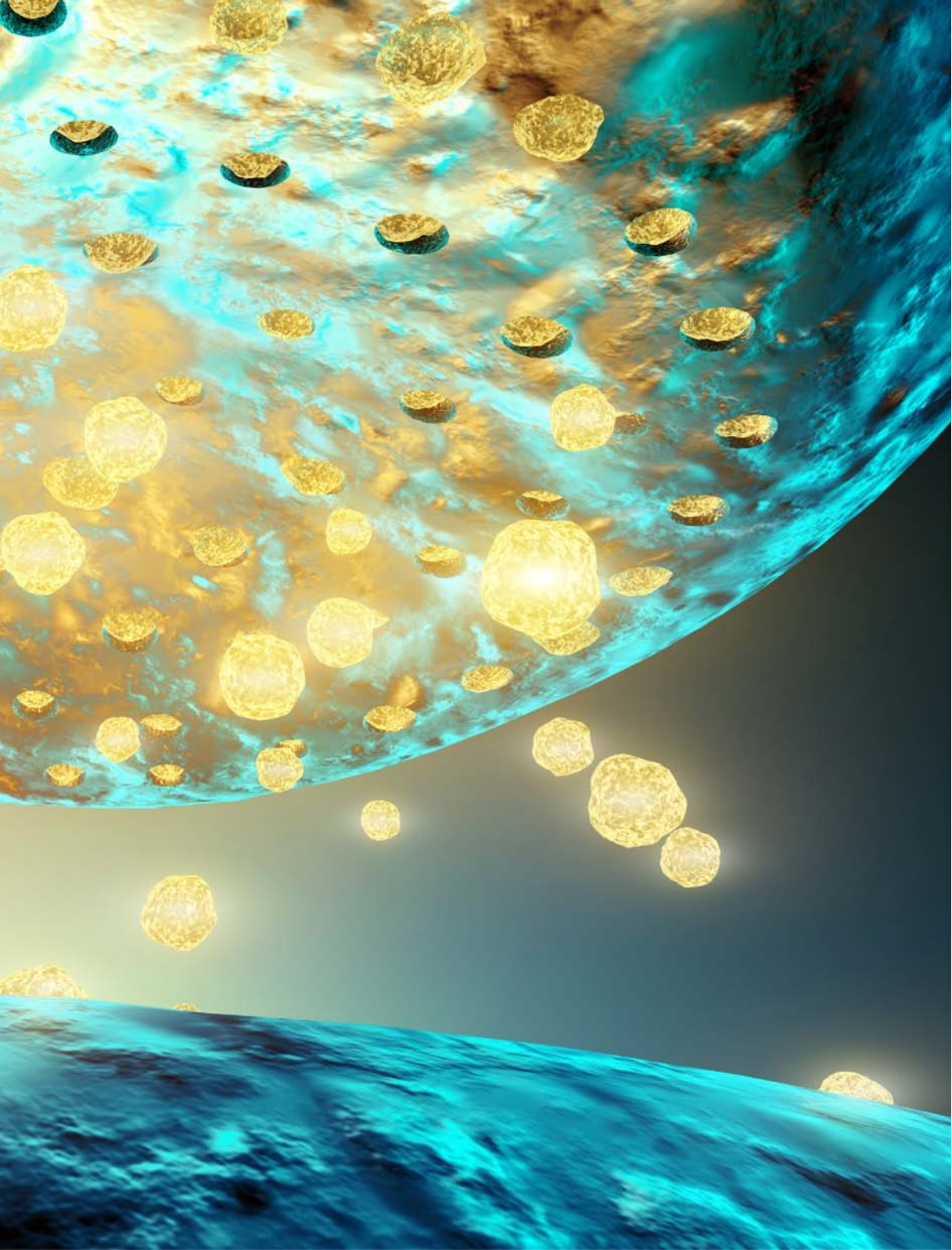
DRUG-INDUCED PSYCHOMIMETIC EFFECTS AS A MODEL FOR PSYCHOSIS

Daniël Kleinloog



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PROEFSCHRIFT

Drug-induced psychomimetic effects as a model for psychosis

PROEFSCHRIFT
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CHAPTER 1

Introduction

The first antipsychotic drug, chlorpromazine (marketed as Largactil in Europe -Figure 1- and as Thorazine in the United States), was synthesised in 1950 as an anaesthetic drug (Bennet, 1998). Its benefit in the treatment of psychosis was discovered in 1952, in the Parisian mental hospital Saint Anne (Delay et al., 1952a,b; Bennet, 1998; Kapur and Mamo, 2003).

At the time of the introduction of chlorpromazine, Jean Thuillier was a psychiatrist at Saint Anne and a pharmacologist: a rare combination in those days. In the book *Les dix ans qui ont changé la folie* (translated title: *Ten years that changed the face of mental illness;* Thuillier, 1980) he describes his experiences before and after the introduction of chlorpromazine. Before the discovery of chlorpromazine, psychiatry was heavily focused on the psychoanalytical theories put forth by Freud and there was hardly any place for biological psychiatry. Knowledge on neurotransmitters was very limited. The focus of most psychiatrists was more on understanding mental illness than on treating it. Patients with psychosis were put away in mental asylums and treatment options were limited to several types of shock therapies, including induced coma (e.g. insulin-induced), induced convulsions (e.g. electroconvulsive therapy without anaesthesia), and induced fever (e.g. through infection with malaria).

The introduction of chlorpromazine as a treatment for psychosis has been described as 'the French revolution of 1952' (Thuillier, 1980). It led to a remarkable change in the prognosis of patients with psychosis and psychiatric wards were no longer filled with cries of rage. In fact, the effect of chlorpromazine could be measured by recording the sound levels outside a psychiatric ward (Thuillier, 1980). It was also the start of the era of psychopharmacology.

Soon after the discovery of chlorpromazine in Europe, the drug reserpine was developed in the United States (Lehman and Ban, 1997). Reserpine is a plant-derived anti-hypertensive, acting by depletion of monoamines, originating in India. It was introduced into Western medicine in 1949 and psychiatry in 1954 (Kline, 1954; Lehmann and Ban, 1997; Bennet, 1998). The revolutionary changes that were seen after treatment with chlorpromazine

and reserpine, let to the introduction of many more antipsychotic drugs with a similar mechanism of action, which are now referred to as typical antipsychotics (e.g. haloperidol, flupentixol, droperidol).

In 1963, Carlsson and Lidqvist discovered that dopamine acts as a neurotransmitter (Carlsson and Lidqvist, 1963), for which Carlsson received the Nobel Prize of Medicine in 2000. This led to the believe that dopamine antagonism was essential to the mechanism of action of the typical antipsychotics (Carlsson and Lidqvist, 1963; Bennet, 1998; Benes, 2001; Seeman, 2002). This dopamine hypothesis of psychosis was only formed in 1967 by van Rossum and refined in the early 1970s, more than fifteen years after the discovery of chlorpromazine. The similarity between the symptoms elicited by a pharmacological challenge with amphetamine (which increases the release of dopamine into the synaptic cleft) and the symptoms of psychosis further supported the dopamine hypothesis (Lehmann and Ban, 1997; Featherstone et al., 2007). There are four distinct dopaminergic systems (Bennet, 1998; Lieberman, 2004; see also Figure 2): the mesocortical system (from the ventral tegmentum in the mesencephalon to the frontal lobes and cingulate cortex), the mesolimbic system (from the ventral tegmentum to the hippocampus and amygdala), the nigrostriatal system (from the substantia nigra to the striatum) and the tuberoinfundibular system (from the hypothalamus to the posterior pituitary). Although all drugs also affected the serotonergic system, the blockade of dopamine receptors within the nigrostriatal system was considered the primary target of (typical) antipsychotic drugs (Seeman, 2002).

The first clinical trials with clozapine, which has a wide range of receptor effects including a relatively low affinity for dopamine D_2 receptors and high affinity for serotonin 5-HT_2 receptors, were carried out in 1966 (Bennet, 1998). It was withdrawn from the market in 1975, because of the severe side-effects of agranulocytosis (Idänpään-Heikkilä et al., 1975, 1977). However, after a pivotal comparative study between chlorpromazine and clozapine, which showed superior efficacy of clozapine on both positive and negative symptoms, clozapine was again approved for treatment of refractory schizophrenia (Kane et al., 1988).

Following the introduction of clozapine, several other drugs were developed with different, 'atypical', working mechanisms (e.g. olanzapine, risperidone, quatiapine). The definition of 'atypical' is very varied and can refer to the occurrence of extra-pyramidal side effects, the relative affinity for dopamine D_2 versus serotonin 5-HT_2 receptors, or the fast or slow dissociation rate at the dopamine D_2 receptor (Meltzer, 1999; Kapur and Remington, 2001; Seeman, 2002; Kapur and Mamo, 2003). Initially, these drugs were thought to be more effective and have fewer side-effects. However, three large, multi-center trials (Clinical Antipsychotic Trials of Intervention Effectiveness-CATIE; Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study-CUTLASS; European First-Episode Schizophrenia Trial-EUFEST) showed that all antipsychotic drugs are similar in effectiveness (Lieberman et al., 2005, Jones et al., 2006; Kahn et al., 2008). Clozapine is the only drug that has demonstrated superior efficacy (Chakos et al., 2001; Lieberman et al., 2005; Carpenter and Davis, 2012).

Current perspectives

Even though dopamine antagonists have been able to reduce symptoms of psychosis (in particular the positive symptoms), the long-term prognosis of schizophrenia remains poor (Carpenter and Koenig, 2008). All currently available antipsychotics have comparable dopamine occupancy at clinically relevant doses (Farde et al., 1988; de Visser et al., 2001; Seeman, 2002; Agid et al., 2007). It is hypothesized that dopamine dysregulation might be the final common pathway of different pathophysiological pathways of schizophrenia, with other pathophysiological mechanisms as the primary disturbance (Howes and Kapur, 2009). Many drugs targeting other pathways are currently under development, although none has been successful yet (Miyamoto et al., 2012). These include drugs targeting glutamatergic and cannabinoid systems (Ferretjans et al., 2012; Javitt et al., 2012).

Preclinical models for psychosis and schizophrenia

Currently used preclinical models for psychosis and antipsychotic drug action are not able to model the highly complex phenomenon of psychosis and schizophrenia (Carpenter and Koenig, 2008; Nestler and Hyman, 2010). Many models measure the ability to block or reverse a 'hyperdopaminergic' state, which results in pharmacologic isomorphism (novel drugs that pass these models will likely have a similar mechanism of action as the drugs that are currently on the market; models that have different mechanisms of action will likely fail at these models; Carpenter and Koenig, 2008; Nestler and Hyman, 2010). Although many new preclinical models, including mechanistic models, have been developed (reviewed by Pratt et al., 2012), there is no clear standardisation in their use and both positive and negative results are easily ignored because of limited predictive power (Nestler and Hyman, 2010, Jones et al., 2011).

Clinical challenge models

As an alternative for preclinical models, clinical studies with pharmacological challenges can be used. A pharmacological challenge influences a specific regulating system by means of a pharmacological agent. The resulting change in effect provides information about the mediating process (van Gerven, 2005). A pharmacological challenge can also be used to assess the effect of a drug that interacts with a certain regulating system. For example, administration of glucagon will lead to hyperglycaemia and can be used as a model in diabetes research (van Dongen et al., in preparation). The HPA axis can be stimulated when 5-hydroxytryptophan or desmopressin are administered; changes in the concentrations of cortisol and ACTH describe the sensitivity of the HPA axis (Smarius et al., 2008; Jacobs et al., 2011a,b).

Pharmacological challenges for psychosis and antipsychotic drug action

Several pharmacological agents can be used to illicit psychomimetic symptoms in healthy volunteers and patients (reviewed in detail by Gouzoulis-Mayfrank et al., 1998). Systematic scientific research into the properties of hallucinogenic compounds started around 1900 with mescaline, an agonist of both dopaminergic and serotonergic receptors. Administration of mescaline primarily leads to disturbances of perception -predominantly visual hallucinations- and was considered an excellent model for psychosis at the time. When Albert Hofmann discovered the psychomimetic effects of lysergic acid diethylamide (LSD, a serotonergic agonist) in 1943, a new phase of research into drug-induced psychomimetic effects as a model for psychosis started. LSD was not only used to study psychosis-like states in patients or healthy volunteers, but also to let researchers and psychiatrists experience the effects of psychosis. It is interesting to note, that the response in a pharmacological challenge with LSD played a role in assessing the efficacy of both chlorpromazine (Thuillier, 1980) and reserpine (Ban et al., 2010). The development of phencyclidine (PCP) in 1957, brought along a glutamateric agent that could be used as a model for psychosis. PCP was never registered for use in humans, but the pharmacologically related N-methyl-D-aspartate (NMDA) antagonist ketamine is used for this purpose. The abuse of psychedelic drugs in the general population (i.e. the hippie movement) led to major restrictions in the use of these drugs and scientific interest in psychedelics faded in the end of the 1960s. In the last two decades, a renewed interest in the drug-induced psychomimetic effects as a model for psychosis has arosen. The pharmacological mechanisms underlying these effects are of particular interest, as they may elude the underlying pathophysiological mechanisms of psychosis and schizophrenia.

Challenges in drug development for schizophrenia

In general, the market for drug development is not very positive. The cost of developing a single drug had increased to more than 800 million dollars per approved drug in 2003 (DiMasi et al., 2003). Many drugs fail during clinical development, with only 7.9% of all drugs and only 3.8% of drugs with a target in the central nervous system reaching the market (DiMasi et al., 2010). This has in the recent years led to the stop of development of psychiatric drugs in several major pharmaceutical companies (van Gerven and Cohen, 2011; Nutt and Goodwin, 2011). A thorough understanding of the pathophysiology, pharmacology of potential agents and adequate models to test drug efficacy early in development are essential to develop new drugs (especially drugs with a new mechanism of action) in the field of psychopharmacology (Cohen, 2010; van Gerven and Cohen, 2011).

Scope of this thesis

This thesis describes the improvement of two pharmacological challenge models (based on a cannabinoid and glutamatergic mechanism of action) that can be used to model aspects of psychosis. It includes a search to improve the optimal use of outcome measures used to measure effects and an exploration of the influence of individual differences on the measured effect.

Chapter 1 provides an overview of the history of antipsychotic drugs and a general introduction to the use of pharmacologic challenges in drug development. It focuses on challenge models used to model aspects of psychosis and antipsychotic drug action. In **Chapter 2** the effect of a known antipsychotic drug (olanzapine) on the tetrahydrocannabinol (THC) challenge model of psychosis is investigated. It shows that administration of olanzapine inhibits the psychomimetic effects of THC in healthy volunteers.

The subjective effects of THC, as measured using visual analogue scales (VAS), are looked at more closely in **Chapter 3**. These subjective effects can be grouped into three distinct clusters: changes in perception, feelings of relaxation and dysphoric reactions.

Chapter 4 explores the relation between differences in the subjective response to THC and personality traits.

In **Chapter 5** the ketamine challenge as a model of psychosis is investigated. A particular emphasis is placed on the finding of an optimal target concentration and outcome measure.

Chapter 6 investigates a proposed VAS for psychomimetic effects and compares this new VAS to other commonly used outcome measures for subjective effects.

Chapter 7 takes a closer look at resting state functional magnetic resonance imaging (RS-FMRI) responses to different pharmacological challenges. The functional connectivity of resting state networks is related to the subjective effects of different drugs.

In **Chapter 8**, the findings within this thesis are related to the information that was already known.

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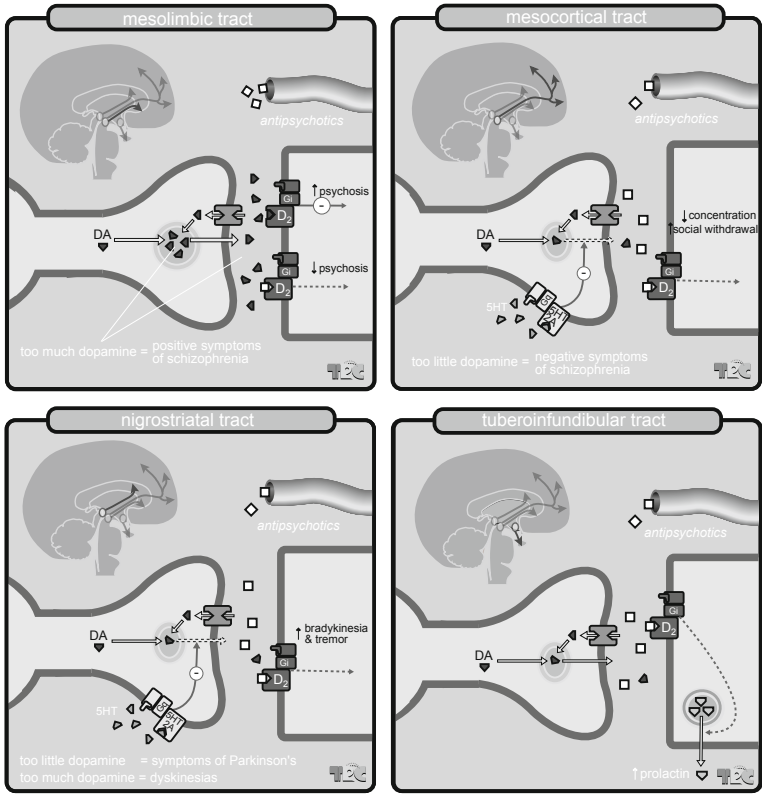
FIGURE 1 Original packaging of Largactil *(reproduced with permission: Boerhaave Museum, Leiden).*

FIGURE 2 Overview of the different dopamine projections in the brain (adapted from: TRC).



CHAPTER 2

Does olanzapine inhibit the psychomimetic effects of Δ^9-tetrahydrocannabinol?

J Psychopharmacol 26(10): 1307-1316

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ABSTRACT

Δ^9-Tetrahydrocannabinol (THC) produces transient psychomimetic effects in healthy volunteers, constituting a pharmacologic model for psychosis. The dopaminergic antagonist haloperidol has previously been shown to reduce these effects. This placebo-controlled, cross-over study in 49 healthy, male, mild cannabis users aimed to further explore this model by examining the effect of a single oral dose of olanzapine (with dopaminergic, serotonergic, adrenergic, muscarinergic and histaminergic properties) or two oral doses of diphenhydramine (histamine antagonist) on the effects of intrapulmonarily administered THC. Transient psychomimetic symptoms were seen after THC administration, as measured on the positive and negative syndrome scale (20.6% increase on positive subscale, $p < 0.001$) and the visual analogue scale for psychedelic effects (increase of 10.7 mm on feeling high). Following the combination of THC and olanzapine, the positive subscale increased by only 13.7% and feeling high by only 8.7 mm. This reduction of THC effects on the positive subscale failed to reach statistical significance ($p = 0.066$). However, one third of the subjects did not show an increase in psychomimetic symptoms after THC alone. Within responders, olanzapine reduced the effects of THC on the positive subscale ($p = 0.005$). Other outcome measures included pharmacokinetics, eye movements, postural stability, pupil/iris ratio, and serum concentrations of cortisol and prolactin.

Introduction

Schizophrenia and other forms of psychosis result from a complex and extensive disruption of the central nervous system (CNS). The pathophysiologic mechanism is not entirely understood and the symptoms of schizophrenia are very heterogenous. Currently available animal models for psychosis and antipsychotic action are not able to adequately model this complex phenomenon (Nestler and Hyman, 2010; Jones et al., 2011).

As an alternative to animal models, several in-human models for psychosis and antipsychotic action have been developed, using psychotropic agents to induce psychomimetic symptoms. Dopaminergic (e.g. amphetamine, see Strakowski et al., 1996), serotonergic (e.g. psilocybin, see Vollenweider et al., 1998) and glutamatergic (e.g. ketamine, see Krystal et al., 1994), as well as cannabinoid compounds (reviewed by Sewell et al., 2010) have been used for this purpose. This idea itself is not new: in the late 1950s one of the registered indications of the partial 5-HT_{2A} agonist lysergic acid (LSD, Delysid®) was to 'experience the nature of psychosis' in normal subjects, patients and psychiatrists (as described by Blewett and Chwelos, 1959).

D'Souza et al. (2004) were the first to show that intravenous administration of Δ^9-tetrahydrocannabinol (THC) induces a transient increase in psychomimetic symptoms in healthy volunteers. These psychomimetic symptoms were measured using the positive subscale of the Positive and Negative Syndrome Scale (PANSS). The results were replicated in healthy volunteers (D'Souza et al., 2008a; Morrison et al., 2009) and patients with schizophrenia (D'Souza et al., 2005).

Subsequently, the potential of the THC-model to measure antipsychotic activity of pharmacologic agents was investigated for the dopamine antagonist haloperidol. In a study by D'Souza et al. (2008a), co-administration of a single dose of haloperidol seemed to show a reduction in psychomimetic symptoms of THC as measured on the PANSS, although this result was not statistically significant. In a study by Liem-Moolenaar et al. (2010b) a significant reduction of THC-induced psychomimetic symptoms as measured on

the PANSS was shown following co-administration of a single dose of haloperidol. The effect of THC on the visual analogue scale (VAS) feeling high was not altered significantly by haloperidol in either study.

The use of THC as a model for psychosis can be supported by the relation between the endocannabinoid system and psychosis. In addition to frequently published links between cannabis use and psychosis (reviewed by D'Souza et al., 2009 and McLaren et al., 2010), several observations connect the endocannabinoid system to the pathophysiology of schizophrenia. Leweke et al. (1999) found increased concentrations of the endocannabinoid anandamide in cerebrospinal fluid of antipsychotic-naïve patients with schizophrenia, which was later replicated by the same group (Giuffrida et al., 2004). It was also found that cerebrospinal fluid concentrations of anandamide were not increased in patients with affective disorders or dementia. Levels of anandamide in cerebrospinal fluid in patients with schizophrenia correlated inversely with the severity of psychotic symptoms as assessed on the PANSS (Giuffrida et al., 2004), suggesting that the endocannabinoid system might be upregulated as a protective mechanism in patients with schizophrenia.

Cerebrospinal fluid concentrations of anandamide were normalized in patients with schizophrenia who were treated with 'typical' (dopaminergic) antipsychotics, but not in patients on 'atypical' (both dopaminergic and serotonergic) antipsychotics (Giuffrida et al., 2004). The authors suggested that 'typical' and 'atypical' antipsychotics may have different effects on the activity of the endocannabinoid system in schizophrenia. The notion that the endocannabinoid system is involved in the pathophysiology of schizophrenia is further supported by neuroimaging studies that found higher expression of cannabinoid type 1 receptors in patients with schizophrenia (Wong et al., 2010; Dalton et al. 2011). In the study by Wong et al. (2010), cerebral expression of CB_1 cannabinoid receptors was correlated with positive symptoms. Additionally, the cannabinoid antagonist rimonabant may reduce positive symptoms in patients with schizophrenia (Kelly et al., 2011).

The pharmacological effect of haloperidol, like most typical antipsychotics, is largely attributed to dopamine receptor antagonism (Agid et al., 2007). Although dopamine antagonism is also important for the activity of atypical antipsychotics, these drugs have a broader pharmacological profile, which includes modulation of serotonergic, glutamatergic, muscarinergic, histaminergic and adrenergic receptors. Among the atypical antipsychotics, olanzapine has relatively low affinity for dopaminergic receptors. However, the antipsychotic efficacy of olanzapine is comparable to that of haloperidol and its effect is assumed to originate from the combination of dopaminergic and serotonergic action. It was hypothesized that the model would be predictive of antipsychotic activity (regardless of the mechanism of action of the antipsychotic drug). The pharmacological differences between olanzapine and haloperidol provide an opportunity to further explore the pharmacologic basis of the PANSS effects of THC as a human psychosis model.

The main objective of the study was to investigate whether olanzapine modulates the (psychomimetic) effects of THC. This could, in addition to the other studies described earlier, further validate the THC model of psychosis. The study also aimed to investigate the influence of drug-induced sedation on psychomimetic symptoms, which is a well known side-effect of olanzapine that is attributed to histamine H_1 receptor antagonism. To this end, the antihistaminergic drug diphenhydramine was incorporated in the design. Also, the effects of THC and olanzapine on the CNS were examined individually.

Methods

Participants

Healthy male subjects aged between 18 and 45 years (inclusive) and with a body mass index between 18 and 30 kg/m^2 (inclusive) were recruited by the Centre for Human Drug Research. Subjects had to be mild cannabis users,

defined as self-reported use of cannabis no more than once a week on average in the last year. After providing written informed consent, subjects received a medical screening within 3 weeks prior to study participation. Clinically relevant abnormalities (in particular a personal or family history of clinically relevant psychiatric illness and/or abnormalities on psychiatric examination) were considered reason for exclusion. More specific: a personal history of attention deficit disorder without the use of medication was allowed; a personal history of depression or psychotic symptoms was not allowed; a family history of psychosis in first or second degree relatives and bipolar disorder in first degree relatives was not allowed. The use of medication and agents (including recreationally used drugs such as cannabis) that were expected to affect central nervous system performance or the pharmacokinetics of the study medication was not allowed during the study period. Subjects were tested for the use of recreational drugs (in urine) and alcohol (in breath) before each study day. Following the medical screening, subjects were trained for the study procedures.

Study design

This was a randomized, double-blind, placebo-controlled, five-way crossover interaction trial with a washout period of minimally two weeks. The study was performed in accordance with Good Clinical Practice and the Dutch Medical Research Involving Human Subjects Act and was approved by the Independent Ethics Committee of the Leiden University Medical Centre.

