudate is implicated in reward prediction error [102]. In the current reward task, a cue is presented that is associated with a reward. Subjects cannot predict winning or not winning, so a missed target may be regarded as a reward prediction error. It might be possible that addicted subjects have more difficulty in learning this reward prediction error and therefore show more caudate activation. However, this is highly speculative.

Importantly, there was no difference in activity in the nucleus accumbens (which was activated by reward anticipation) between cannabis users and smokers. This indicates that anticipation activity in this region was attenuated by both cannabis and nicotine, possibly through an effect on dopamine transmission [103]. In contrast, the caudate nucleus (anticipation) and the putamen (outcome) appear to be affected more by cannabis than by nicotine, which may be due to down-regulation and desensitization of CB1-receptors due to chronic use [104]. Chronic cannabis use has been shown to induce tolerance in the striatum, cerebellum and limbic forebrain, through desensitization and down-regulation of CB1-receptors in animals [90, 104]. Cannabis tolerance occurs differentially across different brain areas, in time-course as well as in magnitude [18, 105]. In the nucleus accumbens, Romero and colleagues only found a trend for cannabinoid receptor binding reduction in THC-tolerant rats, whereas binding was mildly but significantly reduced in the lateral caudate-putame[105]. The present results are in agreement with these findings in that the effect of cannabis involved the striatum but both cannabis and nicotine affected the nucleus accumbens.

Both nicotine [106] and cannabis [30] have long-lasting effects on the nucleus accumbens dopamine system. Cannabis acutely enhances dopamine transmission in the nucleus accumbens, like nicotine [34, 107] and other drugs of abuse [43, 82]. Both long-term cannabis use and long-term nicotine use appear to cause an attenuation of dopamine transmission in the nucleus accumbens, albeit via different mechanisms [108, 109], which may explain the finding that there is no difference in reward activity in the nucleus accumbens between cannabis and nicotine users.

Even though the effect of smoking was assessed in the present study, the goal of the study was not to measure the effects of nicotine. No nicotine abstinence was required, to prevent additional complicating factors. Consequently, it is difficult to disentangle the effects of cannabis and nicotine. These substances pharmacologically interact when consumed together [92-94], leading for example to a less pronounced down-regulation of CB1-receptors in animals. Therefore, the possibility that the main effect we found in the striatum is a consequence of an interaction effect of cannabis and nicotine, instead of an effect of cannabis alone, cannot be excluded, especially since no nicotine abstinence was required. Although the smoking control group consumed more nicotine than the cannabis users, effects did not change when analyses were repeated with subgroups matched for number of cigarettes smoked last year. Moreover, rerunning the analysis covarying for smoking did not change the effects in the ROI analysis. It would, however, be interesting

to include another group, consuming only cannabis and no nicotine, to investigate this in more detail.

An alternative explanation for attenuated brain responses in cannabis users and smokers might be that it reflects a motivational difference between groups, that is, cannabis users and smokers could be less driven to perform the task. Kirsch et al. [45] found reward-related activity in the nucleus accumbens and substantia nigra when money could be won, but not when only verbal feedback was given. They interpret this result as an indication that highly motivating stimuli lead to stronger activation compared to less motivating stimuli. In a study of Martin-Soelch and co-workers, opiate addicts were compared with normal controls while performing a reward task during H_2O PET imaging [110]. The same task was used to compare smokers with non-smokers. Opiate addicts and smokers showed fewer activated areas during reward than (non-smoking) healthy controls. In addition, non-monetary reward caused no reward-related activation in addicted subjects, but in healthy controls non-monetary reward did cause reward-related activation. This indicates that non-monetary reinforcement may have insufficient motivational value for addicts. Translating this to the current study, one could argue that reduced reward anticipation activity in cannabis and nicotine users means that monetary reward is less motivating for drug users than for non users. However, responses to rewarding targets were faster than to neutral targets in all groups, and reaction times did not differ between groups. Since reaction time is strongly affected by motivation, and all groups performed equally, it is rather unlikely that the imaging results can be attributed to differences in motivation.

In summary, this study showed attenuated brain responses to anticipated monetary rewards in reward-related areas in cannabis users. In some regions, this attenuation may (partly) be due to tobacco use. This suggests that both chronic cannabis and nicotine use reduce the sensitivity of some regions of the reward system to rewarding stimuli. This may be attributed to an attenuation of dopamine transmission in the nucleus accumbens and supports the concept of a mesolimbic reward system that is hyporesponsive to non-drug rewards (reward deficiency hypothesis) [91, 111]. Interestingly, a similar result was obtained in abstinent alcoholics [112], where monetary reward yielded reduced activation in the ventral striatum, which was related to craving (not assessed in the present study). The concept of a hyporesponsive mesolimbic reward system (concerning non-drug reward) is thus supported by both studies. It has, however, been questioned by another study where abstinent alcoholics exhibited normal monetary reward anticipation activity, but an elevated response in the nucleus accumbens to reward outcome [113]. Possible explanations for the opposing findings in abstinent alcoholics may be the different paradigms used and possibly the comorbidity of the latter group of patients, as well as a different mechanism of action of alcohol compared to cannabis. Our study introduces a potentially important role of nicotine in the various findings, in that in both alcohol studies patients smoked much more than controls. One could speculate that nicotine dependence may have contributed to the Wrase findings, as their patients were allowed to continue smoking, but this is not

straightforward as the Bjork study patients were also allowed to smoke before scanning (personal communication). Interestingly, neither any of our subject groups nor the healthy controls in the Bjork study displayed a significant accumbens response to the reward outcome, suggesting that perhaps this response is selective for alcohol dependence. With regard to paradigm differences, it seems worthwhile to assess effects of smoking and cannabis use on the brain response to reward magnitude and to monetary loss.

As mentioned in the introduction, we cannot exclude that other factors than chronic cannabis or nicotine use (e.g. pre-existing characteristics) may have contributed to the results. A longitudinal study with measurements before first cannabis use would certainly help in discovering whether differences in the reward systems of addicted subjects compared to controls are a cause or a consequence of using the substance, and whether or not other factors are involved.

Concluding, cannabis users show differences in reward processing in the caudate nucleus, putamen, thalamus, inferior and superior frontal gyrus, and the parahippocampal gyrus, areas that are involved in reward processing, motor control, and cognitive aspects of behavior. This is the first study that shows an effect of chronic cannabis use on reward processing in humans, which may be important in further research regarding the involvement of cannabis use, or the endocannabinoid system, in diseases such as addiction, depression, and schizophrenia.

Chapter 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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International Journal of Methods in Psychiatric Research 2011: 20(1): 10-27

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 that prove feasibility of the method. Core elements in the methodology of PhICS are the administration method (THC is administered by inhalation using a vaporizing device) and the innovative approach of pharmacological Magnetic Resonance Imaging (phMRI) using convergent imaging techniques like 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, the used fMRI paradigms demonstrate activation of expected networks of brain regions in the cognitive domains of interest. Extending scientific knowledge on the endocannabinoid system will greatly contribute to further research and development of therapeutic opportunities of cannabinoid ligands. Newly developed cannabinoids may, in future, reinforce the pharmaceutical arsenal to combat brain disorders such as cognitive decline, psychiatric disorders, and addiction.

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 [23,26,48]. 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 CB1-receptors are the most important and they are widely distributed throughout the brain (see for extensive reviews on the eCB-system [18,114,115]). 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 [114,115]. 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 [23,26,48].

Modulation of the eCB-system by administering exogenous cannabinoids such as Δ9-tetrahydrocannabinol (THC), the main psychoactive constituent of cannabis [116], produces a diverse range of acute effects by activating the CB1-receptor. Apart from the euphoriant effect or "high" [33,117,118], THC also induces impairments in working memory [117-119], episodic memory [120] (see for a review [23]), and attention [121-123]. THC also affects impulse control [22,55]. High-dose intoxication with cannabis can result in acute psychosis, usually of a transient nature [124,125].

THC possesses rewarding properties: it is self-administered by monkeys [126] and enhances striatal dopamine levels in both animals [127] and humans [33] (see for a review [48]).

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 [128,129] and worsens its clinical outcome [130,131]. Further, patients with schizophrenia demonstrate both enhanced CB1-receptor densities in cortical regions [132-134] and increased levels of endogenous cannabinoids in cerebral spinal fluid [135,136] and plasma [137]. 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 [138,139]).

The eCB-system is involved in different aspects of drug addiction, including reward, withdrawal and relapse (see for reviews [11,28,140]). For example, animal studies have shown that addictive properties reflected in behaviors such as self-administration or conditioned place preference of opiates, nicotine and alcohol are absent or attenuated in cannabinoid CB1-receptor knockout mice and after the administration of the CB1-antagonist rimonabant [11]. Further, CB1-agonists reinstate drug seeking behavior of drugs of abuse, whereas rimonabant blocks this effect [28,140]. In humans, clinical trials are performed to investigate the effect of rimonabant on the cessation of smoking nicotine [141] and in the reduction of food intake in obesity [142].

Key symptoms of Attention Deficit Hyperactivity Disorder (ADHD) are disturbed impulse regulation and attention [143]. Preclinical studies indicate that the eCB-system is involved in impulse regulation, since CB1-receptor agonists and antagonists, as well as inhibiting fatty acid amide hydrolase (FAAH), the enzyme responsible for the degradation of the endogenous cannabinoid anandamide, affect impulsivity [53,144]. Impaired performance on attention tasks after administration of cannabinoids to both animals and humans indicates the involvement of the eCB-system in attention [123,145]. The cognitive deficits in ADHD may be caused by a dysregulation of dopaminergic frontal-subcortical circuits, also affecting the reward system [146,147].

In depression, the role of the eCB-system is less straightforward (see for reviews [26,148]). Preclinical studies have demonstrated that both facilitation [149,150] and inhibition [151,152] 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 [142].

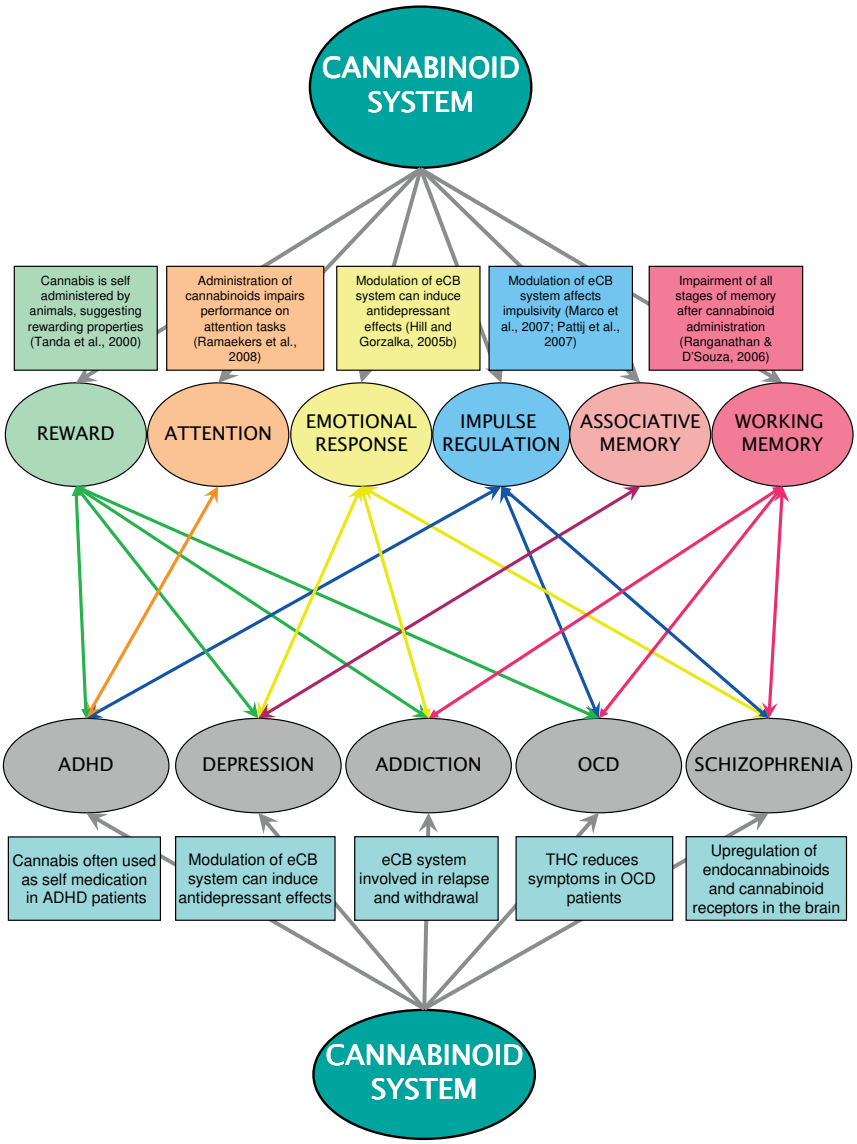

Figure 3.1 A schematic presentation of the rationale behind the PhICS study. There is evidence for involvement of the endocannabinoid (eCB) system in both psychiatric disorders (lower part) and different domains of cognition (upper part). Impairments in cognition are significant symptoms in psychiatric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

In Obsessive Compulsive Disorder (OCD) impairments in working memory, attention and impulse regulation are core symptoms [153]. As mentioned before, there are several indications that the eCB-system is involved in these symptoms (see for a review [139]). Interestingly, treatment with THC reduces obsessive compulsive symptoms in patients with Gilles de la Tourette-Syndrome [154] and OCD [155].

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 [66]). PhMRI is a powerful tool to map direct modulation of brain function by psychopharmacological agents, in this case the CB1-agonist THC. By comparing acute effects of THC administration on brain function between psychiatric patients with specific cognitive impairments and healthy controls, we explore the role of the eCB-system in the regulation of cognitive brain function in these populations. The purpose of this paper is to present the background and methodology of the PhICS study.

Methods

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 Centre 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 [156]. 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.

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 [157]
• 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/m2 or >28 kg/m2
• 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)

Table 3.1 In- and exclusion criteria for participants

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 [158,159]. 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 [158,159], 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 [38].

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 [160]. For OCD patients the Y-BOX questionnaire is used. The ADHD rating scale is used for assessing symptomatology in ADHD patients [161], the Beck Depression Inventory for depressive patients [162], and the Fägerstrom Test for Nicotine Dependence (FTND) for smokers [163]. 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) and the Behavioral Inhibition Scale / Behavioral Activation Scale (BIS/BAS) 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 SENSE-PRESTO scan protocol is used for all fMRI tasks, as well as the Resting State scan [164] (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 pre-processed and analyzed using SPM5 (Wellcome Trust Centre for Neuroimaging, London, UK). Pre-processing of data includes realignment of functional images and co-registration 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 [143,146], 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.

Working memory

Working memory is assessed using a modified version of the Sternberg recognition task [165]. 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 [166-168].

Reward

To activate reward circuitry an adapted version of the monetary incentive delay task as developed by Knutson and colleagues is used [78]. 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 and colleagues [169] 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) [169,170].

Impulse regulation

As a measure of impulsivity, brain activity underlying inhibitory motor control is assessed with a stop signal task [81,171]. 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 [58,81,171].

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 [172]. 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 [172,173].

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 [100, 101].

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

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

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 Labelling (ASL) permits the non-invasive quantification of global and regional brain perfusion (see for a review [174]). 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 (M_0) and also for segmentation purposes, an inversion recovery sequence is acquired with the same geometry and resolution as the ASL sequence (inversion times 100–1900 ms with 200-ms intervals, preceded by a saturation pulse at -1680 ms) [175].

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 [74]. 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 [176]. Psychedelic effects are assessed using an adapted version of a 13-item visual analogue rating scale, originally described by Bowdle and colleagues [39, 177]. 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 [39]. 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. [39].

Neuropsychological tests

After scanning, neuropsychological tests are applied to measure the acute behavioral effects of THC on cognitive task performance. The results of these tests are related to the fMRI results. This provides insight in the behavioral correlates of brain activity findings and improves our understanding of the neurophysiological basis of the CB1-mediated behavioral effects of THC. Testing is done using a comprehensive set of 8 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. 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 [173,178]. However, in these studies THC was administered orally. Since THC plasma concentrations are much higher after intrapulmonary compared to oral administration [33,39], 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 [33,39] and to detect differences in cerebral blood flow between patients and healthy controls using ASL [179]. 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.

	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

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.

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 illustrate 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).

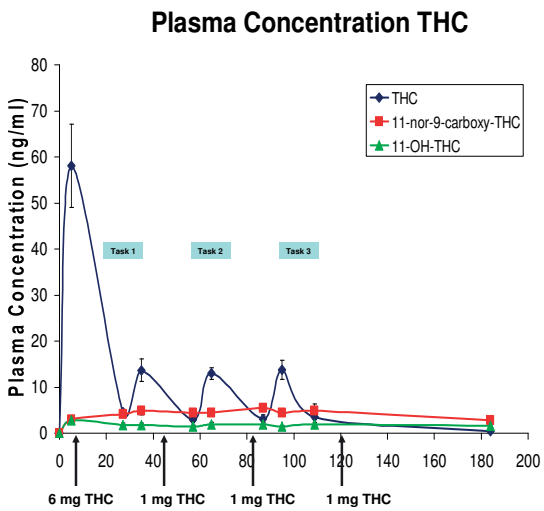

Figure 3.3 Plasma concentrations of Δ9-tetrahydrocannabinol (THC) and its main metabolites 11-OH-THC and 11-nor-9-carboxy-THC after inhalation of 6, 1, 1 and 1 mg THC with 30 minutes intervals (mean ± standard error of mean (SEM); n = 13). At the X-axis vertical arrows indicate the time points of THC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

Heart rate

Heart rate was measured at 20 timepoints during the test days. Per subject, heart rate scores (beats per minute (bpm)) were mean corrected, for placebo and THC sessions separately. Figure 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 ($F(1,11) = 10.2$, $p < 0.01$).

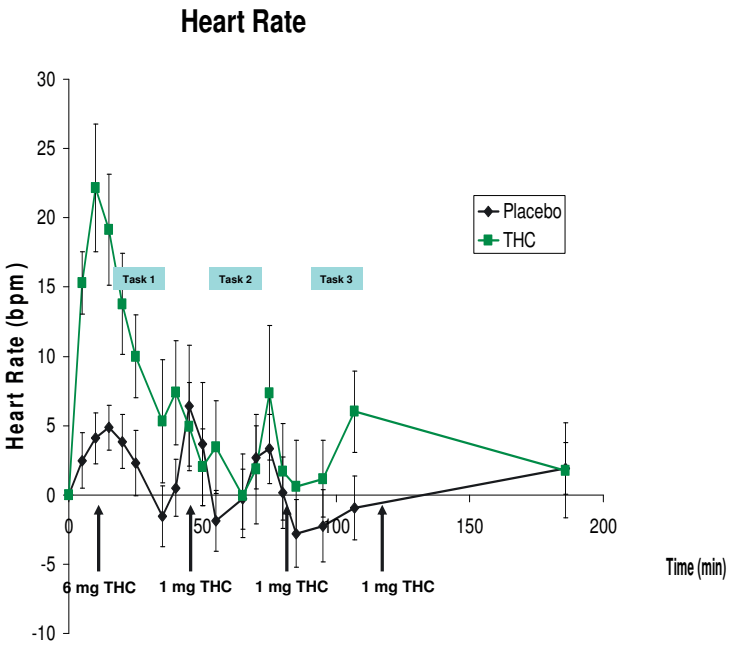

Figure 3.4 Mean corrected heart rate in beats per minute (bpm) over time. Error bars denote standard errors of mean (SEM). At the X-axis vertical arrows indicate the time points of THC administration. The task blocks in the figure indicate time blocks when fMRI tasks were administered in randomized order.

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.

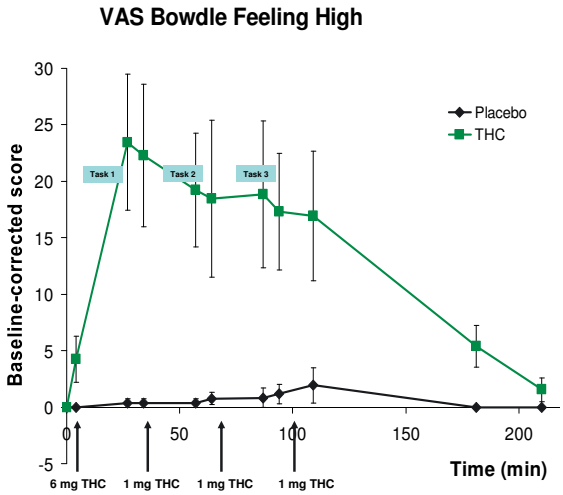

Figure 3.5 Mean corrected VAS scores of Feeling High over time in minutes. Error bars denote standard errors of mean (SEM). At the X-axis vertical arrows indicate the time points of THC administration. The task blocks in the figure indicate time blocks when fMRI tasks were administered in randomized 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 found in previous fMRI studies from our lab using the same task paradigm in different groups of subjects [101,180,181].

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 [166,168,182]. 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 [183,184].

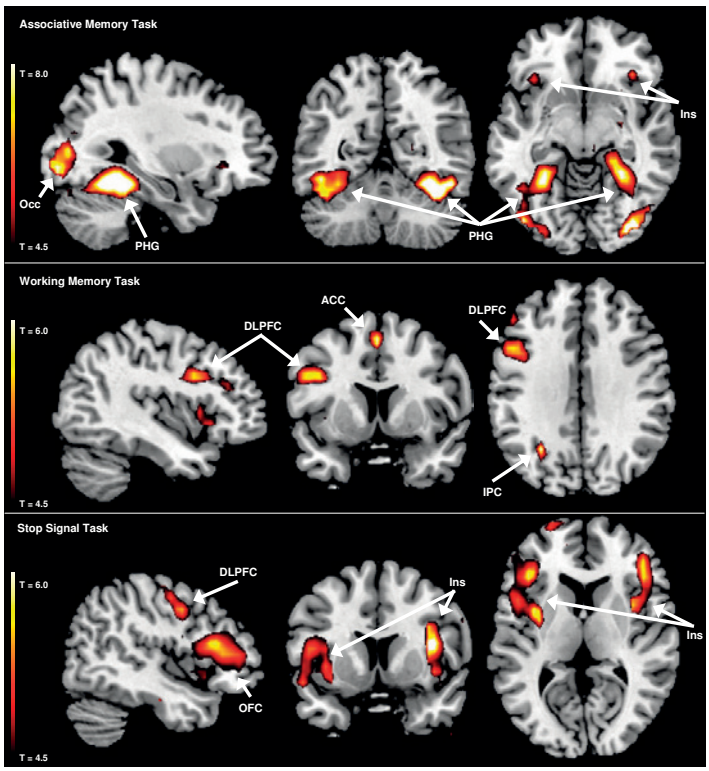

Figure 3.6 Overview of brain regions critically involved in associative memory, working memory and impulse regulation as measured with the task paradigms used in PhICS. All activation maps reflect suprathreshold ($T > 4.5$, $p < 0.05$, corrected for multiple comparisons) group brain activity during placebo 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.

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 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 neuro- psychological 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 Blood-Oxygen-Level Dependent (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.

Relatedly, 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 driven by important ethical and theoretical considerations. For one, incidental users, without known negative implications (e.g. bad trip, cannabis-induced anxiety or psychosis) can a priori be expected to tolerate the THC challenge used in this experiment with a minimal risk for adverse reactions. Secondly, incidental users, as opposed to frequent users, will show minimal long-term neuroadaptation in the brain as a consequence of long-term frequent cannabis use. The choice for incidental cannabis users (both healthy volunteers and patient groups) may limit generalization of the findings. Still, as the primary focus of the PhICS study is on the role of the eCB system in the (cognitive) symptomatology of the involved psychiatric disorders (e.g. the endophenotype) and groups are matched on history of cannabis use, we feel the external validity of the design remains large. Second, in the presented study design, the effects of the pharmacological challenge (THC) likely provides 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" [185]. 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 [186,187] and in both disorders there is tentative evidence for the

involvement of the eCB-system in emotional dysregulation. 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 – possibly related to a malfunctioning endocannabinoid system. The topic of the present methodological paper (i.e. describing procedures for all patient groups) leaves little room for detailed hypotheses. Instead, we give some 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. 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 [117]. 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 [130, 188]. 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 [189].

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.

Acknowledgements

The PhICS study is performed within the framework of Top Institute Pharma, project number T5-107. We like to thank Storz & Bickel for their generous support in supplying the Volcano vaporizer. Competing interests: The authors have no competing interests.

Chapter 4

Evidence for involvement of the insula in the psychotropic effects of THC in humans
a double-blind, randomized pharmacological MRI study

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International Journal of Neuropsychopharmacology 2011: 14: 1-12

Abstract

The main reason for recreational use of cannabis is the 'high', the primary psychotropic effect of Δ^9-tetrahydrocannabinol (THC). This psychoactive compound of cannabis induces a range of subjective, physical and mental reactions. The effect on heart rate is pronounced and complicates bloodflow-based neuroimaging of psychotropic effects of THC. In this study we investigate the effects of THC on baseline brain perfusion and activity in association with the induction of feeling high. Twenty-three subjects participated in a pharmacological MRI study, where we applied Arterial-Spin-Labelling (ASL) to measure perfusion, and resting-state functional MRI to assess BOLD signal-fluctuation as a measure of baseline brain activity. Feeling high was assessed with a Visual Analogue Scale and was compared to the imaging measures. THC increased perfusion in the anterior cingulate cortex, superior frontal cortex, and insula, and reduced perfusion in the postcentral and occipital gyrus. Baseline brain activity was altered, indicated by increased amplitude of fluctuations in resting-state functional MRI signal after THC administration in the insula, substantia nigra and cerebellum. Perfusion changes in frontal cortex were negatively correlated with ratings of feeling high, suggesting an interaction between cognitive control and subjective effects of THC. Concluding, an acute THC-challenge altered baseline brain perfusion and activity, especially in frontal brain areas involved in cognitive and emotional processes, and the insula, associated with interoceptive awareness. These changes may represent the THC-induced neurophysiological correlates of 'feeling high'. The alterations in baseline brain perfusion and activity also bear relevance for studies on task-related effects of THC on brain function.

Registered at the Nederlands Trials Register
Name: The Role of the Endocannabinoid System in Psychiatric Disorders and Symptoms: a Pharmacological fMRI study
URL: http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=1787
Registration Number: 1787

Introduction

Cannabis is one of the most widely used drugs in the world. Its main psychoactive constituent, Δ^9-tetrahydrocannabinol (THC), produces a number of acute, dose-dependent psychotropic effects, such as feeling high, relaxation and euphoria which are regarded as the main reason why people use cannabis [190]. In addition, feeling high has been reported as the most stable subjective effect of THC or cannabis administration, as recently reviewed by Zuurman et al. [191]. Effects on cognition are also frequently reported, such as memory impairments [192,193]. In addition to these, THC has prominent effects on blood pressure and heart rate, and causes cerebral vasodilatation [194]. All of these effects can be expected to reflect changes in brain function and perfusion. Brain imaging studies have measured acute effects of cannabis (THC) on baseline brain perfusion in humans, using Positron Emission Tomography (PET) [68,69], the main outcome of which is that THC administration increases regional cerebral blood flow (rCBF) in prefrontal, insular, and anterior cingulate regions. Changes in rCBF have been associated with many aspects of THC-induced behavioural effects such as a changed time perception, increased anxiety, intoxication levels and arousal [195-201]. Yet, the specific neurophysiological correlate of the main reason for recreational use of THC, the 'feeling high', remains to be elucidated.