Interventions

This study investigated the effect of a single oral dose of olanzapine (10 mg tablet, Eli Lilly™) on the psychomimetic effects of THC. A dose of 10 mg olanzapine was expected to show an occupancy rate of dopamine D_2 receptors of 60 to 70% (Kapur et al., 1998). Purified THC was administered intrapulmonary using the Volcano™ vaporizer (Storz-Bickel, Tuttlingen, Germany)

as described in more detail by Zuurman et al. (2008) in three consecutive dosages of 2, 4 and 6 mg with 90 minute intervals. The first dose of THC or placebo was administered 4 hours after olanzapine was given, when the plasma concentration of olanzapine was expected to reach its maximum (Kassahun et al., 1997). Diphenhydramine was used as a positive control for the sedative effects of H_1 antagonism, given as two separate oral doses of 15 mg at 1 and 3 hours after olanzapine administration, to mimic the average expected time-concentration profile of olanzapine.

All treatments and doses were placebo and double-dummy controlled. All subjects were randomized to receive all of the following five treatment arms in a random order: THC + olanzapine (olanzapine 10 mg + placebo diphenhydramine + THC 2, 4 and 6 mg); olanzapine alone (olanzapine 10 mg + placebo diphenhydramine + placebo THC); THC alone (placebo olanzapine + placebo diphenhydramine + THC 2, 4 and 6 mg); placebo (placebo olanzapine + placebo diphenhydramine + placebo THC); and THC + diphenhydramine (placebo olanzapine + diphenhydramine 2 x 15 mg + THC 2, 4 and 6 mg). A treatment arm with diphenhydramine alone was not added as this would increase the burden of the study and diphenhydramine was only added as a positive control.

Outcome measures

Psychomimetic symptoms were measured using the PANSS, as described by Kay et al. (1987). This clinically validated rating scale is based on a structured clinical interview. The interviews were performed four times during a study day: once before the administration of olanzapine (or placebo) and again after each THC (or placebo) administration. To adjust the interview for the repetition of interviews, time frames for symptoms evaluation were limited to 'since this morning' or 'since the last interview'. All interviews were recorded on video and rated by a second blinded person. The PANSS consists of 30 items that are scored on a seven-point scale. The PANSS is subdivided into three subscales: positive, negative and general. The positive subscale,

which consists of 7 items resulting in a total score ranging from 7 to 49, was predefined as the main evaluation endpoint.

To determine other potentially confounding CNS effects, an extensive test battery (NeuroCart) was used, which included VAS, eye movements, postural stability, pupil / iris ratio, Stroop colour word test and the visual verbal learning test (VVLT), extended with measurements of serum cortisol and prolactin. All these measurements were performed repeatedly throughout the study day, including two baseline measurements. The timing of measurements was very similar to the scheme used by Liem-Moolenaar et al. (2010b).

VAS are widely used to quantify subjective effects. In this study, the composite scales described by Bond and Lader (1974) were used to measure alertness, mood and calmness and those described by Bowdle et al. (1998) for psychedelic effects. The VAS for psychedelic effects (VAS Bowdle) is subdivided into the clusters 'internal perception' (5 items), 'external perception' (6 items) and 'feeling high' (1 item). The scores on each individual VAS item can range from 0 to 100 mm.

Both saccadic and smooth pursuit eye movements were recorded through three electrodes placed on the forehead and next to both lateral canthi. The stimulus for saccadic eye movements had amplitude of approximately 15 degrees to either side, with interstimulus intervals varying randomly between 3 and 6 seconds. Smooth pursuit eye movements were stimulated in a sinusoidal manner at frequencies ranging from 0.3 to 1.1 Hz with amplitude of 22.5 degrees to either side. Eye movements are described in greater detail by Zuurman et al. (2008). Saccadic peak velocity is one of the most sensitive parameters for sedation (van Steveninck et al., 1991). The percentage time in which the eye movements are in smooth pursuit of the target is a parameter for motor coordination.

The body sway meter records body movements in a single (sagittal) plane during two minutes while the subjects close their eyes, providing a measure of postural stability, which can be used as a biomarker for drug effect (Liem-Moolenaar et al., 2010a).

The ratio between the diameter of the pupil and the iris forms a measure of the activity of the autonomous nervous system. Diameters were determined using digital photography with flash after adaptation in ambient lighting (Twa et al., 2004).

In the Stroop colour word test, names of colours are formatted in a congruent or incongruent colour. Subjects have to provide the formatted colour of the presented word. Stroop interference effects are helpful in understanding attention, perception and reading (Laeng et al., 2005).

The VVLT is a memory test that uses 30 words in three consecutive trials: immediate recall, delayed recall and delayed recognition. During each study day and at the training, different parallel versions of the test were used to prevent learning effects (Schmitt et al., 2000).

Serum prolactin and cortisol concentrations were measured using electrochemiluminescence immunoassay (ECLIA) as a biomarker for dopaminergic activity.

Repeated blood samples were drawn to determine pharmacokinetic profiles of THC and its main metabolites, olanzapine and diphenhydramine. Samples were analyzed using high performance liquid chromatography with tandem mass spectrometric detection (HPLC-MS).

Sample size

The sample size of the study was based on a power calculation, using the results of a previous study with a comparable design (Liem-Moolenaar et al., 2010b). This study examined the effect of a single oral dose of haloperidol 3 mg on psychomimetic effects of THC. Co-administration of haloperidol was found to reduce the effects of THC as measured on the positive subscale of the PANSS by 1.11 points with an estimated standard deviation of 2.374 points. Using nQuery Advisor v5.0 (Statistical Solutions Ltd, Cork, Ireland) a sample size of 38 was calculated to have 80% power to detect a difference in means of -1.11, assuming a standard deviation of 2.374, using a paired t-test with a 0.05 two-sided significance level. Randomization using Williams

squares for five treatments requires multiples of ten subjects for each group, which required the total number of subjects to be increased to 40. Subjects who did not complete at least three out of five study days were replaced.

Statistical analyses

All pharmacodynamic endpoints were analyzed using a mixed-model analysis of variance (using SAS PROC MIXED). Subject, subject by treatment and subject by time were used as random effects; treatment, study day, time and treatment by time as fixed effects; and the average baseline value as covariate. Parameters of the PANSS, body sway, and neuroendocrine parameters did not have a normal distribution and were analyzed after log-transformation. After analysis these parameters were back-transformed, where the results can be interpreted as percentage change. All outcome measures are presented as estimated means using the least squares method. Parameters of the VAS Bowdle showed a non-normal distribution that could not be corrected by log-transformation. This was largely due to the fact that psychedelic effects are not present under placebo and that a major proportion of the subjects did not show any effect on this outcome measure after THC administration. Results for VAS Bowdle are presented over time as mean ± standard deviation and in a bar graph as mean ± standard deviation for both the average effect and the maximum effect. To further explore the effect of co-administration of olanzapine and diphenhydramine, an exploratory analysis for VAS feeling high was performed using only the treatment arms that included THC administration and only the subjects that showed any response on the VAS feeling high. Within the subpopulation of responders, effects on VAS feeling high showed a normal distribution after log-transformations.

To describe the pharmacokinetic characteristics of THC, olanzapine and diphenhydramine, plasma concentrations were evaluated by data driven compartmental analysis and simulation using NONMEM software (version 7.2.0, Globomax LLC, Ellicot City, MD, USA).

Results

Subjects

A total of 49 subjects were included, 33 (67%) of whom completed all five study days. One subject stopped during the first study day, 5 (10%) stopped after the first study day, 3 (6%) after the second, 5 (10%) after the third and 2 (4%) subjects after the fourth study day. Subjects who did not complete at least three study days were replaced. Premature withdrawal was due to non-compliance in 13 (27%) subjects (two had used cannabis, one did not understand study procedures, seven could no longer attend the planned study days and three did not show up without a reason) and due to adverse events in 3 (6%) subjects (one had paranoid thoughts after THC administration and was discontinued, one had an anxiety attack after THC administration and was discontinued, and one felt too sedated after olanzapine and THC administration and decided to discontinue). All subjects who received at least one administration of THC or its placebo were included in the analysis.

Positive and negative syndrome scale

The least square means (LSM) of the scores on the different subscales of the PANSS are presented in Table 1 and the differences for the main contrasts are presented in Table 2. Compared to placebo, administration of THC induced an average increase on the positive subscale of the PANSS of 20.6% (95%CI 13.1-28.6%; $p < 0.001$). Co-administration of THC and olanzapine caused an average increase on the positive subscale of 13.7% (95%CI 6.5-21.3%; $p < 0.001$). This apparent average reduction of psychomimetic symptoms as measured on the positive subscale of the PANSS by olanzapine did not reach statistical significance in the whole group ($p = 0.066$). However, it appeared that a considerable number of subjects did not show any increase of positive PANSS scores after THC. When no increase was seen, an effect floor occurred

that obviously could not be reduced by any intervention. Therefore, a secondary analysis was performed on the PANSS scores of responders only. For this purpose, responders were conservatively defined as subjects who showed at least one point increase on the positive subscale compared to baseline in any of the measurements following THC administration. In these 33 (67%) responders, THC induced an average increase on the positive subscale of 25.1% (95%CI 16.6-34.1%; $p < 0.001$). Co-administration of olanzapine reduced this average increase to 13.2% (95%CI 5.4-21.5%; $p = 0.001$). This reduction of positive PANSS-increases was highly significant ($p = 0.005$, THC + olanzapine compared to THC alone). The psychomimetic effects as measured on the positive subscale of the PANSS for responders are presented in Figure 1. Co-administration of olanzapine did not significantly alter the effects induced by THC on the general and negative subscale. Diphenhydramine did not affect the effects of THC on any of the subscales of the PANSS.

Visual analogue scales

Table 1 provides an overview of the LSM and differences between contrasts for the different clusters on the VAS. The average score on VAS feeling high increased from 0.0 mm (SD 0.2; range 0.0-1.0) under placebo condition to 10.7 mm (SD 11.9; range 0.0-38.3) following THC administration. Co-administration of olanzapine led to an average score on VAS feeling high of 8.7 mm (SD 14.0; range 0.0-67.4). Olanzapine seemed to mildly inhibit the effects of THC, but this could not be formally tested due to data skewness (because many subjects did not report a high-effect). In an exploratory analysis, using only treatment arms that contained THC and subjects who showed any response on the VAS feeling high, the reduction of VAS feeling high caused by olanzapine was statistically significant ($p = 0.020$). Co-administration of diphenhydramine did not influence the effects of THC on VAS feeling high. The effects are presented in Figure 2. The Bowdle

cluster 'internal perception' increased mildly from an average composite score of 0.0 mm (SD 0.0; range 0.0-0.2) under placebo conditions to 0.4 mm (SD 0.9; range 0.0-4.2) following THC administration. The average score on the cluster 'external perception' increased mildly from 0.0 mm (SD 0.0; range 0.0-0.2) under placebo condition to 1.7 mm (SD 2.5; range 0.0-9.5) following THC administration. The effect of THC on 'internal perception' and 'external perception' was not affected by co-administration of either olanzapine or diphenhydramine.

VAS alertness decreased following administration of THC alone (-2.2 mm; 95%CI -3.8--0.6; $p = 0.006$) and olanzapine alone (-5.4 mm; 95%CI -7.0--3.9; $p < 0.001$). The additional effect of olanzapine to THC administration (-6.2 mm; 95%CI -7.8--4.7; $p < 0.001$) was larger than that of diphenhydramine (-1.4 mm; 95%CI -2.9-0.2; $p = 0.078$). VAS calmness increased mildly after THC alone (+1.7 mm; 95%CI 0.6-2.7; $p = 0.003$) and olanzapine alone (+1.2 mm; 95%CI 0.2-2.3; $p = 0.024$) administration. No significant additional effect of co-administration of either olanzapine or diphenhydramine was found. VAS mood increased non-significantly by 1.1 mm (95%CI -0.0-2.2; $p = 0.054$) following THC alone administration, and decreased by 0.7 mm (95%CI -1.8-0.4; $p = 0.217$) with olanzapine alone. Co-administration of olanzapine and THC decreased VAS mood by 1.1 mm (95%CI -2.2-0.1; $p = 0.068$) compared to placebo and 2.2 mm (95%CI 1.1-3.3; $p < 0.001$) compared to THC alone.

Eye movements

Olanzapine alone caused a decrease in saccadic peak velocity of 98 deg/s (95%CI 85-110; $p < 0.001$), an increase in saccadic reaction time of 28 msec (95%CI 19-36; $p < 0.001$), an increase in saccadic inaccuracy of 2.3% (95%CI 1.6-3.0%; $p < 0.001$) and a decrease in smooth pursuit of 9.4% (95%CI 5.7-13.1%; $p < 0.001$) compared to placebo. THC alone increased saccadic inaccuracy by 0.7% (95%CI 0.1-1.3%; $p = 0.033$) compared to placebo. Other eye movement parameters were not affected by the other treatments.

Body sway

Administration of THC alone increased the body sway by 56% (95%CI 39-76%; $p < 0.001$) compared to placebo. Olanzapine alone increased body sway by 121% (95%CI 95-150%; $p = 0.001$). Co-administration of THC and olanzapine increased body sway by 177% (95%CI 139-221%; $p < 0.001$) compared to placebo and by 77% (95%CI 54-104%; $p < 0.001$) compared to THC alone. Diphenhydramine did not significantly affect the effects of THC on body sway.

Pupil size

Olanzapine alone caused a decrease in pupil / iris ratio of 0.27 (95%CI 0.25-0.29; $p < 0.001$) compared to placebo. The other treatments did not significantly affect pupil size.

Stroop colour word test

THC and olanzapine did not affect the Stroop test by themselves. The number of incorrect answers increased following co-administration of THC and olanzapine (+0.5; 95%CI 0.1-0.8; $p = 0.010$) compared to administration of THC alone.

Visual verbal learning test

Olanzapine caused a strong reduction on the outcome on the VVLT, for the immediate recall, the delayed recall and the recognition trials, both alone when compared to placebo and when co-administration with THC was compared to THC alone. THC alone reduced delayed recall by 2.0 (95%CI 0.4-3.6; p 0.016) compared to placebo. There were no significant differences for other contrasts.

Prolactin and cortisol

THC alone induced a decrease in serum prolactin concentrations by 17% (95%CI 9-24; $p < 0.001$) compared to placebo. Olanzapine alone caused an increase in serum prolactin concentrations by 255% (95%CI 224-289; $p < 0.001$) compared to placebo. Co-administration of THC and olanzapine decreased prolactin concentrations by 10% (95%CI 1-17; $p = 0.026$) compared to olanzapine alone. Compared to placebo, serum cortisol concentrations increased by 21% (95%CI 3-44; $p = 0.024$) following THC alone, and decreased by 45% (95%CI 35-54; $p < 0.001$) after olanzapine alone, and by 25% (95%CI 11-36; $p = 0.001$) when olanzapine was co-administered with THC compared to THC alone. The serum prolactin concentrations are presented in Figure 3.

Pharmacokinetics

The pharmacokinetics of THC were best described using a two compartment linear model with zero-order absorption. The apparent volume of distribution was 10.3 L and the apparent clearance 149 L/hr. The pharmacokinetics of olanzapine could be described with a one compartment model with buffered absorption and an apparent volume of distribution of 7 L and apparent clearance of 0.3 L/hr. The pharmacokinetics of diphenhydramine required a one compartment model with linear absorption and an apparent volume of distribution of 805 L and apparent clearance of 106 L/hr. An overview of the pharmacokinetic parameters is provided in Table 3 and the pharmacokinetic profiles are presented in Figure 4.

Discussion

THC induced a transient psychomimetic effect in healthy volunteers, as measured on the positive subscale of the PANSS and VAS feeling high. This

effect was reduced by olanzapine, but not diphenhydramine. This induction of psychotic-like symptoms and their suppression by anti-psychotic treatments suggests that the model bears resemblance with clinical psychotic symptoms.

The level of induction of the effects on the positive subscale of the PANSS of THC is comparable to previous studies (D'Souza et al., 2004, 2008a, 2008b; Morrison et al., 2009; Bhattacharyya et al., 2010; Liem-Moolenaar et al., 2010b; Barkus et al., 2011). Co-administration of olanzapine leads to a 33.5% reduction (47.4% in responders) of psychomimetic symptoms. This is a smaller reduction than found with haloperidol by Liem-Moolenaar et al. (2010b), but larger than D'Souza et al. (2008a) reported for haloperidol.

THC did not only produce increases in positive PANSS scores, but also well-known euphoric ('high') feelings and other subjective and objective CNS effects. Some of these were also affected by olanzapine. In particular, VAS feeling high effects of THC seemed to be diminished by olanzapine, in apparent contrast to what has previously been reported for haloperidol (D'Souza et al., 2008a; Liem-Moolenaar et al., 2010b). It is difficult to compare the absolute increase on VAS feeling high with previous results. The proportion of non-responders caused considerable skewness of scores on VAS feeling high (leading to many scores of 0, both in the placebo and THC conditions), making even non-parametrical analyses difficult. It is unclear if other studies experienced similar statistical incongruities, but these may have contributed to the differences in analytical approaches that are encountered in the literature. D'Souza et al. (2008a) used a non-parametric approach to describe the effects on VAS feeling high. They found a somewhat stronger effect of THC on VAS feeling high, which can possibly be explained by differences in dose or administration route. Liem-Moolenaar et al. (2010b) used log-transformation to normalize the distribution of the VAS feeling high outcomes and subsequently used a mixed-model analysis of variance. When the same method was applied to data of the current study, a similar increase was found following THC administration. In previous studies, haloperidol did not have a statistically significant influence on

the effects of THC on VAS feeling high (D'Souza et al., 2008a; Liem-Moolenaar et al., 2010b). However, the graph in the publication by Liem-Moolenaar et al. suggests some blunting of the effect. In the results of the current study, olanzapine seems to reduce the effects of THC on VAS feeling high, although this was only formally tested in a post hoc exploratory analysis. The use of different statistical approaches interferes with a quantitative comparison between studies, but it cannot be excluded that antipsychotic drugs can diminish high feelings to some extent.

The VAS calmness was mildly increased following administration of THC. This is the opposite effect as described by Crippa et al. (2009) in a review on the relation between cannabis / THC use and anxiety. During the current study, one subject experienced anxiety and was discontinued for this reason. No other subjects reported anxiety. The absence of THC-induced anxiety could be explained by the selection criteria for this study. Symptoms of anxiety are most common in cannabis-naive individuals (in particular in people who are vulnerable for psychiatric symptoms), whereas people with cannabis dependence typically have higher state anxiety levels and a reduction in anxiety with cannabis use (Crippa et al., 2009). For this study, mild cannabis users (with previous exposure to cannabis and no cannabis dependence) were selected and subjects with a vulnerability for psychiatric symptoms (psychosis, anxiety or other) were excluded during the medical screening.

As is the case with every model, this model also has its limitations. The THC model only represents a small part of the clinical spectrum of schizophrenia, which is a complex chronic syndrome with a range of negative, positive, cognitive, behavioural and emotional symptoms. The absolute increase in psychomimetic symptoms is transient and small, but statistically highly significant. This can be an ethical advantage in studies with healthy subjects, where the induction of psychomimetic symptoms should be reproducible, but mild, safe and rapidly reversible.

The variability and distribution of the outcome measures of the THC model posed some statistical problems with the analysis and interpretation

of the effects. One third of the participants did not show an increase on the PANSS or on the VAS Bowdle and the distribution of the VAS Bowdle among responders is not normal. This may in part be due to the subjective nature of effects like 'feeling high', which can be interpreted differently by different subjects, but it may also reflect individual differences in sensitivity to psychotic decompensation following cannabis (over)use. Differences in proportions of responders and non-responders complicate the design and interpretation of studies using THC or cannabis to induce psychotic-like symptoms or euphoric ('high') feelings, even if regular (albeit non-frequent) users are recruited. In practice, this can be overcome by selecting subjects based on their response to a test-dose of THC, prior to their inclusion into an actual drug-interaction study.

THC induced mild sedation, as measured on the VAS Bond and Lader. There was also a mild decrease in attention as measured by saccadic inaccuracy and the VVLT and in postural stability as measured by the body sway. These effects are comparable with those previously found with THC (Zuurman et al., 2008; Liem-Moolenaar et al., 2010b). Olanzapine showed clear sedative effects, as measured subjectively on the VAS Bond and Lader and objectively through saccadic and smooth pursuit eye movements, comparable with those previously described (Morrens et al., 2007). Furthermore, strong effects were seen on the pupil / iris ratio, body sway and VVLT, suggesting effects on the autonomic nervous system, decreased postural stability and impaired memory or attention. The sedative effects were expected because olanzapine is a potent histamine H_1 receptor antagonist. The sedative and other non-psychomimetic effects of THC and olanzapine were cumulative when both drugs were administered together.

The possibility was considered that the sedative effects of olanzapine 10 mg could have a non-specific impact on its potential antipsychotic-like effects. For this reason, diphenhydramine was added to the study as a positive control. Unfortunately, the dose that we selected for this histamine H_1 antagonist caused less sedation than olanzapine, although the time profile of exposure to both drugs was similar and constant during the

pharmacologic tests. This apparent lack of sedative equipotency was unintended, but the consequence is that we are unable to determine how much of olanzapine's positive PANSS reduction was caused by sedation, and how much by a true antipsychotic effect. However, in the study by Liem-Moolenaar et al. (2010b), haloperidol seemed to cause less sedation and somewhat more PANSS reduction than olanzapine did in the current experiment. This suggests that the contribution of sedation to anti-psychotic-like effects of olanzapine in this model is limited, although an additional contribution of non-specific CNS effects cannot be fully excluded.

The clear and reproducible psychomimetic effects of THC in various studies support the involvement of the endocannabinoid system in psychosis. It should however be acknowledged that the exogenous administration of THC does not necessarily translate to the effect of endocannabinoid system. Most findings of THC challenge studies and many other reports in the literature suggest that dopamine activation by THC plays an essential role in its psychosis-like effects. THC has been shown to increase the release of dopamine in the human striatum, through activation of cannabinoid type 1 (CB_1) receptors, although not all imaging studies have been able to confirm this. Bossong et al. (2009) found a moderate decrease in striatal [^{11}C]-raclopride binding following intrapulmonary THC administration, which suggests an increased dopamine release that would constitute a logical mechanism of action for antidopaminergic antipsychotics. Stokes et al. (2009) however did not find a change in [^{11}C]-raclopride binding after oral administration of THC and Barkus et al. (2011) did not find a change in [^{123}I]-IBZM binding after intravenous administration of THC. This difference might in part be explained by differences in study designs (route of administration, dose, timing of scans) and/or non-response of participants. Interestingly, Bhattacharyya et al. (2010) showed decreased striatal activation on FMRI during a verbal memory task, which was correlated with the severity of psychomimetic symptoms as measured on the PANSS. THC has also been shown to decrease [^{11}C]-raclopride binding in extrastriatal regions, although it can be debated if this represents indirect dopamine release (Stokes et al., 2010).

The degree of dopamine release in the study of Bossong et al. (2009) was only moderate when compared to other drugs (amphetamine, cocaine, alcohol and nicotine), even at a relatively high dose of THC. This might also explain the absence of dopamine release in other THC studies and could represent a mild and indirect effect of CB_1 activation on dopamine release. The moderate degree of dopamine release is consistent with the moderate absolute increase on the positive subscale of the PANSS, which is much less than observed during clinical psychotic episodes.

The neuroendocrine effects that were found in this study provide further support for the involvement of dopamine systems in THC-induced psychomimetic effects. Dopamine has long been known as a 'prolactin inhibiting factor', and an increase in serum prolactin concentrations constitutes an established biomarker for dopamine antagonism (de Visser et al., 2001). Consequently, olanzapine induces a large increase in serum prolactin concentrations, both when administered alone and with THC. THC, however, causes a mild decrease in serum prolactin concentrations compared to placebo, and it reduces olanzapine-induced prolactin release after co-administration. It should be noted that serum prolactin concentrations can more easily be increased than decreased (a so-called 'floor-effect'). These effects on serum prolactin demonstrate that dopamine release in tuberopituitary pathways is increased by THC and support the notion that other dopaminergic systems in the brain are involved in the psychomimetic effects of cannabinoids.

In previous studies, the dopamine antagonist haloperidol reduced the THC-induced increase on the positive subscale of the PANSS (D'Souza et al., 2008a; Liem-Moolenaar et al., 2010b). We used olanzapine to examine the effect of an atypical antipsychotic with a broader relevant pharmacological spectrum than primarily on dopamine alone. Olanzapine has a relatively low affinity for dopamine compared to other neurotransmitter systems, although the antipsychotic efficacy of all currently available antipsychotics, including olanzapine, can be related to their dopaminergic activity (de Visser et al., 2001; Agid et al., 2007). Dopaminergic activation also seems to

be essential in the THC model described in this study. However, olanzapine has several other pharmacological (histaminergic, serotonergic) effects, which may have blunted the psychomimetic effects of THC. Investigations using other antipsychotic drugs, like quetiapine (which also has a relatively low affinity for dopamine receptors, but less affinity for histamine and serotonin receptors than olanzapine), may improve the understanding of the role of different pharmacological systems in the THC psychosis model and the influence of non-specific pharmacological and functional effects.

In conclusion, the current study provides further support for the use of THC in healthy volunteers as a model for psychosis and anti-psychotic drug action in both pathophysiological and pharmacologic research. This model could be used in early phase clinical trials to predict the clinical efficacy of novel antipsychotic drugs.

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TABLE 1 Least square means for pharmacodynamic outcome measures.

	placebo	olanzapine alone	THC alone	THC + olanzapine	THC + diphenh.
Positive PANSS	7.4	7.7	8.9	8.4	9.0
Positive PANSS (responders)	7.4	7.6	9.3	8.4	9.1
Negative PANSS	8.1	11.4	8.8	12.6	9.0
General PANSS	17.2	19.9	18.3	21.5	19.1
VAS feeling high (mm)	0.0	0.8	10.7	8.7	11.8
VAS internal perception (mm)	0.0	0.3	0.4	0.7	0.6
VAS external perception (mm)	0.0	0.4	1.7	1.4	1.7
VAS alertness (mm)	51.0	45.5	48.8	42.5	47.4
VAS calmness (mm)	51.2	52.4	52.9	53.4	52.9
VAS mood (mm)	52.1	51.4	53.2	51.1	52.6
Saccadic peak velocity (deg/s)	480	382	481	373	470
Saccadic inaccuracy (%)	6.4	8.7	7.1	8.7	7.4
Saccadic reaction time (ms)	200	228	207	251	209
Smooth pursuit (%)	48.1	38.7	45.7	34.9	46.4
Body sway (mm)	220	486	345	610	347
Pupil / iris - ratio left	0.55	0.27	0.54	0.28	0.54
Serum cortisol (µmol/L)	0.23	0.13	0.28	0.21	0.24
Serum prolactin (µg/L)	9.0	32.0	7.5	28.9	7.7
VVLT - First recall (No correct)	9.5	8.5	8.7	6.1	8.4
VVLT - Second recall (No correct)	14.1	10.5	13.3	9.1	12.8
VVLT - Third recall (No correct)	17.0	13.2	15.6	10.6	15.0
VVLT - Delayed recall (No correct)	13.8	8.7	11.9	5.8	10.8
VVLT - Recognition (No correct)	24.9	22.4	24.2	21.3	22.7
Stroop (No incorrect)	0.1	0.4	0.3	0.8	0.1
Stroop (reaction time, ms)	74	57	72	79	69

TABLE 2 Estimates of difference, 95% confidence intervals and p-values for main contrasts.

	olanzapine alone	THC alone	THC + olanzapine
	vs	vs	vs
	placebo	placebo	THC alone
Positive PANSS	+3.4%	+20.6%	-5.7%
	-3.0 - +10.3%	+13.1 - +28.6%	-11.5 - +0.4%
	0.305	<0.001	0.066
Positive PANSS (responders)	+2.4%	+25.1%	-9.5%
	-4.7 - +9.9%	+16.6 - +34.1%	-15.5 - -3.1%
	0.516	<0.001	0.005
Negative PANSS	+41.0%	+9.5%	+42.5%
	+28.1 - +55.2%	-0.5 - +20.5%	+29.7 - +56.5%
	<0.001	0.063	<0.001
General PANSS	+15.6%	+6.2%	+17.4%
	+9.6 - +22.0%	+0.7 - +12.0%	+11.4 - +23.7%
	<0.001	0.027	<0.001
VAS alertness (mm)	-5.4	-2.2	-6.2
	-7.0 - -3.9	-3.8 - -0.6	-7.8 - -4.7
	<0.001	0.006	<0.001
VAS calmness (mm)	+1.2	+1.7	+0.5
	+0.2 - +2.3	+0.6 - +2.7	-0.6 - +1.6
	0.024	0.003	0.351
VAS mood (mm)	-0.7	+1.1	-2.2
	-1.8 - 0.4	-0.0 - +2.2	-3.3 - -1.1
	0.217	0.054	<0.001
Saccadic peak velocity (deg/s)	-98	+1	-108
	-110 - -85	-11 - +13	-121 - -95
	<0.001	0.884	<0.001
Saccadic inaccuracy (%)	+2.3	+0.7	+1.7
	+1.6 - +3.0	+0.1 - +1.3	+1.0 - +2.4
	<0.001	0.033	<0.001
Saccadic reaction time (ms)	+28	+6	+44
	+19 - +36	-2 - +14	+35 - +52
	<0.001	0.111	<0.001

Smooth pursuit (%)	-9.4	-2.4	-10.8
	-13.1 - -5.7	-6.1 - +1.3	-14.4 - -7.1
	< 0.001	0.208	< 0.001
Body sway (mm)	+121	+56	+77
	+95 - +150	+39 - +76	+54 - +104
	< 0.001	< 0.001	< 0.001
Pupil / iris - ratio left	-0.27	-0.01	-0.27
	-0.29 - -0.25	-0.02 - +0.01	-0.28 - -0.25
	< 0.001	0.558	< 0.001
Serum cortisol (µmol/L)	-45%	+22%	-25%
	-54 - -35	+3 - +44	-36 - -11
	< 0.001	0.024	0.001
Serum prolactin (µg/L)	+255%	-17%	+286%
	+224 - +289	-24 - -9	+253 - +321
	< 0.001	< 0.001	< 0.001
VVLT - First recall (No correct)	-1.0	-0.8	-2.6
	-2.2 - +0.1	-2.0 - +0.3	-3.8 - -1.4
	0.083	0.157	< 0.001
VVLT - Second recall (No correct)	-3.6	-0.7	-4.2
	-5.2 - -2.0	-2.3 - +0.8	-5.8 - -2.6
	< 0.001	0.341	< 0.001
VVLT - Third recall (No correct)	-3.8	-1.4	-5.1
	-5.5 - -2.1	-3.0 - +0.3	-6.8 - -3.4
	< 0.001	0.098	< 0.001
VVLT - Delayed recall (No correct)	-5.2	-2.0	-6.0
	-6.8 - -3.6	-3.6 - -0.4	-7.7 - -4.4
	< 0.001	0.016	< 0.001
VVLT - Recognition (No correct)	-2.5	-0.7	-2.9
	-4.4 - -0.6	-2.6 - +1.2	-4.9 - -0.9
	0.010	0.472	0.004
Stroop (No incorrect)	+0.3	+0.2	+0.5
	-0.0 - +0.7	-0.1 - +0.5	+0.1 - +0.8
	0.076	0.272	0.010
Stroop (reaction time, ms)	-18	-2	+7
	-42 - +6	-26 - +22	-17 - +31
	0.149	0.854	0.568

TABLE 3 Estimated pharmacokinetic parameters (± SE) for THC, olanzapine and diphenhydramine.

	THC	Olanzapine	Diphenhydramine
V_D/F (L)	10.3 (0.8)	7.0 (0.2)	805 (56)
CL/F (L/hr)	149.0 (5.0)	0.3 (0.0)	106 (5)
K_A	-	5.6 (0.8)	18.9 (3.4)
lag time (hr)	-	1.47 (0.06)	0.98 (0.00)
V_2 (L)	34.5 (2.2)	-	-
Q (L/hr)	62.9 (3.5)	-	-

FIGURE 1 Effect on the positive subscale of the PANSS for responders over time (mean ± SD).

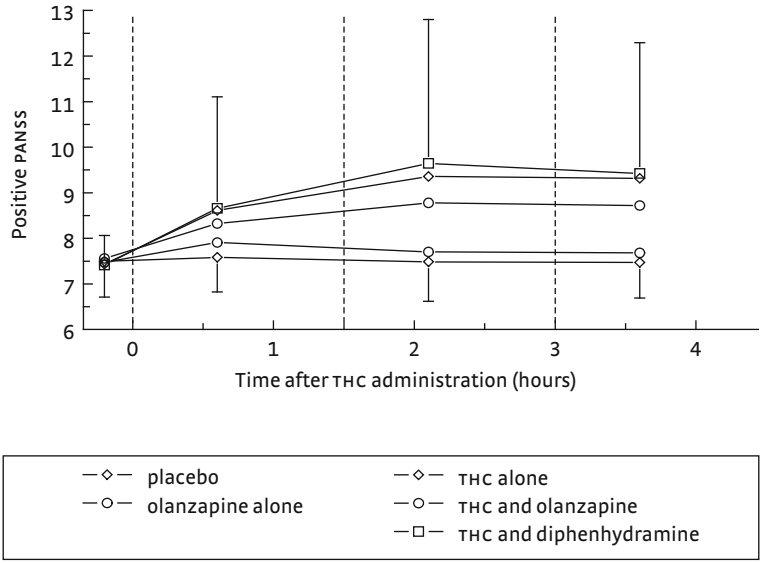


FIGURE 2 Effect on visual analogue scale feeling high over time (mean ± SD).

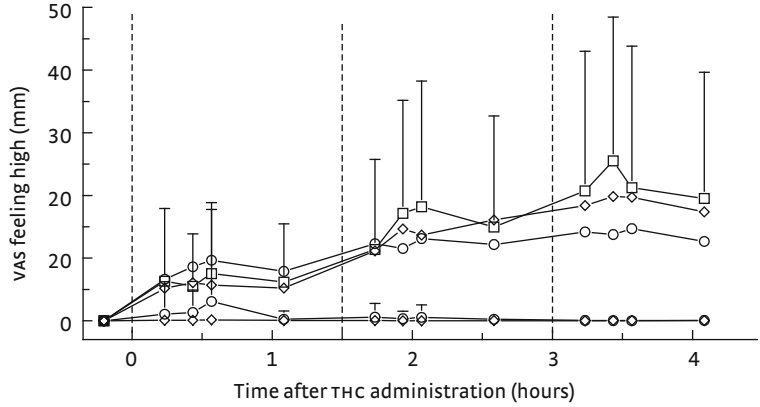


FIGURE 3 Prolactin concentrations in serum (mean ± SD).

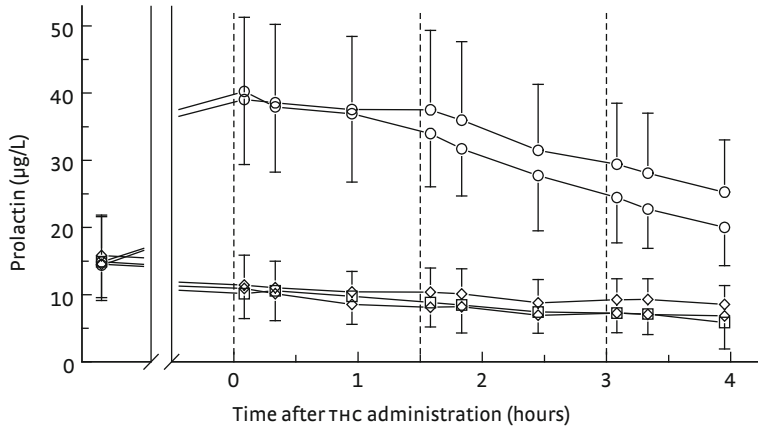
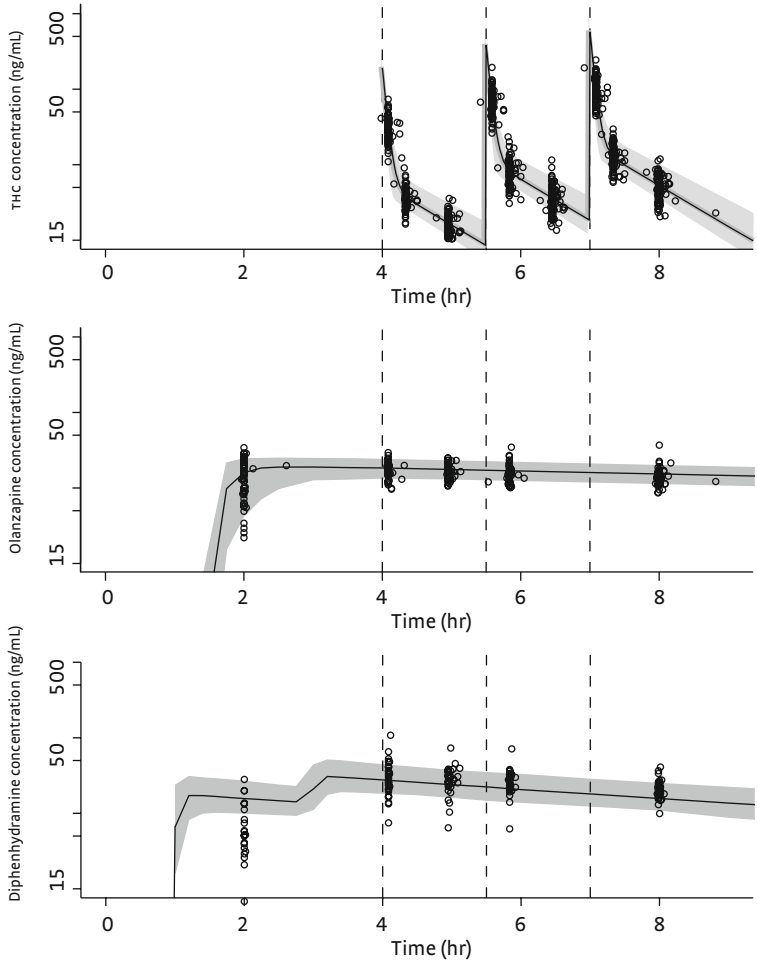


FIGURE 4 Simulated (median, 95%CI) and observed concentrations for THC, olanzapine and diphenhydramine pharmacokinetics.



CHAPTER 3

Profiling the subjective effects of Δ^9-tetrahydrocannabinol using visual analogue scales

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ABSTRACT

The subjective effects of cannabis and its main psychoactive component THC have played an important part in determining the therapeutic potential of cannabinoid agonists and antagonists The effects mainly consist of feeling high, changes in perception, feelings of relaxation and occasionally dysphoric reactions. These effects are captured by two of the most frequently used visual analogue scales (VAS) in clinical (pharmacologic) research to measure subjective effects: VAS Bond and Lader (alertness, calmness and mood) and VAS Bowdle (psychedelic effects). In this analysis, the effects of THC on these VAS scales were compared within a total of 217 subjects who participated in ten different studies. Not surprisingly, the item feeling high was found to be the best predictor for the effect of THC. Three separate clusters that describe the spectrum of subjective effects of THC were identified using different statistical methods, consisting of VAS 'time', 'thoughts' and 'high' ("perception"), VAS 'drowsy', 'muzzy', 'mentally slow' and 'dreamy' ("relaxation") and VAS 'voices', 'meaning' and 'suspicious' ("dysphoria"). These results provide experimental evidence that THC can evoke different classes of effects. These distinct subjective clusters could represent effects on various systems in the brain, which can be used to further differentiate the involvement of endocannabinoid systems in health and disease.

Introduction

Cannabis is best known as a recreational drug that is widely used throughout the world, although its use is illegal in most countries. The main psychoactive component of cannabis is Δ^9-tetrahydrocannabinol (THC), which is an agonist at cannabinoid CB_1 and CB_2 receptors. Both cannabis and THC have been used in preclinical and clinical research to investigate the effects on pain (reviewed by Lynch and Campbell, 2011), food intake (reviewed by Berry and Mechoulam, 2002), pain and spasticity in multiple sclerosis (reviewed by Karst et al., 2010 and Zajicek and Apostu, 2011) and as models of psychosis (D'Souza et al., 2004; Liem-Moolenaar et al., 2010; Kleinloog et al., 2012). Alternatively, CB_1-antagonists (e.g. rimonabant) have been shown to block the anti-nociceptive effects of THC (Compton et al., 1996), reduce food intake and facilitate weight loss (van Gaal et al., 2005), and there is literature that suggests a relation between CB_1-antagonism and the development of multiple sclerosis (van Oosten et al., 2004) and improvement of symptoms in schizophrenia (Kelly et al., 2011). Many of these investigative indications have been inspired by the subjective effect profile of cannabis and THC. These subjective effects, which are largely attributable to THC, can be quite diverse. The main effect is a 'high' feeling, which is described by Ashton (2001) as "a feeling of intoxication, with decreased anxiety, alertness, depression and tension and increased sociability". Less frequently, there can also be effects that are reminiscent of psychosis, like distorted perceptions of colour, space and time. Other effects are impairments of reaction time, short-term memory and motor coordination. Dysphoric reactions, described by Ashton (2001) as "anxiety and panic, paranoia and psychosis" can also occur. In addition, cannabis can induce feelings of appetite ("munchies"). The factors that determine the intensity of each of these effects have not been investigated in detail, but the subjective effects of THC seem to differ among users, and probably also between occasions of use or doses and modes of administration. There are many ways in which the (subjective) effects of cannabis and THC can be measured.

In a review by Zuurman et al. (2009) feeling high was shown to be the most sensitive central nervous system (CNS) biomarker for the effects of cannabis, essentially irrespective of how this was measured. A frequently used tool to measure subjective feelings is the visual analogue scale (VAS). A VAS typically consists of a 100 mm long line, with two extremes on the sides. A subject is asked to indicate his or her current feelings somewhere on the line between the two extremes. Bowdle et al. (1998) described a composite scale for psychedelic effects (hereafter VAS Bowdle, see Table 1), consisting of 13 questions with the extremes of 'not at all' and 'extremely', and validated this scale in a group of healthy volunteers who received ketamine. Zuurman et al. (2008) used the VAS Bowdle to measure the subjective effects of THC. Based on cluster analysis and factor analysis, they suggest the use of two distinct composite scales, which were classified as "internal perception" (VAS 'reality', 'voices', 'meaning', 'suspicious' and 'anxious') and "external perception" (VAS 'body', 'surroundings', 'time', 'thoughts', 'colours' and 'sounds'), in addition to the item 'high' (Zuurman et al., 2008). This same study also reported dose-related effects on subjective alertness, which were assessed using the VAS described by Bond and Lader (1974). These authors identified 16 combinations of two subjective states (Table 1), combined in clusters of alertness (VAS 'drowsy', 'feeble', 'muzzy', 'clumsy', 'lethargic', 'mentally slow', 'dreamy', 'incompetent' and 'bored'), calmness (VAS 'calm' and 'relaxed') and mood (VAS 'contented', 'tranquil', 'happy', 'amicable' and 'gregarious'), based on a principal component analysis (PCA) on response in a group of healthy volunteers, without intervention. Norris (1971) had previously subdivided these 16 items into four categories of four items each, based on a conceptual framework. These categories are "mental sedation or intellectual impairment" (items 'drowsy', 'muzzy', 'mentally slow' and 'dreamy'), "physical sedation or bodily impairments" (items 'feeble', 'clumsy', 'lethargic' and 'incompetent'), "tranquillization or calming effects" (items 'calm', 'contented', 'tranquil' and 'relaxed') and "other types of feelings or attitudes" (items 'happy', 'amicable', 'bored' and 'gregarious').

In combination, the 13 visual analogue scales described by Bowdle and the 16 scales used by Bond and Lader cover most of the subjective effects of cannabis and THC that were summarized by Ashton (2001), with the exception of the effects on appetite. The subjective effects of cannabis and THC are relevant, considering their putative therapeutic potential and pathophysiological significance. The aim of the current analysis is to identify distinct profiles within the spectrum of characteristic subjective effects of THC as measured using well-known sets of VAS. Such distinct effect profiles could provide quantitative information on different neurophysiological effects of THC, and on different sensitivities of individuals to such effects. When these profiles are combined in composite scales, they can be used in the design and interpretation of studies assessing the effect of THC or cannabis and to improve our understanding of the endocannabinoid system in health and disease. For example, the relation between these subjective effects and personality or genetic constitution could be examined (van Winkel et al., 2011), or the relationship between activation of certain brain regions in neuroimaging studies and subjective response patterns of THC or cannabis (e.g. Atakan et al., 2013). Individual VAS items are compared on sensitivity to the effects of THC, including a possible dose-response relationship. Also, different multivariate techniques were employed to examine if the clustering of different VAS items elicited distinct response patterns.

Methods

Data collection

Data from ten studies performed by CHDR in which THC was administered to a total of 217 healthy volunteers were selected to perform an exploratory analysis on the measurements of the subjective effects of THC. The time points and measurements of VAS Bond and Lader and VAS Bowdle, as

well as the time points of drug administration and administered dose were used for the analysis. All the studies had a randomised, cross-over, placebo-controlled design and were approved by the local Ethics Committee. Some studies were interaction studies, but only the treatment arms that involved administration of either THC alone or placebo alone were taken into account. An overview of the studies and their references is provided in table 2. All healthy volunteers who participated in the studies were mild cannabis users, defined as a frequency of cannabis use of maximum once a week in the past year.

THC challenge

In nine out of ten studies (94.5% of subjects) purified THC was inhaled using the Volcano™ vaporizer (Storz-Bickel, Tuttlingen, Germany). This method is described in more detail by Zuurman et al. (2008). In all these studies, several administrations were given during a study day to prolong the effect of THC. This was typically an increasing dose (2 mg, 4 mg, 6 mg) with 60 to 90 minute intervals, although the actual dosing regimen was different throughout the studies (see Table 2). One study used single doses of oral or sublingual tablets of purified THC. This study was included as a check of the notion that THC effects are determined by individual sensitivity and brain concentrations, and not by administration route.

Quality control

Prior to the analysis, a visual quality check of the available data was performed. In this regard, for each VAS item the data of the placebo condition from different studies were presented as boxplots. As no subjective effects are expected during the placebo condition, the scores for the items of the VAS Bond and Lader were expected to be distributed around the middle and the scores for the items of the VAS Bowdle close to a score of 0 mm. A few studies showed a distribution during the placebo condition that was

distinctly different from the other studies (based on visual comparison), and these were excluded from further analysis.

Item sensitivity

Measurements performed in the first 60 minutes after THC administration were pooled to identify items that are sensitive to THC. Items that showed a significant difference between THC and placebo were selected for further analysis. A Kruskal Wallis test was used as the distribution was not normal. A *p*-value of 0.05 / 29 (Bonferroni correction for number of VAS items) was considered significant.

Defining responders

Not all subjects showed a response on the VAS after administration of THC. Subjects were therefore classified as responder or non-responder for each individual VAS item. To make this classification, the distribution of observed scores for the overall placebo condition was examined, and the values within the 95% observation interval during placebo were considered indicative for the absence of a response. Conversely, subjects were considered a responder for a specific VAS item if they showed a response outside this 95% limit during any measurement in the THC condition.

Dose-response relationships

To determine possible dose-response relationships for the different VAS items that are sensitive for the effect of THC, the studies that used intrapulmonary administration of THC were selected. Most of these studies had a design where multiple doses of THC were administered on each study day, with a fixed time interval between administrations. As the times of measurements were different for each study, the maximum response after each administration was used. All individual studies were designed to include

measurements around the expected maximum effect (T_{max}). Possible relationships were tested using a Kruskal Wallis test and a *p*-value of 0.05/n (Bonferroni correction) was considered significant. Since most studies included several different consecutive doses on each individual study occasion, it was possible to assess a dose-response relationship, which was performed in two steps. Initially, only the first administration of THC during each study day was taken into account, which assured the absence of carry-over effects and tolerance. Subsequently, all administrations during each study day were considered if the dosing interval was at least 60 minutes, which covered more observations and a larger dose range. Both steps were repeated within subjects who were identified as responders.

Cluster selection

For the items that showed a significant dose-response relationship, different methods were applied to determine the combination of (weighted) items that could best describe 'the subjective effect of THC'. The combinations of items found with these different methods were then compared on their ability to predict the drug condition (THC or placebo). To find clusters within the dataset, multiple correspondence analysis (MCA), principal component analysis (PCA), factor analysis (FA), k-means cluster analysis (KCA), hierarchical cluster analysis (HCA), variable clustering (VC) and discriminant analysis (DA) were used. Each technique has its own advantages and should more or less lead to the same conclusion if the clusters are the result of an underlying construct. The final cluster selection was based on what the different clustering methods have in common. MCA is an exploratory technique that uses logical indicators (true or false), which makes it more suitable for data that is not normally distributed or categorical (Greenacre, 2007). For MCA, the dataset was recoded into responders and non-responders, and the final item selection was based on the inertia of the items. PCA is the most commonly used tool in exploratory data analysis (Jolliffe, 2002). FA is a technique similar to PCA, but it only focuses on the variability that is shared with

another item, whereas PCA takes all variability into account (Jolliffe, 2002). Both techniques can be applied to a dataset that is jointly normally distributed and are sensitive to the relative scaling of the original variables. PCA and FA were therefore performed using the maximum response in the THC condition, after a mean subtraction for all VAS items and log-transformation for VAS Bowdle items. KCA is a disjoint clustering method, in which all items are distributed within a pre-defined number of separated clusters to minimize within-cluster variability and maximize between-cluster variability (Hartigan and Wong, 1979). Within HCA and VC, clusters are organised to identify a hierarchical structure based on similarity between items, which is typically presented as a dendrogram (Jain et al., 1999). Linear stepwise DA is another method to find combinations of items that are able to predict the subjective effect of THC. For this analysis, the maximum response following intrapulmonary administration of THC was used to select items and the data from the study that was performed most recently were exclusively used for cross-validation. A stopping criterion of 0.1% improvement was used for forward and combined analysis and a stopping criterion of -0.5% improvement (either any improvement or a maximum of 0.5% worsening) for backward analysis.

Inverse predictive check

An inverse predictive check was performed for the individual items and the possible combinations of items to compare the probability to identify the original treatment. In this regard, predictive values were calculated for each individual VAS item, the clusters described by Bond and Lader (1974) and Zuurman et al. (2008), and the combinations of items found in the current analysis. The predictive value describes the chance that the score on a certain item (or combination of items) correctly identifies the given treatment (THC or placebo). As there are two possible outcomes (THC or placebo), the *a priori* predictive value is 50%. Again, the data from the study that was most recently performed were exclusively used for cross-validation.

Statistical software

The open source statistical software package R (version 2.14.0, www.r-project.org) was used for the analyses.

Results

Quality control

Based on a visual check of the distribution of scores under placebo conditions and prior to other evaluations, three studies (with a total of 74 (34.1%) subjects) were excluded from further analysis, based on the scores under placebo on all VAS items. One study showed a slightly different placebo profile compared with the other studies, which was a PET-study with administration of [^{11}C]-raclopride and PET-measurements during THC administration in both study arms. It was decided to include the information from this study with the use of placebo correction.