Recent advances in MRI techniques have provided the Arterial Spin Labeling (ASL) technique, which offers a quantitative and non-invasive measure of CBF [174]. ASL employs blood as an endogenous tracer by magnetically labelling arterial blood water [72,73]. This technique can be administered repeatedly within a scan session, making it very attractive for studies involving a pharmacological challenge [71].

Perfusion, however, does not immediately reflect brain activity when a drug is administered with potential effects on cerebrovasculature, such as THC. An additional measure of brain activity would thus be informative for assessing effects of THC on baseline brain function, the most obvious being functional MRI. Both techniques can be applied within the same scan session, allowing for a rich assessment of effects of a pharmacological challenge. Moreover, it constitutes an advancement in study design as compared to the mentioned PET studies [195-201] because it allows for a combination of within-subject placebo-drug comparisons (multiple sessions) without exposing subjects to radioactivity twice, with pre- and post-administration measurements for corrections of baseline values, thereby improving internal validity of the data.

Blood-oxygen-level-dependent (BOLD) functional Magnetic Resonance Imaging (fMRI) is currently the preferred technique for studying the acute effects of THC on specific brain functions. fMRI studies have reported THC-induced alterations in brain activity during emotional, inhibitory, and memory processes [178,192,202]. These pharmacological imaging studies used task-dependent fMRI, i.e. fMRI in combination with a cognitive challenge. So far, these have not addressed the effects that THC may have on baseline brain activity during rest. Here we present a pharmacological MRI (phMRI) study aimed

at bridging this knowledge gap by investigating the acute psychotropic effects of THC on brain neurophysiology. We combined a randomized, double-blind, placebo-controlled design with ASL and resting-state fMRI techniques to assess the intrinsic effects of THC administration on global and regional perfusion and on BOLD signal fluctuation as a measure of baseline brain activity. Additionally, subjective effects of THC were measured with Visual Analogue Scales, and heart rate and respiration were continuously monitored. We expected that THC administration, in line with findings from PET studies, would increase brain perfusion in a region-specific manner, predominantly in frontal and limbic areas.

For analysis of the resting-state data we chose to apply a robust measure of signal fluctuations, and to not rely on assumptions in terms of specific fluctuation frequencies (amplitude of low-frequency fluctuations (ALFF)[203]) or in terms of specific networks (independent component analysis (ICA)[74]). A reliable measure of fluctuation amplitude of the BOLD signal over time is the temporal signal-to-noise ratio (tSNR), computed by dividing the mean BOLD signal over a period of time by its standard deviation. The tSNR is first computed per voxel, and then averaged across all voxels in the brain. Given the fact that the distribution of power across frequencies in fMRI follows a 1/frequency relationship [204], the tSNR is dominated by lower frequencies. Hence, a high tSNR corresponds largely to low ALFF and vice versa. As the tSNR is affected by all frequencies, it reflects all physiological fluctuations [203]. Given that an increase in brain activity is accompanied by increased spontaneous signal fluctuations [205], we hypothesized that the psychotropic effects of THC would be reflected by reduced tSNR in the involved regions.

Materials and Methods

This study is part of the Pharmacological Imaging of the Cannabinoid System (PhICS) study, a comprehensive research project on the role of the endocannabinoid system in the regulation of cognitive brain function in healthy volunteers and patients with psychiatric disorders. Methods and study protocol are reported in detail in a methodological paper [206]. Here we focus on ASL and resting-state fMRI measurements.

Subjects

Twenty-six male subjects were scanned at the University Medical Centre Utrecht after the inhalation of either placebo or THC, on two separate study days. In- and exclusion criteria are described by van Hell et al. [206], the most important being that subjects needed to be occasional cannabis users (i.e. use at least four times a year but at most once a week) who never had negative experiences after cannabis use (e.g. bad trip, cannabis-induced psychosis). Subjects were excluded if they or their first degree relatives were diagnosed with a psychiatric disorder according to DSM IV criteria. All volunteers gave written informed consent before entry into the study and were paid 250 euros for participation. For the progress of subjects in the study, see the CONSORT diagram in figure 4.1. The

study was approved by the Ethical Committee of the University Medical Centre Utrecht in accordance with the Declaration of Helsinki 2008.

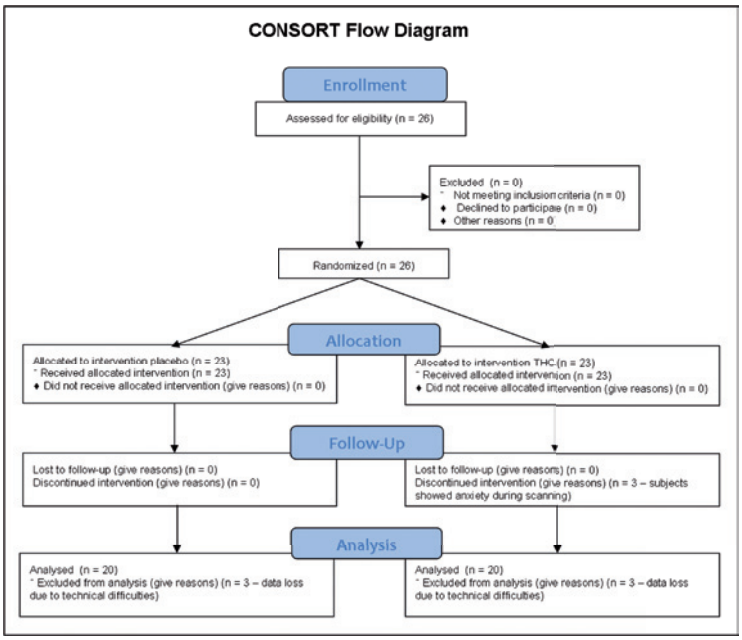

Figure 4.1 CONSORT flow diagram to illustrate the progress of all subjects in the study. Twenty-six patients were included, three subjects did not finish study procedures due to feelings of anxiety during scanning, and for both ASL and resting-state, data of three subjects were lost due to technical difficulties.

Procedure

At a practice session, subjects completed personality questionnaires and performed the Dutch Adult Reading Test (DART) to estimate verbal intelligence. Subsequently, the procedure of drug administration (inhalation) was practiced and participants were familiarized with the scan protocol in a mock scanner to reduce stress effects on the following test days. The actual study consisted of two test days, separated by at least two weeks to allow for complete clearance of drugs. Subjects were instructed not to use cannabis from two weeks before the first test day until study completion. Clearance of drugs was tested by means of a urine sample at the beginning of each test day. Additionally, no alcohol was permitted in the 48 hours preceding a test day, and subjects needed to refrain from smoking, eating and drinking during four hours preceding a test session. A standard breakfast or lunch was provided at the beginning of each test day, to ensure equal states of metabolism on both test days. A catheter was placed in the arm for venous blood sampling. On test days subjects received THC or placebo by means of a Volcano® vaporizer [39] at several timepoints. Vehicle (ethanol only) was used as a placebo. The first dose consisted of 6 mg THC or placebo. To maintain equal levels of intoxicating effects throughout the

experiment, upload dosages of 1 mg were used, 30 minutes apart. Two ASL scans were performed, one before and one after the first administration of THC or placebo. Resting-state fMRI was measured after the 2nd or 3rd upload dose of THC or placebo (see figure S4.1, and [206]).

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. [39]. Subjective effects were measured before and after each task and throughout the test day using self-reported Visual Analogue Scales (VAS) [39,176,177]. Psychedelic effects were assessed using an adapted version of a 13-item VAS, originally described by Bowdle and colleagues [39,177]. The VAS "Feeling High" (defined as the specific psychological effects experienced after THC or cannabis intake) was analyzed individually and composite scores of "External Perception" and "Internal Perception" were 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 [39]. A computerized version of both rating scales was performed consecutively (see also [206]). Furthermore, heart rate and respiratory function were monitored continuously during scanning. Heart rate was assessed by measuring the electrocardiogram using four electrodes attached to the subject's chest, and respiratory function was assessed by measuring the expansion of a respiration band around the subject's abdomen. The cardiac and respiratory signal were sampled at a frequency of 500 and 100 Hz, respectively.

Scanning parameters

Image acquisition was performed on a Philips Achieva 3.0 Tesla MR scanner with a Quasar dual gradient set.

ASL

Pseudo-continuous labeling was performed by employing a train of Radio Frequency (RF) pulses (duration 0.5 ms) with an interpulse pause of 0.5 ms in combination with a balanced gradient scheme [207, 208]. The duration of labeling was 1650 ms. The control situation was achieved by adding 180° to the phase of every other RF pulse. ASL imaging was performed in combination with background suppression, which consisted of a saturation pulse immediately before labeling and inversion pulses at 1680 and 2830 ms after the saturation pulse. Imaging was performed with single-shot echo planar imaging (EPI) in combination with parallel imaging (SENSE factor 2.5). In total, 17 slices of 7 mm slice thickness were acquired in ascending fashion with an in-plane resolution of 3*3 mm^2. Imaging was performed 1525 ms after labeling stopped. The total scan time for a pair of control and label images was 8 sec. For measurement of the magnetization of arterial blood (M_0) and also for segmentation purposes, an inversion recovery sequence was 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) [175].

Resting-state fMRI

For the BOLD resting-state scan, a single run of 400 volumes was obtained over a period of 4 minutes using a SENSE-PRESTO scan protocol [164] (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; acquisition time per volume 0.6075 s; 40 slices; sagittal orientation). A high-contrast volume with a flip angle of 27° (FA27) was scanned for registration purposes. Before the functional imaging run, a high-resolution whole brain anatomical scan was 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).

During the ASL scans as well as the BOLD fMRI resting-state scan, subjects were instructed to lie still and keep their eyes open.

Analysis

Behavioral and physiological measures

VAS feeling high scores were corrected for baseline values and analyzed using repeated measures ANOVA with drug and time as within-subject factors [39]. Mean heart rate was calculated for every scan (i.e., ASL before administration, ASL after administration, and BOLD fMRI resting-state, for placebo and THC sessions separately).

ASL

ASL perfusion images were motion-corrected in SPM5 (Wellcome Trust Centre for Neuroimaging, London, UK) prior to subtraction of control images from perfusion-weighted images. The subtraction images were subsequently averaged. Quantitative CBF maps were calculated in mL/100mL/min from the ASL images using a formula described by van Osch and colleagues [175].

CBF maps were realigned, normalized, and spatially smoothed (FWHM = 8 mm) in SPM5. Global CBF was calculated for every individual separately, by averaging CBF values in the whole brain excluding cerebrospinal fluid. Individual CBF maps were normalized for global CBF. Group results were analyzed using paired-sample t-tests in SPM5 (placebo versus THC).

Resting-state fMRI

fMRI preprocessing

Resting-state data were pre-processed and analyzed using SPM5. Pre-processing of data included realignment of functional images and co-registration with the anatomical scan using the FA27 volume. Subsequently, functional scans were normalized into standard MNI space and smoothed (FWHM = 8 mm).

Before statistical analyses, the BOLD signal was corrected for cardiac and respiratory measures using the RETROICOR method (for details see [209]). Cardiac and respiratory phases were calculated per image. A trigger marked peaks in the cardiac signal. Cardiac phase was

assumed to advance linearly from 0 to 2π during each peak-to-peak interval and was reset to 0 for the next cycle. For the respiratory phase, both time and amplitude of respiration were accounted for. While inhaling, the phase advanced from 0 to π, and during expiration the phase was negated. A transfer function was used to relate the amplitude of respiration to the phase of respiration (see [209]). For each functional image, the cardiac and respiratory phases were calculated. RETROICOR then modelled the relationship between the cardiac and respiratory phases and the BOLD signal, and corrected the BOLD signal accordingly. All further analyses were done on these corrected data.

Temporal signal-to-noise

To assess fluctuations in the resting-state BOLD signal, the temporal signal-to-noise ratio (tSNR) was calculated per voxel by dividing the mean signal of the timeseries (400 BOLD fMRI resting-state scans) by the standard deviation (see Supplemental Materials for an in-depth explanation of the use of tSNR). For further analysis the tSNR was averaged across all voxels. For comparison between THC and placebo, tSNR values were compared with a paired-sample t-test in SPM5.

Multiple Regression

Multiple regression analysis was used to determine the relationship between imaging data and (psycho)physiology (heart rate and subjective measures of feeling high). Regions of interest were defined based on a group comparison of THC and placebo maps for the ASL and BOLD data separately, selecting those regions that showed significant differences in perfusion ($T > 3.6$, $p < 0.001$ uncorrected) or resting-state BOLD signal ($T > 3.0$, $p < 0.005$ uncorrected) between THC and placebo conditions. For those regions, difference scores were calculated by subtracting placebo data from THC data.

For the ASL, both heart rate and feeling high scores were used as regression factors, to assess the amount of variance explained. For the BOLD resting-state data, only feeling high scores were used as regression factor, as effects of heart rate had been filtered out during pre-processing (i.e. RETROICOR method).

Results

Demographic characteristics are presented in table 4.1. Three subjects did not complete both sessions due to anxiety during scanning and were excluded from the study. Subjects were on average 21± 2.2 (SD) years old and had an IQ of 106 ± 5.2. Cannabis was used on average on 19 ± 11.2 occasions per year (median = 17.5; range 4 – 52). Subjects smoked on average 3.7 ± 8.4 cigarettes per week (median = 0, range 0 - 30) and 16.3 ± 10.2 units of alcohol were used per week (median = 14.5; range 2 – 40). Hard drugs were used on 2.3 ± 3.4 occasions life time (median = 1.0; range 0 – 15), and last use was more than 6 months prior to participation in the study.

Characteristics	mean ± SD	range
Age	21.1 ± 2.1	18 – 27
IQ	105.7 ± 5.2	94 – 113
Nicotine use last year (cigarettes / week)	3.7 ± 8.4	0 – 30
Alcohol use last year (units / week)	16.3 ± 10.2	2 – 40
Cannabis use last year (no. occasions)	19.0 ± 11.2	4 – 52
Hard drug use lifetime (no. occasions)	2.3 ± 3.4	0 – 15

Table 4.1 Demographic characteristics and patterns of drug use

Physiological and subjective measures are presented in table 4.2. THC plasma concentration reached a maximum of 85.3 ± 52.8 ng/ml 4 min after inhalation of 6 mg THC and decreased rapidly thereafter. Heart rate significantly increased after THC administration compared to placebo ($p < 0.001$) during the post-administration ASL scan. No significant difference in heart rate was found between THC and placebo during the BOLD resting-state scan. Subjective measures of feeling high were significantly increased after THC administration compared to placebo, after both the post-administration ASL scan and the BOLD resting-state scan ($p < 0.01$).

A correlation analysis showed that peak THC plasma concentration (at t = 4 minutes) correlated significantly with peak feeling high after THC (at t = 27 minutes; $r = 0.50$; $p < 0.05$), external perception ($r = 0.47$; $p < 0.05$) and internal perception ($r = 0.52$; $p < 0.05$)).

	Placebo	THC	p
Peak plasma concentration THC (ng/ml)	-	84.1 ± 52.6	n.a.
Heart rate ASL pre administration (bpm)	67.6 ± 14.5	69.2 ± 10.3	n.s.
Heart rate ASL post administration (bpm)	67.3 ± 9.8	84.3 ± 23.7	< 0.001
Heart rate BOLD (bpm)	70.6 ± 20.8	78.0 ± 20.5	n.s.
Feeling High ASL pre administration	0	0	n.a.
Feeling High ASL post administration	2.9 ± 10.3	30.3 ± 29.7	< 0.01
Feeling High BOLD	2.1 ± 8.6	24.9 ± 29.2	< 0.01

Table 4.2 Physiological and behavioral effects of placebo and THC. Bpm: beats per minute; n.s.: non-significant; n.a.: not applicable

ASL

For three subjects one or more of the pre- or post-administration ASL scans were lost due to technical malfunction of the scanner. Twenty subjects completed the pre- and post-administration ASL scans on both test days and were included in the analyses. For each session pre-administration scans were subtracted from post-administration scans to obtain difference-images. A paired-sample t-test on the difference-images (see figure 4.2) revealed that THC increased perfusion compared to placebo in the anterior cingulate

cortex, the left superior frontal cortex, and in the left and right insula ($T > 3.6$; $p < 0.001$ uncorrected, clustersize > 10 voxels; see table 4.3). THC showed significantly decreased perfusion compared to placebo in the right postcentral gyrus, as well as in the left and right occipital gyrus ($T > 3.6$; $p < 0.001$ uncorrected, clustersize > 10 voxels; see table 4.3). Effects of feeling high and heart rate (both computed as the baseline-corrected values for THC minus placebo) in the regions showing a difference between THC and placebo were assessed by means of regression analysis. Multiple regression analysis showed that the regression model containing these two factors explained a significant part of the variance in perfusion changes (THC versus placebo) in left superior frontal cortex ($r^2 = 0.48$, $p = 0.01$) and the anterior cingulate cortex ($r^2 = 0.35$, $p = 0.04$). In the right occipital gyrus this effect was near-significant ($r^2 = 0.31$, $p = 0.07$). To further break down effects of heart rate and feeling high, regression analyses were performed with either as dependent variable, and all seven regions as independent variables. No effects were observed for heart rate on model fit or separate regressors (regions). Feeling high was significantly explained (model fit $p= 0.005$) and this was mainly due to the left superior frontal cortex ($\beta = - 0.70$, $p=0.002$), and to some extent the left insula ($\beta = 0.42$, $p=0.045$). Thus, feeling high was correlated strongly negative with superior frontal cortex, and moderately positive with left anterior insula. Finally, regions were compared directly to one another, revealing only a positive correlation between the anterior cingulate and the right insula ($r = 0.704$, $p = 0.001$).

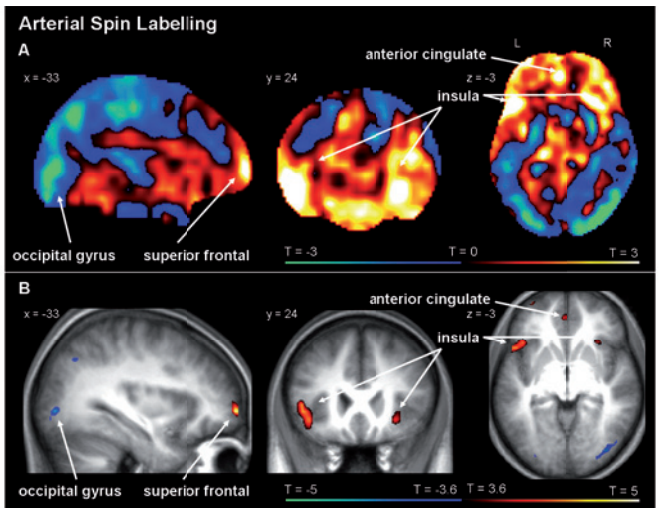

Figure 4.2 ASL difference maps between THC and placebo sessions. In red/yellow: areas showing increased perfusion during THC compared to placebo, and in blue/green: areas showing increased perfusion during placebo compared to THC. A: overall differences between THC and placebo; B: areas that are significantly different between THC and placebo ($T>3.6$, $p < 0.001$ uncorrected, cluster size > 10 voxels). L = left, R = right.

Area	Cluster size (no. of voxels)	Peak t-value	p-value clusterlevel (uncorrected)	Location of t-value		
				x	y	z
THC > Placebo						
Anterior Cingulate Cortex	16	6.31	0.007	-3	42	28
Superior Frontal Cortex L	12	5.56	0.017	-33	63	0
Insula L	59	4.92	0.000	-51	21	7
Insula R	16	4.24	0.009	30	24	-7
Placebo > THC						
Post central gyrus R	11	5.63	0.021	63	-3	21
Occipital gyrus R	50	5.97	0.000	42	-78	0
Occipital gyrus L	12	5.30	0.017	-36	-87	0

Table 4.3 Areas in which THC and Placebo show significant differences in perfusion ($T > 3.6$; $p < 0.001$ uncorrected; cluster size > 10 voxels)

Resting-state fMRI

For three subjects one of the resting-state scans was lost due to technical problems. Twenty subjects completed the resting-state scan on both test days and were included in the analysis.

Signal fluctuations in the BOLD resting-state data were assessed by comparing the tSNR-images between THC and placebo with a paired-sample t-test (see figure 4.3). THC reduced tSNR in the right insula, the left cerebellum, and the left substantia nigra ($T > 3.0$, $p < 0.005$ uncorrected; cluster size > 10 voxels; see table 4.4) compared to placebo. No areas showed significantly higher tSNR after THC administration compared to placebo. This indicates that BOLD fMRI signal fluctuations increased after THC administration. Multiple regression analysis showed no significant correlation between THC effects on tSNR and feeling high scores. Comparing THC effects in the ASL and the tSNR regions, only a trend for a negative correlation was found in the insula (ASL insula vs tSNR right insula, $r=-0.45$, $p=0.06$). Further, tSNR values were assessed in the regions where ASL showed differences between THC and placebo. In these regions, tSNR values were similar for THC and placebo.

Area	Cluster size (no. of voxels)	Peak t-value	p-value clusterlevel (uncorrected)	location of t-value		
				x	y	z
Placebo > THC						
Substantia Nigra L	16	5.72	0.022	-12	-12	-12
Insula R	19	4.59	0.014	40	16	-4
Cerebellum L	15	3.78	0.026	-12	-44	-32

Table 4.4 Areas in which THC and placebo show significant differences in temporal signal-to-noise ratio (tSNR) ($T > 3.0$; $p < 0.005$ uncorrected; cluster size > 10 voxels)

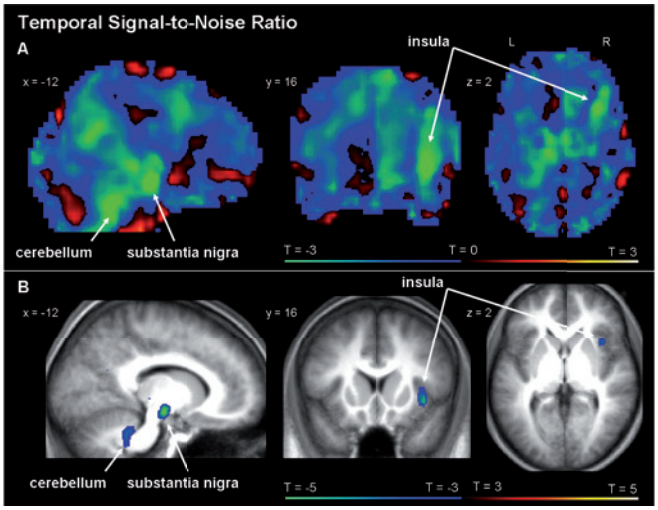

Figure 4.3 Fluctuations in the fMRI BOLD signal; difference maps of signal-to-noise ratio (tSNR) between THC and placebo sessions. In red/yellow: areas showing higher tSNR during THC than during placebo, and in blue/green: areas showing higher tSNR during placebo than during THC. A: overall differences between THC and placebo; B: areas that are significantly different between THC and placebo ($T > 3$, $p < 0.005$ uncorrected; cluster size > 10 voxels). L = left, R = right.

Discussion

This study investigated the acute effects of THC on resting-state brain perfusion and activity, measured with ASL and BOLD-fMRI respectively, in association with the psychotropic effect (feeling high). Compared to placebo, THC increased perfusion in the anterior cingulate cortex, the superior frontal cortex, and the insula, whereas perfusion was decreased in the post-central gyrus and the occipital gyrus. The feeling high effect of THC was negatively correlated with changes in perfusion in the left superior frontal cortex. THC increased fluctuation of the resting state BOLD signal in the insula, the substantia nigra and the cerebellum, reflecting increased baseline activity.

Effects of THC were observed on both perfusion and on resting state signal fluctuation in one specific region, the right anterior insula. This is a structure integrating visceral, autonomic and hedonic information [210], which is represented in its interconnections with the amygdala, hypothalamus, anterior cingulate cortex and orbitofrontal cortex [211]. The insula is implicated in many cognitive brain functions, such as emotion, attention, and motivation, and has recently been shown to play an important role in addiction [212]. Insular activity is associated with interoceptive awareness, a conscious representation of self [213] and self-reflection [214], essential for generating a mental image of one's physical state [215]. Also, the insula is regarded as the primary sensory cortex for inner body feelings like hunger and thirst, autonomic processes and awareness thereof [216]. A recent report on epileptic seizures originating in the insula indicated that this brain region plays an important role in emotion

and bodily feelings in relation to subjective awareness. It was observed that increased insular activity due to epileptic activity was associated with feelings of intense well-being [217]. Hence, our finding of increased perfusion and elevated signal fluctuation in the insula after THC administration could signify an increase in interoceptive awareness, as well as an increase in well-being and/or euphoria. The moderate correlation between feeling high and perfusion in (left) anterior insula supports this notion. Interestingly, perfusion was decreased by THC in somatosensory cortex, and in visual cortex which has been reported before [218]. It is not directly clear how this related to the psychotropic effects of THC, but it is tempting to associate it with the typically reported altered sensory perception [190]. Still, this needs further investigation.

The anterior cingulate cortex, associated with various functions including attentional processes and emotional control [219,220], evidenced elevated perfusion after THC administration which was correlated with that in right insula. These two regions are highly interconnected and are often activated together during emotional processing or bodily awareness [215,221]. Taylor and colleagues [222] showed strong resting-state functional connectivity between these regions and proposed that this may reflect integration of interoceptive information with emotional salience to form a subjective image of the bodily state. Craig [211,221] proposed a model stating that the (anterior) insula is more implicated in the sensory aspects of emotional processing, while the anterior cingulate cortex is part of the motor limbic cortices that constitutes motivation and initiates behaviour. Given that acute effects of THC include changes in bodily awareness, in motivational drive and in emotionality, our finding of correlated perfusion increases in these two regions suggests increased activity commensurate with the model of Craig, extending it with the concept of a related psychotropic effect of THC.

A strong increase in left superior frontal cortex perfusion following THC is associated with a relatively low rating of feeling high, and vice versa. This region matches the 'lateral rostral prefrontal cortex' described as being connected to anterior cingulate and anterior insula [223]. Given the notion that this network is primarily involved in tasks that require cognitive control [219,223], our data suggest that the superior frontal cortex may serve to *suppress* the feeling of high. Activity in this region may then reflect an individual's abililty to resist or overcome the sensory and perceptual effects in the service of maintaining control over one's mental state.