Item sensitivity

VAS Bond and Lader items 'contented', 'tranquil', 'happy', 'amicable' and 'bored' were the only items that did not show a statistically significant different score between THC and placebo conditions (after Bonferroni correction). All these items are part of Bond & Lader's "mood" cluster, with the exception of VAS 'bored'.

Selection of responders

Table 2 shows the upper and lower limits of the 95% observation interval for VAS Bowdle and VAS Bond and Lader respectively, during all placebo occasions. Individual scores outside of these limits during THC occasions were

considered to be indicative of a drug response. The percentages of subjects who were classified as item responders after THC administration are presented per item.

Dose-response relationships

When taking into account all administrations and all subjects, VAS 'drowsy', 'feeble', 'clumsy' and 'dreamy' of Bond and Lader and all VAS Bowdle items except 'voices' and 'anxious' showed a significant dose-response relation using a *p*-value of 0.05 / 96 (Bonferroni correction for four times 24 items; the five items that did not differ significantly between placebo and THC were not taken along). The items that showed a significant dose-response relation for all administrations and all subjects were used for cluster selection. It should be noted that the dose interval (mostly between 60 and 90 minutes) is likely to have resulted in an accumulation of effect. The dose level of 8 mg is not included in the dose-response analysis, as only 8 observations were available for this dose (compared to 150, 110, 118 and 101 observations for the other doses).

Multiple correspondence analysis

The outcome of the MCA is presented in Figure 1. The closer two data points are to one another, the more likely they are to show a response at the same time. Items that are relatively closer to the right side of the map are items that are more likely to show a response than items that are closer to the left side of the map. The relative inertia of the items is provided in Table 2. Inertia is a measure of how much the item contributes independent of other items (comparable to *eigenvalues*).

Principal component analysis and factor analysis

Following parallel analysis, two components were selected for the varimax rotated principal component analysis based on the observation of a 'sharp

break' in the scree plot. Another way of determining the optimal number of components is comparing the *eigenvalues* of the possible components in the dataset with those obtained from a random, simulated dataset of the same size. Using this approach, three components would have been selected. Figure 2 presents a map of the rotated principal component analysis and the factor loadings are presented in Table 2. For factor analysis (based on maximum likelihood), parallel analysis suggested the use of five factors, which are presented in Figure 3.

Cluster analysis

Within *k*-means cluster analysis, all items were assigned to one of three clusters. This technique gives no indication as to how well the variable fits into the cluster. Although items were scaled to allow for better comparison, all items of VAS Bond and Lader grouped into one cluster and VAS Bowdle items 'surroundings', 'colours', 'sound' and 'suspicious' were separated from the remaining items. The results of hierarchical cluster analysis are presented in Figure 4. Variable clustering had similar results. Because all these clustering methods will place all items within a cluster, the items that do not cluster consistently throughout the methods (e.g. item 'drowsy' of VAS Bowdle) are likely irrelevant.

Discriminant analysis

Forward, backward and combined discriminant analyses were performed. The results from the combined DA were equal to the results of forward DA and are therefore not presented separately. Forward DA identified VAS 'calm', 'dreamy', 'incompetent' and 'high' as most predictive for the effect of THC and backward DA identified VAS 'lethargic', 'relaxed', 'incompetent', 'bored', 'gregarious', 'thoughts' and 'high'. As VAS 'high' was expected to have a large impact on the outcomes, the analysis was repeated without this

item, resulting in a combination of VAS 'lethargic', 'dreamy', 'thoughts' and 'colours' in case of forward DA and the same items together with VAS 'sound' in case of backward DA.

Inverse predictive check

To calculate the predictive value of the different combinations of items, two methods were used: a composite (average) score of the items and a combination of the individual scores on the different items. Individually, VAS 'high' has the best predictive value (83.6%), followed by VAS 'thoughts' (77.1%), VAS 'mentally slow' (75.8%) and VAS 'time' (75.2%), as presented in Table 2. From the different combinations of items, only those found with DA resulted in a better predictive value as a composite scale.

Discussion

THC and cannabis have a rather broad range of effects, which can differ between subjects, doses and use circumstances. The effect patterns can give insight into the many different functions, therapeutic areas and diseases in which the cannabinoid system has been implicated. This analysis explored the characteristics of the scope of subjective effects of THC as measured on different visual analogue scales. As THC is the main component of cannabis and the elicited subjective effects of THC are comparable to the subjective effects of cannabis described in the literature, the findings might be applicable to cannabis. However, the other components of cannabis (i.e. cannabidiol) might distort the subjective effect patterns. It is important to note that different preparations of cannabis have different levels of THC and cannabidiol. The analysis examines the effects of THC in mild cannabis users. Results might be relevant to other groups of people (heavy users, non-users), although further research is needed.

VAS Bond and Lader and VAS Bowdle are frequently used in CNS drug research and capture most of the subjective effects that have been described with cannabis or THC. Not surprisingly, the analysis indicated that feeling high was the most predictive item for the effects of THC, which confirms the literature review of Zuurman et al. (2009) that showed a statistically significant 'high' effect in 96% of cannabis studies. The other items that had high individual predictive values describe effects on time perception and cognitive functions (controlling of thoughts and mental slowness), which are also well known and frequent effects of cannabis and THC (Ashton, 2001). The items with high predictive values could be grouped into three distinct factors of effect. Table 3 presents an overview of the proposed composite scale that measures these factors.

The first common factor that was found using the different methods of cluster selection consists of VAS 'time', 'thoughts' and 'high'. VAS 'colours' and 'sound' showed a relation with this factor. Together, this factor can be described conceptually as a measure of feeling high and changes in perception ("perception"). All these items were a part of the cluster "external perception" as described by Zuurman et al. (2008), except 'high' which was treated by Zuurman et al. as a separate cluster because of its predominance. This clustering of feeling high and the other items follows the description of the most typical THC effects by Ashton (2001).

VAS 'drowsy' (from VAS Bond and Lader), 'muzzy', 'mentally slow' and 'dreamy' constitute the second common factor. VAS 'feeble' and 'clumsy' showed a relation with this factor. All these items are included in the "alertness" clusters as described by Bond and Lader (1974). The main four items can be seen as mental aspects of sedation ("relaxation"), whereas the two related items are more physical aspects of sedation.

VAS 'voices', 'meaning' and 'suspicious' are included in the third common factor within the effects of THC. These items may represent what Ashton (2001) describes as 'dysphoric reactions' ("dysphoria"). Zuurman et al. (2008) included these items in the "internal perception" cluster. Not many subjects show an effect on these items, but if these scales are affected, the effects

seem to be clear. Even though this cluster of items does not seem highly predictive of the effect of THC, this aspect might correlate with other important predictors for the effect of THC such as the occurrence of adverse events. Henquet et al. (2005) suggest these 'dysphoric reactions' could also reflect a predisposition for the development of psychosis.

Within the PCA, these three factors ("perception", "relaxation" and "dysphoria") were shown to represent two principal components. The majority of the variation was explained by "relaxation" (aligned with the horizontal component in Figure 2). The remaining variation could be explained by "dysphoria" (aligned with the vertical component in Figure 2), which appears to be the opposite of mental relaxation. The "perception" effects (the more 'typical' effects of THC) were not a part of the two components, but rather seem to represent a separate component that is a vector of the other two clusters and therefore represents the main underlying effect. As described earlier, another way of determining the number of components would have resulted in three components.

The items that did not show a significant difference between THC and placebo are a part of the "mood" cluster in the VAS Bond and Lader, with the exception of VAS 'bored' (a scale that does seem to relate to mood). This would suggest that THC does not affect mood in a stricter sense, which is consistent with the review by Zuurman et al. (2009). The effect of THC on appetite is not measured by VAS Bond and Lader or VAS Bowdle and therefore not taken along in the current analysis. Given the relevance of THC (and the endocannabinoid system) on appetite (Farrimond et al., 2011), it would have been interesting to observe how additional VAS scales of hunger and appetite would have behaved in relation with the other clusters. For use in future studies, the authors would recommend the addition of scales for hunger and appetite.

A more complete assessment of the different effect dimensions of THC could aid in the exploration of the various pharmacological and physiologic aspects of cannabinoid systems, in health and disease. For example, the interactions of different constituents of cannabis (i.e. THC and cannabidiol)

could be disentangled, by measuring the effects of each component and different combinations of components (Bhattacharyya et al., 2010). Also, the effect profiles can help in quantifying the dose-response relationships for different THC-effects, for instance to discriminate peripheral and central cannabinoid type 1 antagonists (Klumpers et al., 2013b). Other applications of the dimensional scales could be in exploring the brain structures underlying different effects of THC (Atakan et al., 2013), as well as examining the influence of genetic factors (i.e. polymorphisms) on the subjective effects of THC or cannabis (van Winkel et al., 2011). The dimensional quantification of cannabinoid effects can also help in exploring the relation between subjective effects to cannabis and clinical risk of psychosis, for instance by demonstrating that patients (or people at risk) show relatively strong "perception" effects, compared to the other effects of a cannabinoid challenge (Henquet et al., 2010).

The skewed distribution of VAS Bowdle is unfavourable for statistical analysis. The finding that a combination of items of the VAS Bond and Lader, which has an approximately normal distribution, explains most of the variation in effect is therefore important. The clear separation between items from VAS Bond and Lader and from VAS Bowdle that was seen with most methods is interesting. This could be caused by the differences in distribution that are characteristic for the scales, which is the result of (1) the use of two-sided versus one-sided scales and (2) the use of effects that are present and absent under 'normal' circumstances (i.e. it is normal to have fluctuations in mood and alertness, but not in psychedelic effects). However, the separation could also be caused because the psychometric properties of the scales (i.e. what they measure) are different.

In summary, the current analysis provides experimental evidence that the subjective effects of THC in mild cannabis users have three main dimensions (consistent over a variety of statistical techniques). The main subjective effects of THC consist of feeling high and changes in perception. In addition, mental relaxation or dysphoric reactions can occur more or less independently. These findings correspond with previous descriptions

of the subjective effects of THC and cannabis. The three dimensions can be used as the basis of an evidence-based composite scale (see Table 4, which could also include effects on hunger and appetite), to further explore and differentiate the involvement of endocannabinoid systems in health and disease and to quantify the subjective effects of THC and cannabis in clinical research. There seems to be a subset of individuals (even among occasional cannabis users) who respond to THC with dysphoric reactions and another small group of individuals who do not experience the typical 'high' effects of THC. Further exploration of the genetic or psychological profiles of these individuals and the relation with subjective effect patterns could shed more light on the role of the cannabinoid system in health and (mental) disease.

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TABLE 1 Description of visual analogue scales.

	Item	Name	Full description	
VAS Bowdle	1	Body	My body or body parts seemed to change their shape or position.	
	2	Surroundings	My surroundings seemed to change in size, depth, or shape.	
	3	Time	The passing of time was altered.	
	4	Reality	I had feelings of unreality.	
	5	Thoughts	It was difficult to control my thoughts.	
	6	Colours	The intensity of colours changed.	
	7	Sound	The intensity of sound changed.	
	8	Voices	I heard voices or sounds that were not real.	
	9	Meaning	I had the idea that events, objects, or other people had particular meaning that was specific for me.	
	10	Suspicious	I had suspicious ideas or the belief that others were against me.	
	11	High	I felt high.	
	12	Drowsy	I felt drowsy.	
	13	Anxious	I felt anxious.	
	Item	**Name**	**First extreme**	**Second extreme**
VAS Bond and Lader	1	Drowsy	Alert	Drowsy
	2	Calm	Calm	Excited
	3	Feeble	Strong	Feeble
	4	Muzzy	Muzzy	Clear-headed
	5	Clumsy	Well-coordinated	Clumsy
	6	Lethargic	Lethargic	Energetic
	7	Contented	Contented	Discontented
	8	Tranquil	Troubled	Tranquil
	9	Mentally slow	Mentally slow	Quick witted
	10	Relaxed	Tense	Relaxed
	11	Dreamy	Attentive	Dreamy
	12	Incompetent	Incompetent	Proficient
	13	Happy	Happy	Sad
	14	Amicable	Antagonistic	Amicable
	15	Bored	Interested	Bored
	16	Gregarious	Withdrawn	Gregarious

TABLE 2 Overview of original studies.

Reference	N	Doses	Interval	VAS timepoints
Bossong et al. 2009	7	8 mg inhalation	N/A	7, 12, 17, 32 and 105 min after dose
Van Hell et al. 2011	26	6 + 1 + 1 + 1 mg	30 min	27 and 34 min after first dose
Kleinloog et al. 2012	49	2 + 4 + 6 mg	90 min	13, 25, 33 and 64 min after each dose
Klumpers et al. 2012a	12	5, 6.5 or 8 mg	Oral	19, 36, 51, 65, 95 min after dose
Klumpers et al. 2012b	22	2 + 6 + 6 mg	90 min	29, 59 and 83 min after each dose
Klumpers et al. 2012c	30	2 + 4 + 6 + 6 mg	60 min	23 and 41 minutes after each dose
Klumpers et al. (2013)	34	5 x 4 mg	≥ 150 min	10, 24 and 115 min after each dose
Liem-Moolenaar et al. 2010	37	2 + 4 + 6 mg	90 min	22, 34 and 61 min after each dose
Zuurman et al. 2008	12	2 + 4 + 6 + 8 mg	90 min	22 and 47 min after each dose
Zuurman et al. 2010	36	2 + 4 + 6 + 6 mg	60 min	23 and 41 min after each dose

TABLE 3 Overview of different outcome measures

VAS item	LLOI	ULOI	% Responders	% Resp. (0 mg)	% Resp. (2 mg)	% Resp. (4 mg)	% Resp. (6 mg)	MCA: % inertia	PCA: comp. 1	PCA: comp. 2	Predictive value
Drowsy	36	61	62	13	21	37	521	5.30	0.80	0.20	70.8
Calm	35	54	56	7	8	11	18	2.89	0.28	0.41	57.6
Feeble	37	56	52	7	17	30	451	5.56	0.82	0.27	68.3
Muzzy	44	64	69	9	20	26	51	5.01	0.85	0.29	74.4
Clumsy	38	53	69	12	34	47	561	5.25	0.80	0.30	71.5
Lethargic	35	62	66	7	9	16	20	0.60	-0.15	0.11	47.7
Contented	34	53	48	35	44	58	61	3.54	0.42	0.16	63.6
Tranquil	48	66	68	19	19	25	36	1.27	0.37	0.44	71.6
Mentally slow	42	64	69	9	22	36	54	5.53	0.84	0.23	75.8
Relaxed	46	66	53	11	15	21	34	2.13	0.52	0.12	72.4
Dreamy	38	61	64	5	23	41	551	5.31	0.82	0.26	72.3
Incompetent	48	64	62	13	12	16	31	2.65	0.73	0.37	66.4
Happy	32	52	49	7	15	13	32	2.91	0.59	0.33	56.3
Amicable	48	68	47	20	26	20	27	0.59	0.47	0.09	47.6
Bored	37	62	34	6	6	14	18	1.26	0.68	0.04	53.8
Gregarious	47	69	50	16	17	15	30	1.34	0.68	0.38	61.8
Body	0	1	42	5	15	24	311	3.71	0.26	0.69	55.9
Surroundings	0	1	40	1	15	25	371	4.60	0.37	0.76	65.1
Time	0	1	63	4	27	48	571	5.00	0.49	0.65	75.2
Reality	0	1	48	7	24	34	461	4.63	0.37	0.77	67.6
Thoughts	0	1	74	7	39	63	661	5.30	0.55	0.60	77.1
Colours	0	1	46	3	19	29	421	3.76	0.27	0.69	63.9
Sound	0	1	49	3	20	31	391	4.52	0.46	0.66	68.5
Voices	0	1	25	3	7	14	19	1.91	-0.01	0.84	54.2
Meaning	0	1	22	2	7	10	171	1.71	0.09	0.76	53.8
Suspicious	0	1	21	4	7	12	161	1.50	0.01	0.84	52.8
High	0	2	88	1	58	80	871	4.63	0.58	0.46	83.6
Drowsy	0	3	58	7	30	40	611	4.63	0.61	0.46	66.6
Anxious	0	1	35	4	11	19	30	2.95	0.29	0.78	56.8

(1: significant dose-response relationship).

TABLE 4 Overview of suggested composite scale.

Subscale Perception
Time perception
Change in control of thoughts
Feeling high
Subscale Relaxation
Feeling drowsy
Feeling muzzy, not having a clear head
Mental slowness
Feeling dreamy
Subscale Dysphoria
Hearing voices
The idea that events, objects or people have a special meaning
Suspicious ideas or beliefs
Subscale Appetite
Feelings of hunger
Feelings of appetite

FIGURE 1 Map of multiple correspondence analysis. The final cluster selection based on all methods has been highlighted (squares: dysphoria; triangles: perception; closed circles: relaxation). *For colour figure see inside of front cover.*

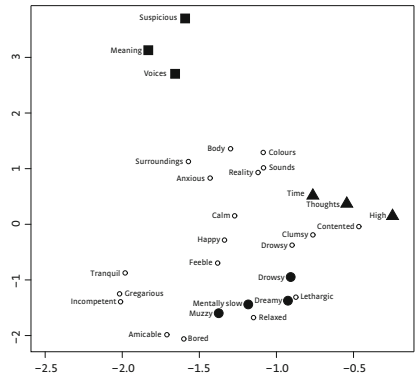


FIGURE 2 Plot of rotated principal component analysis. *For colour figure see inside of front cover.*

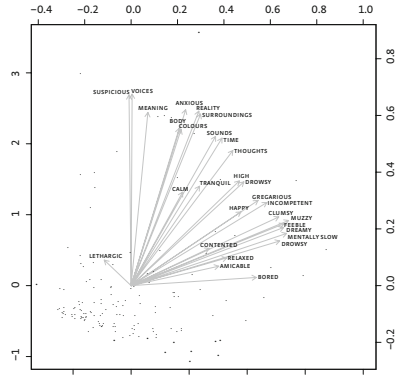


FIGURE 3 Overview of factor analysis. (light grey: relaxation; middle grey: dysphoria; dark grey: perception).

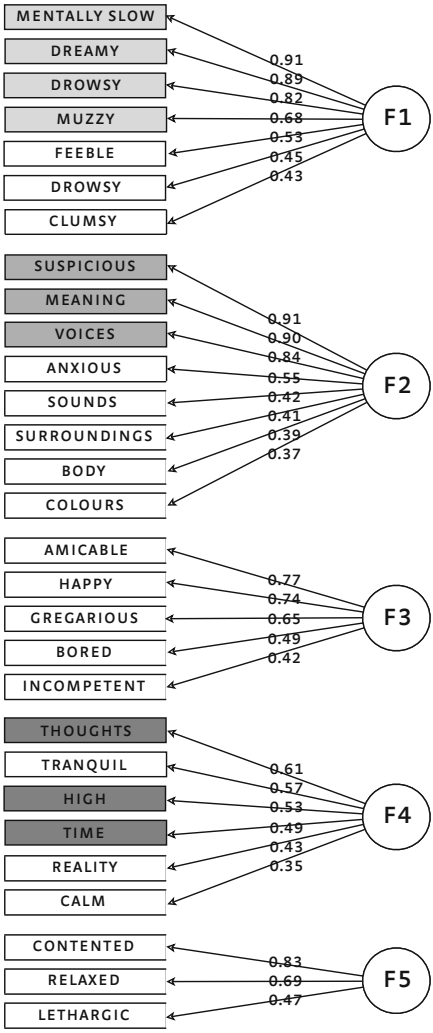
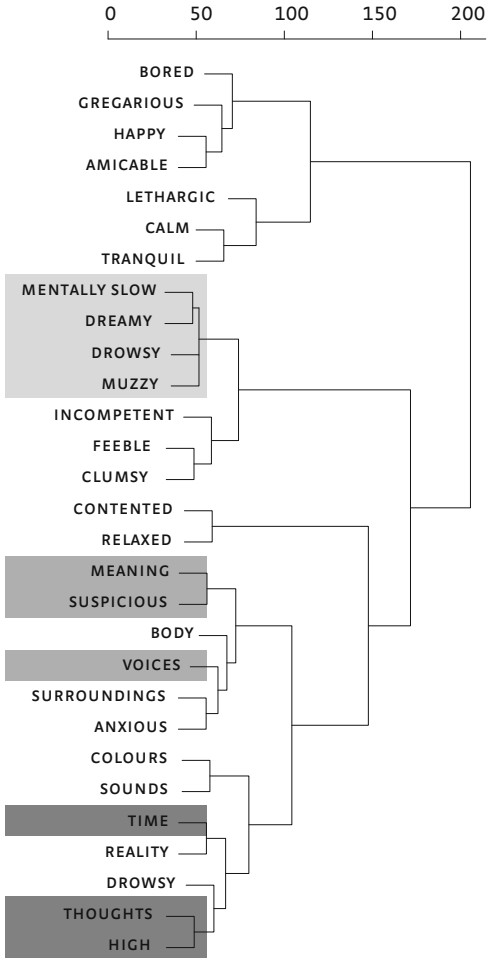


FIGURE 4 Overview of hierarchical cluster analysis. The final cluster selection based on all methods has been highlighted (light grey: relaxation; middle grey: dysphoria; dark grey: perception).



CHAPTER 4

The influence of personality on the sensitivity to subjective effects of Δ^9-tetrahydrocannabinol

Psychiatric Res (submitted for peer review)

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ABSTRACT

The effects of drugs are not only determined by their pharmacological action, but also by user characteristics. This analysis explored the influence of personality on the differences in subjective effects in response to a standardized pharmacological challenge with the cannabinoid CB_1/CB_2 agonist Δ^9-tetrahydrocannabinol (THC).

To express the sensitivity to THC, pharmacokinetic-pharmacodynamic (PK-PD) non-linear mixed effects modelling was applied to the subjective response of 184 healthy subjects to a pharmacological challenge with inhalation of THC. The subjective effects were measured using visual analogue scales and described by three clusters: 'perception', 'relaxation' and 'dysphoria'. The sensitivity for THC (described as EC_{50}) was related to scores on Cloninger's temperament and character inventory (TCI) using multiple linear regression.

Effect compartment models were used to describe the PK-PD relations of THC. Within the multivariate model, 'harm avoidance' was significantly correlated with changes in 'perception', and 'self-transcendence' with changes in 'dysphoria'.

Within the psychobiological model of personality, 'harm avoidance' is related to serotonergic systems. Subjects with either very low (easy-going) or very high (cautious) scores were less sensitive to THC-induced changes in 'perception'. 'Self-transcendence' relates to schizotypy. Subjects with more schizotypy were more sensitive to the dysphoric subjective effects of THC.

Introduction

Cannabis sativa is a plant consumed all over the world for its psychomimetic properties. The active ingredients of cannabis exert their effect through interaction with cannabinoid receptors (Ashton, 2001). Two distinct types of receptors have been identified: CB_1 receptors are mostly found in the central nervous system (CNS) and CB_2 receptors are mainly found peripherally. Several endogenous ligands (named endocannabinoids) have been discovered that interact with cannabinoid receptors, including arachidonylethanolamide (anandamide) and 2-arachidonylglycerol (2-AG; Ashton, 2001; Grotenhermen, 2003; Kano et al., 2009).

The main active ingredient of cannabis is Δ^9-tetrahydrocannabinol (THC), which is an agonist of both cannabinoid CB_1 and CB_2-receptors (Grotenhermen, 2003). The two main metabolites of THC are the active 11-hydroxy-tetrahydrocannabinol (11-OH-THC) and the inactive 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH).

The main subjective effect of cannabis is a 'high' feeling, which is described by Ashton (2001) as a euphoric effect with "a feeling of intoxication, with decreased anxiety, alertness, depression and tension and increased sociability". This 'high' feeling is often associated with changes in perception, such as brighter colour vision and distorted spatial and time perception. Occasionally, and more frequently in naive or psychologically vulnerable users, dysphoric reactions (including severe anxiety and panic, paranoia and psychosis) can occur.

In a pharmacological challenge test, a drug with a known pharmacological mechanism of action is administered. A pharmacological challenge provides insight into underlying (patho-) physiological systems related to the mechanism of action (Gijsman et al., 2004). The use of THC as a pharmacological challenge test of the cannabinoid system has previously been used to assess the action of cannabinoid antagonists (i.e. Klumpers et al., 2013a) and as a model for psychosis (i.e. Liem-Moolenaar et al., 2010; Kleinloog et al., 2012). Cannabis contains many other constituents then THC, some with

opposing effects, such as cannabidiol (CBD). Moreover, the administered dose of THC can be controlled more accurately by inhalation of vaporized pure THC than by smoking cannabis. Therefore, the use of purified THC for a pharmacologic challenge has advantages over the use of cannabis extract.

To extract information of the underlying biological system, the relation between the drug given during a pharmacological challenge and the resulting changes in an outcome measure can best be described using pharmacokinetic-pharmacodynamic (PK-PD) relations (Danhof et al., 2008). Within such a model, the exact time course of the input (the time-concentration profile of the drug) is mathematically related to the exact time course of the output (the time-effect profile of the outcome measure).

There are only a few models that describe the PK-PD relations of the subjective effects of THC, all based on a limited amount of data. Independent of the method of administration, a counter-clockwise hysteresis is observed over time, suggesting a delay in effect, most likely due to delayed distribution to the target site (Grotenhermen, 2003). Chiang and Barnett (1984), Harder and Rietbrock (1997) and Strougo et al. (2008) described an effect compartment model with sigmoid E_{max} curve for the subjective effects of THC. Data by Ohlsson et al. (1980; Hollister et al., 1981; Grotenhermen, 2003) were consistent with the model described by Chiang and Barnett (1984).

The concept of personality can be defined as a set of characteristics in behaviour that define how a person deals with adjustments to their environment. Many different instruments have been developed that measure personality. Factor analyses (Eysenck, 1990) show three consistent dimensions of personality that are present in the general population: neuroticism (vs emotional stability), extraversion (vs introversion) and psychoticism (vs super-ego control). However, these three dimensions do not correspond with the descriptions of personality disorder. Moreover, drugs that reduce neuroticism (e.g. alcohol) also reduce introversion, suggesting a common underlying biological pathway. That is why Cloninger (1987, 1993, 1994) describes a construct of personality that is based on a psychobiological model. The described personality dimensions are hypothesised to be

correlated with specific neurotransmitter systems and are therefore more in line with the field of psychopharmacology then the dimensions described by Eysenck et al. (1985).

Although several studies have been performed that describe the difference in personality between cannabis users and non-cannabis users, there are few studies that examined the influence of personality on the effects of THC. Ashton et al. (1981) found a clear interaction between personality traits and subjective effects and electroencephalography (EEG) signals after administration of THC. Subjects with higher introversion scores showed a larger magnitude in EEG signal and subjects with lower neuroticism scores showed larger subjective effects of 'intoxication'. Klapper et al. (1972) describe a relation between personality traits and cognitive performance in response to THC. Barkus et al. (2006; 2008) report increased subjective responses (psychosis-like and dysphoric effects) for subjects with higher schizotypy scores.

The current analysis aimed to examine the influence of personality traits on the variability in response in subjective effects following a pharmacological challenge with THC. To fulfill this aim, a PK-PD model of the subjective effects of THC was developed and the parameter for drug sensitivity (EC_{50}) was compared to scores on Cloninger's Temperament and Character Inventory (TCI). The relationship between personality and sensitivity may be useful for the interpretation of drug effects and may provide clues to underlying neuropharmacological processes.

Methods

Data collection

Data from seven studies performed by the Centre for Human Drug Research (CHDR) that included repeated administration of THC were selected (see Table 1). All studies had a double-blind, placebo-controlled, cross-over

design and were conducted in physically and mentally healthy volunteers who were occasional users of cannabis (maximum of once a week). Most studies investigated a pharmacological interaction between THC and another compound. The analysis was restricted to the treatment arms that contained only THC. The wash-out period between two study days was at least two weeks and subjects were not allowed to use cannabis during the study period (which was confirmed using urinalysis at the start of each study day). All these studies, as well as the current analysis, were approved by the local Ethics Committee.

Administration of THC

All of the selected studies used the Volcano™ vaporizer to administer THC by inhalation (Hazekamp 2006, Zuurman 2008). A purified THC solution is vaporized using a flow of hot air. The resulting vapour is collected in a balloon to which a valved mouthpiece is attached. The subjects then inhale the contents of the balloon, hold their breath for 10-20 seconds and exhale. This is repeated until the balloon is empty (typically 4-6 inhalations are required). To reduce the risk of losing inhaled vapour through nasal expiration, a nose clip was used in the later studies (specified in Table 1). All studies used a design in which multiple (3-5) doses of THC were administered throughout a study day, with an interval between 60 and 150 minutes (see Table 1).

Quantification of THC and its main metabolite 11-OH-THC

Throughout the study days, venous blood samples were collected repeatedly to measure the concentration of THC and its main metabolites. Samples were put in ice water directly after collection and handled within one hour, without exposure to direct light. Concentrations of THC, 11-OH-THC and THC-COOH in plasma were determined using high pressure liquid chromatography mass spectrometry (HPLC-MS) and the lower limit of quantification (LLOQ) varied across studies (specified in Table 1).

Quantification of the subjective effects of THC

In all studies, the subjective effects of THC were measured using visual analogue scales. A visual analogue scale is a 100 mm long line with two subjective states on both ends of the lines (e.g. 'alert' and 'drowsy' or 'not at all high' and 'extremely high'). During each study day, each subject was asked repeatedly to indicate somewhere on this line how he or she felt at that time. A total of 29 visual analogue scales were used: 16 measuring effects on alertness, calmness and mood (as described by Bond and Lader, 1974) and 13 measuring psychedelic effects (as described by Bowdle et al., 1998). A recent cluster analysis identified three distinct clusters within the subjective effects of THC: the cluster 'perception' consists of changes in time perception, the control over one's thoughts and a 'high' feeling, the cluster 'relaxation' consists of feeling drowsy, muzzy (the opposite of clear-headed), mentally slow and dreamy and the cluster 'dysphoria' (which occurs in only a subset of subjects) consists of hearing voices, the idea that events, objects or people have a different meaning and suspicion (Kleinloog et al., in press).

Quantification of personality traits

To assess personality, subjects were asked to complete a validated Dutch version of the TCI, which is based on the Cloninger model of personality (Cloninger et al., 1993; Duijsens et al., 2000). The TCI consists of four dimensions for temperament (novelty seeking, harm avoidance, reward dependence and persistence), which are determined mainly by genetic factors, and three dimensions for character (self-directedness, cooperativeness and self-transcendence), which are determined mainly by environmental factors (Cloninger et al. 1993, 1994). There is substantial variation on each of these dimensions within the general population and the distribution of this variation follows a normal distribution (Cloninger et al., 1987; 1993). Scores within the study population were compared to the normative values of the Dutch general population included in the TCI manual (Duijsens et al., 2000).

Pharmacokinetic / pharmacodynamic model

Nonlinear mixed effect modelling in NONMEM, version 7.2.0 (ICON plc, Hanover, Maryland, USA) was used to describe pharmacokinetic and pharmacodynamic relations. A sequential PK-PD approach was applied, using the structure and parameters of the pharmacokinetic model described by Heuberger and co-workers (unpublished results) as basis. The structural models were built under ADVAN 13 and first order conditional estimation with interaction (FOCEI) was used for all final models with a convergence criterion in the parameter estimates of 3 significant digits. Population parameters (θ) describe the central tendency of the population. To describe individual differences in parameters, the population parameters may be weighted with a factor η, which is the individual parameter drawn from a normal distribution with a mean of zero and a variance of ω^2. The residual errors of each measurement have a normal distribution with a mean of zero and a variance of σ^2. Exponential functions for θ and η were explored during model development. Drug concentrations below the LLOQ were not included in the modelling.

To assess the performance of the three-compartmental pharmacokinetic model of THC (Heuberger and co-workers, unpublished results), a visual predictive check (VPC) was performed (Post et al., 2008) for each study. The pharmacokinetic profile of each study was simulated using the dosing regimen with 500 replications. This simulation was used to plot the predicted pharmacokinetic profile (median and 95% prediction interval), which was visually compared with the actually observed pharmacokinetic data per study (data not shown). If the Heuberger model was considered to perform well (i.e. describe the observed data correctly), the structure and population parameters (θ) for the pharmacokinetics of THC were fixed, and the parameters for inter-individual variation on elimination rate constant (k_E) and volume of distribution (V_D) included in the model were estimated for the new population. If the model would not describe the observed data correctly, population parameters would be estimated and/or changes to

the structure could be explored. The pharmacokinetic profile of the active metabolite 11-OH-THC was fitted with the same initial structural model as the parent compound.

For each of the three clusters of subjective effects (perception, relaxation and dysphoria), several structural models were evaluated: the effect compartment model which is used in all currently available models for subjective effects of THC (Chiang and Barnett, 1984; Harder and Rietbrock, 1997; Grotenhermen, 2003; Strougo et al., 2008); the addition of one or more transit compartments (Savic et al., 2007); and an indirect response model (Jusko et al., 1995). All of these models include parameters for baseline effect (E_0), maximum effect (E_{max}), half maximal effective concentration (EC_{50}) and diverse rate constants (e.g. k_{e0} for equilibration rate constant and K_E or K_{out} for elimination rate constant).

Each model fit was evaluated using the minimal value of the objective function (MVOF), goodness-of-fits plots, parameter distributions and individual predicted profiles of all subjects (compared to the observations, including drug concentrations below LLOQ). A covariance matrix (describing the uncertainty of the estimations) was required before accepting a model.

Assessing the relation between personality and the response to THC

It was expected that the relation between personality and sensitivity to THC would be complex, due to a bias in selection (i.e. subjects who participate in a clinical trial were expected to have low scores on 'harm avoidance' compared to the general population and there were specific requirements regarding the frequency of cannabis use for the study population) and adaptation (i.e. the occurrence of negative experiences will influence the frequency of subsequent cannabis use and sensitivity to THC in experienced users may be influenced by the frequency of use -tolerance-). The relation between the personality subscales and sensitivity to THC was therefore assessed using multiple linear regression, using both linear and quadratic relations

as described by Kabacoff (2011). The individually estimated EC_{50} was used as a measure for sensitivity to THC. Multiple linear regression was performed using the statistical software package R, version 3.0.1 (R Core Team, Vienna, Austria). As the relations found with multiple linear regression are described in multidimensional space, the significant relations between a subscale and the EC_{50} within the multiple linear model were presented as separate graphs, using a corrected measure (the modelled EC_{50} if all personality traits would have had a mean score). Post-hoc univariate linear regression analyses for each personality subscale were performed. To further explore the relationship between personality and sensitivity to THC, an exploratory analysis into the influence of the frequency of cannabis use was performed for significant relationships between personality and sensitivity to THC.

Results

Sample size

A total of 220 subjects were included in the selected studies. Subjects that did not complete the study day on which they only received THC were excluded. For the pharmacokinetic and pharmacodynamic models, a total of 184 (83.6%) subjects were used, of whom 127 (69.0%) subjects completed the personality questionnaire. The number of observations was 2497 for all pharmacokinetic parameters and 2776 for all pharmacodynamic parameters.

Pharmacokinetic model

For all studies, but three, the population pharmacokinetic model for THC (Heuberger and co-workers, unpublished results), correctly described the observations and was not adapted. In three studies (Klumpers et al., 2013b; Zuurman et al., 2008, 2010), the predicted pharmacokinetic profile (slightly) exceeded the observed drug concentrations. This was attributed to the fact

that these earlier studies did not use a nose clip during the inhalation of THC. Therefore, all population parameters (θ) for THC were fixed, with the exception of bioavailability for the studies that used inhalation without a nose clip. The individual parameters for inter-individual variation (η) showed a strong correlation between THC and 11-OH-THC and therefore one η was estimated for k_E (for both THC and 11-OH-THC) and one η was estimated for V_D. Table 2 provides an overview of the pharmacokinetic parameter estimates.

Pharmacodynamic models

Because the inter-individual variation within the pharmacokinetic model is the same for THC and 11-OH-THC, the predicted time profile of 11-OH-THC is fully dependent of the predicted time profile for THC. It is therefore impossible to mathematically distinguish the relative contribution of THC and its metabolite on the effect. For that reason, the effect model is only driven by the individual THC concentration-time profiles.

For the cluster perception, an effect compartment model best described the time-effect profile. All scales included in the perception cluster range from 'not at all' (0 mm) to 'extremely' (100 mm). However, estimating the parameter for maximum effect resulted in unrealistically high values for maximum effect, without improving the model fit. This was found to be related to the wide and skewed distribution of the scores on this cluster. Almost all measurements before drug administration scored 0 and some subjects reached scores close to 100. For this reason, the parameter for baseline effect (E_0) was fixed to 0 and the parameter for maximum effect (E_{max}) was fixed to 100. The parameters for EC_{50} and k_{e0} were estimated and allowed for intra-individual variation.

The effect on the cluster relaxation was best described using an effect compartment model with multiple transit compartments. This resulted in better individual predictions than a traditional effect compartment model. Because of these transit compartments, the estimate for EC_{50}, which refers to the concentration in the effect compartment, appears to be lower than

for the other two subjective effects. All parameters in the final model were estimated and all parameters except for the number of compartments included intra-individual variation.

As expected, many subjects did not show any response within the cluster dysphoria. For that reason, a PK-PD model with all subjects could not be developed and the final model was only based on responders. Response was conservatively defined as 'any subject who had at least one score other than 0 during the study day', resulting in 89 (48.4%) responders. An effect compartment model best described the effect on dysphoria in responders. The parameter for baseline effect was again fixed at 0 and all other parameters (E_{max}, EC_{50} and k_{e0}) were estimated and allowed for intra-individual variation.

The parameter estimates and their confidence intervals of the best models are included in Table 2. The predicted subjective effects can be related to the THC concentration in the effect compartment (C_E) using the formula: $E(t) = E_0 + E_{max} \cdot C_E / (EC_{50} + C_E)$. In Figure 1, the observations and the predicted time profile for the pharmacokinetics and subjective effects are presented for three typical individuals.

Distribution on personality subscales

The study population was found to have relatively high scores on novelty seeking (mean 24.6; SD 5.3; range 13-37), relatively low scores on harm avoidance (mean 8.5; SD 4.9; range 0-22) and relatively low scores on self-transcendence (mean 7.0; SD 5.1; range 0-29) compared to the normative group (means of 17.9, 15.2 and 12.3 respectively).

The relation between personality and sensitivity to THC

None of the multiple linear regression models showed a statistically significant relationship betwween the subjective effects and sensitivity to THC (perception: R^2 0.1526, p 0.1464; relaxation: R^2 0.0670, p 0.8799; dysphoria:

R^2 0.3217, *p* 0.3794). Within the multiple linear regression models, the cluster of perception was significantly associated with harm avoidance and dysphoria with self-transcendence. The relationships between these subjective effects and personality traits are presented graphically in Figure 2 and the coefficients of the full multivariate regression model are presented in Table 3. In post-hoc univariate linear regression models with linear and quadratic components, the only model that showed a significant relation was between perception and harm avoidance (R^2 0.0615, *p* 0.0195). No relation was found between the self-reported frequency of cannabis use and sensitivity to the subjective effects of THC.

For the cluster dysphoria, only responders were included in the final PK-PD model. Therefore, no individual PK-PD parameters can be extracted for the non-responders. Because the probability of being a responder can be related to personality factors as well, a Mann-Whitney U test was performed to compare each personality trait between responders and non-responders (see Table 4). Again, only the score on self-transcendence was found to be significantly different.

Discussion

For the current analysis, the response on three clusters of subjective effects of THC was described using mathematical PK-PD models. These models include a parameter to describe the sensitivity to drug effect (the EC_{50}). This individually estimated parameter was used to explore a relation between personality traits and the subjective effects of THC. The personality trait harm avoidance was found to be associated with changes in perception and the personality trait self-transcendence with dysphoria.

Several studies that used a standardized pharmacological challenge with THC were combined. Therefore, the number of evaluated subjects was very high (184 subjects) compared to other studies that have modeled the PK-PD relations of THC, which are often based on 8 to 12 subjects. Since all

studies used administration of THC via inhalation, the results cannot necessarily be applied to the subjective effects following oral or intravenous administration of THC, although the use of a PK-PD model makes it more likely that the results will also apply to other modes of administration. Of particular interest in this regard is the relative contribution of the active metabolite 11-OH-THC to the effect. In the current model, the effect of the metabolite could not be distinguished from the parent compound. This was also true for other PK-PD models described in the literature (Chiang and Barnett, 1984; Harder and Rietborck, 1997; Strougo et al., 2008). The model could be improved by incorporating data from other methods of administration (i.e. oral). Sole administration of the metabolite could provide valuable information on the relative contribution of 11-OH-THC to the effect of THC.

As an outcome measure for subjective effect, visual analogue scales (VAS) were used. This tool is easy to use and can be measured repeatedly throughout the study day without much burden on the subject (Bond and Lader, 1974). The use of an outcome measure that can be measured repeatedly throughout the study day will result in a more reliable estimation of the time-course of the effect (Samara and Granneman, 1997), which makes the VAS a useful tool to model the time-effect relationship. Once a PK-PD model has been established, individual parameters can be estimated even with limited (or sparse) sampling. The VAS is continuous in its design, and therefore allows for measurement of small changes (Bond and Lader, 1974). The three clusters of subjective effects that were used (perception, relaxation and dysphoria) have been shown to describe different aspects of the subjective effects of THC (Kleinloog et al., in press).

The selection of the final PK-PD models was based on a combination of the objective function, individual visual predictive checks and the confidence interval of the parameter estimates. This combination allowed for a more flexible approach in model selection (compared to objective function alone) and resulted in final models that were more suitable for the aim of the analysis. The PK-PD model for perception described the data well, given the large differences in response between subjects. These large differences

are reflected by the relatively large contribution of the parameter for intra-individual variability on sensitivity (EC_{50}). The development of the PK-PD model for dysphoria was burdened by many non-responders. Even within the subjects currently classified as responder, many subjects showed minimal response and even clear responders exhibited a large variation over time (for example the second subject in Figure 1). Altogether, this resulted in a systematic underprediction of the model, which could have been improved if an increased threshold for response was used. However, an increased threshold would be arbitrary and lead to a smaller subpopulation of responders and therefore would not serve the aim of the current analysis. The shape of the predicted time-effect profile seemed proportional to the observed response (see Figure 1). This might have affected the absolute value of the estimated EC_{50}, but proportional for all subjects. The relative distribution of the EC_{50} (which is used for the subsequent analysis) will not have been affected.

The study population, consisting of mild cannabis users, was found to have higher scores on novelty seeking and lower scores on harm avoidance and self-transcendence, compared to the normative group. These findings are not unexpected and consistent with previous research into the personality structure of volunteers in clinical studies (Pieters et al., 1992).

None of the multivariate linear regression models showed a significant overall relation between personality and sensitivity to THC. This shows that the relationship between personality and sensitivity to THC is very complex and non-linear. In a further exploration of the relationship between personality and sensitivity to THC, the clusters of perception and dysphoria were found to have a significant relation with one personality trait each (harm avoidance and self-transcendence respectively) within the multivariate linear regression models. It should be noted that some personality traits had a higher estimated effect size (e.g. novelty seeking in relation to perception, reward dependence in relation to relaxation or persistence in relation to dysphoria), but they did not reach statistical significance due to large variation.

The personality trait harm avoidance was significantly correlated to the subjective effect cluster perception. Harm avoidance describes the tendency to respond to aversive stimuli and to learn inhibiting behaviour to avoid punishment, novelty and non-reward (Cloninger et al., 1987). Individuals with a higher score on harm avoidance are more cautious, tense, apprehensive, fearful, inhibited, shy and fatigable, whereas individuals with a lower score are more confident, balanced, relaxed, optimistic, carefree, uninhibited, outgoing and energetic. It can be argued that subjects with high harm avoidance scores, who are cautious and inhibited, do not 'give in' to the subjective effects of THC and are therefore less sensitive to the changes in perception. On the other hand, people with lower harm avoidance scores, who are more balanced, easy-going and carefree, might apply different definitions as to what constitutes 'extreme' changes in perception, which would also affect their EC_{50}. According to the psychobiological model of Cloninger (1987, 1994), harm avoidance is mediated through serotonergic processes. Harm avoidance is a temperament, and therefore considered stable over time and related to biological structures (Cloninger et al., 1993). The biological structures that are hypothesized to be related to harm avoidance include serotonergic neurons that ascend from the raphe nuclei to the limbic system, including the septum, hippocampus and prefrontal cortex (Cloninger et al., 1987). Stimulation of the serotonin-mediated hypothalamus-pituitary-adrenal axis results in activation of the endocannabinoid system, and alternatively, stimulation of cannabinoid CB_1 receptors by endocannabinoids reduces the release of serotonin in the prefrontal cortex and hippocampus (Haj-Dahmane and Shen, 2011). These projections have been suggested to function as a check between predicted and actual events and to adapt behaviour when registered events do not match expected events. It can therefore be expected that this retrograde feedback is less efficient in subjects with a less sensitive cannabinoid system (i.e. higher EC_{50} values in the THC challenge). It is conceivable that a less appropriate endocannabinoid-induced feedback can be associated with extremes of harm avoidance. The cluster perception represents the most sensitive effects of

THC and includes changes in perception of time and space and an increase in perception effects corresponds to a mismatch in registered and actual events (Kleinloog et al., in press). A non-linear relation between harm avoidance and serotonin has been previously suggested (Peirson et al., 1999).

The cluster of dysphoria was associated with the self-transcendence trait. The relationship between dysphoria and self-transcendence as presented in Figure 2, is not as clear as the relationship between perception and harm avoidance. It was also not found to be significant in the univariate linear regression model. Subjects who reported dysphoria (responders) had higher scores on self-transcendence than subjects who did not show any response on dysphoria (non-responders). Within subjects who did show a response on dysphoria, the EC_{50} was negatively correlated with the self-transcendence score and therefore subjects with a higher self-transcendence-score were more sensitive to the dysphoric effects of THC. Based on the distribution presented in Figure 2, the linear relation appears more pronounced than a quadratic U-shaped relation. Self-transcendence describes the spontaneous feeling of participation in one's surroundings as a unitive whole (Cloninger et al., 1994). Individuals with high scores on self-transcendence are more creative and spiritual, more inclined to apply 'magical thinking' and (in Western society) perceived as 'naive'. Low scores on self-transcendence are associated with limited imagination, and a rational, down-to-earth perspective on life. The primarily negative correlation between self-transcendence and EC_{50} for dysphoria in our study suggests that subjects who are more 'grounded' are less likely to experience dysphoric effects of THC, which is consistent with findings by Barkus et al. (2006, 2008). Self-transcendence is a character trait, which means it is considered to be subject to development and maturation over time. It is likely influenced by previous experiences and learned adaptive mechanisms. Naive and psychologically vulnerable people are more likely to experience dysphoric reactions (Ashton, 2001), which is in agreement with the suggested relation between dysphoria and self-transcendence.

The psychobiological basis of the Cloninger model is largely based on theory. Not all studies investigating the underlying relations with neurotransmitter systems have shown consistent results (Paris, 2005). However, a series of neuroendocrine challenges (Gerra et al., 2000), as well as neuroimaging studies (Sugiura et al., 2000; Moresco et al., 2002; Gardini et al., 2009), have shown supportive evidence for the psychobiological basis of the Cloninger model. Following the psychobiological model, it was expected that the dopaminergic system would be more prominently involved in the psychomimetic effects of THC (in particular for perception and dysphoria), given the role of dopamine in the positive symptoms of psychosis (Seeman, 2002). Within the psychobiological model of Cloninger (1987, 1994), the novelty seeking trait is thought to be regulated by dopaminergic systems. Individuals with higher scores on novelty seeking are impulsive, exploratory, excitable, quick-tempered and extravagant. They readily engage in new interests and activities, but are quickly distracted or bored. Individuals with lower scores are not easily engaged in new interest and often preoccupied with details and requiring considerable thought before decision making. For both perception and dysphoria the estimated slope for novelty seeking was relatively large (in both cases positive), but did not reach statistical significance.

An explanation as to why these personality traits show a relation with the sensitivity to the subjective effects of THC might be difficult to provide. Using positron emission tomography (PET), Moresco et al. (2002) showed an inverse relation between harm avoidance and serotonergic activity in the frontal and left parietal cerebral cortex and Van Laere et al. (2009) showed an inverse correlation between cannabinoid CB_1 receptor density and the personality trait novelty seeking. This could mean that personality, or the characteristic manner in which a person responds to his or her environment, is (in part) regulated by the cannabinoid system. The cannabinoid CB_1 receptors are widely distributed across the central nervous system and provide an auto-regulation and fine-tuning function for different pharmacological systems through retrograde neurotransmission, mediating

short-term and long-term synaptic plasticity (Di Marzo, 2006; Kano et al., 2009; Haj-Dahmane and Shen, 2011; Pamplona and Takahashi, 2012). A higher sensitivity of this system may be associated with a larger capacity to maintain homeostasis during mental and physical perturbations. Psychologically, a more sensitive endocannabinoidsystem could be associated with a more stable personality. This could explain why some of the more extreme temperament and character traits seem to be associated with relatively low cannabinoid sensitivities.

In conclusion, a correlation was found between personality traits and the sensitivity to subjective effects of THC. Changes in perception (including the typical 'high' feeling) showed a significant, albeit non-linear, correlation with the serotonin-mediated trait of harm avoidance. Subjects with either very low (easy-going) or very high (cautious) scores were less sensitive to THC-induced changes in perception. This relationship can be explained by the interaction between the cannabinoid and serotonergic system, which mainly seems to be auto-regulatory. The personality trait of self-transcendence, describing schizotypy, was associated with the occurrence and intensity of dysphoric reactions. Subjects with higher levels of schizotypy were generally more sensitive to the dysphoric subjective effects of THC.

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TABLE 1 Overview of included studies.