In literature, subjective measures of intoxication and of depersonalization due to THC are reportedly associated with magnitude of CBF increase in frontal regions [198,200]. Whether these disagree with our findings is not clear, because in those studies subjects rated 'intoxication' as opposed to 'feeling high'. Intoxication covers a mix of components including the feeling of being in a different mental state, euphoria, anxiety, drowsiness and changes in bodily awareness. Zuurman et al. [191] recently reviewed the literature that assesses acute effects of cannabis or THC, and indicated that the most reliable and stable effects that are found after acute THC or cannabis administration are elevated heart rate

and subjective effects (i.e. feeling high, stoned or euphoric). In addition, they differentiate between the scales 'high' and 'drug effect', where 'drug effect' represents 'strength of drug effect, feelings of intoxication, or subjective psychological effects'. The scales assessing 'drug effect' were not as sensitive as the scales for 'high', and addressed subjective changes that are less specific for THC or cannabis.

Differences in designs of the studies (e.g. between-subject comparisons as opposed to within-subject comparisons in the current study) or in the methods used (PET versus ASL) may also contribute to the seeming discrepancy with the present study. In the present study we corrected for changes in heart rate in the ASL group analysis and in preprocessing of the resting state data. Although in the PET studies corrections are applied for global CBF, regional effects of heart rate may not be fully corrected for. The general effect of THC in the present study (elevated perfusion in frontal regions) is, however, in line with previous PET studies [68-70]. Regardless, the observed alterations in brain neurophysiology during rest may reflect physical and emotional sensations (euphoria, feeling high) that form the psychotropic effects of THC.

One would expect higher CBF to increase restingstate fluctuations. However, direct comparison between baseline perfusion measures and restingstate tSNR showed that CBF may not simply amplify restingstate fluctuations, as tSNR values were strikingly similar for THC and placebo in the regions where ASL showed differences between the two drugs. This is worth further investigation with direct manipulations of CBF in a separate study.

THC increased signal fluctuation in the right insula, substantia nigra, and cerebellum. As these regions also exhibit high densities of cannabinoid receptors [15,18], it is plausible that THC directly affects neuronal activity by influencing endocannabinoid signalling in these regions, rather than indirectly affecting neuronal activity by changing neurotransmission through cannabinoid alteration of dopaminergic or glutamatergic signalling. The elevation of signal fluctuation is then most likely caused by increased spontaneous activity or by increased amplitude of low frequencies (which dominate our measure of signal fluctuation). This is supported by the fact that the cannabinoid system has a neuromodulatory role in the central nervous system and is capable of modulating regional synaptic activity [114,224]. Interestingly, the feeling high effect of THC is not affected by the dopamine antagonist haloperidol, supporting the notion of a direct endocannabinoid basis [225,226].

The present study has several limitations. For one, the effects were not overly strong, so a rather liberal threshold was used when comparing THC and placebo in the imaging analyses. It may be that correction for pre-administration limited sensitivity for changes. Further, the resting-state fMRI scan was made during a relatively short period of time (i.e. 4 minutes), where most studies use at least 8 minutes of rest [74,227]. The tight THC administration scheme [206] did not allow for a longer scan between upload doses. Thus, more regions may be involved in the psychotropic effects of THC than what is reported here. The ASL scans did not cover the cerebellum, perfusion of which is likely to be affected by THC given findings in earlier PET studies [69].

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. We tried to minimize the influence of expectancy by using a randomized cross-over design. All subjects received THC and placebo on two separate sessions. By randomizing the order of administration between subjects (50% of the subjects receive THC first, 50% placebo first), expectancy effects were balanced across sessions. Also, even though most subjects identified the administered drug correctly, some subjects did not notice on which test day they had received THC. Still, we cannot exclude that expectancy effects may have affected our results to some extent.

Taken together, our findings indicate that THC increases perfusion and signal fluctuations in anterior insula. Given the functions attributed to the anterior insula, we postulate that it plays an important role in the effects of THC that lead up to feeling high, which may be related to enhanced interoceptive awareness. Engagement of the left prefrontal cortex could serve to suppress this effect. Given the reported role of the prefrontal cortex in activities that require top-down control over cognitive processes [223], it may well be that engagement of this region enables one to resist the subjective effects of THC, including the feeling high. Our findings also indicate usefulness of baseline measurements in pharmacological MRI studies. THC-induced changes in fMRI brain activity during a cognitive challenge may not solely be cognitive in nature, as THC exerts subjective and physiological effects that can interact with cognitive and emotional brain function. In conclusion, the findings raise the possibility that the primary psychotropic effect of THC, feeling high, results from an interplay of regions involved in subjective interoceptive awareness and in cognitive control.

Acknowledgements

This study was performed within the framework of Top Institute Pharma, project number T5-107. We thank Storz and Bickel for kindly supplying the Volcano vaporizer and Annelies Brouwer, Emi Saliasi, Joep van der Graaf, Erik Oudman, David Terburg, Estrella Montoya and Mitul Mehta for help with data acquisition and analysis.

Supplementary Material

Methods

The relationship of tSNR to frequency amplitude is as follows. Frequency amplitudes can be quantified as power in frequency bands (in fact the square root thereof). Power across all frequencies in the timeseries constitute a power spectrum. The total area under the curve of the power spectrum divided by the power at 0 Hz is the same as the normalized variance of a timeseries. tSNR is proportional to the inverse of the square root of this value. Hence, given the 1/f property of fMRI timeseries [204], an increase in low frequency fluctuations translates to a decrease in tSNR. The inverse of the tSNR reflects the magnitude of signal fluctuation corrected for mean signal intensity. As the latter depends on scanner settings, it is not informative in comparisons across sessions. The relative fluctuation is, however, a relevant measure because the BOLD effect is itself a measure relative to mean signal intensity: the fluctuations in BOLD signal are mainly caused by changes in T2*, which is a measure of relative loss of signal intensity due to deoxyhemoglobin. Thus, tSNR is a robust estimate of the fluctuations in local concentration of deoxyhemoglobin.

Supplementary figure

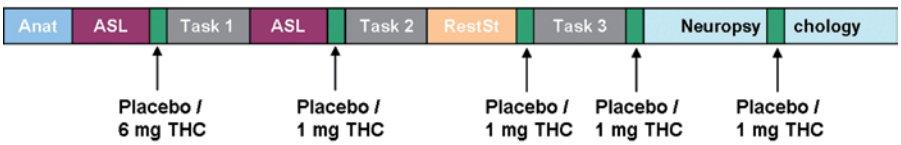

Figure S4.1 Outline of a scanning session comprising three functional MRI scans during a cognitive challenge. In addition, an anatomical scan (Anat), a resting-state scan (RestSt) and Arterial Spin Labeling (ASL) scans are acquired. Subjects receive subsequent doses of THC or placebo. The first THC dose is 6 mg, followed by four doses of 1 mg each to maintain equal levels of CNS effects. Intervals between administrations are 30 minutes. After the scanning session a neuropsychological test battery is performed outside the scanner.

Chapter 5

Involvement of the endocannabinoid system in reward processing in the human brain

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Psychopharmacology 2011: Aug [Epub ahead of print]

Abstract

Rationale: Disturbed reward processing in humans has been associated with a number of disorders, such as depression, addiction, and attention-deficit hyperactivity disorder (ADHD). The endocannabinoid (eCB) system has been implicated in reward processing in animals, but in humans the relation between eCB functioning and reward is less clear.

Objectives: The current study uses functional Magnetic Resonance Imaging (fMRI) to investigate the role of the eCB system in reward processing in humans by examining the effect of the eCB agonist Δ^9-tetrahydrocannabinol (THC) on reward-related brain activity.

Methods: Eleven healthy males participated in a randomized placebo-controlled pharmacological fMRI study with administration of THC to challenge the eCB system. We compared anticipatory and feedback-related brain activity after placebo and THC, using a monetary incentive delay (MID) task. In this task subjects are notified before each trial whether a correct response is rewarded ('reward trial') or not ('neutral trial').

Results: Subjects showed faster reaction times during reward trials compared to neutral trials, and this effect was not altered by THC. THC induced a widespread attenuation of the brain response to feedback in reward trials, but not in neutral trials. Anticipatory brain activity was not affected.

Conclusions: These results suggest a role for the eCB system in the appreciation of rewards. The involvement of the eCB system in feedback processing may be relevant for disorders in which appreciation of natural rewards may be affected such as addiction.

Introduction

Disturbed reward processing is associated with a number of psychiatric disorders in humans, such as depression [41], addiction [12], and attention-deficit hyperactivity disorder (ADHD) [42]. Animal studies have indicated that the endocannabinoid (eCB) system in the brain plays an important role in reward processing [228]. This system consists of eCB receptors and eCB ligands that work on these receptors, and has a retrograde synaptic effect on the release of various neurotransmitters, such as GABA, glutamate, and dopamine [14]. High densities of eCB receptors are found in brain structures associated with reward processing, including the ventral tegmental area (VTA), the nucleus accumbens, and prefrontal cortex [18,32]. Endocannabinoid agonists have rewarding effects, as has been shown in animals [32,228]. Also, blocking the eCB system with the antagonist rimonabant has been shown to reduce the rewarding effects of drugs of abuse such as opiates, nicotine, alcohol, and cocaine, indicating that the eCB system is involved in in the neurobiological mechanism underlying drug addiction [11,28]. Further, the cannabinoid agonist Δ^9-tetrahydrocannabinol (THC) has rewarding properties by increasing dopamine transmission in the nucleus accumbens [32]. However, whether these findings can be extrapolated to humans is unclear.

The location of eCB receptors in the human brain suggests that the eCB system is also involved in human reward processing [229,230]. An extensive network of brain regions is involved, including limbic structures (notably striatum) and frontal regions [46,79,231,232]. Chronic cannabis use has been shown to blunten the response of the striatum in anticipation of a reward [233]. Similarly, treatment with the eCB antagonist rimonabant in healthy volunteers resulted in reduced striatal brain activity during reward processing [234]. Rimonabant has been demonstrated to reduce overweight and smoking and to cause depression, but the potential involvement of the reward system is unclear [141]. However, use of rimonabant in healthy subjects is thwarted by the withdrawal of this drug from registration, following an increased risk of depression and suicide in obese patients [235]. This essentially precludes further use of this drug for elucidating the role of eCB in human brain function. An alternative approach, where the eCB system is challenged with THC, can also provide a powerful tool for studying its role in reward processing in humans. THC is the main psychoactive constituent of cannabis and possesses rewarding as well as addictive properties [14]. Human studies using Positron Emission Tomography (PET) have shown both increased dopamine transmission [33] and no change in striatal dopamine transmission after THC administration [236,237].

Here, a pharmacological fMRI study is presented that examines the involvement of the eCB system in anticipation to a reward as well as reception of the reward. A monetary incentive delay (MID) task is applied [78], an established reward paradigm which provides a measure of sensitivity to anticipation of reward as well as sensitivity to notification that the reward has been won [238]. Previous studies using this paradigm have indicated that anticipation of a reward activates the ventral striatum, and especially the nucleus accumbens, while

the reward itself activates frontal brain areas [46,78]. It was expected that THC would increase baseline dopamine transmission in the reward system. As a result, it was expected that the fMRI response to a natural reward would be decreased, especially in regions in which eCB receptors are densely distributed, such as the nucleus accumbens and prefrontal cortex.

Methods

This study is 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 cognitive brain function in healthy volunteers and patients with psychiatric disorders. Methods of the entire study are reported in detail in a methodological paper [206]. The study is registered in both the EudraCT database (2007-004247-30) and the Dutch Trial Register (NTR1787).

Subjects

Fourteen healthy male subjects participated in a randomized placebo-controlled cross-over pharmacological MRI study with THC administration. For ethical reasons, subjects needed to be occasional cannabis users (at least four times a year and at most once a week) who never had negative experiences after cannabis use. Subjects were in good health as assessed by medical history, physical examination, electrocardiogram (ECG), and routine laboratory tests. In- and exclusion criteria are described in further detail in Van Hell et al. [206]. All volunteers gave written informed consent before entry into the study and were compensated for their participation. The study was approved by the Ethical Committee of the University Medical Centre Utrecht in accordance with the Declaration of Helsinki 2008. Results are reported on eleven out of the fourteen included subjects. Two data sets were incomplete, due to respectively a technical malfunction of the scanner, and feelings of anxiety during the second scanning session. One subject was excluded from analysis due to movement artefacts. Subject characteristics are summarized in table 5.1.

Subject characteristics (n = 11)	mean ± SD	range
Age	21.7 ± 2.3	18 – 27
IQ	104.5 ± 6.0	94 – 111
Nicotine use last year (cigarettes / week)	3.0 ± 8.4	0 – 28
Alcohol use last year (units / week)	16.7 ± 8.7	2 – 40
Cannabis use last year (no. occasions)	17.9 ± 13.4	5 – 52
Illicit use lifetime (no. occasions)	1.3 ± 1.6	0 – 4

Table 5.1 Demographic characteristics and patterns of drug use

Procedure

At a training session, subjects practiced the procedure of drug administration (inhalation) and participants were familiarized with the scan protocol in a mock scanner to reduce stress effects on the following test days. The actual study consisted of two test days, separated by at least two weeks to allow for complete clearance of drugs. A standard breakfast or lunch was provided at the beginning of each test day, to ensure equal states of metabolism on both test days. Subjects were instructed not to use cannabis from two weeks before the first test day until study completion. Clearance of drugs was tested by means of a urine sample at the beginning of each test day. Additionally, no alcohol was permitted in the 48 hours preceding a test day and subjects needed to refrain from smoking, eating and drinking during four hours preceding each session.

Drug administration

On test days subjects received THC or placebo by means of a Volcano® vaporizer [39,206] at four time points. The first dose consisted of 6 mg THC or placebo. To maintain average stable levels of intoxicating effects throughout the experiment, upload dosages of 1 mg were used, 30 minutes apart, as predicted from previously described dose-effect relationships [38]. After the first three administrations of THC or placebo, subjects performed several cognitive tasks during which fMRI scans were obtained. After the last dose of THC or placebo, a battery of neuropsychological tasks was performed (see also [206]). Here, results are reported for the monetary incentive delay (MID) task, results of other assessments are reported elsewhere.

Drug effects

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 [39].

Subjective effects were measured at baseline and before and after each task and throughout the test day using self-reported Visual Analogue Scales (VAS) [176,177]. Heart rate and respiratory function were monitored continuously during scanning. Heart rate was assessed by measuring the electrocardiogram using four electrodes attached to the subject's chest, and respiratory function was assessed by measuring the expansion of a respiration band around the subject's abdomen.

Task

The MID task was based on the paradigm described by Knutson and colleagues [78] (see also [233] and figure 5.1). The task consisted of 48 trials, each lasting eight seconds on average (range 6–12 sec). At the beginning of each trial a cue was presented signalling a trial in which a reward could be won ('reward trial', a circle) or a trial that was never rewarded ('neutral trial', a square). After the cue, a target was presented for a very short time, and

subjects had to press a button before the target disappeared. After each reward trial feedback was given which indicated a successful ('hit') or unsuccessful response ('miss') with the amount of money won (respectively '2 euro' or '0 euro'), as well as the total reward. Anticipation time (the time between cue and target) and inter trial interval were varied (4.3-10.3 sec; mean 6.6 sec, and 0-30 sec; mean 4.2 sec, respectively).
Prior to the experiment, ten practice trials were presented to familiarize subjects with the task. From the practice data, the shortest reaction time to a target was used to determine an individual threshold. Half of the targets in reward trials were presented 200 ms longer than the individual threshold, and half of the trials 150 ms shorter to ensure a close to equal number of correct and incorrect responses, to achieve optimal statistical power, as well as a similar total monetary reward for all subjects. Neutral trials were presented with an identical distribution as reward trials.

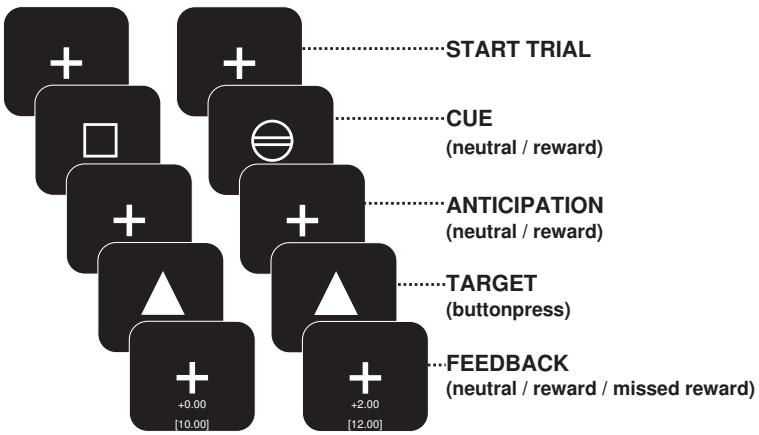

Figure 5.1 MID paradigm (see also Van Hell et al. 2010)

Scanning parameters

Image acquisition was performed on a Philips Achieva 3.0 Tesla MR scanner with a Quasar dual gradient set. Functional imaging was performed using a SENSE-PRESTO scan protocol [164]; 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, 1182 volumes). A high contrast volume with a flip angle of 27° (FA27) was scanned for registration purposes. Before the functional imaging run, a high-resolution whole brain anatomical scan was 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).

Analysis

Behavioral and physiological measures

VAS scores were corrected for baseline values and analyzed using repeated-measures ANOVA with drug and time as within-subject factors [39,206]. Mean heart rate during the MID task was calculated for placebo and THC sessions separately.

Task Performance

Reward task performance was measured using reaction times (RT) on neutral and rewarding trials. A repeated-measures analysis with drug (two levels: THC and placebo) and condition (two levels: reward and neutral) was performed to analyze differences between THC and placebo, and rewarding and neutral trials.

fMRI

Functional MRI data were pre-processed and analyzed using SPM5 (Wellcome Trust Centre for Neuroimaging, London, UK). Pre-processing of data consisted of realignment of functional images and co-registration with the anatomical volume using the FA27 volume. After realignment, functional scans were spatially normalized into standard MNI space and smoothed (FWHM = 8 mm) to reduce the effect of between-subject spatial variability in activation.

For each individual subject, regression-coefficients for each voxel were obtained from a general linear model regression analysis using a factor matrix that contained factors representing event-related changes time-locked to anticipation and feedback of neutral and reward trials (hits and misses modelled separately). All factors were convolved with a canonical hemodynamic response function. For anticipation, the variable anticipation period was used as the expected duration of brain activity. For feedback, a fixed period (duration of feedback period) was used as the expected duration of brain activity. To reduce the presence of slow trends in the signal, a high-pass filter with a cut-off frequency of 0.007 Hz was applied to the data.

As this is the first study exploring the effects of THC administration on reward processing in the brain, we chose to perform Region of Interest (ROI) analyses on areas that were involved in this particular task. This approach has been described previously as a powerful approach to explore data in a complex design (see [239]). Group activation maps were created for placebo and THC sessions separately. ROIs were calculated based on two contrasts that were sensitive for signal changes related to reward. The first group map contrasted anticipation of rewarding targets versus anticipation of neutral targets (denoted as 'ANT'). The second group map contrasted feedback of rewarded targets versus feedback of missed targets (denoted as 'FB'). ROIs were constructed by clustering neighbouring voxels that reached threshold in either the placebo or the THC session (ANT thresholded at $t>3.2$, $p<0.005$; FB (thresholded at $t>4.1$, $p<0.001$). Constructing the ROIs based on the highest

values in either the THC or the placebo session prevents bias towards our hypothesis [240,241]. Mean signal change for each ROI, each subject and each condition were based on beta values averaged over voxels in each ROI, extracted using Marsbar [242].

All hypothesis tests were performed using SPSS 15. To measure THC effects on anticipation, an overall repeated-measures MANOVA was performed on ANT ROIs with drug (two levels: THC and placebo), condition (two levels: reward and neutral) and ROI (fourteen levels) as within-subjects factors. Follow-up ANOVA analyses were performed for every ROI separately with drug and condition as within-subjects factors. To measure effects of THC on reward feedback activity, repeated-measures MANOVA were performed on FB ROIs, for neutral and reward trials separately, with drug (two levels), condition (two levels: hits and misses), and ROI (ten levels) as within-subjects factors. Follow-up ANOVA analyses were again performed for every ROI.

Results

Behavioral results

THC plasma concentration reached a maximum of 60.1 ± 33.7 ng/ml five minutes after inhalation of 6 mg THC and decreased rapidly thereafter (also see [206]). Repeated-measures ANOVA with drug (two levels) as within-subject factor showed that THC administration increased subjective scores of 'feeling high' ($F(1,10) = 10.4$, $p < 0.01$) and heart rate ($F(1,10) = 8.0$, $p < 0.02$). THC decreased 'alertness' ($F(1,10) = 6.6$, $p < 0.03$) and induced a trend towards increased 'internal perception' (reflecting inner feelings that do not correspond with reality) ($F(1,10) = 3.6$, $p < 0.09$) (see table 5.2).

	Placebo	THC	p
Heart rate (beats per minute)	68.9 ± 8.5	83.8 ± 20.8	0.018*
VAS Feeling High	0.2 ± 0.8	19.5 ± 19.9	0.009*
VAS Internal Perception	-0.4 ± 1.5	1.3 ± 2.7	0.086
VAS External Perception	1.5 ± 2.5	6.7 ± 9.6	0.13
VAS Alertness	-1.5 ± 7.8	-10.6 ± 9.7	0.028*
VAS Contentedness	-1.5 ± 4.7	-4.2 ± 4.8	0.11
VAS Calmness	1.5 ± 7.9	1.8 ± 10.7	0.94

Table 5.2: Physiological and behavioral effects of placebo and THC (mean ± SD). * significant difference between THC and placebo

Performance MID task

Repeated-measures ANOVA with drug (two levels) and condition (two levels) as within-subject factors showed a significant effect of condition ($F(1,10) = 22.0$, $p = 0.001$), indicating

that subjects were faster on reward trials than on neutral trials, and a near significant effect of drug ($F(1,10) = 4.5$, $p = 0.06$), indicating that subjects were slower after THC administration compared to placebo (see figure 5.2). A post-hoc paired-t test per condition indicated that this effect was most pronounced during reward trials ($t = 2.2$, $p = 0.051$).

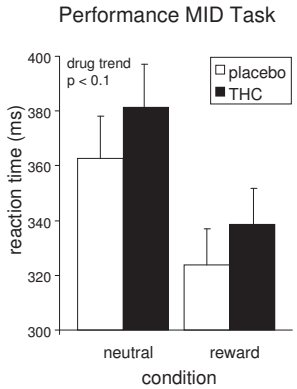

Figure 5.2 Performance of the MID task. Error bars denote Standard Error of the Mean (SEM)

fMRI results

Anticipation

ANT ROIs included left and right caudate nucleus, left and right parietal cortex, middle cingulate, left and right cerebellum, left pre/postcentral gyrus, left insula, anterior cingulate / supplementary motor area, right inferior orbitofrontal gyrus, right middle and inferior frontal gyrus, and the thalamus / brain stem (see figure 5.3a and figure S5.1 and table S5.1a).

Repeated-measures MANOVA revealed no significant effect of drug ($F = 0.01$, $p = 0.9$) or drug by condition ($F = 0.03$, $p = 0.9$) (see figure 5.4a). A significant drug by condition by ROI interaction effect ($F = 2.5$, $p < 0.05$) indicated that drug by condition effects differed between ROIs. Follow-up analysis per ROI (not corrected for multiple comparisons) revealed no significant drug effect in individual ROIs. One ROI, the right inferior orbitofrontal gyrus, showed a significant drug by condition interaction effect ($F = 7.9$, $p < 0.05$; see figure 5.4b and table S5.1b). This interaction was a result of a larger signal increase in anticipation of a reward after THC administration than after placebo administration. However, the individual ROI effect did not survive correction for multiple comparisons.