Reference	Dose	Interval	Clip	N	LLOQ
Kleinloog et al. (2012)	2 + 4 + 6 mg	90 min	yes	49	1.0 ng/mL
Klumpers et al. (2012)	2 + 6 + 6 mg	90 min	yes	22	1.0 ng/mL
Klumpers et al. (2013a)	2 + 4 + 6 + 6 mg	60 min	no	30	0.5 ng/mL
Klumpers et al. (2013b)	5 x 4 mg	150 min	yes	34	1.0 ng/mL
Liem-Moolenaar et al. (2010)	2 + 4 + 6 mg	90 min	yes	37	1.0 ng/mL
Zuurman et al. (2008)	2 + 4 + 6 + 8 mg	90 min	no	12	1.0 ng/mL
Zuurman et al. (2010)	2 + 4 + 6 + 6 mg	60 min	no	36	1.0 ng/mL

TABLE 2 Population PK-PD parameters

	Parameter	Unit	Value	95% CI	Variability	S.E.
THC	v_D	L	6.17 †	5.30 - 7.18 †	0.1870	0.0252
	κ_e	h-1	6.30 †	5.78 - 6.85 †	0.0939	0.0155
	k23	h-1	1.30 †	1.06 - 1.59 †	-	-
	k32	h-1	0.04 †	0.03 - 0.05 †	-	-
	k24	h-1	4.10 †	3.76 - 4.46 †	-	-
	k42	h-1	1.04 †	0.87 - 1.24 †	-	-
	Finhalation with noseclip	%	28.4 †	24.2 - 32.5 †	-	-
	Finhalation without noseclip	%	19.2	16.9 - 21.6	-	-
	Proportional error	-	-	-	0.0959	0.0051
	Additional error	-	-	-	0.0480	0.0153
11-OH-THC	v_D	L	30.6	8.8 - 104.8	0.1870	0.0252
	κ_E	h-1	5.93	1.70 - 20.72	0.0939	0.0155
	k56	h-1	1.99	0.65 - 6.11	-	-
	k65	h-1	0.17	0.13 - 0.21	-	-
	k57	h-1	33.4	7.0 - 160.3	-	-
	k75	h-1	7.85	6.13 - 9.99	-	-
	Proportional error	-	-	-	0.1240	0.0097

† = described by Heuberger et al. (in preparation), variability presented as ω^2 for intra-individual variability of parameters and as σ^2 for residual error

Perception	E_0	mm	0	-	-	-
	E_{max}	mm	100	-	-	-
	EC_{50}	ng/mL	678	521 - 880	2.38	0.23
	K_{e0}	h-1	0.95	0.76 - 1.19	0.49	0.14
	Additional error	-	-	-	82.4	10.4
Relaxation	E_0	mm	47.4	45.0 - 49.8	0.09	0.03
	E_{max}	mm	23.1	13.6 - 32.7	0.58	0.26
	EC_{50}	ng/mL	39.3	16.2 - 95.5	3.97	1.96
	Transit compartments	n	7.5	1.2 - 13.9	-	-
	ktransit	h-1	2.2	0.5 - 3.9	0.09	0.02
	K_{e0}	h-1	1.6	0.9 - 2.2	0.59	0.14
	Additional error	-	-	-	28.3	3.6
Dysphoria	E_0	mm	0	-	-	-
	E_{max}	mm	4.53	2.05 - 10.02	1.40	0.584
	EC_{50}	ng/mL	276	101 - 752	0.198	0.079
	K_{e0}	h-1	1.24	0.92 - 1.68	65.1	98.6
	Proportional error	-	-	-	2.21	0.503

† = described by Heuberger et al. (in preparation), variability presented as ω^2 for intra-individual variability of parameters and as σ^2 for residual error

TABLE 3 Coefficients (intercept and slopes) from multiple linear regression.

	Perception		Relaxation		Dysphoria	
	Estimate	p	Estimate	p	Estimate	p
Intercept	$3.0 \cdot 10^{2}$	0.9442	$-3.1 \cdot 10^{2}$	0.2189	$-1.9 \cdot 10^{5}$	0.1160
Novelty Seeking	$3.0 \cdot 10^{2}$	0.0804	$8.8 \cdot 10^{0}$	0.3737	$8.9 \cdot 10^{2}$	0.1995
(squared)	$-5.4 \cdot 10^{0}$	0.1163	$-1.7 \cdot 10^{-1}$	0.3835	$-1.7 \cdot 10^{1}$	0.2035
Harm Avoidance	$-1.8 \cdot 10^{2}$	0.0423	$1.2 \cdot 100$	0.8083	$2.6 \cdot 10^{2}$	0.2462
(squared)	$9.2 \cdot 10^{0}$	0.0347	$-3.5 \cdot 10^{-2}$	0.8890	$-8.7 \cdot 10^{0}$	0.4179
Reward Dependence	$-7.1 \cdot 10^{1}$	0.7668	$1.8 \cdot 10^{1}$	0.2069	$6.1 \cdot 10^{2}$	0.3598
(squared)	$-1.2 \cdot 10^{-1}$	0.9885	$-6.7 \cdot 10^{-1}$	0.1538	$-2.0 \cdot 10^{1}$	0.3819
Persistence	$-4.4 \cdot 10^{2}$	0.0894	$9.7 \cdot 10^{0}$	0.5155	$1.1 \cdot 10^{3}$	0.1663
(squared)	$5.1 \cdot 10^{1}$	0.0684	$-7.1 \cdot 10^{-1}$	0.6594	$-1.1 \cdot 10^{2}$	0.1832
Self-Directedness	$-7.9 \cdot 10^{1}$	0.6317	$-1.9 \cdot 10^{0}$	0.8406	$3.6 \cdot 10^{2}$	0.3756
(squared)	$1.7 \cdot 10^{0}$	0.5332	$5.4 \cdot 10^{-2}$	0.7274	$-6.9 \cdot 10^{0}$	0.2945
Cooperativeness	$1.9 \cdot 10^{1}$	0.9168	$8.4 \cdot 10^{0}$	0.4254	$-2.3 \cdot 10^{2}$	0.6143
(squared)	$-5.4 \cdot 10^{-1}$	0.9859	$-1.3 \cdot 10^{-1}$	0.4628	$5.9 \cdot 10^{0}$	0.4438
Self-Transcendence	$-1.8 \cdot 10^{1}$	0.3795	$2.9 \cdot 10^{-1}$	0.9393	$-4.7 \cdot 10^{2}$	0.0168
(squared)	$2.6 \cdot 10^{0}$	0.3799	$-2.7 \cdot 10^{-2}$	0.8770	$1.6 \cdot 10^{1}$	0.0237

TABLE 4 Estimates of Mann-Whitney U Test comparing responders and non-responders for 'dysphoria'.

Scale	Estimate	95% CI	Statistic	p value
Novelty Seeking	1	-1 - 3	2086	0.3464
Harm Avoidance	1	-1 - 3	2092	0.3295
Reward Dependence	1	0 - 2	2181	0.1551
Persistence	0	-1 - 1	1855	0.8363
Self-Directedness	-1	-3 - 1	1781	0.5682
Cooperativeness	0	-2 - 2	1902	0.9781
Self-Transcendence	2	0 - 4	2305	0.0415

FIGURE 1 Observed (dots) and simulated (line) time profiles for three typical subjects.

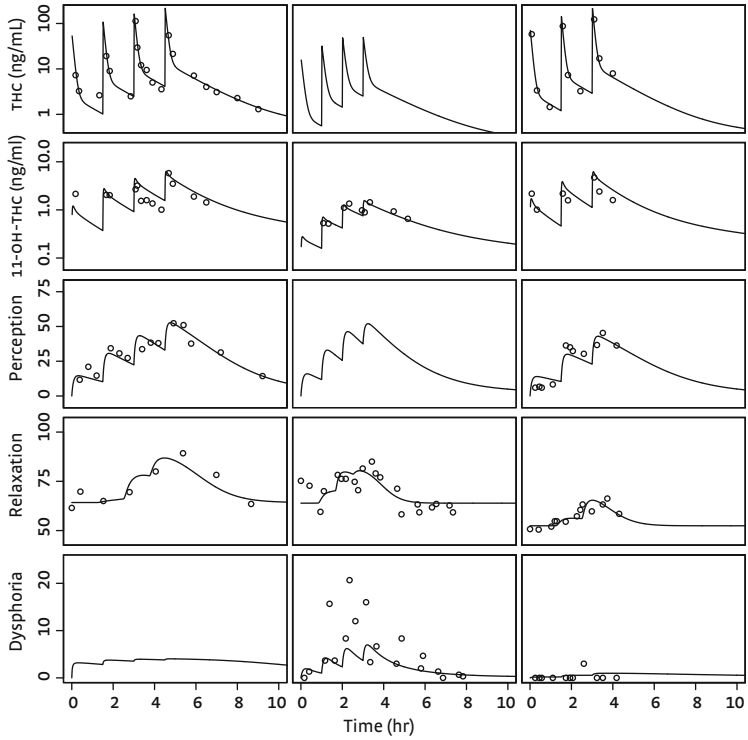
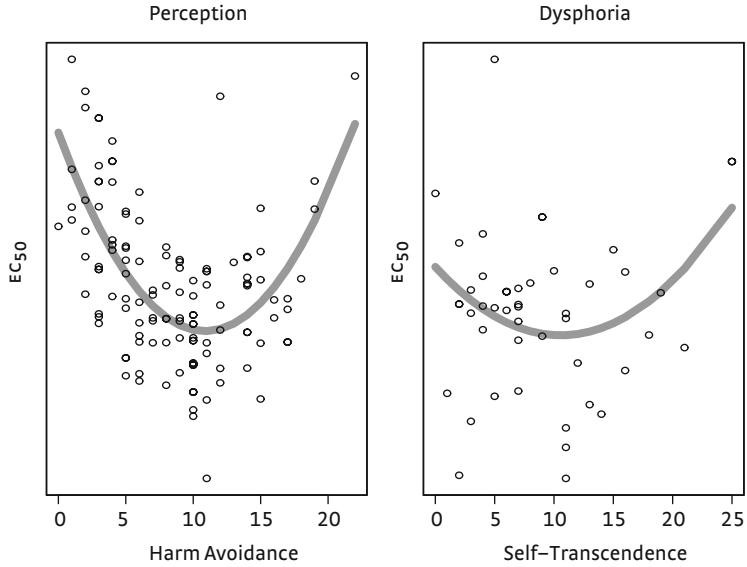


FIGURE 2 Relation between corrected EC_{50} and personality traits.



CHAPTER 5

Optimizing the glutamatergic challenge model for psychosis: using S(+)-ketamine to induce psychomimetic symptoms in healthy volunteers

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ABSTRACT

The psychomimetic effects that occur after acute administration of ketamine can constitute a model of psychosis and antipsychotic drug action. However, the optimal dose / concentration has not been established and there is a large variety in outcome measures. In this study, a total of 36 healthy volunteers (21 males and 15 females) received infusions of S(+)-ketamine or placebo to achieve pseudo-steady state concentrations of 180 and 360 ng/mL during two hours. The target of 360 ng/mL induced more intense effects than expected, after which the targets were reduced to 120 and 240 ng/mL, which were considered tolerable. There was a clear, concentration-dependent psychomimetic effect as shown on all subscales of the positive and negative syndrome scale (PANSS) and different visual analogue scales (VAS). The startle reflex was inhibited (prepulse inhibition, PPI) by both main target concentrations to a similar extent, suggesting a maximum effect. More general measures of drug effect (including eye movements and prolactin concentrations) were also affected by ketamine in a concentration-related manner. Ketamine was found to constitute a robust model for induction of psychomimetic symptoms and the optimal concentration range for a drug interaction study would be between 100 and 200 ng/mL.

Introduction

All currently available pharmacologic treatments for schizophrenia and psychosis are related to the dopamine hypothesis of schizophrenia (Kapur and Mamo, 2003). Many agents also target other receptors, such as the serotonergic 5-HT_{2A} receptor, but contrary to these targets the occupancy of the dopamine D_2 receptor is comparable for all drugs at clinically relevant doses (Farde et al, 1988; de Visser et al., 2001; Seeman, 2002; Agid et al., 2007). However, the dopamine hypothesis alone does not seem sufficient to explain all signs and symptoms of schizophrenia, and other neurotransmitter systems, including the glutamatergic system, have been implicated in the pathophysiology of schizophrenia and psychosis (Ackenheil and Weber, 2002; Sodhi et al., 2008; Moghaddam and Javitt, 2012).

There are currently several drugs in development with non-dopaminergic mechanisms of actions (Miyamoto et al., 2012). These include drugs with glutamatergic (e.g. glycine transport inhibitors; Umbricht et al., 2010; and $mGlu_{2,3}$ receptor agonists; Patil et al., 2007), serotonergic (e.g. 5-HT_6 antagonists; Liem-Moolenaar et al., 2011), nicotinergic (e.g. α_7 nicotinic receptor agonists; Freedman et al., 2008), tachykinin (e.g. NK_3 antagonists; Meltzer et al., 2004; Liem-Moolenaar et al., 2010a) and cannabinoid targets (e.g. CB_1 antagonists; Kelly et al., 2011). Where the currently available drugs mainly affect the positive symptoms of schizophrenia (such as hallucinations and delusions), these novel pharmacotherapeutic strategies aim to improve other aspects (such as cognition and negative symptoms) as well (Carpenter and Koenig, 2008).

The pharmaceutical industry has found it very difficult to test drugs for psychiatric diseases, resulting in a recent drop in compounds that are being developed. Some companies have announced that they would cease research in psychiatry completely. This trend is referred to as "the shrinking pipeline". The reasons for this drop in development can be found in the lack of understanding of pathophysiology, the difficulty to predict clinical outcome, the lack of experimental disease models and biomarkers and the

increasing costs of drug development (van Gerven and Cohen, 2011; Nutt and Goodwin, 2011). Drug development in psychiatry requires a tailor-made approach, which includes testing for efficacy early in clinical development (Macaluso et al., 2011). Improving the predictability of models early in drug development would improve the risk / benefit ratio for drug development. This tailor-made approach would include the development of specific biomarkers and pharmacologic challenges.

Most models that are currently in use are more predictive of dopamine antagonism, rather than reduction of psychotic symptoms per se (Carpenter and Koenig, 2008). The United States National Institute of Mental Health (NIMH) suggests that psychiatric drug development should be based on an understanding of pathophysiology, rather than mimicking the mechanism of action of current drugs (Brady and Insel, 2012). As an alternative to preclinical models, several models have been developed that involve the induction of psychomimetic symptoms in healthy volunteers. These models have used administrations of THC or cannabis (e.g. D'Souza et al., 2004; Liem-Moolenaar et al., 2010b; Kleinloog et al., 2012), LSD or psilocybin (e.g. Vollenweider et al., 1998; Carhart-Harris et al., 2012), ecstacy or amphetamines (e.g. Strakowski et al., 1996) and ketamine (e.g. Krystal et al., 1994; Malhotra et al., 1996; Vollenweider et al., 2000; Duncan et al., 2001; Abel et al., 2003; Gouzoulis-Mayfrank et al., 2005; Covington et al., 2007; Stone et al., 2008) to induce psychomimetic effects. When used in early phase clinical trials (phase I-II), these models have great potential in predicting clinical efficacy of novel drugs.

However, the currently available literature that focuses on the use of ketamine as a model for psychosis and antipsychotic drug action is limited by several factors. First of all, there is no consensus regarding the optimal dose or concentration for induction of psychomimetic symptoms. Different studies use different dosing schemes and most studies do not use different dose levels to detect a dose-effect relation. Concentrations of ketamine in the blood are useful to explain variation within and between subjects and between studies, but are generally not reported. Whether racemic ketamine

or the S(+)-isomer is used is not always clearly described, while S(+)-ketamine has been shown to be up to four times as potent as R(-)-ketamine (Øye et al., 1992). There is also much variety in the outcome measures that are used to quantify psychomimetic effects.

Several studies have examined the effect of the known antipsychotics haloperidol and clozapine on ketamine-induced psychomimetic symptoms in either healthy volunteers (Malhotra et al., 1997b; Krystal et al., 1998 and 1999; Oranje et al., 2000 and 2002; Lahti et al., 2003) or patients with schizophrenia (Lahti et al., 1995 and 2003; Malhotra et al., 1997a and 1997b). In some of these studies, a mild effect of treatment was seen, although this effect in general is inconclusive, possibly due to the previously described limitations of the application of the ketamine model. Prior to performing an interaction study using the ketamine model of psychosis and a known antipsychotic, we wanted to optimize the model. Therefore, this study aimed to explore the relation between the concentration of ketamine and the psychomimetic, general and adverse effects of ketamine. This would allow the selection of a target concentration that induces psychomimetic effects at a sufficient level to allow for a reduction in effect by an antipsychotic drug, whilst still being tolerable for the subjects. This information could be used for decisions on study designs and ketamine infusion rates. Furthermore, different outcome measures for psychomimetic effects were compared on sensitivity and variability.

Methods

Participants

Healthy subjects aged between 18 and 45 years (inclusive) and with a body mass index between 18 and 30 kg/m^2 (inclusive) were recruited by the Centre for Human Drug Research. Recreational experience with ketamine was not required, but subjects had to be mild cannabis users to ensure they

were familiar with induced psychomimetic symptoms. Mild cannabis use was defined as use of cannabis at least four times in the last year and no more than once a week on average. After providing written informed consent, subjects received a medical screening within 3 weeks prior to study participation. Clinically relevant abnormalities, in particular a personal or family history of clinically relevant psychiatric illness and/or abnormalities on psychiatric examination were considered reason for exclusion. The use of medication and agents that were expected to affect central nervous system performance or the pharmacokinetics of the study medication was not allowed during the study period. Female subjects were tested for pregnancy (in urine) and all subjects were tested for the use of recreational drugs (in urine) and alcohol (in breath). All subjects were required to have negative drug screens on all occasions. Following the medical screening, subjects were trained for the study procedures.

Study design

This was a randomized, double-blind, placebo-controlled, three-way cross-over trial with a washout period of minimally two days. The study was performed in accordance with Good Clinical Practice and the Dutch Medical Research Involving Human Subjects Act and was approved by the Independent Ethics Committee of the Leiden University Medical Centre.

Interventions

To achieve a pseudo-steady state in effect, S(+)-ketamine (Ketanest®, Pfizer) or matching placebo (0.9% sodium chloride) was administered using individualized two-hour intravenous infusion regimens to achieve pseudo-steady plasma concentrations. Target concentrations were selected based on the dosing schedules of previous studies using ketamine as a model for psychosis (Vollenweider et al., 2000; Duncan et al., 2001; Abel et al., 2003;

Gouzoulis-Mayfrank et al., 2005; Covington et al., 2007; Stone et al., 2008). As these studies did not describe the pharmacokinetic profile of S(+)-ketamine or the active metabolite S(+)-norketamine, plasma concentrations were simulated to select target concentrations using the deSolve package for R (www.r-project.org), as shown in Figure 1. This simulation was based on the pharmacokinetic model described by Sigtermans et al. (2009). Two target concentrations were selected, based on the simulated pharmacokinetic profiles, to examine a concentration-effect relation. Subsequently, individualized infusion regimens were calculated based on the pharmacokinetic model described by Sigtermans et al. (2009), adapted to sex and weight (which were the covariates in the model). In order to create optimal blinding circumstances, the S(+)-ketamine concentration within the syringes prepared by the pharmacy was different for each study day (2.50 mg/mL, 1.25 mg/mL or placebo), which allowed for the use of the same infusion rates during each study day and thus aided study blinding.

Outcome measures

The effect of S(+)-ketamine was measured using the positive and negative syndrome scale (PANSS), prepulse inhibition of the startle reflex (PPI) and an extensive test battery for CNS effects (NeuroCart) consisting of several visual analogue scales (VAS), saccadic and smooth pursuit eye movements, pupillometry, adaptive tracking and body sway. All tests were measured repeatedly throughout each study day, including two baseline measurements before the start of infusion, two measurements during infusion and one measurement several hours after infusion. For the PANSS, only one baseline measurement was performed and for the PPI an additional measurement was performed 45 minutes after the stop of infusion. In addition, blood samples for cortisol and prolactin concentrations in serum and concentrations of S(+)-ketamine and the active metabolite S(+)-norketamine in plasma were taken repeatedly throughout the study day.

Positive and negative syndrome scale

The primary method to measure psychomimetic effects was the PANSS, described by Kay et al. (1987). The PANSS is a clinically validated scale to longitudinally measure changes in psychotic symptoms and is based on a structured clinical interview. To adjust for the repetition of interviews, time frames for symptoms evaluation were limited to 'since this morning' for the baseline assessment or 'since the last interview' for all other assessments. All interviews were recorded on video and rated independently by a second blinded person. For the analysis, the geometric mean of the two scores was used. All team members performing the interviews and rating the videos were certified PANSS raters™ (by the PANSS Institute LLC, New York). The PANSS consists of 30 items that are scored on a seven-point scale. The PANSS is subdivided into three subscales: positive, negative and general. The positive subscale, which consists of 7 items resulting in a total score ranging from 7 to 49, was predefined as the main evaluation endpoint.

Prepulse inhibition

As a more objective measure of psychomimetic symptoms, PPI was used as described by Braff et al. (2001). The startle reflex consists of a contraction of the skeletal and facial muscles in response to a sudden, relatively intense stimulus. Subjects were exposed to a background noise level of 70 dB starting 600 ms before the first trial and lasting until 400 ms after the last trial, presented binaurally through stereo headphones. Each trial consisted of either a pulse alone or a prepulse followed by a pulse. The startle-eliciting stimulus (pulse) was 115 dB during 40 ms. If used, the prepulse was 85 dB during 20 ms, with a prepulse-to-pulse interval of 120 ms or 240 ms. Each measurement consisted of 32 trials, presented in a random order and separated by random inter-trial intervals of 10-20 s (average 15 s). Randomisation was subdivided in two blocks of 16 trials to allow for an analysis of the sensitivity of test blocks of different length. The eyeblink component of the startle was

measured using electromyography (EMG) of the orbicularis oculi muscle. PPI was calculated as the percentage of inhibition of the area under the curve (AUC) of the startle response following prepulse plus pulse, compared to the AUC of the startle response of pulse alone. PPI is a ubiquitous and robust experimental phenomenon that is present even at the first exposure to the stimulus and is not affected by habituation or extinction following multiple trials (Braff et al., 2001).

Visual analogue scales

VAS are widely used tools to quantify subjective effects. VAS are 100 mm long lines with two extreme subjective states on each end (e.g. 'drowsy' and 'alert' or 'not at all' and 'extremely'). Subjects indicate their current feelings on these lines, resulting in a score between 0 and 100 mm. The current study included the composite scales for mood, calmness and alertness (described by Bond and Lader, 1974), the composite scales for psychedelic effects (described by Bowdle et al., 1998), three scales for general drug effect ('feel drug', 'like drug' and 'dislike drug' with 'not at all' to 'extremely' as extremes, referred to as VAS drug rating) and a newly developed set of scales for psychedelic effects (the development and evaluation of this new VAS will be described elsewhere).

Saccadic and smooth pursuit eye movements

Both saccadic and smooth pursuit eye movements were recorded through three electrodes placed on the forehead and next to both lateral canthi. The stimulus for saccadic eye movements had a fixed amplitude of approximately 15 degrees to either side, with interstimulus intervals varying randomly between 3 and 6 seconds. Smooth pursuit eye movements were stimulated in a sinusoidal manner at frequencies ranging from 0.3 to 1.1 Hz with amplitude of 22.5 degrees to either side. Eye movements are described in greater detail by Zuurman et al. (2008). Saccadic peak velocity is one of the most

sensitive parameters for sedation by a wide range of different causes (van Steveninck et al., 1991). The percentage time in which the eye movements are in smooth pursuit of the target is a parameter for motor coordination.

Pupillometry

The ratio between the diameter of the pupil and the iris is determined by the autonomous nervous system activity. Diameters were determined using digital photography with flash after adaptation in ambient lighting (Twa et al., 2004).

Adaptive tracking

For the adaptive tracking test, subjects had to keep a dot inside a moving circle using a joystick. If the subject was successful, the speed of the moving circle increased and if a subject was unsuccessful, the speed decreased. Adaptive tracking was performed using customised equipment and software (as described by Borland and Nicholson, 1984) and was measured during three minutes (after a run-in period of 30 seconds). Subjects received three training sessions to minimize learning effects. Adaptive tracking is a pursuit task that is very sensitive to drug effects.

Body sway

The body sway meter records body movements in a single (sagittal) plane during two minutes, while the subjects close their eyes, providing a measure of postural stability, which can be used as a biomarker for drug effect (Liem-Moolenaar et al., 2010a).

Hormones

Serum prolactin and cortisol concentrations were measured using electrochemiluminescence immunoassay (ECLIA) as a biomarker for dopaminergic activity.

Pharmacokinetics

Concentrations of S(+)-ketamine and its main active metabolite S(+)-norketamine were determined in plasma, using high performance liquid chromatography with ultraviolet detection (HPLC-UV), with a lower limit of quantification of 10 ng/mL.

Safety

In addition to the pharmacokinetic and pharmacodynamic measurements, several tests were performed for safety reasons. Subjects were tested for drugs (urine), pregnancy (urine, females only) and alcohol (breath) at the start of each study day. Electrocardiograms (ECGs) and vital signs (blood pressure, heart rate and oxygen saturation) were repeatedly recorded throughout the study day. All subjects were frequently asked if they had any symptoms and all symptoms were recorded, whether assumed to be related to the study drug or not.

All subjects had breakfast two hours prior to the start of infusion and lunch half an hour after the infusion. Subjects remained in the research unit until approximately four and a half hours after the stop of infusion. Prior to infusion, subjects were informed that they could ask for the premature termination of the infusion at any time if they had negative experiences. During the study period (from two weeks prior to the first dose), subjects were not allowed to use recreational drugs or any medication that has a potential influence on the pharmacokinetics of ketamine. From 24 hours before the study day until the end of the study day, subjects were instructed not to smoke, exert heavily physical exercise or use alcohol, xanthine or grapefruit juice.

Sample size

As this was an exploratory study, no formal power calculation has been performed. For equal randomisation, the number of subjects had to be a

multiple of six and males and females were distributed in an even fashion. A total of 12 male and 12 female subjects were required to complete the study.

Statistical analyses

All pharmacodynamic outcome measures were analysed within SAS (version 9.1.3) using a mixed model analysis of covariance (ANCOVA) with treatment, study period, time, gender, treatment by time, treatment by gender, gender by time and treatment by gender by time as fixed factors and subject, subject by treatment and subject by time as random factors and the average baseline value as covariate. The body sway was analysed without taking gender into account and without treatment by time as random factor, because of many missing observations (the body sway was performed at the end of each testing round and was cancelled if there was a delay more than 10%). Results of the body sway, PANSS scores, cortisol and prolactin concentrations were log-transformed prior to analysis, based on expected log-normal distribution of the data. VAS Bowdle items 8 (voices), 9 (meaning) and 10 (suspicious) did not show any variation under any condition and could not be analysed. VAS Bowdle items 1 (body), 2 (surroundings), 6 (colours), 7 (sound), 12 (drowsy) and 13 (anxious) were log-transformed after the addition of 2 points (to prevent log-transformation from 0). VAS Bowdle items 3 (time), 4 (reality), 5 (thoughts) and 11 (high) did not need transformation. As all items from both VAS Bowdle and VAS drug rating did not show any variation under placebo, the placebo condition was not included in the analysis of these scales.

Results are presented as least square means (LSM), which are calculated within the ANCOVA and result from the minimisation of the sum of squared residuals. LSM are based on the data and estimations for missing observations and are less sensitive for an unbalanced design than geometric means. Therefore, LSM are more likely to represent the estimated population means. Data that were log-transformed prior to analysis are presented after back-transformation. Differences are to be interpreted as percentage

change. As two points were added to the VAS Bowdle scores prior to transformation, the percentage difference refers to score (a+2) and score (b+2), rather than (a) and (b).

To test the suitability of the pharmacokinetic model (by Sigtermans et al., 2009) simulations were performed using the deSolve package within R (version 3.0.2). The simulations used the population parameter estimates (theta's), parameters for inter-subject variability (eta's), parameters for intra-subject variability (sigma's) and the uncertainty of the population parameter estimates (covariance matrix). For each individual subject (using body weight, sex and the individualized infusion regimen), a simulation was performed with 1000 permutations, resulting in a mean predicted pharmacokinetic profile and a 95% prediction interval. The actual measurements were presented using standardised scores (actual measurement - mean predicted / standard deviation of prediction) over time. Approximately 95% of the actual measurements are expected to have a standardised score between -2 and 2.

Results

Selection of target concentration

Based on the simulated pharmacokinetic profiles of the available literature (presented in Figure 1), target concentrations of 180 ng/mL and 360 ng/mL were initially selected for the study. These concentrations were chosen to represent a wide range of subanaesthetic concentrations in which psychomimetic effects were expected while subjects would still remain responsive. To achieve the pseudo-steady state, the infusion regimen was determined as a bolus of 32 µL/kg (target concentration x volume of central compartment / concentration in syringe), followed by subsequent infusion rates of 0.56 mL/kg/hr for 7 minutes, 0.36 mL/kg/hr for 23 minutes and 0.24 mL/kg/hr for another 90 minutes. As the clearance within the model by Sigtermans et

al. (2009) was higher for females and the relative contribution of clearance increases over time, the three infusion rates were increased for females by 5%, 10% and 15%, respectively. This infusion regimen was predicted to yield the same pharmacokinetic profile for a wide range of body weights in both males and females. The mean simulated concentration of S(+)-ketamine stayed within 10% of the target concentration throughout the infusion. As the size of effect of the active metabolite S(+)-norketamine on psychomimetic symptoms is unknown, this effect was not incorporated in the determination of the infusion regimen.

Adverse events

Within the first six subjects (all male) who were dosed, three adverse events were seen that were more severe than expected. One subject had a presyncope 2 minutes after the stop of infusion for the 180 ng/mL target concentration. The same subject had altered consciousness after 29 minutes of infusion for the 360 ng/mL target, for which the infusion was stopped immediately (symptoms subsequently improved rapidly and had disappeared after 22 minutes). Another subject experienced anxiety and an altered mood with the 360 ng/mL target after 70 minutes of infusion (the infusion was stopped 10 minutes later, after which symptoms improved rapidly and disappeared completely within 20 minutes after the stop of infusion). Because of these unexpected intense effects, the study was temporarily halted. Other adverse events that occurred were expected and included feeling high, changes in perception, nausea, hypoaesthesia and somnolence, all of mild or moderate intensity. One subject vomited during the 180 ng/mL target and another subject vomited during the 360 ng/mL target. The intensity of adverse effects appeared to increase during the infusion and improved rapidly after the stop of infusion. Due to the temporary interruption of the study, only two of these initial six subjects (33%) completed all three study days according to the protocol, one subject (17%) completed two study days (ketamine 180 and 360 ng/mL) and three subjects (50%) completed one study

day (two subjects received placebo, one ketamine 360 ng/mL). The available data of these subjects is presented graphically, but was not taken along in the statistical analysis due to the limited number and truncation of the observations. The average response is indicated separately in the figures.

Adjustment of target concentration

Based on the experience with the first subjects, the rationale for selection of target concentrations was revisited. As we aimed to establish a concentration that showed maximum psychomimetic effects, whilst being easily tolerated by the subjects, the target concentrations were reduced to 120 ng/mL and 240 ng/mL respectively. The intensity of effects slowly increased during the infusion, which was hypothesized to be related to the formation of the active metabolite S(+)-norketamine. Although the size of the effect in humans is unknown, animal studies suggest that norketamine is roughly one third as potent for CNS effects as ketamine (White et al. 1975). Within the adjusted infusion regimen, the effect of S(+)-norketamine was incorporated by targeting a stable level of [S(+)-ketamine concentration + 1/3 S(+)-norketamine concentration]. The infusion regimen was set to a bolus of 0.21 µL/kg and infusion rates of 0.34 mL/kg/hr for 15 minutes, 0.22 mL/kg/hr for 25 minutes and 0.12 mL/kg/hr for the last 80 minutes. Infusion rates were again increased for females with 5%, 10% and 15% respectively.

Adverse events with adjusted target concentrations

All adverse events that occurred after the adjustment of target concentrations were expected and of mild or moderate intensity. However, the infusion was stopped prematurely due to adverse events (either at the subject's wish or judgement by the investigator) in 15 subjects (56%) on the 240 ng/mL target (after 8 to 105 minutes), 1 subject (4%) on the 120 ng/mL target (after 25 minutes) and 1 subject (3%) on placebo (after 96 minutes). The most important reason for stopping the infusion prematurely on the 240 ng/

mL target was vomiting (7/15, 47%). Other adverse events included feeling high, changes in perception, derealisation, nausea, bradyphrenia, dizziness, hypoaesthesia and somnolence. Of the thirty subjects who were enrolled after the adjustment in target concentrations, 24 subjects (80%) completed all three study days, 2 subjects (7%) completed two study days (both placebo and the 240 ng/mL target; both stopped due to adverse events) and 4 subjects (13%) completed only one study day (3 on placebo and 1 on ketamine 240 ng/mL; 2 stopped due to adverse events and 2 for personal reasons).

Demographics

A total of 21 male and 15 female subjects were included in this study (15 males and 15 females for the target concentrations of 120 and 240 ng/mL). The median age was 23 years (range 18-42) and mean body weight 71.6 kg (range 47.6-95.5). 32 subjects (88.9%) were Caucasian. The median frequency of cannabis use was 10 times / year (range 4-52) and 23 subjects (63.9%) were non-smokers. Of subjects who smoked, the median frequency was 3 cigarettes / day (range 1-5).

Pharmacokinetics

Figure 2 provides a standardised representation of the relation between the simulated concentrations and the measured concentrations over time for ketamine and norketamine. The model is able to describe the measured concentrations adequately. All measured concentrations are between the mean predicted concentration (standard score of 0) and one third the standard deviation below the mean predicted concentration (standard score of -0.33). However, the measured concentrations of ketamine were lower than expected and the standard deviation of the predicted values is much larger than the standard deviation of the measured values, suggesting that the model is not able to accurately predict ketamine concentrations.

Pharmacodynamic effects

The results of the pharmacodynamic endpoints are described below and summarised in tables 1 and 2.

Positive and negative syndrome scale

All subscales of the PANSS showed a robust, dose-dependent increase, as presented in Figure 3. The positive PANSS, which was predefined as the main endpoint, was increased by 43.7% (95%CI 34.4-53.7, $p < 0.0001$) for the 120 ng/mL target compared to placebo and 70.5% (95%CI 59.0-82.8, $p < 0.0001$) for the 240 ng/mL target compared to placebo. These increases represent an average absolute increase on the positive PANSS of 3.1 and 5.1 points, respectively.

Prepulse inhibition

For the 240 ms prepulse-to-pulse interval, a clear inhibition of the startle reflex was seen for both target concentrations (an absolute increase in the full version of 28.5% and 23.9% compared to placebo for 120 and 240 ng/mL respectively). There was no difference between target concentrations, which might be because of a maximum effect or differences in baseline values. However, the effect with the higher concentration seems to persist longer after the stop of infusion. The 120 ms prepulse-to-pulse interval did not result in the same changes in PPI. When only the first 16 blocks (short version) of each measurement were analysed, the differences are comparable to the analysis with all 32 blocks (full version). Therefore, a shorter version of the PPI with a prepulse-to-pulse interval of 240 ms is regarded sufficient for future studies. The results of the short version with a prepulse-to-pulse interval of 240 ms are presented in Figure 4.

Visual analogue scale as described by Bowdle

Items 8 (voices), 9 (meaning) and 10 (suspicious) of VAS Bowdle did not show any variation during measurements and could therefore not be analysed. Items 1 (body), 2 (surroundings), 6 (colours), 7 (sound), 12 (drowsy) and 13 (anxious) were not normally distributed and were log-transformed after addition of 2 points (as 0 cannot be log-transformed). The outcome of the ANCOVA can be interpreted as percentage difference between the original scores (after addition of 2 points). The remaining items 3 (time), 4 (reality), 5 (thoughts) and 11 (high) met the criteria for ANCOVA and were not transformed prior to analysis. Differences for these contrasts are presented as absolute differences. Except for the non-responsive items 8, 9 and 10, all items showed a clear response and this response was stronger for the 240 ng/mL target than the 120 ng/mL target. Figure 4 presents the time profile of item 11 (feeling high).

Zuurman et al. (2008) describe three clusters of VAS Bowdle, based on the response to THC: internal perception (items 4, 8, 9, 10 and 13), external perception (items 1, 2, 3, 5, 6 and 7) and feeling high (item 11). To allow comparison with these and other studies, the results on the clusters internal and external perception are also included in Table 2. As these clusters are created by calculating the mean of log-transformed data (after the addition of 2 points), back-transformation is no longer meaningful and the transformed data are presented as 'units'.

Kleinloog et al. (2013) also describe three clusters of subjective effects in response to THC, based on the items included in VAS Bowdle and VAS Bond and Lader: the cluster 'perception' (Bowdle items 3, 5 and 11, see Table 2), the cluster 'relaxation' (Bond and Lader items 1, 4, 9 and 11, see Table 1), and the cluster 'dysphoria' (Bowdle items 8, 9 and 10, all three items were not responsive and therefore not included in the tables).

Visual analogue scale for drug rating

All items of VAS drug rating ("feel drug", "like drug" and "dislike drug") were analysed without transformation and responded strongly to ketamine. The effect of the drug was experienced as more intense, but less pleasant for the 240 ng/mL target than for the 120 ng/mL target. For both target concentrations the score on VAS like drug (44.8 mm and 37.9 mm) was higher than on VAS dislike drug (13.3 mm and 23.8 mm).

Visual analogue scale as described by Bond and Lader

The most prominent effects on VAS Bond and Lader were seen within the "alertness" cluster. Subjects report a dose-dependent decrease in alertness (i.e. an increase in sedation) following administration of ketamine (-3.7 mm and -13.1 mm). Within the "calmness" cluster, an increase was seen for the 120 ng/mL target (+4.9 mm), but not the 240 ng/mL target. Cluster "mood" was not significantly affected.

Saccadic and smooth pursuit eye movements

Saccadic eye movements were impaired in a dose-dependent manner following administration of ketamine (saccadic peak velocity decreased by 33 deg/s and 76 deg/s for the lower and higher target respectively). Smooth pursuit eye movements (displayed in Figure 4) were impaired in a concentration-related manner (absolute decrease of 9.9% and 13.6%).

Other CNS effects

The pupil/iris ratio was affected by the higher, but not the lower target concentration. As the pupil/iris ratio represents the activity of the autonomic nervous system, this might indicate the start of anaesthetic effect. The body sway, which is a measure for general drug effect, was increased in a

dose-dependent manner (relative increase of 25.6% and 76.4%). Adaptive tracking was affected by the higher (absolute reduction of 7.7%), but not the lower target concentration.

Neuroendocrine parameters

Both cortisol and prolactin were increased in a dose-dependent manner following administration of ketamine. Cortisol increased by 46.5% and 89.8% respectively, whereas prolactin increased by 32.3% and 95.6%. Based on the individual graphs, the increase in prolactin for the 240 ng/mL target concentration was largely associated with premature termination of the infusion due to nausea and vomiting.

Safety

No relevant changes in vital signs, ECG or physical examination were found during the study.

Discussion

The current study aimed to optimise the ketamine model of psychosis and explore the relation between the concentration of ketamine and the psychomimetic, general and adverse effects of ketamine. The target concentrations used in this study represent a broad range of the subanaesthetic level. Very early in the execution of the study, it became clear that the 360 ng/mL target concentration was too high, as it resulted in unwanted adverse effects that can be related to the (anaesthetic) effect of ketamine. The target concentrations between 120 ng/mL and 240 ng/mL were more tolerable for the subjects, although the infusion was terminated prematurely quite frequently (56%) during the 240 ng/mL target. From a point of tolerability, the optimal target concentration would therefore be slightly lower, e.g.

up to 200 ng/mL. All adverse effects that were seen during the study were transient and resolved spontaneously, which is in line with a meta-analysis on the adverse effects of ketamine as a psychosis model (Perry et al., 2007). Within the current study, a total infusion time of two hours was used, during which many tests were performed. Based on the experience of the subjects, the total duration would ideally be shorter and there should be more time in between tests to allow the subjects to experience the effect of ketamine without performing pharmacodynamic tests.

The measured concentrations of ketamine and its metabolite norketamine were within the expected ranges as simulated using the model described by Sigtermans et al. (2009). However, the actual measured concentrations of ketamine were lower than predicted and the standard deviation of the predictions was much larger than the standard deviation of the measured concentrations. It is hypothesized that this is due to uncertainty of the parameter estimation for (intercompartmental) clearance, overestimation of the residual error or the influence of covariates. It is recommended that the pharmacokinetic model will be further improved by adding data from studies with different dosing regimens (including the current study). In that way, the population parameter estimates could be estimated more accurately, which would most likely result in better predictions for future studies.

There was a clear, concentration-dependent psychomimetic effect as shown on all subscales of the PANSS and many VAS scales. As a general rule of thumb, a difference of three points on the positive subscale of the PANSS is considered clinically relevant, although a smaller difference can be acceptable for biomarker research in healthy volunteers. The increase seen for the 120 ng/mL target is similar to this minimum clinically relevant change and the 240 ng/mL target had an even stronger response. Although different study designs make it difficult to compare the dose-related effects with the literature, the absolute increases on the positive and negative subscale that were seen in this study are comparable to previous findings with different exposure levels in healthy volunteers (Krystal et al., 1994, 1998 and 1999;

Malhotra et al., 1996 and 1997b; Breier et al., 1997; Anand et al., 2000; Hetem et al., 2000; Umbricht et al., 2000; Kegeles et al., 2002; Lahti et al., 2003; Aalto et al., 2005; Deakin et al., 2008; Morgan et al., 2006; Nagels et al., 2011; Passie et al., 2005) and findings in patients with schizophrenia (Lahti et al., 1995 and 2003; Malhotra et al., 1997a and 1997b). Chronic treatment with haloperidol (Lahti et al., 1995) and clozapine (Malhotra et al., 1997a) reduces these effects in patients with schizophrenia. In healthy volunteers, ketamine-induced psychomimetic symptoms were reduced by acute treatment with lamotrigine (Anand et al., 2000; Deakin et al., 2008), but not by haloperidol (Krystal et al., 1999), lorazepam (Krystal et al., 1998) or the metabotropic glutamate receptor agonist LY354740 (Krystal et al., 2005). The effects that were seen on the PANSS with the ketamine-model are stronger and more robust than those seen with the THC model of psychosis (D'Souza et al., 2004; Liem-Moolenaar et al., 2010b; Kleinloog et al., 2012) or the psilocybin model of psychosis (Umbricht et al., 2003).

Most other studies that measured VAS feeling high have found an increase of 30-40 mm (Krystal et al., 1998 and 1999; Morgan et al., 2006), similar to the effects of the 120 ng/mL target concentration. Niesters et al. (2012) describe an increase of 60 mm, which is comparable with the 240 ng/mL target concentration.

The startle reflex (PPI) was inhibited by both main target concentrations to a similar extent and consistent with available literature (Oranje et al., 2002; Abel et al., 2003; Heekeren et al., 2007), suggesting a maximum effect. Although PPI is an objective measure, its outcome measure is not as robust as the effects measured on the PANSS and it is more difficult to translate to clinical effect.

Eye movements, in particular non-saccades and anti-saccades, are affected in patients with schizophrenia and their non-affected first-degree relatives (reviewed by Levy et al., 2010). They therefore seem a good, objective biomarker for psychomimetic effect and possibly antipsychotic drug action. In our study, visually guided saccadic and smooth pursuit eye movements were affected by ketamine administration. This effect

was dose-dependent for saccadic eye movements and appeared to have reached a maximum effect for smooth pursuit eye movements. The effect on saccadic eye movements is more likely to be related to sedation. There is also a large body of literature that supports the use of antisaccades as a biomarker for schizophrenia, but antisaccades were not measured in this study.

Both cortisol and prolactin were increased in a dose-dependent manner. The effects on these neuroendocrine parameters were similar to other studies (Krystal et al., 1994, 1998 and 1999; Oranje et al, 2002; van Berckel et al., 1998).

Overall, ketamine administration resulted in a dose-dependent increase in biomarkers for psychomimetic effect (i.e. the PANSS, VAS, PPI, eye movements). This included not only the typical, positive symptoms, but also negative and cognitive symptoms. Most currently available antipsychotic drugs (with the exception of clozapine) are only effective on positive symptoms and therefore, there is a large unmet clinical need to treat the negative and cognitive symptoms of schizophrenia (Carpenter and Koenig, 2008). Many of the drugs that are currently in development for the treatment of schizophrenia have novel mechanisms of action and would potentially influence these symptoms as well. As ketamine induces positive, negative and cognitive psychomimetic symptoms, this model might be able to predict the efficacy of novel antipsychotic drugs in these domains.

Biomarkers that can be related to the onset of the anaesthetic effect of ketamine (i.e. pupil size ratio and adaptive tracking) were mainly affected by higher concentrations of ketamine, whilst body sway (a biomarker of general drug effect) increased dose-dependently. This supports the use of a maximum target concentration of 200 ng/mL, which is consistent with the suggested limit from a tolerability point of view.

A pharmacologic challenge with ketamine provides a great potential as psychosis model in healthy volunteers. The current study improved the understanding of the optimal range of drug concentrations to induce psychomimetic effects, whilst being tolerable. Also, the use of an infusion

regimen based on a pharmacokinetic model has been shown to be useful in maintaining pseudo-steady state plasma concentrations. However, the model's usefulness to predict antipsychotic drug action remains unclear. Future studies should explore the predictability of clinical antipsychotic effect using the ketamine model of psychosis.

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TABLE 1 Overview of least square means by treatment and differences for contrasts (Δ = absolute or relative difference; sac. = saccadic).

		Least square means			120 ng/mL vs placebo			240 ng/mL vs placebo			240 ng/mL vs 120 ng/mL		
Outcome	unit	pla	120	240	Δ	95% CI	p	Δ	95% CI	p	Δ	95% CI	p
PANSS positive	points	7.2	10.3	12.3	+43.7%	34.4 - 53.7	<0.0001	+70.5%	59.0 - 82.8	<0.0001	+18.6%	10.3 - 27.6	<0.0001
PANSS negative	points	7.7	8.8	11.1	+14.6%	4.0 - 26.4	0.0072	+44.0%	30.3 - 59.1	<0.0001	+25.6%	12.9 - 39.7	<0.0001
PANSS general	points	16.5	20.0	25.1	+21.6%	13.6 - 30.2	<0.0001	+52.3%	41.8 - 63.6	<0.0001	+25.3%	16.4 - 34.8	<0.0001
PANSS total	points	31.4	39.2	48.7	+24.9%	17.4 - 33.0	<0.0001	+55.2%	45.4 - 65.7	<0.0001	+24.2%	16.2 - 32.8	<0.0001
PPI 120 ms, full	%	28.3	38.9	35.0	+10.6	0.0 -21.1	0.0491	+6.7	-4.8 - 18.3	0.2471	-3.9	-16.1 - 8.3	0.5228
PPI 120 ms, short	%	28.0	38.0	28.2	+10.0	-2.9 - 22.9	0.1256	+0.2	-13.2 - 13.7	0.9736	-9.8	-24.2 - 4.7	0.1794
PPI 240 ms, full	%	-8.2	20.3	15.7	+28.5	10.6 - 46.4	0.0025	+23.9	4.6 - 43.3	0.0164	-4.6	-25.1 - 16.9	0.6554
PPI 240 ms, short	%	-5.9	21.6	18.5	+27.5	9.5 - 45.5	0.0037	+24.4	5.2 - 43.7	0.0140	-3.0	-23.8 - 17.7	0.7692
VAS mood	mm	52.5	55.9	54.6	+3.3	-0.3 - 7.0	0.0739	+2.1	-1.9 - 6.1	0.3005	-1.2	-5.5 - 3.0	0.5651
VAS calmness	mm	52.4	57.3	54.3	+4.9	2.3 - 7.5	0.0005	+1.9	-1.0 - 4.9	0.1988	-3.0	-6.1 - 0.1	0.0596
VAS alertness	mm	50.2	46.6	37.2	-3.7	-7.3 - 0.0	0.0503	-13.1	-17.0 - -9.1	<0.0001	-9.4	-13.6 - -5.2	<0.0001
VAS relaxation	mm	49.8	55.7	66.1	+6.0	1.9 - 20.8	0.0052	+16.3	11.9 - 20.8	<0.0001	+10.4	5.7 - 15.0	<0.0001
Sac. peak velocity	deg/s	478	445	402	-33	-45 - -20	<0.0001	-76	-91 - -62	<0.0001	-43	-58 - -28	<0.0001
Sac. reaction time	ms	185	191	203	+6	-1 - 13	0.1027	+18	10 - 27	<0.0001	+12	4 - 21	0.0064
Sac. inaccuracy	%	6.9	6.8	7.7	-0.1	-1.1 - 0.8	0.7673	+0.8	-0.3 - 1.9	0.1681	+0.9	-0.3 - 2.1	0.1229
Smooth pursuit	%	45.1	35.1	31.5	-9.9	-12.7 - -7.1	<0.0001	-13.6	-16.6 - -10.6	<0.0001	-3.7	-6.9 - -0.5	0.0248
Pupil / iris ratio	-	0.47	0.45	0.42	-0.02	-0.06 - 0.02	0.3035	-0.05	-0.10 - -0.00	0.0425	-0.03	-0.08 - 0.02	0.2907
Body sway	mm	295	371	521	+25.6%	2.7 - 53.5	0.0277	+76.4%	28.1 - 143.0	0.0009	+40.5%	0.2 - 97.1	0.0489
Adaptive tracking	%	25.1	23.6	17.4	-1.5	-3.4 - 0.4	0.1259	-7.7	-10.2 - -5.3	<0.0001	-6.3	-8.7 - -3.8	<0.0001
Cortisol	ng/mL	0.30	0.44	0.57	+46.5%	29.4 - 65.8	<0.0001	+89.8%	68.2 - 114.2	<0.0001	+29.6%	13.7 - 47.7	0.0002
Prolactin	ng/mL	6.40	8.46	12.51	+32.3%	14.7 - 52.6	0.0003	+95.6%	69.7 - 125.5	<0.0001	+47.9%	27.1 - 72.1	<0.0001

TABLE 2 Overview of least square means (LSM) by treatment, without placebo (Δ = absolute or relative difference).

Outcome	unit	120	240	Δ	95% CI	p
VAS body (1)	mm	1.1	6.7	+182%	68 - 373	0.0005
VAS surroundings (2)	mm	1.9	18.6	+424%	256 - 670	< 0.0001
VAS time (3)	mm	10.5	34.0	+23.5	13.5 - 33.5	0.0001
VAS reality (4)	mm	13.9	48.6	+34.7	24.9 - 44.5	< 0.0001
VAS thoughts (5)	mm	10.6	34.3	+23.8	13.5 - 34.1	< 0.0001
VAS colors (6)	mm	1.3	7.5	+189%	77 - 365	0.0004
VAS sound (7)	mm	1.5	6.9	+155%	46 - 346	0.0023
VAS high (11)	mm	27.4	61.8	+34.4	19.4 - 49.3	0.0001
VAS drowsy (12)	mm	2.8	11.9	+190%	66 - 407	0.0008
VAS anxious (13)	mm	-0.0	1.1	+54%	12 - 113	0.0107
VAS internal	units	0.42	0.60	+0.18	0.12 - 0.24	< 0.0001
VAS external	units	0.60	1.11	+0.50	0.38 - 0.63	< 0.0001
VAS perception	mm	16.0	43.9	+27.8	19.2 - 36.4	< 0.0001
VAS feel drug	mm	52.4	84.0	+31.6	18.9 - 44.2	< 0.0001
VAS like drug	mm	44.8	37.9	-6.9	-19.6 - 5.8	0.2726
VAS dislike drug	mm	13.3	23.8	+10.5	1.9 - 19.1	0.0208

FIGURE 1 Simulated pharmacokinetic profiles of selected literature, including initial target concentrations.