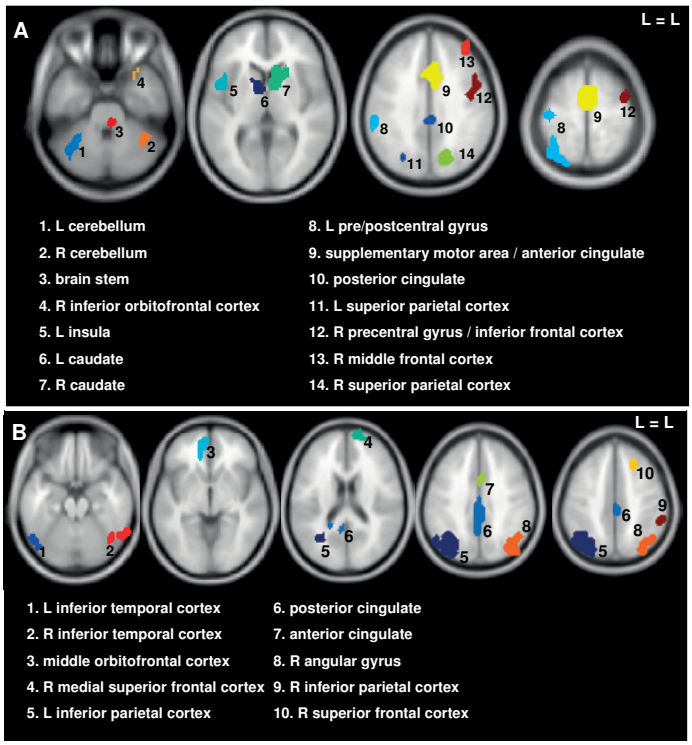

Figure 5.3 Regions of Interest; based on pooled group activation maps of THC and placebo; a: reward anticipation; b: reward feedback. L = left, R = right

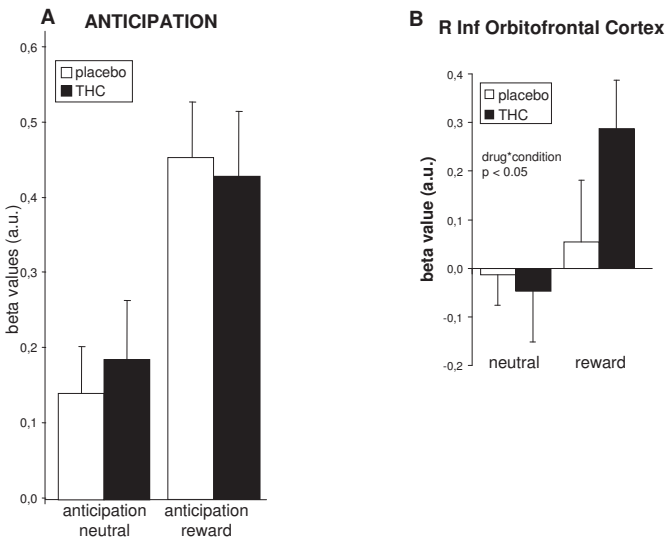

Figure 5.4 Brain activity during anticipation. a: brain activity during anticipation averaged across ROIs; b: right inferior orbitofrontal cortex. Error bars denote Standard Error of the Mean (SEM). a.u. = arbitrary units; R = right; Inf = inferior. p-values are not corrected for multiple comparisons

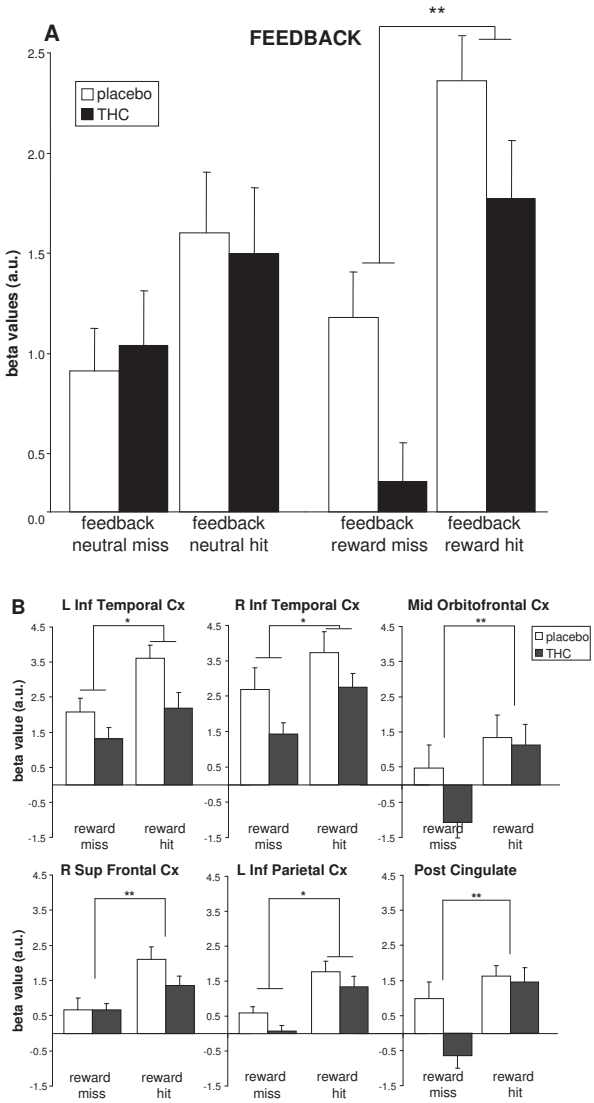

Figure 5.5: Brain activity during feedback. a: brain activity during feedback averaged across ROIs; b: reward feedback in separate ROIs. Error bars denote Standard Error of the Mean (SEM). L = left, R = right; Inf = inferior; Sup = superior; Post = posterior; Cx = cortex; a.u. = arbitrary units. * significant drug effect ($p < 0.05$); ** significant drug by condition interaction effect ($p < 0.05$). p-values are not corrected for multiple comparisons

Feedback

FB ROIs included the inferior parietal and temporal gyrus bilaterally, posterior and anterior cingulate, middle orbitofrontal gyrus, and right superior frontal gyrus (see figure 5.3b and figure S5.2 and table S5.2a). No differences were found in FB ROIs between THC and placebo during neutral feedback (see figure 5.5a). A repeated-measures MANOVA showed

that during reward trials, THC administration caused a significant reduction in reward-related brain activity (drug effect: $F = 13.1$; $p < 0.01$; see figure 5.5a), indicating that THC reduced the signal for hits as well as misses. Further analysis per ROI (not corrected for multiple comparisons; see figure 5.5b and table S5.2b) showed that this main drug effect was present in the left inferior parietal cortex ($F = 5.5$, $p < 0.05$), inferior temporal gyrus bilaterally (left: $F = 8.2$, $p < 0.05$ and right: $F = 7.4$, $p < 0.05$), and at trend level in the posterior cingulate ($F = 4.8$, $p < 0.1$) and right inferior parietal cortex ($F = 4.2$, $p < 0.1$).

In addition, an overall interaction effect of drug by condition by ROI ($F = 4.6$; $p = 0.001$) was found, indicating that the drug by condition effect differed between ROIs. This interaction was a result of the fact that two different drug by condition interaction effects were found in individual ROIs: THC decreased the signal change for a miss but not for a hit in the posterior cingulate ($F = 6.3$, $p < 0.05$), and the middle orbitofrontal cortex ($F = 12.6$, $p < 0.01$), while THC reduced the signal change related to a hit but not a miss in the right superior frontal cortex ($F = 6.7$, $p < 0.05$). A trend for an interaction effect of drug by condition in the left inferior temporal gyrus indicated a larger attenuation of brain activity during hits than misses after THC administration ($F = 3.5$; $p < 0.1$). However, none of these ROI effects survived correction for multiple comparisons.

Discussion

In this study the role of the eCB system in reward processing in humans was examined by assessing the effects of THC administration on brain activity during monetary reward anticipation and feedback. Subjects showed similar behavioral responses to reward during THC and placebo, with faster responses if a reward could be won. THC administration attenuated brain activity during reward feedback compared to placebo. THC did not affect brain activity related to feedback if there was no possibility to win a reward. These results indicate that THC administration predominantly reduces the effect of feedback in situations where there is the possibility to earn a reward, and suggest involvement of the eCB system in appreciation of a received reward. This effect was largest in the inferior parietal and temporal cortex. The inferior parietal cortex is a pivotal part of the attention network [243,244]. More specifically, the inferior parietal cortex is associated with a salience representation of the outside world [245], indicating that attention is directed towards salient- or task-relevant objects. Previous studies have shown that eCB affects inferior parietal cortex function, as decreased activity has been reported after THC administration in association with auditory attention [246], and during emotional processing [202]. Hence, our result suggests a general effect of THC on attentional processes during salient feedback.

Subjects reacted faster on the task when a reward could be won, during both THC and placebo, an effect that has been reported previously [78,79]. This indicates that subjects were motivated to perform the task during both placebo and THC. However, subjects were slower after THC administration during both neutral and reward trials, indicating that

THC has a more generalized motor or attentional effect in addition to the reward-specific effects observed in the present study. Indeed, another study assessing the effects of acute THC administration reported that THC can increase reaction times on a number of tasks assessing either memory, attention or simple reaction times [120].

Analyses of individual ROIs yielded some effects of THC (described in the results section) but none survived the corrected threshold for multiple comparisons. They do suggest that brain activity during feedback was attenuated most in the posterior cingulate, the superior frontal cortex and orbitofrontal cortex. These have all been associated with reward processing [247-252] or effects of THC administration [202,253]. Nevertheless, the most robust finding was a network-wide effect of THC.

The eCB system has been implicated in various aspects of addiction, such as drug-seeking and relapse [28]. Animal studies showed that activating the eCB system with an agonist provoked relapse to use of other drugs, suggesting a generic, but complex role for eCB in reward [54]. Blocking the eCB system with the antagonist rimonabant led to the opposite effect, reducing drug-seeking and relapse [254]. One could argue that if THC activates the eCB system and thereby induces mild elevation of activity of the reward system [33,253], the impact of other rewarding stimuli may be dampened as a result, thus explaining the attenuation of brain activity due to a monetary reward. An indication for such a mechanism can be derived from the effect of chronic cannabis use on motivation in general. Although there is no clear evidence for loss of motivation, the negative effects of chronic cannabis use on school performance and later life outcomes may be seen as an indication [255], but this interpretation warrants further investigation.

In our study, anticipation of a reward activated the striatum, insula, anterior cingulate and frontal brain regions, and feedback of reward activated the posterior and anterior cingulate, inferior parietal cortex, orbitofrontal and superior frontal cortex. This pattern of brain activity is in line with previous reward imaging studies, which have reported striatal activity during anticipation, and frontal activity during feedback of reward [46,79,231,232].

The nucleus accumbens did not show significantly elevated activity during reward anticipation in our study. Additional ROI analyses with the nucleus accumbens as an anatomically defined ROI also did not show a significant effect of THC (data not shown). Factors that may have reduced effects of reward in the striatum and nucleus accumbens include the fact that subjects were paid 250 euro for participation in the study. An extra reward of 24 euro that was won during the reward task may have been too small in comparison. Specifically for the nucleus accumbens, it can be noted that fMRI measurements in this region tend to be less reliable, as it is located close to the nasal cavity, which reduces the BOLD signal to noise ratio.

The significant anticipatory effect of reward in striatum was slightly reduced after THC, but this effect was too small to become significant. This is contrary to what we expected, as the striatum shows high densities of CB1 receptors and THC is known to elicit rewarding and dopamine-elevating effects on the striatum [32,33]. However, recent PET studies in humans

indicated that the role of THC in increasing dopamine levels in the striatum may be limited [236,237]. Effects of THC in the striatum may also have been influenced by the fact that our subjects were occasional users of cannabis (see also [233]). It may also be possible that the effect of THC on striatal activity is domain specific, and limited for anticipatory reward activity. There have not been any previous studies examining effects of THC on striatal reward processing activity to compare our results to, but previous studies using different cognitive paradigms have shown attenuation of ventrostriatal activity after THC during retrieval of memory [192] and an increase in brain activity in the caudate during response inhibition [178], which would be in line with the hypothesis that THC effects may be domain specific.

The results from the current study should be interpreted with due care. Our sample size was small for an fMRI study which compares data between sessions. A larger sample could have revealed more effects of THC, for instance during task anticipation in striatal areas. In addition, 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 cross-over design, thus balancing expectancy effects across sessions. Still, it cannot be excluded that expectancy effects may have affected our results to some extent.

In conclusion, this study provides new arguments for eCB involvement in the reward system in humans. Findings suggest that THC affects appreciation of obtaining a monetary reward. The involvement of the eCB system in feedback processing may be relevant for disorders in which appreciation of natural rewards may be affected such as addiction.

Supplementary Information

Involvement of the endocannabinoid system in reward processing in the human brain.

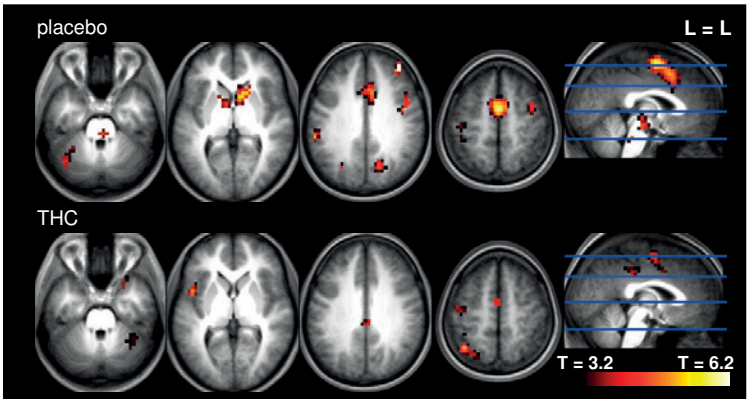

Figure S5.1A: Activated areas during reward anticipation after placebo and after THC administration. T > 3.2, p < 0.005, cluster size > 20 voxels. L = left.
No significant differences were found between THC and placebo when performing a whole brain paired-sample-T-test.

Area	BA	Max T	Cluster size	MNI coordinates		
				x	y	z
After placebo administration						
R middle frontal cortex	46	7.9	30	40	44	32
R precentral gyrus	6	7.1	101	48	0	40
R caudate	-	6.0	58	12	8	0
Supplementary motor area/anterior cingulate	6 / 24	5.8	283	0	-4	60
L precuneus	7	5.3	54	-12	-72	48
L inferior parietal cortex	2 / 40	5.1	33	-60	-36	36
R precuneus	7	4.9	59	12	-72	44
L cerebellum	37	4.6	35	-32	-52	-32
Brainstem	-	4.5	56	0	-20	-12
L postcentral gyrus	3 / 4	4.4	32	-48	-24	56
L caudate	-	4.2	24	-8	4	4
After THC administration						
R inferior orbitofrontal cortex	38	8.7	22	28	24	-24
L inferior frontal operculum	48	6.5	41	-44	12	8
L inferior parietal cortex	40	5.5	72	-40	-60	60
Supplementary motor area	6	4.9	27	-4	-4	56
Middle cingulate	-	4.8	29	-8	-40	48
L pre/postcentral gyrus	4 / 6	4.3	41	-48	-12	56
R inferior temporal cortex	37	4.2	25	44	-48	-24

Table S5.1A: Network of activated areas during reward anticipation after placebo and after THC administration. T > 3.2, p < 0.005. BA = brodmann area; Max T = maximum T-value; R = right; L = left

	Area	Max T	Cluster size	MNI coordinates			Repeated-Measures ANOVA			
							drug		drug*condition	
				x	y	Z	F	p	F	p
1	L cerebellum	4.6	42	-32	-52	-32	0.08	0.8	0.6	0.4
2	R cerebellum	4.2	31	44	-48	-24	0.07	0.8	1.1	0.3
3	Brain stem	4.5	68	0	-20	-12	0.2	0.6	1.0	0.3
4	R inferior orbitofrontal cortex	8.7	27	28	24	-24	1.5	0.2	7.9	0.02
5	L insula	6.5	49	-44	12	8	0.4	0.5	2.6	0.1
6	L caudate	4.2	30	-8	4	4	1.1	0.3	0.001	1.0
7	R caudate	6.0	70	12	8	0	0.06	0.8	0.7	0.4
8	L pre/postcentral gyrus	4.4	209	-48	-24	56	0.03	0.9	0.2	0.7
9	SMA / anterior cingulate	5.8	326	0	-4	60	0.03	0.9	1.4	0.3
10	Posterior cingulate	4.8	36	-12	-72	44	0.6	0.4	3.1	0.1
11	L superior parietal cortex	5.5	65	-40	-60	60	0.7	0.4	0.9	0.4
12	R precentral gyrus	7.1	123	48	0	40	0.1	0.7	1.1	0.3
13	R middle frontal cortex	7.9	37	40	44	32	0.009	0.9	0.8	0.4
14	R superior parietal cortex	4.9	71	12	-72	44	0.06	0.8	0.7	0.4

Table S5.1B: Regions of Interest during reward anticipation. Max T = maximum T-value; R = right; L = left. Results of Repeated-Measures ANOVA per ROI with drug and condition as within-subject factors. Results are not corrected for multiple comparisons.

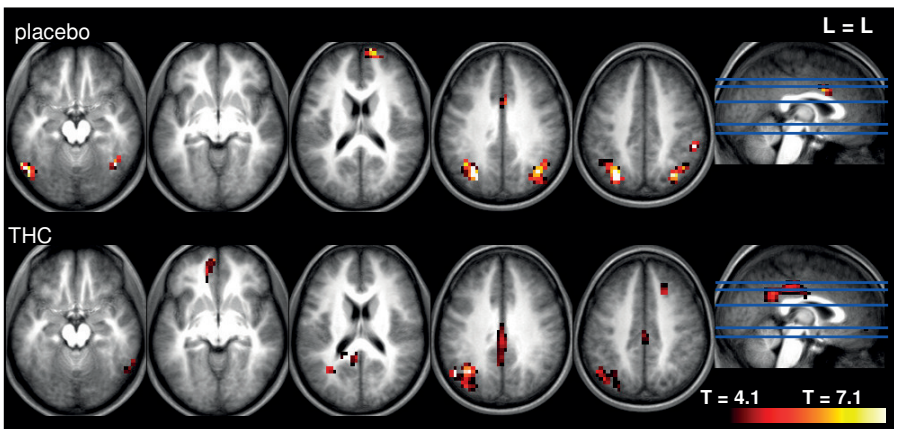

Figure S5.2: Activated areas during feedback of reward after placebo and after THC administration. $T > 4.1$, $p < 0.001$. L = left.
No significant differences were found between THC and placebo when performing a whole brain paired-sample-T-test.

Area	BA	Max T	Cluster size	MNI coordinates		
				x	y	z
After placebo administration						
L inferior parietal cortex	7/19/40	15.4	186	-32	-68	36
R medial superior frontal cortex	10	8.7	25	16	68	16
R inferior parietal cortex	40	8.7	31	56	-40	48
L inferior temporal cortex	37	8.5	36	-56	-64	-20
R angular gyrus	7	8.3	111	40	-68	40
R inferior temporal / fusiform gyrus	37	7.2	22	44	-60	-16
anterior cingulate	24	6.1	25	0	8	32
After THC administration						
R inferior temporal gyrus	37	8.3	20	60	-60	-20
L angular gyrus	39	7.5	192	-52	-64	36
Posterior cingulate	23	6.4	168	-4	-48	28
Middle orbitofrontal cortex	10	6.1	26	-4	60	-4
R superior frontal cortex	8 / 32	5.9	22	16	20	52

Table S5.2A: Network of activated areas during reward feedback after placebo and after THC administration. BA = brodmann area; Max T = maximum T-value; R = right; L = left

	Area	Max T	Cluster size	MNI coordinates			Repeated-Measures ANOVA			
							drug		drug*condition	
				x	y	z	F	p	F	p
1	L inferior temporal cortex	8.5	43	-56	-64	-20	8.2	0.02	3.5	0.1
2	R inferior temporal cortex	8.3	51	60	-60	-20	7.4	0.02	0.7	0.4
3	Middle orbitofrontal cortex	6.1	31	-4	60	-4	2.4	0.2	12.6	0.01
4	Medial superior frontal cortex	8.7	30	16	68	16	1.3	0.3	6.7	0.03
5	L inferior parietal cortex	15.4	350	-32	-68	36	5.5	0.04	0.1	0.7
6	Posterior cingulate	6.4	202	-4	-48	28	4.8	0.05	6.3	0.03
7	Anterior cingulate	6.1	30	0	8	32	0.9	0.4	0.3	0.6
8	R angular gyrus	8.3	132	40	-68	40	4.2	0.07	0.4	0.6
9	R inferior parietal cortex	8.7	39	56	-40	48	0.5	0.5	0.1	0.7
10	R superior frontal cortex	5.9	27	16	20	52	0.7	0.4	3.3	0.1

Table S5.2B: Regions of Interest during reward feedback. Max T = maximum T-value; R = right; L = left. Results of Repeated-Measures ANOVA per ROI with drug and condition as within-subject factors. Results are not corrected for multiple comparisons.

Chapter 6

Acute THC administration attenuates reward processing in nicotine addiction

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Abstract

Introduction: The endocannabinoid (eCB) system has been implicated in both reward processing and the pathophysiology of addiction. Addiction has been associated with altered reward processing in the brain. The aim of the current study was to elucidate the involvement of eCB in altered anticipation or feedback processes related to reward in addiction.

Methods: Thirteen healthy controls and ten subjects with a nicotine addiction participated in a pharmacological MRI study with Δ^9-tetrahydrocannabinol (THC) administration to challenge the eCB system. A Monetary Incentive Delay (MID) task with a small and large reward was used to generate a response of the brain reward system. Activity in the nucleus accumbens and striatum during anticipation and feedback of reward was compared after administration of THC and placebo.

Results: Nicotine users showed blunting of reward anticipation in the nucleus accumbens after THC administration compared to placebo administration. THC did not affect brain activity during anticipation of reward in healthy controls. THC did not affect reward-related feedback activity in healthy controls or nicotine users, but nicotine users did show attenuated feedback activity which was not specific for reward.

Conclusion: Our results show that in nicotine users the nucleus accumbens is insensitive to monetary reward after THC administration. These results suggest that nicotine addiction, and possibly addiction in general, is linked to an eCB system that functions different from that in controls.

Introduction

Drug use is one of the most important causes of health problems and accounts for a substantial proportion of total deaths [2]. Smoking for instance, is the largest cause of avoidable death in the European Union. In the United States 28% of the population use tobacco on a regular basis [3], and the prevalence of adult nicotine addiction in Europe is around 30%.

These numbers illustrate the importance of gaining knowledge about processes in the brain related to addiction. One major hypothesis, called the reward deficiency hypothesis, posits that in subjects prone to substance abuse, non-drug rewards fail to adequately activate the brain's motivational circuitry, while drugs of abuse do activate this system [91,256]. A recent review on brain imaging in addictive disorders has shown that, although results are not conclusive, reward processing is affected in addictive disorders [238]. For one, alcohol dependence has been associated with increased ventral striatal recruitment during notification of monetary reward [257]. Abstinent alcoholics on the other hand, exhibit an attenuated ventral striatal response to reward anticipation [112]. In nicotine addicted subjects, a decreased reward-related brain response in the ventral striatum has been reported repeatedly [110,233,258].

There are compelling reasons to believe that the endocannabinoid (eCB) system could be an important factor involved in altered reactivity of the reward system in addiction. The eCB system, with high receptor densities in frontal cortex as well as striatum [18], is primarily neuromodulatory in nature, affecting many aspects of human cognition [138]. It has been associated with drug addiction in both animal studies and clinical trials in humans [34,235]. From animal studies it is known that blocking CB1 receptors with the antagonist rimonabant prevents self-administration of several drugs of abuse [235,254]. In addition, relapse to cocaine, nicotine and ethanol is reduced in abstinent animals pre-treated with rimonabant [28]. In humans, rimonabant has been shown to facilitate smoking cessation in clinical trials [141].

The involvement of the eCB system in addiction, combined with the indications that addiction may be linked to altered reward processing, leads to the question whether these two findings may be related. Could it be that the eCB system plays a role in abnormal reward processing in addiction? To test this hypothesis we performed a pharmacological functional MRI study with THC administration, comparing subjects with a heavy nicotine addiction (NA) with healthy controls (HC). Reward processing was probed with a Monetary Incentive Delay (MID) task. Image analyses were focused on the nucleus accumbens and striatum, areas that have been implicated in addiction [259], reward processing [103,260], and in the eCB involvement in reward processing in animals [44]. We expected NA subjects to exhibit attenuated brain activity during anticipation of receiving a reward (anticipatory reward processing), based on earlier studies in our lab [233]. In line with the reward deficiency hypothesis [256], we expected NA subjects to be less responsive to a monetary reward after receiving THC in terms of striatal activity, in particular in the nucleus accumbens, than healthy controls.

Methods

This study is part of the Pharmacological Imaging of the Cannabinoid System (PhICS) study. Methods of the entire study are reported in detail in a methodological paper [206].

Subjects

Thirty two healthy male subjects participated in a randomized placebo-controlled cross-over pharmacological MRI study with THC administration. The NA group included fourteen subjects while eighteen subjects were included in the HC group. All subjects needed to be occasional cannabis users (use at least four times a year but at most once a week) who never had negative experiences after cannabis use (for instance a bad trip or cannabis-induced psychosis). Subjects were excluded if they or their first degree relatives were diagnosed with a psychiatric disorder, as assessed using the Mini International Neuropsychiatric Interview for DSM-IV clinical disorders (M.I.N.I: Translated Dutch Version 5.0.0 [96]). In- and exclusion criteria are described in further detail in [206]. All volunteers gave written informed consent before entry into the study and were paid 250 euro for participation. The study was approved by the Ethical Committee of the University Medical Centre Utrecht in accordance with the Declaration of Helsinki 2008.

Two HC and one NA subject did not complete the entire study procedure due to feelings of anxiety during one of the scanning sessions. One HC did not show elevated plasma levels of THC and was excluded from the analysis. One NA was excluded from analysis due to high blood pressure during the first test day. Two HC and two NA were excluded from analysis due to movement artefacts in the fMRI data. Results are therefore reported on thirteen HC and ten NA. Subject characteristics are summarized in table 6.1.

Subject characteristics	healthy controls		nicotine users	
	mean ± SD	range	mean ± SD	range
Age	21.1 ± 2.6	18 – 26	25.6 ± 6.7	20 – 40
IQ	105.2 ± 4.7	98 – 113	107.0 ± 5.0	98 – 114
Nicotine use last year (cigarettes / week)	3.2 ± 8.3	0 – 30	120.5 ± 36.0	70 – 203
FTND	0.0 ± 0.0	0	4.3 ± 1.1	3 – 6
Alcohol use last year (units / week)	15.2 ± 10.4	3 – 40	14.4 ± 9.3	2 – 30
Cannabis use last year (no. occasions)	21.9 ± 11.2	4 – 52	23.5 ± 18.4	5 – 52
Illicit use lifetime (no. occasions)	2.2 ± 4.4	0 – 15	2.4 ± 4.2	0 – 13
Peak Plasma THC concentration (ng / ml)	97.5 ± 43.8	30 – 189	81.8 ± 38.9	27 – 178

Table 6.1: Demographic characteristics

Procedure

At a training session, subjects practiced the procedure of drug administration (inhalation) and participants were familiarized with the scan protocol in a mock scanner to reduce stress

effects on the following test days. The actual study consisted of two test days, separated by at least two weeks to allow for complete clearance of drugs. A standard breakfast or lunch was provided at the beginning of each test day, to ensure equal states of metabolism on both test days. Subjects were instructed not to use cannabis from two weeks before the first test day until study completion. Clearance of drugs was tested by means of a urine sample at the beginning of each test day. Additionally, no alcohol was permitted in the 48 hours preceding a test day, and subjects needed to refrain from smoking, eating and drinking during four hours preceding a test session.

Drug administration

On test days subjects received THC or placebo by means of a Volcano® vaporizer [39,206] at four time points. Vehicle (ethanol only) was used as a placebo. The first dose consisted of 6 mg THC or placebo. To maintain equal levels of intoxicating effects throughout the experiment, upload dosages of 1 mg were used, 30 minutes apart. After the first three administrations of THC or placebo, subjects performed a cognitive task during which fMRI scans were obtained. After the last dose of THC or placebo, a battery of neuropsychological tasks was performed. Here we report on the results of the Monetary Incentive Delay (MID) task. Results of other assessments are reported elsewhere (see also [206,261]).

Drug effects

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 [39].

Subjective effects were measured at baseline and before and after each task and throughout the test day using self-reported Visual Analogue Scales (VAS) [176,177]. Heart rate and respiratory function were monitored continuously during scanning. Heart rate was assessed by measuring the electrocardiogram using four electrodes attached to the subject's chest, and respiratory function was assessed by measuring the expansion of a respiration band around the subject's abdomen.

Task

The MID task consisted of 60 trials (duration range 6 – 12 sec). At the beginning of each trial a cue was presented signalling a potentially neutral, rewarding (big or small) or loss trial (see figure 6.1). After the cue, a target was presented to which subjects had to respond as fast as possible by pressing a button. Prior to the experiment, ten practice trials were presented to familiarize subjects with the task. From these practice data, the shortest reaction time to the target was used to determine the individual time limit allowed for responses to the target during the task. Hence, in case of a reward cue subjects could win 10 cents (small) or 5 euro (big) when responding within the time limit. A loss cue indicated that subjects avoided a 5 euro loss by responding within the time limit. The function of

the loss trials was primarily to prevent adaptation to reward trials and is not used in group analyses. In addition, the task was designed to deliver reward (or avoid loss) in fifty percent of the trials for each condition, to ensure that all subjects received the same number of rewards, in both sessions. This was achieved by increasing the time limit with 200 ms in half of the trials to make sure subjects would be fast enough to win the trial, and decreasing the time limit with 150 ms in the other half of the trials to make sure subjects would miss the trial. Anticipation time (the time between cue and target) and inter trial interval were varied (4.3-10.3 sec; mean 6.6 sec, and 0-30 sec; mean 4.2 sec, respectively).

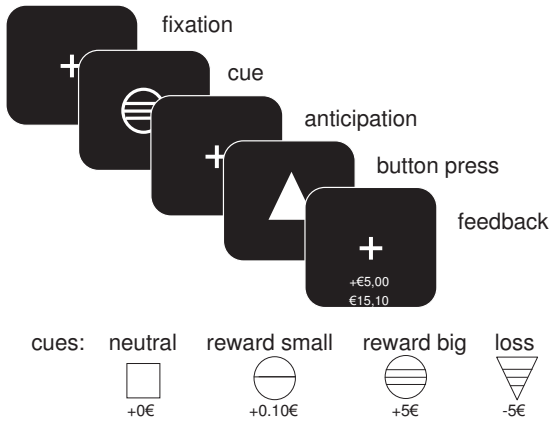

Figure 6.1: Task paradigm

Scanning parameters

Image acquisition was performed on a Philips Achieva 3.0 Tesla MR scanner with a Quasar dual gradient set (Philips Medical Systems, Best, The Netherlands). Functional imaging was performed using a SENSE-PRESTO scan protocol [164] (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, 1182 volumes). A high-contrast volume with a flip angle of 27° (FA27) was scanned for registration purposes. Before the functional imaging run, a high-resolution whole brain anatomical scan was 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).

Analysis

Behavioural and physiological measures

VAS scores were corrected for baseline values and analyzed using repeated-measures ANOVA with drug and time as within-subject factors [206] and group as between-subjects factor. Mean heart rate during the MID task was calculated for placebo and THC sessions separately and analyzed with a repeated-measures ANOVA with drug as within-subject factor and group as between-subjects factor.

Task Performance

Reward task performance was measured using reaction times (RT). A repeated-measures ANOVA with drug and condition (three levels: reward big, reward small, and neutral) as within-subject and group as between-subject factor was performed to analyze differences between THC and placebo, groups, and conditions.

fMRI

Functional MRI data were pre-processed and analyzed using SPM5 (Wellcome Trust Centre for Neuroimaging, London, UK). Pre-processing of data consisted of realignment of functional images and co-registration with the anatomical volume using the FA27 volume. After realignment, functional scans were normalized using parameters obtained from spatial normalization into standard MNI space of the anatomical volume. After normalization, functional scans were spatially smoothed with a Gaussian filter (FWHM = 8 mm).

For each individual subject, regression-coefficients for each voxel were obtained from a general linear model regression analysis using factors time-locked to task events, convolved with a canonical hemodynamic response function. For anticipation, the variable anticipation period was used as the expected duration of brain activity. The feedback conditions were modelled as events (hits and misses modelled separately). To correct for drifts in the signal, cosine-based regressors were added to the model.

To test whether THC altered brain activation in striatum and nucleus accumbens in HC compared to NA during the MID task, a region of interest (ROI) analysis was performed. We defined the nucleus accumbens by drawing a sphere with a diameter of 5 mm around MNI coordinates -12, 14, -8 (left) and 12, 14, -8 (right), and combined these ROIs. In addition, striatal brain activity was measured by defining left and right caudate nucleus and putamen using the WFU Pick Atlas, and combining these ROIs to assess striatal activity during ANT and FB.

For anticipation, we performed a repeated-measures ANOVA (linear contrast) with condition (three levels: neutral, small reward, and big reward) as within-subject factor for HC and NA during THC and placebo separately. To test for effects of THC we performed a repeated-measures ANOVA (linear contrast) with drug (two levels: THC and placebo) and condition (three levels: neutral, small reward and big reward) as within-subject factors for HC and NA separately.

To directly test for differences in reward processing between NA and HC, we performed a repeated-measures ANOVA (linear contrast) for placebo and THC separately.

For feedback, a similar set of tests were performed, using the contrast between hits and misses for the two reward conditions.

Results

Behavioural and physiological results

THC increased feeling high ($F = 24.4$, $p < 0.001$) and external perception ($F = 7.8$, $p = 0.01$) compared to placebo (see table 6.2). Alertness ($F = 27.2$, $p < 0.001$), contentedness ($F = 18.8$, $p < 0.001$), and calmness ($F = 10.1$, $p < 0.005$) significantly decreased after THC administration compared to placebo. In addition, a drug by group effect was found for calmness, indicating that HC were less calm after THC compared to NA ($F = 5.0$, $p < 0.05$). THC administration increased heart rate overall ($F = 36.0$; $p < 0.001$).

THC plasma concentration reached an average of 90.7 ng/ml. No differences between groups were found (see table 6.2). Significant correlations (although not corrected for multiple comparisons) were found between THC plasma concentration and subjective ratings of feeling high ($r = 0.51$, $p = 0.01$), external perception ($r = 0.48$, $p = 0.02$), internal perception ($r = 0.44$, $p = 0.03$), and alertness ($r = -0.48$, $p = 0.02$).

VAS score	placebo		THC		Effect	F	p
	pre	post	pre	post			
feeling high	1.5 ± 5.0	2.4 ± 11.3	24.4 ± 26.8	33.8 ± 34.2	drug	24.4	<0.001**
internal perception	0.2 ± 0.8	0.4 ± 1.3	3.0 ± 10.0	5.5 ± 13.6	drug	2.4	0.13
external perception	0.7 ± 1.7	1.3 ± 4.9	5.9 ± 14.5	12.2 ± 17.7	drug	7.8	0.01*
alertness	-5.8 ± 7.9	-6.8 ± 7.7	-18.7 ± 15.4	-22.6 ± 14.7	drug	27.2	<0.001**
contentedness	-2.6 ± 6.4	-4.2 ± 7.4	-9.8 ± 9.8	-12.7 ± 9.7	drug	18.8	<0.001**
calmness	5.4 ± 11.2	3.6 ± 13.1	-7.1 ± 20.2	-8.3 ± 15.8	drug	10.1	<0.005*
					drug*grp	5.0	<0.05*

Table 6.2: Subjective effects as measured using Visual Analogue Scales. Significant difference between THC and placebo (* $p < 0.05$; ** $p < 0.001$). pre = measured before reward task; post = measured after reward task

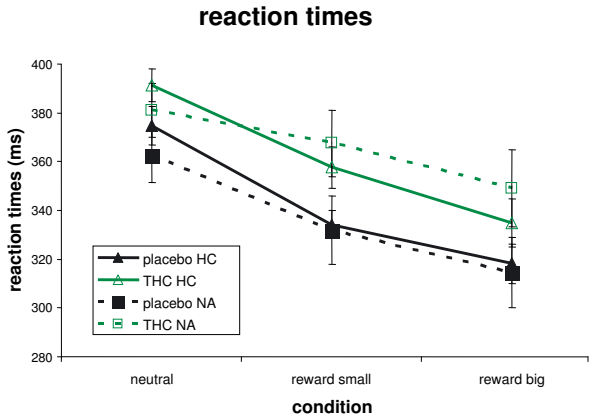

Figure 6.2: Reaction times during the MID task. Solid lines: healthy controls (HC); dotted lines: subjects with nicotine addiction (NA). Black: placebo; Green: THC. Error bars denote standard error of the mean (SEM).

Task Performance

Reaction times showed a linear effect of condition ($F = 53.4$; $p < 0.001$), indicating that reaction times decreased with increasing value of reward, and a significant effect of drug ($F = 10.5$; $p < 0.005$), indicating that subjects were slower after THC administration compared to placebo, irrespective of condition or group (see figure 6.2). No main or interaction effects with group were found between NA and HC (interaction effect of drug by condition by group: $F = 0.4$, $p = 0.5$; drug by group: $F = 0.5$, $p = 0.5$; condition by group: $F = 1.6$, $p = 0.2$; main effect of group: $F = 0.004$, $p = 1.0$).

fMRI

Anticipation

Results for anticipation are shown in figure 6.3, and tables 6.3 and 6.4. For the nucleus accumbens ROI, in HC we found a near significant effect of condition ($F = 4.2$, $p = 0.06$) during placebo, and this effect was significant during THC ($F = 10.4$, $p = 0.007$). In the striatum (Table 6.4), HC showed a strong linear condition effect during placebo ($F = 22.4$, $p < 0.001$) and during THC ($F = 35.6$, $p < 0.001$). No significant drug by condition interaction effect was found in the nucleus accumbens ($F = 0.2$, $p = 0.7$), nor in the striatum ($F = 1.5$, $p = 0.3$). In NA, we found a significant effect of condition after placebo administration ($F = 7.0$, $p = 0.03$), but after THC this effect was eliminated ($F = 1.2$, $p = 0.3$). In addition, a linear drug by condition interaction effect was found in the nucleus accumbens ($F = 10.3$, $p = 0.01$), indicating that NA showed blunted nucleus accumbens brain activation after THC compared to placebo (see table 6.3 and figure 6.3 and 6.4a). Similarly, in the striatum of NA, we found a trend towards a condition effect ($F = 4.7$, $p = 0.06$) for placebo, whereas during THC, no condition effect was found ($F = 0.2$, $p = 0.7$). The drug by condition interaction effect in the striatum showed a trend towards significance ($F = 2.8$, $p = 0.1$) (see table 6.4).

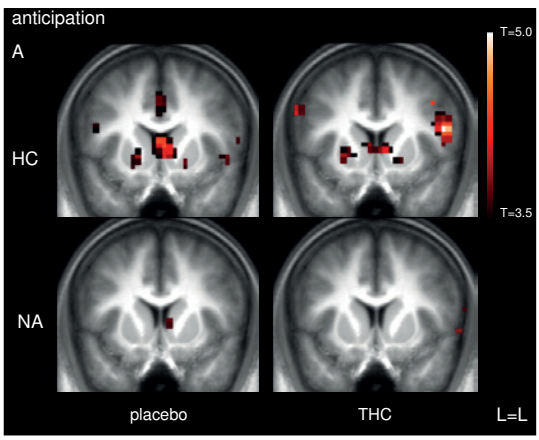

Figure 6.3a Brain activity during anticipation of reward ($T > 3.5$; $p < 0.001$ uncorrected, clustersize > 20 voxels). Upper pannel: healthy controls (HC), lower panel: subjects with nicotine addiction (NA). Left: after placebo administration; right: after THC administration. L = left

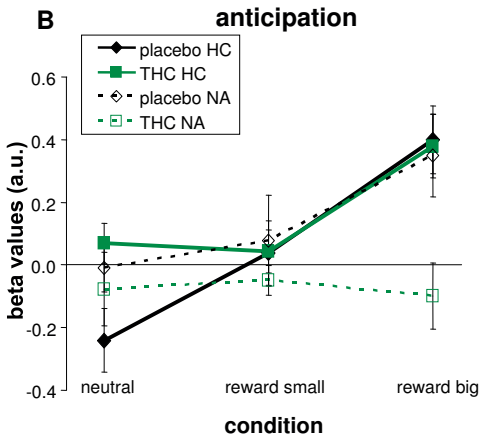

Figure 6.3b Reward activity during anticipation in the nucleus accumbens. Solid lines: healthy controls (HC); dotted lines: subjects with nicotine addiction (NA). Black: placebo; Green: THC. Error bars denote standard error of the mean (SEM).

Next, we explored the differences between groups for the placebo and THC sessions separately. No group by condition effect was found during placebo, ($F = 0.1$, $p = 0.7$), indicating that HC and NA behaved similarly during placebo. A significant group by condition interaction effect was found during THC ($F = 5.0$, $p < 0.05$), indicating a blunted nucleus accumbens response to reward in NA compared to HC. The striatum showed similar results, with a linear effect of group by condition during THC ($F = 7.3$, $p = 0.01$), but not during placebo ($F = 2.3$, $p = 0.14$).

Nucleus accumbens			**ANT**		**FB**	
			F	**p**	**F**	**p**
HC	condition	placebo	4.2	0.06 *	4.3	0.06 *
		THC	10.4	0.007 ***	1.1	0.3
	condition*drug		0.2	0.7	0.4	0.6
NA	condition	placebo	7.0	0.03 **	0.4	0.5
		THC	1.2	0.3	0.06	0.8
	condition*drug		10.3	0.01 **	0.05	0.8
Placebo	condition*group		0.1	0.7		
THC	condition*group		5.0	0.04 **		

Table 6.3: Results of repeated-measures ANOVA (Huynh-Feldt corrected) in the nucleus accumbens comparing THC and placebo administration during neutral, small reward and large reward anticipation (ANT) and small and large reward feedback (FB), between HC and NA. * $p < 0.1$: trend towards significance; ** $p < 0.05$; *** $p < 0.01$.

Feedback

Results for feedback are shown in figure 6.4, and tables 6.3 and 6.4. In HC, there was no drug by condition interaction effect in the nucleus accumbens. There was a trend towards

an effect of condition in HC (F = 4.3, p = 0.06) after placebo administration, but not after THC administration (F = 1.1, p = 0.3). Similarly, NA also showed no interaction between drug and condition (F = 0.0, p = 1.0), and there was no effect of condition during placebo (F = 0.4, p = 0.5) or THC (F = 0.1, p = 0.8) (see table 6.3 and figure 6.3b). In NA, a trend towards an effect of drug (F = 3.8, p = 0.08) indicated that brain activity was attenuated after THC administration, irrespective of condition.

In the striatum, feedback effects were similar to those in the nucleus accumbens (see table 6.4). HC showed no drug by condition interaction effects. A trend towards an effect of condition was found in HC (F = 4.5, p = 0.06) after placebo administration, but not after THC administration (F = 1.5, p = 0.2). In NA, no drug by condition interaction effect was found (F = 0.2, p = 0.7), nor a condition effect after placebo (F = 0.04, p = 0.9) or THC (F = 0.1, p = 0.7). No overall drug effects were found in HC (F = 0.7, p = 0.4), nor in NA (F = 2.9, p = 0.1).

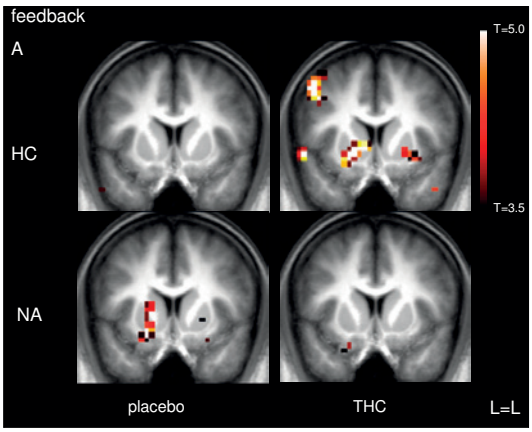

Figure 6.4a: Brain activity during feedback of reward (T > 3.5; p < 0.001 uncorrected, clustersize > 20 voxels). Upper pannel: healthy controls (HC), lower panel: subjects with nicotine addiction (NA). Left: after placebo administration; right: after THC administration. L = left

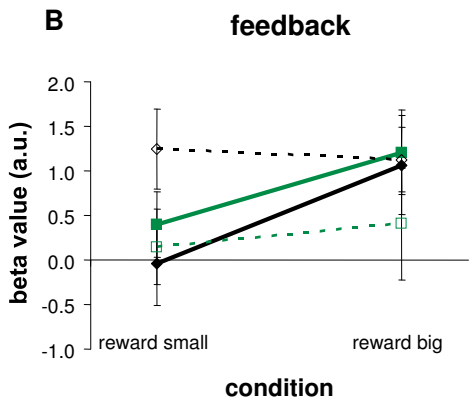

Figure 6.4b: Reward activity during feedback in the nucleus accumbens. Solid lines: healthy controls (HC); dotted lines: subjects with nicotine addiction (NA). Black: placebo; Green: THC. Error bars denote standard error of the mean (SEM).

Striatum			ANT		FB	
			F	p	F	p
HC	condition	placebo	22.4	< 0.001 ***	4.5	0.06 *
		THC	35.6	< 0.001 ***	1.5	0.2
	condition*drug		1.5	0.3	0.1	0.8
NA	condition	placebo	4.7	0.06 *	0.04	0.9
		THC	0.2	0.7	0.1	0.7
	condition*drug		2.8	0.1	0.2	0.7

Table 6.4: Results of repeated-measures ANOVA (Huynh-Feldt corrected) in the striatum comparing THC and placebo administration during neutral, small reward and large reward anticipation (ANT) and small and large reward feedback (FB), between HC and NA. * $p < 0.1$: trend towards significance; ** $p < 0.05$; *** $p < 0.01$.

Discussion

Motivation for the present study was to test the hypothesis that the eCB system plays an important role in addiction. In this study, a THC challenge was applied to nicotine dependent subjects, and the hemodynamic response in key regions of the reward system to natural probes was measured.

The key finding of the study was a reduced nucleus accumbens response to monetary reward anticipation after THC administration in nicotine users. Importantly, there was no sign of a similar effect in the control group, suggesting that nicotine addiction is linked to an eCB system that functions different from that in controls. There was also an indication that in nicotine users nucleus accumbens activity during feedback of a reward was attenuated after THC administration. However, this attentuation was independent of reward size. THC did not affect reward feedback brain activity in the nucleus accumbens in healthy controls. These findings could be explained when assuming that THC maximizes activity in the nucleus accumbens in nicotine users throughout the scan session, thereby abolishing the ability to respond to natural stimuli. The fact that accumbens activity was low in nicotine users after THC, does not contradict this notion, because activity is measured relative to the baseline condition during which activity would also be maximized. Interestingly, blocking the eCB system by chronic administration of rimonabant in healthy non-smoking controls also causes reduced ventral striatal activity compared to placebo in a reward paradigm [234]. Although having a similar effect of an eCB agonist and an antagonist on nucleus accumbens activity seems contradictory at first, assuming that rimonabant blocks accumbens reactivity, as is suggested by the Horder study [234], reduced accumbens activity to natural rewards for both rimonabant and THC would then be caused by different mechanisms.

The main finding of this study supports the hypothesis that altered reward processing, as has been observed in nicotine addicts [110,233,258,262,263], is associated with altered

interaction between the eCB system and reward processing. Results of the current study are supportive of the reward deficiency hypothesis of addiction [91,238,256]. In line with this hypothesis, a non-drug reward (money) did not induce activity in the nucleus accumbens, after administration of a drug reward (THC). Following the reward deficiency theory, this result suggests that in nicotine users, drug-related cues are especially salient, and have biased the individual toward drug-related stimuli [238].

It is not clear whether the absence of a nucleus accumbens response to natural rewards in smokers after THC administration is associated with cause or with consequence of nicotine use. Chronic nicotine use can decrease the density of striatal dopamine receptors [264,265], which may have interacted with the acute effects of perturbation of the eCB system by means of THC. Further, there are indications that nicotine can enhance the effects of THC [92-94]. Thus, recent nicotine use may have increased the effects of THC in the group of nicotine users. Alternatively, the nucleus accumbens response to natural rewards in smokers after THC may be related to pre-existing differences in eCB function, for example through genetic variations. These differences may also have had an effect on proneness for nicotine addiction. Thus, our results suggest the possibility that certain variations in eCB function may form a risk factor for nicotine addiction. If future research provides confirmation of this result, it could be an important new factor in nicotine addiction prevention.

Addiction to nicotine causes similar striatal adaptations as addiction to other drugs of abuse [264,265]. Animal studies have shown that chronic exposure to nicotine, alcohol, and THC (but not to cocaine) increased levels of endocannabinoids in the limbic forebrain, a brain region that comprises, among others, the nucleus accumbens [109,266]. These results suggest a general mechanism of eCB involvement in drug addiction. Thus, the eCB system may be involved in addiction in general, through the reward system. However, these interpretations are somewhat speculative. Whether our results can be extrapolated to general addiction warrants further investigation.

A mechanism through which the eCB system could exert its effects on reward processing in addiction is through alterations in dopamine transmission. Endocannabinoid activation induces increases in dopamine transmission in the nucleus accumbens in animals [32]. Results in humans are less clear, finding either mild dopamine increase [33] or no differences in dopamine transmission [236,237]. Still, dopamine is an important neurotransmitter involved in reward processing [103,267]. Acute administration of drugs of abuse typically causes an increase in dopamine transmission in the nucleus accumbens [268]. In drug addiction, dopamine release is attenuated and dopamine receptors in the striatum appear to be down-regulated [265]. Solinas et al. [44] proposed that the eCB system is involved in modulation of reward processes through fine-tuning of dopaminergic activity. The dopamine response in nicotine users may be maximized, because less dopamine receptors are available, where the dopamine response in healthy controls is relatively smaller, since there are more dopamine receptors present. This explanation, however speculative, would be in line with our findings.

The results of the placebo session can be compared with previous studies that examined differences in reward processing between nicotine users and healthy controls, without THC administration. These studies have predominantly shown a blunted response of the nucleus accumbens or ventral striatum to a rewarding, typically monetary, stimulus [110,233,258,262]. The current placebo results seem to deviate from these studies, as we did not find a significant difference between healthy controls and nicotine users. We did, however, find a small difference in anticipation activity in the current study which was in the same direction as in the previous studies. Possible reasons that this difference did not become significant in the current study may be that the control group included infrequent nicotine users, in contrast to van Hell et al. [233] and the studies by Martin-Soelch and colleagues [110,258], where healthy controls were completely nicotine-free. This could not be prevented, because the ethics protocol required participants to have some experience with THC, and this in turn is typically mixed with tobacco, thus although healthy controls were not regarded as addicted, they were infrequently exposed to nicotine. There are indications that even limited nicotine exposure may already have long-lasting effects on synaptic processes [95]. We have previously shown that chronic cannabis use attenuates reward-related brain activity in the nucleus accumbens [233]. Therefore, even though current subjects were infrequent cannabis users, as opposed to the very frequent users as in the van Hell study, previous use of cannabis in healthy controls may have influenced our results.

Results of this study should be interpreted with care, as there are several limitations. First, nicotine users were abstinent for about three hours when performing the experimental task. This was enough to ensure an absence of acute effects of nicotine, but we cannot exclude the possibility that subjects were in withdrawal at the time of testing. Second, group sizes were relatively small (although typical for intensive pharmacological challenge studies), limiting the statistical power.

In conclusion, our results indicate that in nicotine users the nucleus accumbens is insensitive to natural rewards during THC intoxication, suggesting that nicotine addiction, and possibly addiction in general, is linked to an eCB system that functions different from that in controls. These results may have implications for prevention as well as treatment of nicotine addiction. They possibly identify a risk factor, and suggest new treatment possibilities through pharmacological challenges of the eCB system.

Acknowledgements

The PhICS study is performed within the framework of Top Institute Pharma, project number T5-107. This study is registered in the EudraCT database (2007-004247-30) and the Dutch Trial Register (NTR1787). We would like to thank Storz and Bickel for kindly supplying the Volcano vaporizer, and Annelies Brouwer, Erik Oudman, Joep van der Graaf, David Terburg and Estrella Montoya for help with data acquisition and analysis.

Chapter 7

The endocannabinoid system in response inhibition in addiction

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Manuscript in preparation

Abstract

Addiction has been associated with increased impulsivity in humans and animal studies. An important aspect of impulsivity is the ability to withhold a response, or response inhibition. One of the neurotransmittersystems that may modulate the process of response inhibition is the endocannabinoid system (eCB). This system has been associated with both impulsivity and addiction in animal studies and human studies, but the relation between eCB functioning and impaired response inhibition in addiction has never been investigated in humans.

The current study used functional Magnetic Resonance Imaging (fMRI) to elucidate the role of the eCB system in response inhibition in addiction by examining the effect of the partial eCB agonist Δ^9-tetrahydrocannabinol (THC) on brain activity during a stop-signal task in heavy smokers and matched controls.

Fifteen healthy males participated in a randomized placebo-controlled pharmacological fMRI study with administration of THC to challenge the eCB system, of which seven were healthy controls and eight were nicotine addicts. We contrasted brain activity during anticipation of a stop signal (go-stop) with brain activity during a simple reaction time task (go-only), and we compared THC with placebo. In addition, twenty six subjects performed a similar task outside of the scanner, also after THC and placebo administration. Nicotine addicts were compared to healthy controls in both performance of the stop signal task, and striatal brain activity during response inhibition.

THC increased reaction times during go-only trials, and increased errors in the fMRI task. In the neuropsychological task, THC increased stop signal reaction time (SSRT), indicating increased impulsive responding. No differences were found between nicotine addicts and healthy controls in either performance, or striatal brain activity during response inhibition. These results suggest that inhibitory function addressed with the well-known stop signal task does not depend on the eCB system, nor is it affected in nicotine addiction.

Introduction

Addiction is associated with increased impulsivity [50]. Impulsivity is a cognitive trait that is involved in several psychiatric disorders, such as addiction [50, 269-272] and attention-deficit hyperactivity disorder (ADHD) [143,273]. Impulsivity is often defined as acting without forethought, although many studies have indicated that impulsivity is heterogeneous in nature, and consists of neuroanatomically and neuropharmacologically distinct processes [50-52]. Two important aspects of impulsivity have been identified and thoroughly investigated, the first of which is *impulsive choice*, or choosing a small, immediate reinforcer above a delayed, bigger reward. The second, and subject of the current study, is *impaired response inhibition*, or the inability to withhold a prepotent response [50,52]. Response inhibition is often measured using a stop signal task (SST), first described by Logan et al. [80]. In this task, subjects need to perform a simple reaction time task, pressing a button as quickly as possible when a cue appears. The motor act can be cancelled up to a certain point after it is initiated. The task measures this point by occasionally presenting a cancellation cue ('stop signal') at varying time points after the button press cue and assessing when cancellation fails. Poor performance is reflected by an early time point, signifying a poor ability to interrupt an initiated act. The task is thought to measure an individual's ability to inhibit prepotent responses or impulses. Poor performance is associated with pathological impulsivity, which in turn is thought to play a role in development and maintenance of addiction, and in relapse [50].

In this paper, nicotine addiction is used as a model for general addiction. The reason for choosing nicotine addiction is two-fold. First, subjects with a nicotine addiction are least likely to abuse other drugs as well, which decreases the chance of pharmacological confounds. Second, nicotine users are unlikely to have cognitive problems related to their drug abuse. Although nicotine addiction may not be representative of all addictions, it shares many features with general addiction, including those that form the basis of the criteria of Substance Dependence as described in the DSM IV (such as tolerance, inability to quit, use despite the knowledge of adverse consequences).

Increased impulsivity has indeed been reported in addiction [50,274,275]. Conclusions of a recent review on impulsivity and addiction indicated that addiction may be due to increased impulsiveness following loss of fronto-cortical inhibition of impulses [274]. Further, impulsive behaviour has been linked to drug use, both as a determinant and as a consequence [275]. Mitchell [271] found that nicotine users showed increased impulsivity as measured with delay aversion tasks (impaired impulsive choice), as well as with personality questionnaires. In an animal study addressing the relation of different aspects of impulsivity with different aspects of addiction, Diergaarde et al. [269] found that increased impulsive action is related to increased initiation and continuation of nicotine self administration. In contrast, impaired impulsive choice was associated with failure to inhibit nicotine seeking during abstinence, and with increased relapse. These phenomena were related to dopamine imbalances in

the frontostriatal circuit [269]. In humans, nicotine users displayed elevated inhibition errors during a go/no-go task, and during an antisaccade task [272].

One of the neurotransmitter systems that may be involved in regulating response inhibition is the endocannabinoid (eCB) system. This system consists of cannabinoid receptors and endogenous ligands, and is ubiquitously present in the brain [16]. Challenging the eCB system in humans with the partial CB1 receptor agonist Δ^9-tetrahydrocannabinol (THC), the main psychoactive component in cannabis, results in impaired response inhibition, reflected in increased stop-signal reaction time (SSRT) [22,55]. Further, in animal studies, the eCB agonist WIN55,212-2 has shown to impair inhibitory control, as the number of premature responses increased in the five-choice serial reaction time task (5-CSRTT). The eCB antagonist rimonabant decreased impulsive responding in the same task [53]. These data suggest an eCB role in impulsivity (see also [52]). A functional Magnetic Resonance Imaging (MRI) study on response inhibition using the go/no-go task found that THC significantly attenuated brain activity in areas involved in response inhibition, such as the right inferior frontal cortex and the anterior cingulate [178].

Given evidence that impulsivity plays a role in addiction, and that the eCB system is involved in impulsivity, we here address the question whether the eCB system is involved in response inhibition in addicted people, by investigating how THC affects response inhibition processes as measured with the stop signal task. We used functional MRI to measure brain activity during response inhibition, and focussed specifically on the striatum, as this is an important area in addiction [5], response inhibition [59] and in the eCB system [18]. In addition, a neuropsychological stop signal task without imaging was used to investigate whether we could replicate earlier findings [22,55].

We expected that THC would increase impaired response inhibition in addiction, as reflected in a higher SSRT compared to placebo in the stop signal task, and would attenuate brain activity in areas that are important for response inhibition (see [178]). We expected THC administration to induce stronger effects in nicotine users compared to healthy controls, both in performance and in brain activation due to increased sensitivity of the eCB system. We focus especially on the striatum, and nucleus accumbens as subregion of the striatum, due to its importance in both response inhibition [58,59], and addiction.

Methods

This study is part of the Pharmacological Imaging of the Cannabinoid System (PhICS) study. Methods of the entire study are reported in detail in a methodological paper [206].

Subjects

Thirty five healthy male subjects participated in a randomized placebo-controlled cross-over pharmacological MRI study with THC administration. Twelve subjects were smokers and twenty three were healthy controls. Subjects needed to be occasional cannabis users

(use at least four times a year but at most once a week) who never had negative experiences after cannabis use (for instance a bad trip or cannabis-induced psychosis). Subjects were excluded if they or their first degree relatives were diagnosed with a psychiatric disorder, as assessed using the Mini International Neuropsychiatric Interview for DSM-IV clinical disorders (M.I.N.I: Translated Dutch Version 5.0.0 [96]). In- and exclusion criteria are described in further detail in van Hell et al. [206]. All volunteers gave written informed consent before entry into the study and were paid 250 Euros for participation. The study was approved by the Ethical Committee of the University Medical Centre Utrecht in accordance with the Declaration of Helsinki 2008.

Of the initial thirty five subjects, two healthy controls and one smoker did not complete both testdays due to feelings of anxiety during one of the scanning sessions. One healthy control did not show elevated plasma levels after THC administration and was excluded from the analysis. One smoker was excluded from analysis due to high blood pressure during the first test day.

Ten healthy controls and ten smokers completed the fMRI task on both test days. Two healthy controls and two smokers were excluded after analyzing the data, because they performed below chance level. One healthy control was excluded from analysis due to movement artefacts during the scanning session. Seven healthy controls and eight smokers were included in the analysis for the fMRI task.

Seventeen healthy controls and nine smokers completed the CANTAB task on both test days and were included in the analysis. Subject characteristics are summarized in table 7.1.

Procedure

At a training session, subjects practiced the procedure of drug administration (inhalation) and participants were familiarized with the scan protocol in a mock scanner to reduce stress effects on the following test days. The actual study consisted of two test days, separated by at least two weeks to allow for complete clearance of drugs. A standard breakfast or lunch was provided at the beginning of each test day, to ensure equal states of metabolism on both test days. Subjects were instructed not to use cannabis from two weeks before the first test day until study completion. Clearance of drugs was tested by means of a urine sample at the beginning of each test day. Additionally, no alcohol was permitted in the 48 hours preceding a test day, and subjects needed to refrain from smoking, eating and drinking during four hours preceding a test session.

Drug administration

On test days subjects received THC or placebo by means of a Volcano® vaporizer [39,206] at four time points. Vehicle (ethanol only) was used as a placebo. The first dose consisted of 6 mg THC or placebo. To maintain equal levels of intoxicating effects throughout the experiment, upload dosages of 1 mg were used, 30 minutes apart. After the first three administrations of THC or placebo, subjects performed a cognitive task during which fMRI

scans were obtained. After the last dose of THC or placebo, a battery of neuropsychological tasks was performed. Here we report on the results of the Stop Signal Task (SST). Results of other assessments are reported elsewhere (see also [206,261]).

Subject characteristics	healthy controls		nicotine users	
	mean ± SD	range	mean ± SD	range
Age	21.3 ± 2.4	18 – 26	26.3 ± 6.7	20 – 40
IQ	105.5 ± 5.1	98 – 113	107.1 ± 5.1	98 – 114
Nicotine use last year (cigarettes / week)	2.7 ± 7.5	0 – 30	118.3 ± 37.8	70 – 203
Alcohol use last year (units / week)	13.9 ± 10.1	3 – 40	16.4 ± 10.1	2 – 30
Cannabis use last year (no. occasions)	19.3 ± 8.3	4 – 52	27.6 ± 16.8	5 – 52
Illicit use lifetime (no. occasions)	3.0 ± 5.4	0 – 15	2.5 ± 4.4	0 – 13
Peak Plasma THC concentration	98.4 ± 50.2	30 – 189	82.8 ± 41.1	27 – 178

Table 7.1 Subject's characteristics

Drug effects

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. [39].

Subjective effects were measured at baseline and before and after each task and throughout the test day using self-reported Visual Analogue Scales (VAS) [176,177]. Heart rate and respiratory function were monitored continuously during scanning. Heart rate was assessed by measuring the electrocardiogram using four electrodes attached to the subject's chest, and respiratory function was assessed by measuring the expansion of a respiration band around the subject's abdomen.

Task

fMRI

Impulse regulation related activity was measured using the stop signal task (SST) [80]. Each trial consisted of a visual stimulus (a cross or a circle) with a duration of 500 ms, after which the subject had to press a left or right button (depending on the stimulus) with their right hand thumb as quickly and accurately as possible (go trial). In 30% of the trials, a square appeared around the stimulus after a variable delay, indicating subjects had to cancel or withdraw their ongoing response (stop trial). The stop signal started at 250 ms and was adapted to performance, such that approximately half of the stop trials were successful. An error decreased the delay with 50 ms, successful inhibition prolonged the delay with 50 ms. The inter-trial interval was jittered between 1500 and 2500 ms and the trial order was pseudo-randomized. To measure baseline performance, two blocks of 30 go trials (go-only) were presented. In total, the task consisted of 300 trials (see figure 7.1).

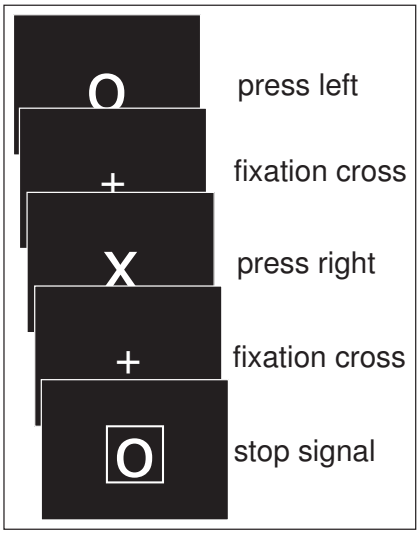

Figure 7.1 Task paradigm

CANTAB

Subjects needed to press a left or right button when seeing a left- or right-pointing arrow. Occasionally, an auditory signal (a beep) followed the stimulus onset, and then, subjects needed to withhold their response and not press the button.

Scanning parameters

Image acquisition was performed on a Philips Achieva 3.0 Tesla MR scanner with a Quasar dual gradient set (Philips Medical Systems, Best, The Netherlands). Functional imaging was performed using a SENSE-PRESTO scan protocol [164] (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, 1182 volumes). A high-contrast volume with a flip angle of 27° (FA27) was scanned for registration purposes. Before the functional imaging run, a high-resolution whole brain anatomical scan was 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).

Analysis

Behavioural and physiological measures

VAS scores were corrected for baseline values and analyzed using repeated-measures ANOVA with drug and time as within-subject factors [206] and group as between-subject factor. Mean heart rate during the SST was calculated for placebo and THC sessions separately and analyzed with a repeated-measures ANOVA with drug as within-subject factor and group as between-subject factor.

Task Performance

Performance was analyzed by computing the stop-signal reaction time (SSRT), accuracy (direction errors and misses), the percentage of false alarms (FA), and go-only and go-stop RT. The SSRT estimates the speed at which the stop-signal is processed [58], and is computed by the difference between the mean RT of the go-stop trials and the mean stop-signal delay. A longer SSRT signals an increase in time needed to process a stop-signal, and thus reflects (relatively) impaired response inhibition. The effect of THC on performance was analyzed using repeated-measures ANOVA, including drug (two levels) as within-subject factor, and group as between-subject factor.

Brain activity

Functional MRI data were pre-processed and analyzed using SPM5 (Wellcome Trust Centre for Neuroimaging, London, UK). Pre-processing of data consisted of realignment of functional images and co-registration with the anatomical volume using the FA27 volume. After realignment, functional scans were normalized into standard MNI space and smoothed (FWHM = 8 mm).

For each individual subject, regression-coefficients for each voxel were obtained from a general linear model regression analysis using a factor matrix that contained factors representing event-related changes time-locked to go-only trials, go-stop trials, successful stop trials, unsuccessful stop trials. All factors were convolved with a canonical hemodynamic response function. To correct for drifts in the signal, a high-pass filter with a cut-off frequency of 0.002 Hz was applied to the data.

Brain activity during impulse regulation was defined as activity during go-stop trials contrasted with activity during go-only trials. This measures impulse regulation, as there is a likelihood of a stop signal and thus anticipation of that stop signal during go-stop trials, but not during go-only trials.

To test whether THC altered brain activation in the striatum in healthy controls (HC) compared to subjects with a nicotine addiction (NA) during the stop signal task, an anatomical region of interest (ROI) analysis was performed. Striatal brain activity was measured by defining left and right caudate nucleus and putamen using the WFU Pick Atlas, and combining these ROIs to assess striatal activity during go-only and go-stop trials. In addition, we defined the nucleus accumbens as a subdivision of the striatum by drawing a sphere with a diameter of 5 mm around MNI coordinates -12, 14, -8 (left) and 12, 14, -8 (right), and combined these ROIs. Mean regressor coefficients representing brain activity during go-only and go stop in the striatum and nucleus accumbens were extracted using the Marsbar SPM tool [242].

To test whether the eCB system is involved in response inhibition in addiction, the mean regressor coefficients in the chosen ROIs were analyzed using a repeated-measures ANOVA with drug (two levels: placebo and THC) and condition (two levels: go-only and go-stop) as within-subject factor and group (two levels: HC and NA) as between-subject factor.

Results

Behavioural and physiological results

During the stop signal task in the scanner, THC increased feeling high (F = 15.7, p = 0.002) and external perception (F = 6.3, p = 0.03) compared to placebo (see table 7.2). Internal perception showed a trend towards significance (F = 4.6, p = 0.052). Alertness (F = 6.7, p = 0.02) and calmness (F = 8.0, p = 0.02) significantly decreased after THC administration compared to placebo. THC administration increased heart rate (F = 14.8; p = 0.003). No effects of group were found, indicating that NA and HC experienced similar subjective and physiological effects. During the CANTAB version of the stop signal task, THC showed increased feeling high (F = 19.6, p < 0.001) and external perception (F = 6.2, p = 0.02). Alertness (F = 31.4, p < 0.001), contentedness (F = 11.6, p = 0.002) and calmness (F = 5.9, p = 0.02) were decreased during the CANTAB after THC administration (see table 7.3).

THC plasma concentration reached 92.7 ng / ml on average (see table 7.1). Significant correlation (although not corrected for multiple comparisons) were found between THC plasma concentration and subjective ratings of feeling high (r = 0.62, p = 0.03), external perception (r = 0.67, p = 0.01), internal perception (r = 0.71, p = 0.007), and alertness (r = -0.68, p = 0.01).

VAS score	Placebo		THC		ANOVA		
fMRI	pre	post	pre	post	Effect	F	p
heart rate	71.9 ± 10.5		92.9 ± 15.3		drug	14.8	0.003 *
feeling high	2.3 ± 5.0	4.7 ± 12.3	23.7 ± 22.4	30.7 ± 27.4	drug	15.7	0.002 *
internal perception	0.1 ± 0.4	0.2 ± 0.6	2.4 ± 3.7	6.3 ± 14.1	drug	4.6	0.052
external perception	0.4 ± 1.1	1.4 ± 2.5	5.2 ± 8.9	12.7 ± 21.1	drug	6.3	0.03 *
alertness	-3.7 ± 7.8	-9.5 ± 13.2	-16.0 ± 14.6	-21.1 ± 13.4	drug	6.7	0.02 *
contentedness	-3.0 ± 6.1	-3.8 ± 6.7	-6.5 ± 7.9	-7.8 ± 7.7	drug	2.9	0.11
calmness	2.9 ± 13.3	6.4 ± 9.9	-9.9 ± 19.5	-13.8 ± 21.2	drug	8.0	0.02 *

Table 7.2 Baseline corrected scores of physiologcial and subjective effects of THC during fMRI task. Pre = before performance of the fMRI task; post = after performance of the fMRI task. ANOVA: results of ANOVA with drug as within-subjects factor. * = significant difference between THC and placebo. No differences between healthy controls and nicotine users were found.

Task Performance

fMRI

Repeated-measures ANOVA with drug (two levels) as within- and group (two levels) as between-subject factor showed no differences between THC and placebo in reaction times of go-trials during the go-stop condition, no differences in SSRT, false alarms or errors during the go-only condition (see table 7.3). THC increased reaction times during go-only (F = 5.0, p = 0.04), and errors (misses plus direction errors) during the go-stop condition (F = 16.4, p = 0.001). No group differences were found.

VAS score	Placebo		THC		ANOVA		
CANTAB	pre	post	pre	post	Effect	F	p
feeling high	0.3 ± 2.5	0.3 ± 2.5	32.4 ± 29.5	17.0 ± 21.3	drug	19.6	0.000 *
internal perception	-0.3 ± 0.7	-0.3 ± 0.7	2.4 ± 7.5	0.4 ± 3.2	drug	1.3	0.3
external perception	0.1 ± 0.4	0.01 ± 0.4	7.7 ± 13.9	3.8 ± 6.5	drug	6.2	0.02 *
alertness	3.3 ± 7.2	-0.9 ± 12.3	-20.8 ± 18.2	-13.2 ± 17.7	drug	31.4	0.000 *
contentedness	3.4 ± 6.7	0.7 ± 7.5	-7.8 ± 10.8	-3.2 ± 7.9	drug	11.6	0.002 *
calmness	16.0 ± 14.4	12.5 ± 14.0	1.1 ± 18.1	6.2 ± 15.9	drug	5.9	0.02 *

Table 7.3 Baseline corrected scores of physiologcial and subjective effects of THC during CANTAB task. Pre = before performance of the CANTAB task; post = after performance of the CANTAB task. ANOVA: results of ANOVA with drug as within-subjects factor. * = significant difference between THC and placebo. No differences between healthy controls and nicotine users were found.

CANTAB

Repeated-measures ANOVA with drug (two levels) showed no differences between THC and placebo in errors or reaction times during go-trials. THC significantly increased SSRT ($F = 4.7$; $p = 0.04$). No effects of group were found, indicating that HC and NA performed the stop signal task similarly, both inside and outside the scanner.

	healthy controls		nicotine users		ANOVA	
	placebo	THC	placebo	THC	F	p
fMRI						
RT go only (ms)	553.0 ± 60.9	580.7 ± 106.3	516.2 ± 67.9	548.3 ± 74.2	5.0	0.04 *
RT go stop (ms)	705.7 ± 159.4	670.1 ± 136.2	662.6 ± 161.3	680.3 ± 186.9	0.2	0.7
SSRT (ms)	358.6 ± 45.5	354.3 ± 57.4	334.7 ± 49.8	358.2 ± 62.3	1.3	0.3
FA (%)	39.6 ± 14.0	46.1 ± 12.3	43.3 ± 16.9	42.2 ± 14.8	0.6	0.4
errors go only	2.4 ± 1.7	3.0 ± 2.2	3.4 ± 2.1	3.5 ± 4.2	0.1	0.7
errors go stop	4.7 ± 4.4	12.9 ± 8.2	7.1 ± 6.2	12.4 ± 8.1	16.4	0.001 *
CANTAB						
RT go	338.4 ± 42.8	359.4 ± 31.9	345.5 ± 44.8	342.5 ± 57.4	0.9	0.3
SSRT	181.4 ± 37.5	202.1 ± 45.0	208.8 ± 55.5	223.3 ± 57.8	4.7	0.04 *
errors	6.8 ± 8.3	5.8 ± 3.5	9.8 ± 11.1	14.8 ± 12.0	1.0	0.3

Table 7.4 Performance of stop signal tasks. RT = reaction times; ms = milliseconds; SSRT = stop signal reaction time; FA = number of false alarms; errors = number of errors during the task. * = significant difference between THC and placebo. No differences between healthy controls and nicotine users were found.

Brain activity

Repeated-measures analysis of striatal brain activation with drug and condition as within-subject factors and group as between-subject factor showed a significant effect of

condition (F = 65.5, p < 0.001), indicating involvement of the striatum in response inhibition. A significant effect of group (F = 5.0, p = 0.04) indicated that NA showed a stronger signal increase during the task compared to rest in the striatum compared to HC. There was no effect of drug (F = 0.2, p = 0.6), nor interaction effects between drug by condition (F = 0.1, p = 0.8), or group by drug by condition (F = 0.06, p = 0.8), indicating that THC did not affect brain activity in the striatum during response inhibition (see figure 7.2).

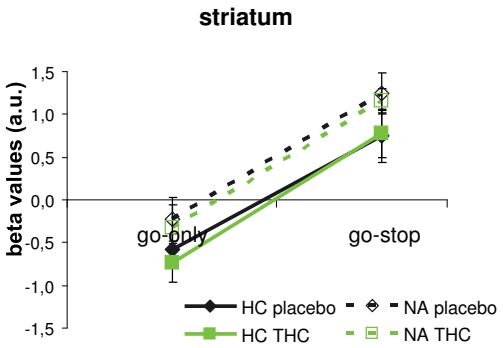

Figure 7.2: Brain activation in the striatum during go-only and go-stop. HC = healthy controls, NA = nicotine addicts; a.u. = arbitrary units. Error bars denote standard error of the mean (SEM).

Similar analysis with the nucleus accumbens also showed a significant effect of condition (F = 23.4, p < 0.001), indicating involvement of the nucleus accumbens in response inhibition. There was no significant effect of group (F = 0.2, p = 0.7) or drug (F = 0.06, p = 0.8), nor interaction effects between drug by condition (F = 0.02, p = 0.9), or group by drug by condition (F = 0.1, p = 0.7; see figure 7.3).

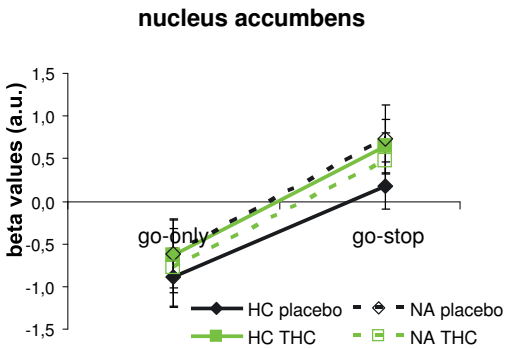

Figure 7.3: Brain activation in the nucleus accumbens during go-only and go-stop. HC = healthy controls, NA = nicotine addicts; a.u. = arbitrary units. Error bars denote standard error of the mean (SEM).

Discussion

In this study we assessed the role of the eCB system in response inhibition in addiction by examining the effects of THC administration on brain activity, and comparing subjects with a nicotine addiction to healthy controls. Subjects performed a stop signal task during which functional MRI scans were obtained. In addition, response inhibition was assessed outside the scanner, using the stop signal task from the CANTAB battery [276]. No differences between nicotine users and healthy controls were found in the effects of THC on response inhibition, neither on performance, nor on striatal brain activity. Moreover, we failed to replicate a performance difference as reported in literature between smokers and non-smokers in the placebo session.

Nicotine users showed increased striatal brain activation compared to healthy controls, irrespective of which drug was administered, but also irrespective of condition. As this effect was similar for both the go-only condition and the go-stop condition, it may either signify non-specific elevation of activity in that region, or diminished baseline activity during rest. In either case, increased striatal activation in nicotine addiction is not specifically associated with response inhibition.

With respect to performance, THC administration induced slight deterioration of performance in the fMRI task, as reflected in longer reaction times during go-only trials, and increased error rate during go trials when subjects expected a stop signal (failures to inhibit the response). In the CANTAB task, THC increased stop signal reaction time, reflecting decreased response inhibition. Hence, THC, as expected, caused a decline in the ability to inhibit responses. We found no differences in inhibitory performance measures between nicotine users and healthy controls. A previous study did report differences between nicotine users and healthy controls on measures of response inhibition in a go/no-go task and an antisaccade task [272], although acute administration of nicotine did not cause changes in inhibitory performance as measured with a stop signal task [277]. This discrepancy suggests that different mechanisms underlie the different tasks, and that elevated impulsivity reported in NA does not extend to all inhibition tasks.

Given the lack of effect of THC on brain activity and the very modest effect on performance, it seems that the eCB system does not play a major role in response inhibition as measured with the stop signal task. This is contrary to what we expected, based on previous reports on behavior [22,55,123] and brain activity [178]. Although we found deterioration in performance (increased SSRT), this was only when subjects performed the task outside the scanner. Behavioral task measures are quite different in the scanner (see table 7.4), suggesting that the scanner environment affects task performance to such a degree that subtle effects of THC go unnoticed. Simple go-only reaction times are, for instance, some 200 ms slower in the scanner. It should be noted that groups were rather small in the fMRI study, while more subjects were included in the CANTAB task. The task that was presented outside the scanner had a longer duration and more trials, so it is also possible that, since effects are

small, increased power may have caused the effects on SSRT to become significant in the CANTAB task, but not in the fMRI task.

Interestingly, response inhibition in animals showed no effect of an eCB agonist (WIN55,212-2) on SSRT, but there was an effect on go reaction time [53]. Our fMRI study also showed increased go reaction time, and no effect on SSRT. With respect to fMRI data on response inhibition, Borgwardt and colleagues [178] did not find differences between THC and placebo in performance measures concerning response inhibition as measured with a go/no-go task, whereas they did find response inhibition related attenuation of brain activity after THC administration. This is different from what we found, in that our study showed slight impairments in performance after THC administration, whereas brain activation did not differ between THC and placebo. Although only results of the striatum are reported here, we did perform a whole-brain analysis, but no differences in brain activity related to response inhibition were found when we compared THC and placebo (data not shown). The discrepancies between the Borgwardt study and the current study may be explained by the fact that go/no-go tasks activate slightly different brain regions (unilateral compared to bilateral), and the Borgwardt study used an oral THC dose, which has different temporal dynamics compared to the intrapulmonary dose which we used in our study. The stop signal task that was used in this study did activate brain regions that are commonly found during response inhibition, such as the pre- and postcentral gyrus, the inferior frontal gyrus, the insula, and the putamen [57,58].

The striatum has been argued to play a role in suppressing brain activity in brain areas necessary to execute, or control, a motor response [59]. Our finding of increased striatal brain activity in nicotine users compared to non-users may reflect that subjects with a nicotine addiction need more brain activity to be able to exert control over a motor response. A recent study by Das et al. [278] showed that nicotine use was significantly related to changes in striatal volume in that the more cigarettes were smoked, the smaller the nucleus accumbens was, and the larger the putamen. The processes behind these structural changes in the striatum may be associated with alterations in striatal function that were found in the current study.

The present study has several limitations. For one, four subjects were excluded from analyses due to bad performance of the task, either after placebo or THC administration. One could argue that subjects should be included no matter how they performed the task. However, when so many errors are made during the task, it is conceivable that subjects were doing or thinking about something totally different in the scanner. Therefore, we chose to exclude the subjects that performed significantly below chance level. Second, group sizes were small. Seven healthy controls were compared to eight subjects with a nicotine addiction. It is possible that no significant differences between THC and placebo were found due to a lack of power.

In conclusion, the present study did not find evidence for an important role of the eCB system in response inhibition as measured with the stop signal task. Moreover, THC had

no differential effect on either performance or brain activity in nicotine addicted subjects as compared to non-smoking controls. Hence we argue that the stop signal task does not address functions dependent on the eCB system, nor does it address mechanisms of elevated impulsivity as postulated in nicotine addiction. More detailed studies on other tasks that address impulsivity seem warranted.

Chapter 8

Summary and Discussion

8.1 Scope of this thesis

The focus of this thesis is the role of the endocannabinoid system in symptoms of addiction, specifically reward processing and response inhibition. The studies described in this thesis are part of the PhICS study, which set out to investigate the involvement of the endogenous cannabinoid system in cognitive functioning, by examining the effects of the cannabinoid agonist THC on brain function during a cognitive challenge.

8.2 Summary of studies

In *chapter two* we compared reward processing in the brain in a group of heavy, chronic cannabis users with a group of healthy controls and a group of nicotine users. As the nucleus accumbens is an important brain area in reward processing, we focussed specifically on that region. We found that chronic cannabis use as well as nicotine use attenuated brain activity during monetary reward anticipation in the nucleus accumbens. Specific effects of cannabis were found in the caudate nucleus, where chronic cannabis users showed an attenuation of brain activity compared to both nicotine users and healthy controls. No effects of reward feedback processing were found in the nucleus accumbens.

In *chapter three* we describe the methods and goals of the Pharmacological Imaging of the Cannabinoid System (PhICS) study. The primary aim of the PhICS study is to clarify the role of the endocannabinoid system in cognitive processing, to elucidate the possibility of eCB involvement in symptoms of psychiatric disorders. Involvement of the eCB system in cognition was assessed using a pharmacological challenge with the well-known cannabinoid (partial) agonist THC, which is the main psychoactive constituent of cannabis [13,14], in combination with functional MRI. Cognitive domains that were examined included memory, attention, emotion, reward and response inhibition. This chapter illustrates several physiological effects of THC, such as elevated plasma levels of THC, the induction of subjective effects, reported as 'feeling high', and increased heart rate. Task paradigms were validated, and the broader framework of the PhICS study was discussed thoroughly.

Chapter four aimed to elucidate the direct effects of THC on brain activity during rest using two separate methods. Brain perfusion was assessed using Arterial Spin Labeling (ASL), and fMRI was used to assess resting state variability of the BOLD signal. Changes in brain activity (either perfusion or BOLD) were correlated with THC-induced changes in physiological measures such as subjective measures of feeling high and heart rate. We found increased perfusion in frontal and insular brain areas, and a significant correlation between subjective effects and perfusion in the superior frontal cortex and insula. Variability of the BOLD signal was specifically increased in the substantia nigra, cerebellum and insula. THC-induced changes in subjective effects or heart rate did not significantly correlate with increased variability of the BOLD signal. We concluded from this study that the psychotropic effects of THC may well originate in the anterior insula. In addition, alterations in baseline brain activity and perfusion after THC administration may have

consequences for interpretation of studies using a cognitive challenge. It makes clear that fMRI paradigms should be carefully controlled, and that certain regions (as described in the paper) may be affected by THC, independent of a particular task.

The role of the endocannabinoid system in reward processing was assessed in *chapter five*. Results presented in this chapter indicated that THC administration caused attenuation of brain activity during feedback of reward, indicating that the appreciation of reward is altered when the endocannabinoid system is activated. As reward processing in the brain is compromised in diseases such as addiction [233], ADHD [146], and depression [279,280], results described in this chapter may have implications for these disorders. Possibly, the endocannabinoid system is involved in altered reward processing in addiction, ADHD, and depression, but this warrants further investigation.

Chapter six describes results of a study that specifically examined the involvement of the endocannabinoid system in altered reward processing in addiction. The results of this study showed that in subjects with a nicotine addiction THC blocked the nucleus accumbens from activating when anticipating a monetary reward, while THC had no effect on reward processing activity in this region in subjects without nicotine addiction. These results suggest a hypersensitivity in nicotine addicts for the rewarding effects of THC, so that other natural rewards (such as monetary rewards) lose their appeal. These results can be interpreted as supportive of the reward deficiency hypothesis [91] that states that subjects with a drug addiction are less interested in and motivated by natural rewards.

Chapter seven described a study in which the role of the endocannabinoid system in response inhibition in addiction was assessed, using a stop signal paradigm. As the study described in chapter six, this study also focused on striatum. In contrast with the results for reward processing, THC did not have a differential effect on response inhibition related activity in striatum in nicotine addicts. This led us to conclude that the inhibitory function addressed with the stop signal task does not involve the endocannabinoid system, nor is it affected in nicotine addiction.

8.3 General discussion

This thesis specifically focuses on the involvement of the endocannabinoid system in cognitive functioning in addiction. Based on the studies in this thesis, we conclude that the endocannabinoid system plays a role in altered reward processing as a symptom of addiction. This conclusion may have consequences for the treatment for subjects with an addiction, but also provides insights in processes behind addiction. It is well possible that the increased sensitivity to an endocannabinoid agonist as we found in subjects with a nicotine addiction precedes development of addiction, and is responsible for the initiation of addiction-related processes. This knowledge may be important for the developments of programs or strategies to prevent people from becoming addicted in the first place. Although the design of the current studies does not allow us to draw conclusions about

the cause or consequence of the alteration in cannabinoid involvement in reward-related processes in addiction, we speculate that differences in cannabinoid processes are pre-existent with respect to the onset of addiction. A high sensitivity of the endocannabinoid system (i.e. a strong response to endocannabinoid ligands) may constitute a risk factor for development of addiction.

In contrast to the effects we found with respect to reward-processing, response inhibition shows limited involvement of the endocannabinoid system. These observations were contrary to our hypothesis, as we expected involvement of the endocannabinoid system in both reward processing and response inhibition, and that this would be altered in subjects with an addiction. Our initial hypotheses were mainly based on animal studies or behavioral studies showing that manipulating the cannabinoid system with either agonists or antagonists altered either reward processing [44] or response inhibition [22,53,55]. The fact that our results show limited involvement of the cannabinoid system in response inhibition stresses the importance of performing these studies in a translational manner, not only in animals, but also in humans.

Interestingly, the nucleus accumbens showed no activation in nicotine users during monetary reward anticipation after THC administration. This could mean that THC administration blocks the nucleus accumbens from activating in general. However, during response inhibition, nicotine users showed significant activation of the striatum, and also the nucleus accumbens, even after THC administration. This indicates that endocannabinoid processing in striatal areas is indeed specifically involved in certain cognitive brain functions, such as reward processing, but not necessarily in response inhibition. The current view on striatal brain areas in addiction is that the ventral striatum, and predominantly the nucleus accumbens, is primarily involved in the rewarding properties of drugs of abuse, whereas the dorsal striatum, consisting of the caudate and putamen, is more involved in the compulsive drug taking in a later stage in addiction [5]. Translating this to the present thesis, the endocannabinoid system seems to be principally involved in ventral striatal reward function, but not in dorsal striatal impulsivity/compulsivity function. Taking into account the theory that the ventral striatum, or nucleus accumbens is involved in the anticipation, or 'craving' phase of reward, while the dorsal striatum is more involved in habit forming and associating cues with stimuli, this would indicate that craving is the most promising stage in which manipulating the cannabinoid system may be effective in preventing or antagonizing addiction. As craving is also connected to the process of relapse to drug use [5], the fact that blocking the cannabinoid system with rimonabant prevents relapse in animal studies [28], supports this theory.

Next to the results on cognitive processes, we found that THC can induce differences in brain activation during rest in *chapter four*. This is important for studies with a cognitive challenge. When alterations are found in brain activity during a cognitive challenge in the same brain regions that show alterations in basal neurophysiology, it is possible that intrinsic psychotropic effects of THC may interfere with other (cognitive) functions of these

regions. If the cognitive functions are the subject of investigation, the interaction between intrinsic, psychotropic effects of THC with the cognitive effects may pose a problem.

8.4 Methodological considerations

The PhICS study aimed to elucidate the role of the eCB system in cognition, by assessing its role in symptoms of psychiatric disorders. The reason behind this approach is the idea that when comparing a normal situation with an impaired situation, and understanding the difference between these situations, this would help us to better understand the normal situation. Although theoretically this is a valid and logic approach, in practice it is difficult to separate 'the role of the endocannabinoid system' from 'the effects of THC'. As the effect of THC is interesting in itself, it needs a shift in the frame of mind to view the THC challenge only as a means by which the endocannabinoid system is challenged. At present, administering THC is the only way of directly challenging the endocannabinoid system in humans, as the use of rimonabant is thwarted by the withdrawal of this drug from registration, following an increased risk of depression and suicide in obese patients in clinical studies, and other cannabinoid agonists or antagonists are still under investigation. An important topic to discuss with respect to the studies presented in this thesis is that by administering THC, functioning of all cannabinoid receptors is challenged. The endocannabinoid system itself works in a more subtle manner, as it is activated on demand and on specific locations. This means that neurotransmission is altered in a more localized way than by administering an exogenous agonist such as THC. Endogenous cannabinoids are able to precisely modulate neuronal functioning by inhibiting release of both excitatory input and inhibitory input [281]. Indirect cannabinoid agonists would therefore be an interesting alternative to investigate the role of the endogenous cannabinoid system. One example of indirect cannabinoid agonists are fatty acid amide hydrolase (FAAH) inhibitors. FAAH is the enzyme that degrades endogenous cannabinoids and inactivates the working of endogenous cannabinoid transmission. Inhibitors of FAAH thus elevate levels of endocannabinoids by prolonging the endogenous tone of the system, making the effects more localized and subtle. These substances are already in use in animal studies [14,282,283], but as of yet, no indirect agonists have been approved for human use. Suggestions for therapeutic use of these indirect cannabinoid agonists have been done for disorders like depression or anxiety disorders [284].

Another matter that is especially relevant in *chapter two*, *six* and *seven* of this thesis, is that by using nicotine users as a model for addiction, we do not take into account the possibility that nicotine addiction has specific characteristics that are not representative for general addiction. Although nicotine addiction resembles addiction to other drugs of abuse such as cocaine, opioids and amphetamines, there are also unique aspects to nicotine addiction, which may have influenced our results. A common effect of drugs of abuse is that acute administration of these substances typically induces dopamine release

in the nucleus accumbens. Chronic administration of drugs of abuse generally decreases dopamine function [285]. This is also the case in chronic nicotine use [264]. In addition, there is a specific interaction between THC and nicotine when used or administered together [92-94], making the results of these studies possibly difficult to extrapolate to addiction in general. Although much is known of the neuromodulatory role of the endocannabinoid system in the transmission of dopamine, GABA, glutamate, serotonin, and others [114,281], we can only speculate about the question of how our results may be caused by specific neurotransmitter systems. By using the technique of functional MRI, we cannot address the role of specific neurotransmitter systems. This is a general limitation of functional MRI studies, but when there is no pharmacological challenge involved, this is usually less relevant. With a neurotransmitter system such as the endocannabinoid system, that modulates the release of so many other neurotransmitters, knowledge of specific alterations of dopamine, GABA or glutamate concentrations in particular brain areas due to cannabinoid activation would greatly contribute to the understanding of the role of the endocannabinoid system in neurotransmission. However, techniques in which cognitive and pharmacological challenges can be combined to assess specific neurotransmitter levels are still in development. Nevertheless, with fMRI paradigms we do have a means of investigating which functions are affected by the endocannabinoid system, and how such involvement relates to psychiatric symptoms.

There are many ways in which functional MRI data can be analyzed. Data are complicated, as one is looking at signal changes in an experimental condition compared to a control condition, during a timeseries in a three dimensional space. Due to the complex nature of the data, choices have to be made in the way to proceed with the statistical analysis. First, a whole-brain analysis gives information about the experimental condition in the entire brain, but, as there are approximately 20,000 voxels to be compared to each other, one needs a very stringent correction for multiple comparisons to reduce the chance of a false positive finding. Second, one can perform a region-of-interest (ROI) analysis. This allows for a more specific analysis, by identifying only the brain areas one is interested in. There are basically two ways to choose from to proceed with an ROI analysis. ROIs can be chosen on the basis of the underlying brain function, which is often referred to as 'functionally defined ROIs' [239]. A particular advantage of this method is that it allows for an exploration of the data, and it is most suitable for a situation in which no brain areas or hypotheses are pre-defined. 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 our studies, the choice to analyze the fMRI data using an ROI-based approach was primarily driven by the advantages of this approach with respect to specificity and sensitivity. The design of the studies described in this thesis was very complicated, with many comparisons even regardless of the complexity of fMRI analysis. Healthy controls were compared to nicotine users, and in both groups a comparison was made between THC and placebo. In addition, during both THC and placebo, control trials were compared

to experimental trials. Therefore, we chose to perform anatomically based ROI analyses, by choosing one specific brain area, the striatum, and mainly the nucleus accumbens, of which the involvement in reward [260], response inhibition [60], the endocannabinoid system [44], and addiction [259] was established.

8.5 Limitations

An important limitation of the studies in which we administered THC, is that subjects who participated in these studies were all chronic, albeit mild, cannabis users. This may have caused adaptations in endocannabinoid functioning. As we showed in *chapter two*, the reward system in cannabis users (but also nicotine users) is altered. Ethical considerations did not permit us to use cannabis naïve subjects. To study the endogenous cannabinoid system, one would ideally study subjects who never challenged their system with an agonist before. However, as THC can induce anxiogenic effects, especially in naïve subjects or in high dosage, administration of THC to naïve subjects is ethically not tenable. Prospective studies may solve this, although such studies are expensive and difficult to accomplish. A similar but additional limitation to the studies in which we compared nicotine users with healthy controls is that apart from the differences in nicotine use, it may well be possible that these two groups differ in many other aspects as well, which may confound our results. For example, nicotine users may show differences in personality traits, such as increased impulsivity and risk-taking, or possibly, differences in socio-economic status account for difference between nicotine users and non-users [286], which also may have consequences for reward processing, or brain activity in general.

Another limiting factor is that in the design of the PhICS study, the subjective effects of THC as experienced by the participants is likely to have undermined blinding. Upon debriefing, most (but not all) subjects correctly identified on which test day they had received THC, and this may have caused expectancy effects. We have tried to minimize the influence of expectancy by the use of a randomized cross-over design. All subjects received both THC and placebo on two separate sessions. By randomizing the order of administration of the psychoactive drug and placebo between subjects, expectancy effects should have been balanced across sessions. Still, we cannot exclude that expectancy effects may affect the results of the study to some extent.

8.6 Future directions

This thesis provides insights that confirm the possibility for the endocannabinoid system to become an important target for pharmacotherapy. Especially disorders in which reward processing is impaired, such as addiction, ADHD and depression, would benefit from additional research into artificial cannabinoid modulation.

With the developments in the area of cannabinoids, indirect agonists of the endocannabinoid system will be on the market before long. The introduction of for

example FAAH-inhibitors for human use would be a great opportunity to study the endogenous working of the cannabinoid system in cognition in humans. Pharmacological MRI studies using these substances will help to gain insight in more subtle involvement of the eCB system in symptoms of psychiatric disorders.

It would be interesting to investigate whether the findings from the studies presented in this thesis could be extrapolated to addiction to other substances, such as cocaine, amphetamines, or opioids. We know from literature that the endocannabinoid system is not only involved in the rewarding and addictive effects of nicotine, but also in other substances. Further, behavioural addictions, such as gambling or gaming, would be a highly interesting topic to do further research on with respect to involvement of the endocannabinoid system.

Other scan techniques such as Single Photon Emission Computed Tomography (SPECT) or PET, each with its own advantages and disadvantages, are perhaps more suitable to address the biological mechanisms responsible for the effects of endocannabinoid activation on neurotransmission of for example dopamine, glutamate or GABA. Until now, these techniques have not often been used in combination with cognitive challenges, but technical progression may enable the use of these techniques to combine pharmacological and cognitive challenges to find out the biological mechanisms behind processes such as memory, attention, reward, and thus diseases in which these processes are impaired, such as addiction, depression and ADHD.

8.7 Conclusion

In this thesis we have shown that the endocannabinoid system plays a role in altered reward-processing, which is a symptom of addiction. Altered reward processing as is found in addiction is associated with increased sensitivity of the cannabinoid system. In contrast, the endocannabinoid system seems to play a limited role in normal reward processing and in response inhibition. Together, these data indicate that the endocannabinoid system is involved in certain aspects of addiction, and possibly other diseases in which reward processing is impaired, such as depression and ADHD, and as such is a valuable target for psychopharmacological therapy.

List of Abbreviations

2-AG	2-arachidonoylglycerol	**ms**	milliseconds
ADHD	attention-deficit hyperactivity disorder	**n.a.**	not applicable
ANOVA	analysis of variance	**n.s.**	not significant
ASL	arterial spin labelling	**OCD**	obsessive compulsive disorder
a.u.	arbitrary units	**PET**	positron emission tomography
BA	brodmann area	**PhICS**	pharmacological imaging of the cannabinoid system
BMI	body mass index	**phMRI**	pharmacological magnetic resonance imaging
BOLD	blood-oxygenated level-dependent	**PRESTO**	principle of echo shifting with a train of observations
CB1	cannabinoid receptor type 1	**R**	right
CBF	cerebral blood flow	**rCBF**	regional cerebral blood flow
DART	dutch adult reading test	**ROI**	region of interest
DSM-IV	diagnostic statistic manual IV	**RT**	reaction times
eCB	endocannabinoid	**SD**	standard devietion
fMRI	functional magnetic resonance imaging	**s / sec**	seconds
FAAH	fatty acid amide hydrolase	**SEM**	standard error of the mean
FOV	field of view	**SENSE**	sensitivity encoding
FWHM	full width at half maximum	**SNR**	signal-to-noise ratio
GABA	γ-aminobutyric acid	**SPECT**	single photon emission computed tomography
GLM	general linear model	**SSD**	stop signal delay
Hz	hertz	**SST**	stop signal task
ICA	independent component analysis	**SSRT**	stop signal reaction time
IQ	intelligence quotient	**THC**	Δ^9-tetrahydrocannabinol
L	left	**TE**	echo time
MANOVA	multivariate analysis of variance	**THC-COOH**	carboxy-tetrahydrocannabinol
MID	monetary incentive delay	**TR**	repetition time
MNI	montréal neurological institute	**tSNR**	temporal signal-to-noise ratio
MRI	magnetic resonance imaging	**VIF**	variance inflation factor
mg	milligrams	**WHO**	world health organization

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Nederlandse samenvatting

In dit proefschrift worden studies beschreven die de rol van het lichaamseigen cannabis-systeem bestuderen bij symptomen van verslaving, in het bijzonder beloningsverwerking en impulsiviteit. Hiervoor is gebruik gemaakt van functionele Magnetische Resonantie Imaging (fMRI). Dit is een techniek waarbij hersenscans gemaakt worden van proefpersonen terwijl zij een cognitieve taak uitvoeren, waardoor hersenactiviteit in kaart kan worden gebracht. Op basis van de resultaten beschreven in dit proefschrift worden veranderde beloningsprocessen in de hersenen, zoals gevonden in verslaving, geassocieerd met een verhoogde sensitiviteit van het lichaamseigen cannabis-systeem. Het lichaamseigen cannabis-systeem lijkt een beperkte rol te spelen in normale beloningsprocessen en in een specifieke maat van impulsiviteit, namelijk respons inhibitie.

Inleiding

Verslaving is gedefinieerd als 'mentaal en fysiek afhankelijk zijn van een stof of gewoonte'. Verslaving is wereldwijd één van de meest voorkomende psychiatrische ziektes, die vaak tot gezondheidsproblemen leidt en een groot deel van het totaal aantal sterfgevallen veroorzaakt. Nicotine verslaving is bijvoorbeeld de grootste oorzaak van vermijdbare sterfte in de Europese Unie. Ongeveer 30% van de volwassen bevolking gebruikt regelmatig nicotine. Deze getallen laten zien dat het effect van verslaving op de publieke gezondheid aanzienlijk is, en benadrukken dat het belangrijk is om meer te weten te komen over de processen in het brein die gerelateerd zijn aan verslaving.

Een belangrijk neurotransmittersysteem dat recentelijk geassocieerd is met verslaving is het lichaamseigen cannabis-systeem, ofwel het endocannabinoïde systeem. Dit systeem is betrokken bij diverse cognitieve functies, zoals emotie, beloning, aandacht en geheugen. Het bestaat uit cannabinoïde receptoren en endogene cannabinoïden die op deze receptoren aangrijpen. Cannabinoïde receptoren komen veel voor in de hersenen, waarbij de meeste receptoren zich bevinden in gebieden zoals de hippocampus, basale ganglia, prefrontale cortex en cerebellum.

Het endocannabinoïde systeem wordt geassocieerd met verslaving, met name doordat de belonende effecten van middelen zoals nicotine, alcohol, en opioiden, door dit systeem gemedieerd lijken te worden. Wanneer het cannabinoïde systeem namelijk geblokkeerd wordt met de cannabinoïde antagonist rimonabant, worden de belonende effecten van de eerder genoemde middelen ook geblokkeerd. Bovendien is het endocannabinoïde systeem bij dierstudies geassocieerd met de hunkering en terugval naar gebruik van cocaïne, nicotine, en alcohol. Bij mensen hebben klinische studies aangetoond dat de cannabinoïde antagonist rimonabant effectief is bij de behandeling voor stoppen met roken.

Doel van het proefschrift

Het doel van dit proefschrift is het in kaart brengen van de rol van het endocannabinoïde systeem in symptomen van verslaving, in het bijzonder het verwerken van beloning en respons inhibitie. De studies die in dit proefschrift beschreven worden zijn deel van de groter opgezette PhICS studie, die tot doel heeft om de rol te identificeren van het endogene cannabinoïde systeem bij cognitief functioneren. Deze rol wordt onderzocht door de effecten van de cannabinoïde agonist THC op hersenfunctie te meten, tijdens het uitvoeren van een cognitieve taak.

De methode die in dit proefschrift gebruikt is om hersenfunctie te meten is functionele Magnetische Resonantie Imaging (fMRI). Deze methode maakt gebruik van een sterke magneet om hersenactiviteit in kaart te brengen, door indirect het zuurstofverbruik in de hersenen te meten. Door deze techniek te combineren met een cognitieve taak kan onderzocht worden welke hersengebieden specifiek met een bepaald cognitief gebied (zoals geheugen, aandacht of beloning) geassocieerd worden. De rol van het cannabinoïde systeem werd onderzocht door het toedienen van de cannabinoïde agonist THC, de meest psychoactieve stof in cannabis. Door het vergelijken van hersenactiviteit na THC toediening en na placebo (een niet-werkend middel) toediening bij zowel een beloningstaak als een impulsiviteitstaak, hebben we de rol van het cannabinoïde systeem bij belangrijke symptomen van verslaving kunnen identificeren.

Samenvatting van de studies

In *hoofdstuk twee* hebben we het verwerken van beloning in de hersenen vergeleken tussen een groep zware, chronische cannabis gebruikers, een groep nicotine gebruikers en een groep gezonde controles. We hebben specifiek naar de nucleus accumbens gekeken, omdat dat een belangrijk hersengebied is dat betrokken is bij het verwerken van beloning. We hebben gevonden dat zowel chronisch cannabis gebruik als nicotine gebruik de hersenactiviteit in de nucleus accumbens verlaagt tijdens de verwachting van een (geld) beloning. Specifieke effecten van cannabis werden gevonden in de nucleus caudatus, waar chronische cannabis gebruikers een verlaging van hersenactiviteit lieten zien vergeleken met zowel gezonde controles als nicotine gebruikers. Er waren geen effecten in de nucleus accumbens van het verwerken van het daadwerkelijk krijgen van de beloning.

In *hoofdstuk drie* beschrijven we de methode en doelen van de ' Pharmacological Imaging of the Cannabinoid System' (PhICS; farmacologische imaging van het cannabinoïde systeem) studie. Het hoofddoel van de PhICS studie is de rol te verduidelijken van het endocannabinoïde systeem in cognitieve processen, om de mogelijkheid van betrokkenheid van het endocannabinoïde systeem bij psychiatrische aandoeningen te verhelderen. Betrokkenheid van het cannabinoïde systeem bij cognitie wordt onderzocht door een farmacologische toediening van de cannabinoïde agonist THC (tevens het belangrijkste psychoactieve bestanddeel van cannabis), in combinatie met functionele MRI. Cognitieve gebieden die onderzocht worden zijn geheugen, aandacht, emotie,

beloning en respons inhibitie. Dit hoofdstuk laat een aantal fysiologische effecten zien van THC, zoals verhoogde plasma waardes van THC, verhoogd hartritme, en subjectieve effecten, zoals een gevoel van 'high'. Verder worden taak paradigma's gevalideerd, en wordt de PhICS studie ingepast in een bredere context.

Hoofdstuk vier heeft tot doel de directe effecten van THC op hersenactiviteit tijdens rust in kaart te brengen, gebruik makende van twee verschillende methodes. Perfusie in het brein werd gemeten door Arterial Spin Labeling (ASL), en functionele MRI werd gebruikt om de variabiliteit van hersenactiviteit tijdens rust te meten. We vonden dat THC de perfusie in het brein verhoogde in met name de frontale gebieden en de insula, en dat die verhoging samenhing met de gevoelens van 'high' die THC veroorzaakte. Variabiliteit van hersenactiviteit was specifiek verhoogd in de substantia nigra, cerebellum en insula. Uit deze studie concludeerden we dat de psychotrope effecten van THC vermoedelijk in de insula verwerkt worden. Bovendien kunnen de veranderingen die wij gevonden hebben in de hersenactiviteit tijdens rust belangrijk zijn in de interpretatie van de studies waarbij we een cognitieve taak gebruiken. De studie maakt duidelijk dat fMRI paradigma's zorgvuldig gecontroleerd moeten worden, en dat bepaalde hersengebieden beïnvloed worden door THC, onafhankelijk van een bepaalde taak.

De rol van het endocannabinoïde systeem in beloningsverwerking werd onderzocht in *hoofdstuk vijf*. De resultaten die beschreven worden in dit hoofdstuk laten zien dat THC toediening een verlaging van hersenactiviteit teweegbrengt tijdens het krijgen van beloning, wat erop wijst dat de waardering van beloning veranderd is wanneer het endocannabinoïde systeem geactiveerd is. Omdat beloningsverwerking veranderd is in ziektes zoals verslaving, ADHD en depressie, hebben de resultaten die beschreven worden in dit hoofdstuk mogelijk implicaties voor deze ziektes. Het endocannabinoïde systeem is mogelijk betrokken bij de veranderde beloningsverwerking in verslaving, ADHD en depressie.

Hoofdstuk zes beschrijft resultaten van een studie die specifiek de betrokkenheid van het endocannabinoïde systeem bij veranderde beloningsprocessen in nicotine verslaving onderzoekt. De resultaten van deze studie lieten zien dat, na toediening van THC, de nucleus accumbens van proefpersonen met een nicotine verslaving niet geactiveerd wordt in antwoord op verwachting van een geldelijke beloning, terwijl THC geen effect had op beloningsverwerking in dit gebied in proefpersonen zonder nicotine verslaving. Deze resultaten suggereren dat nicotine verslaafden overgevoelig zijn voor de belonende effecten van THC, zodat andere beloningen (zoals geld) hun aantrekkelijkheid verliezen. Deze resultaten kunnen worden geïnterpreteerd als ondersteunend voor de beloningsdeficiëntie hypothese, die stelt dat mensen met een (drugs)verslaving minder geïnteresseerd zijn in en gemotiveerd worden door natuurlijke beloningen.

Hoofdstuk zeven beschrijft een studie waarin de rol van het endocannabinoïde systeem in respons inhibitie in verslaving wordt onderzocht, met behulp van een zogeheten 'stop signal' paradigma. Net zoals de studie beschreven in *hoofdstuk zes*, centreert deze

studie zich rond het striatum. In tegenstelling tot de resultaten voor beloningsverwerking heeft THC geen veranderd effect op respons inhibitie gerelateerde hersenactiviteit in het striatum van nicotine verslaafden. De conclusie was dan ook dat het endocannabinoïde systeem geen rol speelt bij inhibitie processen in de hersenen, gemeten met een stop signal taak, en dat deze rol ook niet veranderd is in nicotine verslaving.

Algemene discussie

Dit proefschrift richt zich specifiek op de betrokkenheid van het endogene cannabinoïde systeem in cognitief functioneren in verslaving. Op basis van de studies in dit proefschrift concluderen we dat het cannabinoïde systeem een rol speelt in veranderde beloningsverwerking als symptoom van verslaving. Deze conclusie kan gevolgen hebben voor de behandeling van mensen met verslavingsproblematiek, maar verschaft ook inzicht in processen die ten grondslag liggen aan verslaving. Het is goed mogelijk dat de verhoogde gevoeligheid voor een cannabinoïde agonist, zoals wij vonden in proefpersonen met een nicotine verslaving, voorafgaat aan de ontwikkeling van verslaving, en verantwoordelijk is voor de initiatie van verslavingsgerelateerde processen. Deze kennis kan belangrijk zijn voor de ontwikkeling van programma's of strategieën om te voorkomen dat mensen veslaafd worden. Ondanks het feit dat het ontwerp van de huidige studies ons niet in staat stelt om conclusies te trekken over de oorzaak of gevolg van de verandering van de cannabinoïde betrokkenheid bij beloningsprocessen in verslaving, veronderstellen we dat de verandering in cannabinoïde processen al bestaan voorafgaand aan het begin van verslaving. Een hoge gevoeligheid van het endogene cannabinoïde systeem (dat wil zeggen, een sterke respons op cannabinoïde stoffen) kan een risico factor zijn voor de ontwikkeling van verslaving.

In tegenstelling tot de effecten die we hebben gevonden op het gebied van beloningsverwerking, laat respons inhibitie weinig betrokkenheid van het endocannabinoïde systeem zien. Deze bevindingen waren tegengesteld aan onze hypothese, omdat we verwachtten dat er zowel in beloningsverwerking als in respons inhibitie een rol zou zijn voor het cannabinoïde systeem, en dat deze rol veranderd zou zijn in mensen met een verslaving. Onze initiële hypothese was hoofdzakelijk gebaseerd op dierstudies of gedragstudies die lieten zien dat manipulatie van het cannabinoïde systeem met ofwel een agonist ofwel een antagonist, een verandering teweegbracht van zowel beloningsverwerking als respons inhibitie. Het feit dat onze resultaten weinig tot geen rol voor het cannabinoïde systeem lieten zien in respons inhibitie benadrukt het belang van het uitvoeren van dergelijke studies op een translationele manier: niet alleen in dieren, maar ook in mensen.

De nucleus accumbens liet geen activatie zien in nicotine gebruikers tijdens de verwachting van een (geld-)beloning na THC toediening. Dit kan betekenen dat THC toediening de nucleus accumbens weerhoudt van activeren in het algemeen. Tijdens respons inhibitie echter, lieten nicotine gebruikers significante activatie van zowel het striatum als de

nucleus accumbens zien, zelfs na THC toediening. Dit laat zien dat endocannabinoïde processen in striatale gebieden inderdaad specifiek betrokken zijn bij bepaalde cognitieve hersenfuncties, zoals beloningsprocessen, maar niet noodzakelijkerwijs in respons inhibitie.

De PhICS studie had tot doel om de rol van het endocannabinoïde systeem in cognitie te verhelderen door deze rol te onderzoeken in symptomen van psychiatrische aandoeningen. De reden voor deze benadering is dat het ons kan helpen om meer te begrijpen over de normale situatie: als een normale situatie vergeleken wordt met een verstoorde situatie, kunnen we de verschillen tussen deze situaties duidelijk beschrijven en zo meer licht werpen op de normale situatie. Hoewel deze benadering in theorie valide en logisch is, is het in de praktijk moeilijk om 'de rol van het endocannabinoïde systeem' te scheiden van 'de effecten van THC'. Momenteel is het toedienen van THC in mensen de enige manier om direct in te grijpen in het endocannabinoïde systeem, omdat het gebruik van rimonabant niet meer mogelijk is doordat dit middel van de markt is teruggetrokken vanwege verhoogde risico's op depressie en suïcide in patiënten met obesitas in klinische studies. Andere cannabinoïde agonisten of antagonisten worden nog onderzocht.

Een belangrijk onderwerp om te bespreken met betrekking tot de studies in dit proefschrift is dat door het toedienen van THC het functioneren van alle cannabinoïde receptoren veranderd wordt. Het endogene cannabinoïde systeem werkt subtieler, omdat het enkel waar en wanneer nodig geactiveerd wordt, met als gevolg dat neurotransmissie veel gelocaliseerder gewijzigd wordt dan wanneer een exogene agonist zoals THC toegediend wordt. Daarom zouden indirecte cannabinoïde agonisten een interessant alternatief vormen om het endogene cannabinoïde systeem te onderzoeken. Een voorbeeld van indirecte cannabinoïde agonisten zijn de zogeheten fatty acid amide hydrolase (FAAH) remmers. FAAH is het enzym dat endogene cannabinoïden inactiveert, waardoor de werking van het cannabinoïde systeem (lokaal) beëindigd wordt. Remmers van FAAH zullen dus de aanwezigheid van endocannabinoïden verlengen. Hierdoor wordt de endogene tonus van het systeem verlengd, wat de effecten meer subtiel en gelocaliseerder maakt. FAAH remmers worden al gebruikt in dierstudies, maar tot op heden zijn nog geen indirecte cannabinoïde agonisten goedgekeurd voor gebruik in mensen. Suggesties voor therapeutische meerwaarde van deze middelen zijn gedaan richting aandoeningen zoals depressie of paniek- of angststoornissen.

Een ander belangrijk onderwerp wat met name relevant is voor *hoofdstuk twee, zes* en *zeven* van dit proefschrift is dat door het aanwenden van nicotine gebruikers als model voor verslaving geen rekening gehouden wordt met de mogelijkheid dat nicotine verslaving specifieke karakteristieken heeft die niet representatief zijn voor verslaving in het algemeen. Ook al is nicotine verslaving op veel punten vergelijkbaar met verslaving aan andere middelen zoals cocaine, opioïden, en amfetamines, er zijn ook een aantal unieke aspecten aan nicotine verslaving die onze resultaten wellicht hebben beïnvloed. Een algemeen effect van verslavende middelen is dat acute toediening hiervan dopamine

afgifte in de nucleus accumbens veroorzaakt. Chronische toediening van verslavende middelen verlaagt over het algemeen de dopamine functie. Dit gebeurt ook in chronisch nicotine gebruik, wat het als model voor verslaving bruikbaar maakt. Daarentegen is er een specifieke interactie tussen THC en nicotine wanneer deze middelen tegelijkertijd worden toegediend, wat de resultaten van onze studies mogelijk moeilijk extrapoleerbaar maakt naar verslaving in het algemeen.

Ondanks het feit dat we veel weten over de neuromodulerende rol van het lichaamseigen cannabis-systeem in de transmissie van dopamine, GABA, glutamaat, serotonine en andere neurotransmitters, kunnen we alleen maar speculeren over de vraag hoe onze resultaten veroorzaakt kunnen worden door specifieke neurotransmitter systemen. Door het gebruik van fMRI als techniek kunnen we die rol ook niet onderzoeken. Dit is een algemene limitatie van fMRI studies, maar wanneer er geen farmacologische interventie wordt gebruikt is dit gewoonlijk minder relevant. Met een neurotransmitter systeem zoals het endocannabinoïde systeem, dat de afgifte van diverse andere neurotransmitter reguleert, zou kennis over specifieke veranderingen in dopamine, GABA of glutamaat concentraties in betrokken hersengebieden een belangrijke bijdrage kunnen bieden aan het begrijpen van de rol van het endocannabinoïde systeem bij neurotransmissie in het algemeen. De technieken om cognitieve en farmacologische interventies te combineren om hier meer over te weten te komen zijn nog in ontwikkeling.

Limitaties

Een belangrijke limitatie van de studies waarbij we THC hebben toegediend is dat alle subjecten die meegedaan hebben aan deze studies, milde doch chronische cannabis gebruikers waren. Dit kan veranderingen in endocannabinoïd functioneren tot gevolg hebben gehad. Zoals we hebben laten zien in *hoofdstuk twee* is het beloningssysteem in cannabis gebruikers (maar ook in nicotine gebruikers) veranderd. Ethische overwegingen lieten deelname van cannabis-naïeve proefpersonen niet toe. Om het endogene cannabinoïde systeem te bestuderen zou men idealiter proefpersonen onderzoeken die nooit blootgesteld zijn aan agonisten van dit systeem. Aangezien THC anxiogene effecten kan veroorzaken, vooral in cannabis-naïeve proefpersonen, of bij het gebruik van een hoge dosis, is het ethisch niet verantwoord om naïeve proefpersonen te onderzoeken in dit onderzoek. Prospectieve studies kunnen hier wellicht een oplossing voor bieden, hoewel dergelijke studies duur zijn en moeilijk te bewerkstelligen. Een vergelijkbare maar additionele limitatie van de studies waarin we nicotine gebruikers met gezonde controles hebben vergeleken, is dat behalve de verschillen in nicotine gebruik, deze groepen ook kunnen verschillen in veel andere aspecten, wat onze resultaten lastiger te interpreteren maakt. Nicotine gebruikers kunnen bijvoorbeeld verschillen van gezonde controles in bepaalde karaktereigenschappen, zoals verhoogde impulsiviteit en risicovol gedrag, wat dan ook consequenties heeft voor beloningsprocessen, of hersenactiviteit in het algemeen. Een andere beperkende factor is dat de subjectieve effecten van THC die door de

proefpersonen ervaren werden, de blindering van de proefpersonen in de weg gestaan kan hebben. Na het onderzoek konden de meeste (maar niet alle) proefpersonen correct aangeven op welke dag zij THC hadden gekregen. Dit kan voor verwachtingseffecten gezorgd hebben. We hebben geprobeerd de invloed van deze verwachting te minimaliseren door het gebruiken van een gerandomiseerd cross-over ontwerp van de studies, wat inhoudt dat de verwachtingseffecten gebalanceerd zouden moeten zijn doordat de volgorde van administratie van THC en placebo gerandomiseerd was. Toch kunnen we de mogelijkheid niet uitsluiten dat verwachtingseffecten de studie tot op zekere hoogte beïnvloed hebben.

Toekomstperspectief

Dit proefschrift geeft inzichten die de mogelijkheid bevestigen dat het endogene cannabinoïde systeem een belangrijk doelwit wordt voor farmacotherapie. Vooral aandoeningen waarin beloningsprocessen verstoord worden, zoals verslaving, ADHD en depressie, zouden baat hebben bij onderzoek naar kunstmatige modulatie van het cannabinoïde systeem.

Met de ontwikkelingen die gaande zijn op het gebied van cannabinoïden zullen indirecte agonisten van het cannabinoïde systeem binnenkort beschikbaar komen. De introductie van bijvoorbeeld FAAH-remmers voor het gebruik in mensen is een grote kans voor het onderzoek naar de endogene werking van het cannabinoïde systeem in cognitie in mensen. Farmacologische MRI studies die deze stoffen gebruiken zullen ons helpen om inzicht te verkrijgen in de meer subtiele betrokkenheid van het endocannabinoïde systeem in symptomen van psychiatrische aandoeningen.

Het zou interessant zijn om te onderzoek of de bevindingen in dit proefschrift geëxtrapoleerd kunnen worden naar verslaving aan andere middelen, zoals cocaïne, amfetaminen, of opioïden. Uit de literatuur weten we dat het endocannabinoïde systeem niet alleen betrokken is bij de belonende en verslavende effecten van nicotine, maar ook van andere middelen. Verder zouden gedragsmatige verslavingen, zoals gokverslaving of verslaving aan computerspellen, een bijzonder interessant onderwerp zijn om verder onderzoek te doen met betrekking tot betrokkenheid van het endocannabinoïde systeem. Andere scantechnieken zoals Single Photon Emission Computed Tomography (SPECT) of PET, elk met zijn eigen voor- en nadelen, zijn wellicht meer geschikt om de biologische mechanismen te belichten die verantwoordelijk zijn voor de effecten van cannabinoïde activatie op neurotransmissie van bijvoorbeeld dopamine, glutamaat of GABA. Tot op heden zijn deze technieken zelden gebruikt in combinatie met cognitieve interventies, maar technische vooruitgang geeft ons wellicht de mogelijkheid om deze technieken te combineren met farmacologische en cognitieve interventies, om de biologische mechanismen te identificeren achter processen zoals geheugen, aandacht, en beloning – en dus ziektes waarin deze processen verstoord zijn, zoals verslaving, depressie en ADHD.

Conclusie

In dit proefschrift hebben we laten zien dat het endocannabinoïde systeem een rol speelt in veranderde beloningsprocessen die een symptoom zijn van verslaving. Veranderde beloningsprocessen zoals gevonden in verslaving worden geassocieerd met verhoogde sensitiviteit van het cannabinoïde systeem. Daarentegen lijkt het endocannabinoïde systeem een beperkte rol te spelen in normale beloningsprocessen, en in respons inhibitie. Samen laten deze data zien dat het endocannabinoïde systeem betrokken is bij bepaalde aspecten van verslaving, en mogelijk andere aandoeningen waarin beloningsprocessen zijn verstoord, zoals depressie en ADHD, waardoor het een waardevol doelwit is voor psychofarmacologische therapie.

Dankwoord

Nu ontkom ik er echt niet meer aan – nu moet ik woorden gaan vinden voor het bedanken van de mensen zonder wie dit proefschrift nooit voor u had gelegen. Woorden schieten tekort, maar ik zal toch een poging wagen.

Mijn (co-)promotoren: Nick, begeleider van het eerste uur.. ik weet nog dat je me de allereerste keer dat ik je zag vroeg hoe mijn computervaardigheden waren. Voor iemand die toen niet eens wist dat Linux überhaupt bestond heb ik het toch aardig gered... Bedankt voor je vertrouwen in mij en je begeleiding in de voorzichtige stapjes die ik via stagiaire, onderzoeksassistent, flow manager en uiteindelijk PhD student op de weg tot de wetenschap heb gezet. Het project liep niet altijd even soepel, en niet helemaal zoals gepland, maar uiteindelijk zijn er toch twee proefschriften uitgerold. Ik heb veel geleerd van je vermogen om onverwachte resultaten van alle verschillende kanten te bekijken. Dank!

Gerry, jij bent niet alleen mijn co-promotor geweest, maar ook vooral een mentor op persoonlijk vlak. Van jou heb ik heel veel geleerd over planning, processen, invloed uitoefenen op processen, keuzes maken, vaststellen wat je doel is, en zorgen dat je daar komt. Bedankt voor je wijsheid en betrokkenheid. Jammer dat je wegging ☺; hoewel dat ook weer een leerzaam proces was…

Martijn, begeleider van het laatste uur… Je kwam er pas vorig jaar bij, en bent nog steeds onwillig om daadwerkelijk co-promotor te zijn.. maar als er iemand is die mij veel geleerd heeft over wetenschappelijk nadenken, alles in twijfel trekken, zorgvuldig naar fMRI analyses kijken, altijd teruggrijpen naar de vraag die je wilt beantwoorden en altijd kritisch zijn (zelfs over Star Trek), dan ben jij het. Dank je wel.

René Kahn, we hebben elkaar niet veel gezien, maar van een afstand was u toch altijd betrokken. Bedankt voor de snelle, bondige en nuttige commentaren op mijn artikelen.

Mijn paranimfen: Matthijs.. zonder jou had dit proefschrift er hoogstwaarschijnlijk niet gelegen… We hebben elkaar in Londen pas echt leren kennen, maar ook goed leren kennen, en wat fijn dat het zo klikte. We hebben vaak dezelfde visie, en vaak ook niet; wat voor een synergistisch geheel zorgde en tot twee succesvolle doch stormachtige promotie-trajecten heeft geleid. Jij bent een van de weinige mensen van wie ik maar een half woord hoef te horen om precies te weten waar je het over hebt (beetje eng soms). Bedankt dat je speciaal voor mij een pak wilt aantrekken!

Lieve lieve Rinke.. zonder jou had het proefschrift er al helemáál niet gelegen… Wat heerlijk dat je mijn zus bent, dat ik zonder schroom elke dag (of zonodig elk uur) de telefoon kon pakken in de laatste maanden, dat jij precies begrijpt wat ik bedoel, dat je me met twee woorden weer met beide benen op de grond zet, dat je me onvoorwaardelijk hebt gesteund, zelfs in mijn zeur-tijdperk, en gewoon, dat jij jij bent… can't wait to return the favour!

De stagiaires: Annelies, ik had me geen betere eerste stagiaire kunnen wensen! Bedankt ook voor je bijdrage later als onderzoeksassistent – zonder jou waren al die scansessies

nooit zo soepel ingevuld geweest. Joep, voor jou was het wel teleurstellend dat de studie halverwege 'on hold' gezet werd, en je niet je echte, eigen, vraag kon onderzoeken, maar de eerste stappen op zoek naar THC effecten op het beloningssysteem bij rokers zijn door jou gezet! Kim, jij kwam er ook al gelijk bij binnenkomst achter dat onderzoek doen niet altijd gaat zoals je wilt, en dat je nogal flexibel moet zijn en tegen teleurstellingen moet kunnen. Je hebt het goed opgepakt. En van een afstand: Lindsey, Emi (altijd gezellig, zo'n scandag) en Erik (ik heb je inzet altijd bewonderd!).

Wat ben ik veel mensen tegengekomen in de afgelopen zeven (zeven? Zéven?! Zeven!) jaar. Bij de BCI: wat fijn dat we de laatste paar weken nog even gezellig bij elkaar op de gang zaten… Erik (om de frustratie te delen over teveel bewegende subjecten..), Mariska (je zei laatst zoiets over dat je achteraf bezien eigenlijk niet over elk woord van je proefschrift na had hoeven denken.. daar heb ik nog wel een paar keer aan terug gedacht de afgelopen tijd), Dora (kort als kamergenoot, en toen was je ineens weg, en bleef je ineens weg, en was je ineens met z'n tweeën! Veel geluk!), Martin (bewonder nog steeds het tempo waarmee jij Nederlands hebt geleerd), en later Zac; daarnaast niet van de BCI, maar wel 'van Nick': Floor (ook kort kamergenote, met jou kon ik het heel goed hebben over het relatieve van het promoveren, en vooral het 'door de mand vallen' gevoel.. maar als ik het kan, kun jij het ook, zeker weten!!), Gert (dank voor je nooit aflatende enthousiasme om nieuwe dingen uit te zoeken.. hoop dat je een beetje focus krijgt de komende paar jaar!), Mathijs (bedankt voor je heldere uitleg over fMRI analyse en kritische visie op onze studie), Wouter (ga je nu die vrijdagmiddagborrel toch weer in het leven roepen?), Jeroen (wetenschapper of muzikant? Of toch allebei?!), Natalia (thanks for putting up with me in Canada ☺), en last but not least, de system beheerders.. van een afstand Roger, van dichtbij Pieter (je was een gezellige kamergenoot!), en van héle grote afstand Vincent.. Systeem beheren vanuit Kroatië, je moet het maar kunnen.. zonder jou waren de SPM-analyses helemaal de soep in gelopen.. Dank voor al je wijze Linux-lessen!

Bij Psychiatrie: Matthijs Vink, stage-begeleider, jouw soms ietwat zegmaar directe manier van doen (sommige mensen zouden het misschien zelfs bot noemen) overtuigde mij er destijds van dat toen je zei dat ik een promotietraject wel aan kon, dat ook daadwerkelijk zo was – van een minder direct iemand had ik het waarschijnlijk niet geloofd. Dank voor je begeleiding tijdens mijn stage, ik heb veel Matlab-dingen van je opgepikt.. Bas (altijd behulpzaam, altijd kritisch, altijd in voor iets gezelligs), Geartsje, Maartje, Rachel, Astrid, Jiska, Tamar, Cédric, Bram, Mariët, Kelly, Janna-Marie, Janna (succes in Londen!), Marieke, Martijn M., Sarah (bedankt voor de oefenscanner!), Martijn vdH, Neeltje, Hugo, René Mandl, en iedereen die ik nog vergeet..

De laboranten van Radiologie: bedankt voor jullie geduld, jullie schrokken altijd een beetje als wij weer aankwamen met onze volle kar met THC-spullen, maar in overleg werd dat altijd in goede banen geleid…

Verder zijn er nog hopen mensen die op een directe of indirecte manier meegeholpen hebben aan dit proefschrift, en ik weet niet waar ik moet beginnen… Bij het begin

dan maar: Suzanne, in onze 28-jarige vriendschap hebben we toch al behoorlijk wat meegemaakt samen.. Kleuterschool, basisschool, middelbare school, universiteit, diverse vakanties en weekendjes, enzovoort enzovoort. Ik bewonder enorm wie je bent, wat je kunt en wat je doet, en word altijd blij van jou omdat je zo enthousiast reageert als ik vertel wat ik aan het doen ben. Jasper natuurlijk ook, en jullie twee mannetjes.. bedankt voor jullie vriendschap..

Esther, sinds de middelbare school hebben we uren en uren telefonisch doorgebracht, en los van de rest van de dingen die we allemaal bespraken is dat proefschrift ook erg vaak voorbij gekomen. Dank voor je bemoedigende woorden vaak, en veelvuldig, en jouw volharding daarin… En Aalt, op een andere manier betrokken (vooral nieuwsgierig of je van eeuwenoude botten ook MRI-plaatjes kan maken), en jullie twee bloedjes van kinders natuurlijk ook (tante Erika – klinkt nog steeds raar hoor). Tim en Ruth, ook bedankt voor jullie betrokkenheid! Wat is er veel gebeurd hè, sinds ik op m'n twaalfde voor het eerst bij jullie over de vloer kwam…

Toen.. ging ik naar de universiteit... Ginny, met jou heb ik veeheel lief en leed gedeeld… we hadden allebei dezelfde mening over de studie Farmacie, maar jij, held, ging door en werd, jazeker… de apotheker. Ik bewonder wie je bent, je creativiteit, en je vermogen om voor jezelf te kiezen, ook als dat niet altijd makkelijk is.. hoop nog veel weekendjes weg te kunnen doen! En.. misschien toch nog.. één keertje Stairway? Nies.. wat was het leuk om met jou in huis te wonen.. we hebben elkaar toen goed leren kennen, en wat fijn dat we elkaar altijd konden bellen als er wat was. Fijn dat jij het ook naar je zin hebt bij je nieuwe banen (!).

En dan meer in de familiale sferen… Tante Hanneke, bedankt voor uw betrokkenheid, wat leuk dat we al zo lang gezellige dingen samen doen, soms met z'n drieën, en soms met z'n tweeën.. Dat was vaak een welkome afwisseling van het onderzoek doen!

Ada, lieve lieve Ada. Ik weet eigenlijk geen woorden te geven aan het gevoel wat jij me geeft. Als je het goed beschouwt ben jij verantwoordelijk voor het feit dat papa en mama elkaar hebben leren kennen, dus daarmee ook voor mijn bestaan ☺ Dank je wel voor jouw bestaan, en dat we onze threesome weekendjes in Amsterdam, Tilburg of Bruchem nog maar lang mogen volhouden!

Schone familie: Marie-José, schone moeder, Marian, schone zus, Tante Mien, we spreken elkaar niet vaak, maar voel me altijd welkom bij jullie. Dank voor jullie begrip als ik weer eens een verjaardag (of de kermis!) moest missen door een scandag of een congres…

Lieve Papa en Mama… wat kan ik nog tegen jullie zeggen? Papa, dank voor je steun en lieve kadootjes op sommige moeilijke momenten. Mama, de afgelopen jaren zijn niet makkelijk geweest voor je. Dank dat je met me meeleeft, en dat je, samen met mij, blij bent als het 22 november is.

Jeroen. Bedankt dat jij me altijd de ruimte hebt gegeven. Van jou heb ik geleerd te relativeren, of dat heb ik in elk geval geprobeerd van je te leren. Ik bewonder jouw ongelooflijke eigenheid, je principiële manier van in het leven staan, en het vaststaande

feit dat je de gevolgen van je eigen keuzes accepteert. Jij bent de meest bijzondere persoon die ik ken. Ik houd van je.

En nu stop ik, want: "Hoe meer woorden, hoe meer onzin. En wat heb je daar aan?" (Prediker 6: 11)

List of Publications

Journal Articles

Van Hell HH, Jager G, Bossong MG, Brouwer A, Jansma JM, Zuurman L, van Gerven J, Kahn RS, Ramsey NF. *Involvement of the endocannabinoid system in reward processing in the human brain*. Psychopharmacology 2011: Aug [Epub ahead of print]

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Van Hell HH, Vink M, Ossewaarde L, Jager G, Kahn RS, Ramsey NF. *Chronic effects of Cannabis on the Human Reward System: an fMRI study*. European Neuropsychopharmacology 2010: 20(3): 153-163

Bossong MG, Jager G, **van Hell HH**, Zuurman L, Jansma JM, Mehta M, Kahn RS, Ramsey NF. *Effects of Δ^9-tetrahydrocannabinol (THC) administration on human encoding and recall memory function: a pharmacological fMRI study.* Journal of Cognitive Neuroscience, accepted for publication

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Van Hell HH, Jansma JM, Bossong MG, Kahn RS, Ramsey NF. *Endocannabinoid involvement in brain reward processing in nicotine addiction.* European Neuropsychopharmacology 2011: 21 (Suppl 3): S578 (awarded with travel award)

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Van Hell HH, Bossong MG, Ramsey NF, Jager G. *THC alters resting-state brain activity and brain perfusion.* Journal of Psychopharmacology 2010: (Suppl)

Van Hell HH, Jager G, Brouwer A, Bossong MG, Kahn RS, Ramsey NF. *Addiction proneness: contribution of the endocannabinoid system and reward processing.* European Neuropsychopharmacology 2010: 20 (Suppl 1): S82 (awarded with a young investigator's award)

Van Hell HH, Bossong MG, Jager G, Saliasi E, Kahn RS, Ramsey, NF. *The acute effects of delta-9-tetrahydro-cannabinol (THC) on brain activity during working memory.* European Neuropsychopharmacology 2009: 19 (Suppl 3): S308

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Van Hell HH, Vink M, Ossewaarde L, Jager G, Kahn R, Ramsey NF. *Long-term effects of cannabis on the human reward system: an fMRI study*. Neuroimage 2006: 31 (Suppl 1): S336

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Curriculum Vitae

Erika van Hell was born on the 1st of September 1979 in Zwolle. She graduated from high school at the Lambert Franckens College in Elburg in 1997. She obtained a Master of Science degree in Pharmacy in August 2003, and a second Master of Science degree in Neuroscience in 2005, for which she performed an fMRI study into the effects of chronic cannabis use on the brain reward system. She worked as flow manager and research assistant from August 2005 until April 2007 at the functional MRI department of the University Medical Centre in Utrecht. Her PhD project started in April 2007, in which she investigated the role of the endocannabinoid system in symptoms of addiction. After her thesis defence in November 2011, Erika will work as a Clinical Research Associate at TFS in Berghem.

Erika van Hell werd op 1 september 1979 geboren in Zwolle. Ze slaagde voor haar VWO examen op het Lambert Franckens College in Elburg in 1997. Ze behaalde haar doctoraal in Farmacie in 2003, en behaalde haar master-titel in Neuroscience in 2005, waarvoor ze een fMRI studie deed naar de effecten van chronisch cannabis gebruik op het beloningssysteem in de hersenen. Ze werkte als flow manager en onderzoeksassistent van augustus 2005 tot april 2007 op de functionele MRI afdeling van het Universitair Medisch Centrum in Utrecht. Haar promotieproject begon in april 2007, waarin ze de rol van het lichaamseigen cannabis-systeem onderzocht in symptomen van verslaving. Na het verdedigen van haar proefschrift in november 2011 zal Erika als Clinical Research Associate gaan werken bij TFS in Berghem.