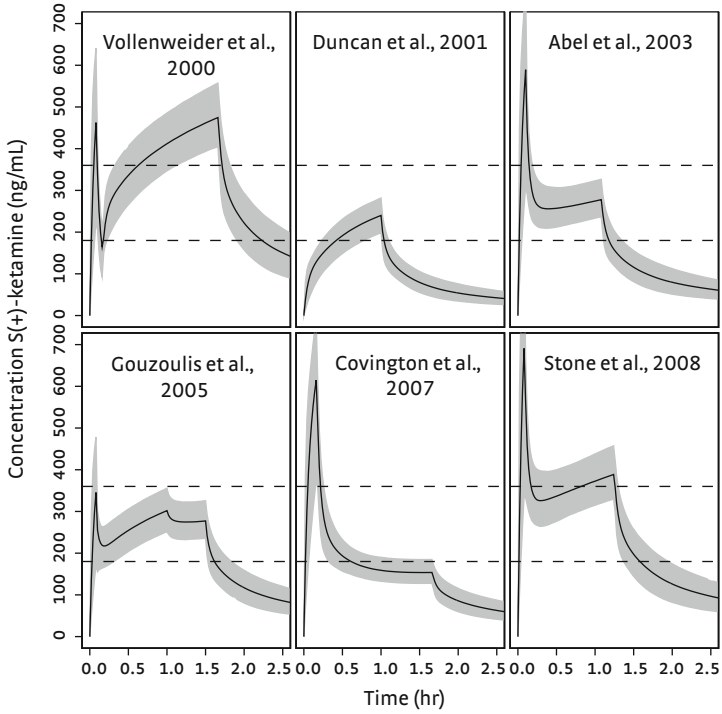


FIGURE 2 Standardized presentation of measured ketamine and norketamine concentrations relative to simulated concentrations.

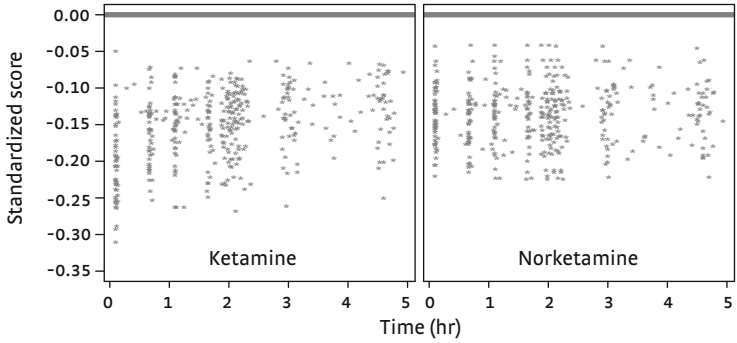


FIGURE 3 Time profile (mean ± SD) for PANSS subscales.

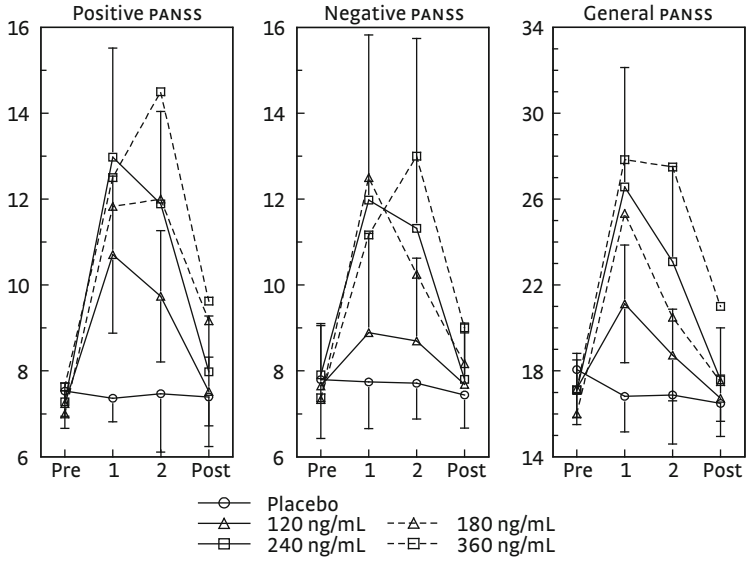
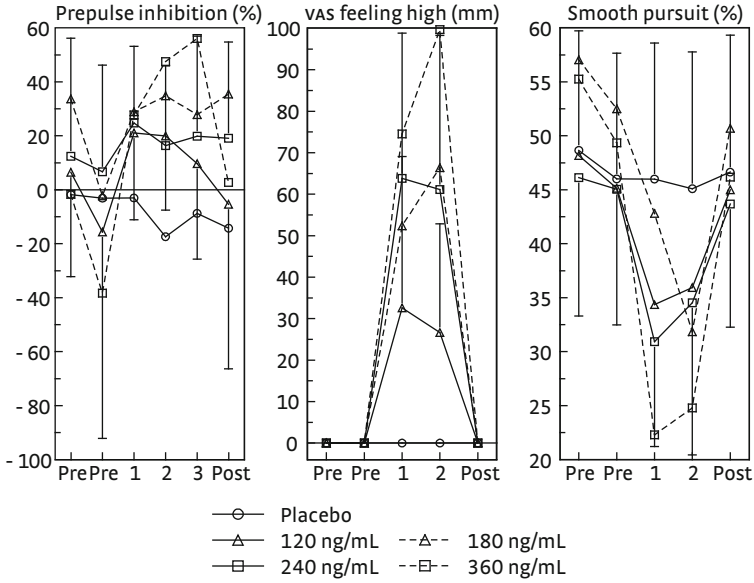


FIGURE 4 Time profile (mean ± SD) for prepulse inhibition (short form, 240 ms interval), VAS feeling high and smooth pursuit eye movements.



CHAPTER 6

Development and evaluation of a new visual analogue scale for psychedelic effects

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ABSTRACT

Visual analogue scales (VAS) are simple instruments to measure drug-induced subjective effects, that can be easily administered. Because the currently used instruments to measure psychedelic effects have several limitations, a new VAS was developed. This proposed VAS was evaluated in a clinical trial that examined the effect of two target concentrations of S(+)-ketamine.

Eleven of the seventeen items of the proposed VAS showed a significant response to treatment. These items could be divided into two clusters measuring 'mental relaxation' and 'physical relaxation'. Although these clusters showed a concentration-dependent increase in subjective effects, these effects were not considered typical psychomimetic effects and the scales were not as sensitive to distinguish between the target concentrations of ketamine as commonly used outcome measures. It is therefore not recommend to replace the commonly used instruments by the proposed VAS.

Introduction

Measuring and quantifying subjective effects within clinical trials can be quite difficult. One method that is widely used is the visual analogue scale (VAS), which typically consists of a 100 mm long line with two subjective states on the extremes. This could be within one subjective state (e.g. experience of 'pain' or 'nausea') with the extremes ranging from 'not at all' to 'extremely' or between two subjective states (e.g. from 'drowsy' to 'alert' or from 'happy' to 'sad'). Throughout a trial, subjects indicate their feelings somewhere on these lines, resulting in a score from 0 to 100 mm. VAS have shown to be sensitive to drug effects.

Bowdle et al. (1998) describe a VAS for drug-induced psychedelic effects, which measures 13 different subjective states, with extremes ranging from 'not at all' to 'extremely'. This scale was validated in a study that involved administration of ketamine to healthy volunteers (Bowdle et al., 1998), and has since then been used to quantify the effects of THC (e.g. Zuurman et al., 2008), alcohol (Khalili-Mahani et al., 2012), ketamine (e.g. Niesters et al., 2012) and scopolamine (e.g. Liem-Moolenaar et al., 2010). Measuring drug-induced psychedelic effects poses a particularly difficult problem in clinical trials, because most of these effects are considered absent under normal circumstances. When the VAS ranges from 'not at all' to 'extremely', this easily results in a score of 0 mm for all items under placebo conditions and even a 'threshold' effect where very minimal effects appear to be rated as 0 mm.

The current article describes the development and validation of a new VAS for psychedelic effects. This VAS was designed to have two subjective states at the extremes, and not range from 'not at all' to 'extremely', with the aim of detecting small variations under normal conditions and maintain a robust, dose-dependent effect following administration of a drug with psychedelic properties.

Methods

Development of a new scale

The new scale was designed to go from one subjective state to another. To select the subjective states, many often-used tools to measure psychomimetic effects (both self-report and interview-based) were examined. One of the problems that was identified with the concept of 'feeling high' is that this subjective phenomenon is interpreted differently by different subjects. It was therefore decided to divide 'feeling high' into different aspects. Several psychologists and clinical pharmacologists with experience in the field of psychopharmacology revised the draft scale (which consisted of 22 pairs of subjective states), resulting in the rewording, deletion and addition of several items. The seventeen remaining items were randomised for order and direction (which subjective state was mentioned first and which last). The resulting scale is presented in Table 1.

Validation in clinical study

To validate the proposed VAS for psychomimetic effects, this VAS was measured within a clinical trial involving the induction of psychomimetic symptoms with the NMDA-antagonist ketamine. The set-up of this study is described in more detail elsewhere (Kleinloog et al., in preparation). In short, healthy volunteers were given a two-hour infusion of S(+)-ketamine to achieve pseudo-steady state concentrations. The clinical study had a three-way cross-over design with placebo, low-dose ketamine (target 120 ng/mL) and high-dose ketamine (target 240 ng/mL) as treatment arms.

The original design included measurement of the positive and negative syndrome scale (PANSS, described by Kay et al. 1987), VAS for mood, calmness and alertness (described by Bond and Lader, 1974), VAS for psychedelic effects (described by Bowdle et al., 1998) and a VAS drug rating (the items 'feel drug', 'like drug' and 'dislike drug' with 'not at all' and 'extremely' as extremes). As

described by Kleinloog et al. (2013), the VAS described by Bond and Lader and Bowdle can be combined into three clusters of subjective effects ('perception', 'relaxation' and 'dysphoria'). In addition to these measurements, the community assessment of psychic experiences (CAPE, described by Konings et al., 2006) and the short form and the LSD subscale of the addiction research centre inventory (ARCI-SF and ARCI-LSD, described by Hill et al., 1963) were taken along with the sole purpose of validating the proposed VAS for psychomimetic effects. All scales were measured twice before the start of infusion (with the exception of the PANSS, which was measured only once), twice during infusion (during which the concentration of ketamine was in pseudo-steady state) and once several hours after the stop of infusion.

Analysis used for validation

Three successive steps were used to validate the proposed VAS for psychomimetic effects. As a first step, the response for each individual item during infusion of ketamine or placebo was compared. As the distribution of the data did not show a normal distribution, a Kruskal Wallis rank test (the non-parametric alternative to an ANOVA) was used for all items.

As a second step, all items that showed a significant difference between treatments were used in a factor analysis to determine if the response on different items can be explained by one or more underlying factors, which could result in the clustering of items. The order of the extremes (left or right) was determined at random and is therefore interchangeable. Prior to the factor analysis, the direction of the items was rearranged to have a positive response on all items. Within the factor analysis, a relatively stringent cut-off value for factor loadings of 0.5 was used to create item clusters.

The third step compared the response on the proposed VAS clusters to other commonly used outcome measures for psychomimetic effects included in the validation study. This was done by comparing the dose-response relationship (placebo, target of 120 ng/mL and target of 240 ng/mL) of the different outcome measures, as well as the sensitivity of each

outcome measure to discriminate between low-target ketamine and high-target ketamine. The dose-response relationship was tested using a Kruskal-Wallis test on the responses during infusion. The discriminatory sensitivity between target concentrations of ketamine was calculated as the chance that the scores on a certain outcome measure during infusion would correctly identify the target concentration. As there are two possible target concentrations, the a priori chance would be 50%. These methods to establish dose-response relationships and discriminatory sensitivity were previously described by Kleinloog et al. (2013).

Results

Clinical study

A total of 30 healthy volunteers (15 male and 15 female) received infusions of S(+)-ketamine with target concentrations of 120 ng/mL (n = 24), 240 ng/mL (n = 25) or placebo (n = 29). There was a clear, concentration-dependent psychomimetic effect as shown on all subscales of the PANSS and different VAS scales. The clinical study is described in more detail elsewhere (Kleinloog et al., in preparation).

Items responding to treatment

The items of the proposed VAS for psychomimetic effects hardly showed any variation under placebo and showed concentration-dependent increases in effect following administration of ketamine. The distribution did not approach a normal distribution and therefore the non-parametric Kruskal-Wallis test was used to identify items that were sensitive to the effects of ketamine. Eleven out of the seventeen items (64.7%) showed a significant response to treatment (see Table 1).

Factor analysis

The eleven items that showed a significant response to treatment were included in the factor analysis. Prior to factor analysis, items that showed a decrease in response following ketamine ('body weight', 'mental control', 'trust', 'distraction' and 'sounds presence') were reversed. Based on parallel analysis (a scree test), two factors were selected. The first factor contained the items 'trust' (loading 1.00), 'safety' (loading 0.64) and 'mental control' (loading 0.59). The second factor contained the items 'bodily control' (loading 0.69), 'speed of thoughts' (loading 0.59) and 'reality' (loading 0.53). The factor loadings for all items with a significant response to treatment are included in Table 2. Figure 1 presents the response by time and treatment for the two clusters that describe factor one and factor two.

Comparison with other outcome measures

As can be seen in Table 3, both factor 1 and factor 2 show a dose-dependent increase, which was statistically significant ($p < 0.0001$). The VAS dysphoria and the subscales of the CAPE did not show a significant dose-response relationship (after Bonferroni correction for multiple comparisons). All three subscales of the PANSS, VAS perception, VAS relaxation and the short form of the ARCI were better able to discriminate between the two target concentrations of ketamine used in the validation study. The VAS 'like drug' and LSD-subscale of the ARCI were less able to detect this difference.

Discussion

The current article describes the development and validation of a new VAS for psychedelic effects. This VAS was designed to have two subjective states at the extremes, and not range from 'not at all' to 'extremely', with the aim of

detecting small variations under normal conditions and maintain a robust, dose-dependent effect following administration of a drug with psychedelic properties.

The effect measured on the seventeen items that were included in the validation, were found to be better described by two underlying factors. Factor 1 was described by the items 'trust' (in the direction of "I trust everyone"), 'safety' (in the direction of "I am safe") and 'mental control' (in the direction of "I easily 'let go'"). This factor appears to be quite similar to the cluster 'relaxation' described by Kleinloog et al. (2013), as can be seen in Figure 2. Factor 2 was described by the items 'bodily control' (in the direction of "my body can't do anything"), speed of thoughts (in the direction of "my thoughts are slow") and 'reality' (in the direction of "what I feel now is not real"). This factor describes a rather physical form of relaxation, which could be related to the cluster of 'Alertness' described by Bond and Lader (1974), as displayed in Figure 2.

In the current evaluation, the proposed VAS did not detect small variations under normal conditions. The effect measured by both factors was dose-dependent, but the mean change was not of the same magnitude as several other outcome measures. Other outcome measures of psychomimetic effects (i.e. the PANSS, VAS perception, and the short form of the ARCI) were shown to be better able to discriminate between the two targets of ketamine. The two identified factors seem to be related to mental and physical relaxation, rather than typical psychomimetic effects. It can therefore be concluded that the proposed VAS does not meet the aims it was designed for.

The current evaluation of the proposed VAS uses the effects induced by S(+)-ketamine to select individual items and underlying factors of the proposed scale. The psychomimetic effects of ketamine are quite pronounced (e.g. Krystal et al., 1994), and it is therefore surprising that factor analysis did not identify these psychomimetic effects as underlying factor. The changes seen on the PANSS, VAS perception and the short form of the ARCI imply that these psychomimetic effects were clearly present in the validation study.

In conclusion, the proposed VAS included two clusters of subjective effect (mental and physical relaxation) that showed a concentration-dependent relationship. These scales were not as sensitive than other commonly used instruments and did not measure typical psychomimetic effects. It is therefore not recommend to replace the commonly used instruments by the proposed VAS.

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TABLE 1 Overview of VAS used in validation study.

REALITY	What I feel now is more real than normal	——	What I feel now is not real
ROOM SIZE	The room around me is small	——	The room around me is large
BODY WEIGHT	My body feels heavy	——	My body feels light
SPEED OF THOUGHTS	My thoughts are fast	——	My thoughts are slow
MENTAL CONTROL	I easily 'let go'	——	I keep control
TRUST	I trust everyone	——	I am suspicious
SAFETY	I am frightened	——	I am safe
BODILY CONTROL	My body can do everything	——	My body can't do anything
DISTRACTION	Everything passes me by	——	Everything is about me
BODILY SENSATIONS	My body feels everything	——	My body feels nothing
SOUNDS INTENSITY	Sounds are intense	——	Sounds are blurred
SOUNDS PRESENCE	Sounds disappear	——	I hear sounds that are not there
TIME	Time goes fast	——	Time goes slowly
HAPPINESS	I am sad	——	I am happy
UNDERSTANDING	I don't understand the world	——	I understand everything in the world
COLOURS	Colours are blurred	——	Colours are intense
COURAGE	I don't dare anything	——	I dare everything

TABLE 2 Overview of item and cluster selection.

			Kruskal-Wallis test		Factor analysis	
		Item	Statistic	p-value	loading F1	loading F2
Significant effect of treatment	Factor 1	Trust	17.8	<0.001	1.00	-0.02
		Safety	6.2	0.045	0.64	-0.12
		Mental control	27.4	<0.001	0.59	0.32
	Factor 2	Bodily control	12.4	0.002	-0.19	0.69
		Speed of thoughts	29.7	<0.001	0.24	0.59
		Reality	8.4	0.015	0.12	0.53
	No factor	Colours	7.7	0.021	-0.09	0.39
		Distraction	17.0	<0.001	0.37	0.18
		Body weight	6.6	0.037	0.20	0.27
		Happiness	12.9	0.002	0.19	-0.09
		Sounds presence	7.6	0.023	0.01	-0.06
No significant effect	Not included	Sounds intensity	5.8	0.056	-	-
		Time	2.3	0.322	-	-
		Understanding	1.8	0.411	-	-
		Courage	1.3	0.533	-	-
		Room size	0.2	0.889	-	-
		Bodily sensations	0.2	0.917	-	

TABLE 3 Overview of dose-response relationship and sensitivity.

	Placebo		120 ng/mL		240 ng/mL		Dose-relation		Sens
Measure	**Mean (SD)**		**Mean (SD)**		**Mean (SD)**		**x^2**	**p**	**%**
Factor 1	50.4	(1.8)	54.4	(8.8)	58.5	(12.3)	32	<0.0001	57.9
Factor 2	49.8	(1.1)	53.9	(5.2)	57.2	(11.7)	32	<0.0001	63.9
Positive PANSS	7.4	(1.1)	10.2	(1.7)	12.5	(2.4)	103	<0.0001	72.6
Negative PANSS	7.7	(0.9)	8.9	(2.2)	11.7	(4.0)	42	<0.0001	70.3
General PANSS	16.8	(2.0)	20.0	(2.7)	25.1	(5.4)	88	<0.0001	72.8
VAS Perception	0.0	(0.0)	18.0	(21.0)	41.2	(28.2)	106	<0.0001	71.8
VAS Relaxation	49.9	(4.3)	56.3	(7.5)	65.2	(15.7)	40	<0.0001	72.0
VAS Dysphoria	0.0	(0.0)	0.1	(0.8)	1.2	(5.6)	6	0.0468	60.5
VAS Feel drug	0.4	(2.0)	53.6	(37.0)	78.6	(28.9)	110	<0.0001	62.8
VAS Like drug	0.0	(0.3)	45.2	(27.1)	40.4	(27.4)	96	<0.0001	54.3
VAS Dislike drug	0.5	(4.1)	11.8	(21.3)	19.4	(23.4)	45	<0.0001	61.8
Positive CAPE	20.4	(0.7)	20.8	(1.3)	20.8	(1.0)	4	0.1670	54.4
Negative CAPE	16.6	(3.5)	18.1	(4.2)	17.7	(3.8)	5	0.0780	56.8
Depression CAPE	9.6	(1.5)	9.8	(1.5)	9.7	(1.4)	0	0.9338	51.9
ARCI LSD	14.7	(1.9)	16.1	(3.0)	17.4	(2.9)	20	<0.0001	55.0
ARCI short	4.3	(0.7)	6.1	(2.1)	8.1	(1.8)	69	<0.0001	70.0

FIGURE 1 Time profile by treatment for selected factors (measurements 3 and 4 are during infusion).

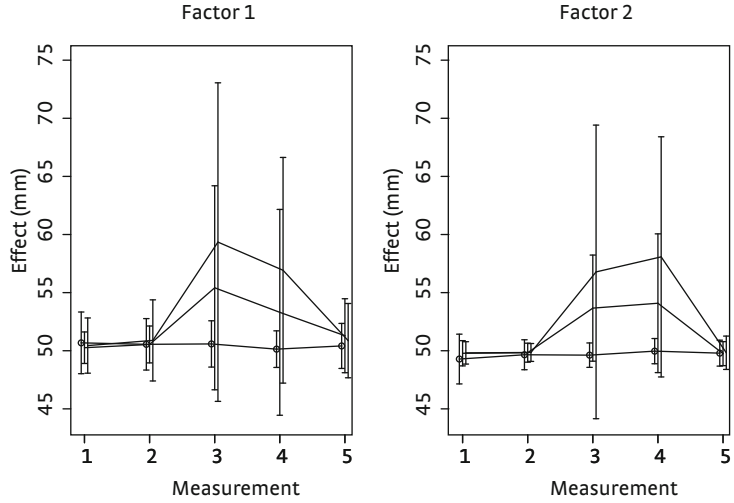
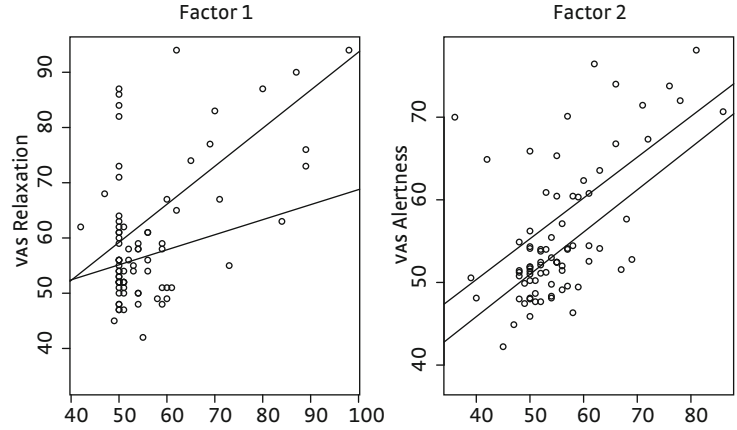


FIGURE 2 Correlation between identified factor 1 and VAS relaxation (left panel) and factor 2 and VAS alertness (right panel).



CHAPTER 7

Subjective effects of ethanol, morphine, Δ^9-tetrahydrocannabinol and ketamine following a pharmacological challenge are related to functional brain connectivity

Neuroimage (submitted for peer review)

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ABSTRACT

This analysis examines the neuronal foundation of drug-induced psychomimetic symptoms by relating the severity of these symptoms to changes in functional connectivity for a range of different psychoactive compounds.

The repeated measures design included 323 resting state FMRI time series and measures of subjective effects in thirty-six healthy male volunteers. Four different pharmacological challenges with ethanol, morphine, Δ^9-tetrahydrocannabinol and ketamine (12 subjects per drug) were applied. A set of ten 'template' resting state networks was used to determine individual connectivity maps. Linear regression was used for each individual subject to relate these connectivity maps to three clusters of drug-induced subjective psychomimetic effects ('perception', 'relaxation' and 'dysphoria') as measured with visual analogue scales. Group-analysis showed that the subjective effects of perception correlated significantly across drugs with the connectivity of the posterior cingulate cortex and precentral gyrus with the sensorimotor network ($p<0.005$, corrected). No significant correlations were found for relaxation or dysphoria.

The posterior cingulate cortex has a role in visuospatial evaluation and the precentral gyrus has been associated with auditory hallucinations. Both the posterior cingulate cortex and the precentral gyrus show changes in activation

in patients with schizophrenia, which can be related to the severity of positive symptoms (i.e. hallucinations and delusions), and have previously been related to changes induced by psycho-active drugs. The similarity of functional connectivity changes for drug-induced psychomimetic effects and for symptoms of psychosis provides further support for the use of pharmacological challenges with psychomimetic drugs as models for psychosis.

Introduction

Pharmacological challenges constitute a useful instrument in clinical pharmacology research. A pharmacological challenge consists of the administration of a drug with a known pharmacology and the (repeated) measurement of quantifiable effects. The relation between the administered drug and the measured effects provides information about the targeted system.

Several drugs have been used to elicit psychomimetic symptoms in healthy volunteers and patients (reviewed in detail by Gouzoulis-Mayfrank et al., 1998). Psychomimetic symptoms are changes in subjective feelings or experiences that bear some resemblance to psychotic symptoms. For example, ketamine (e.g. Krystal et al., 1994; Abel et al., 2003; Gouzoulis-Mayfrank et al., 2005) and Δ^9-tetrahydrocannabinol (THC; e.g. D'Souza et al., 2004; Liem-Moolenaar et al., 2010; Kleinloog et al., 2012) have been examined as models for psychosis. The basis for these models is the resemblance between the drug-elicited psychomimetic symptoms and the symptoms of psychosis and schizophrenia (in particular the so-called 'positive' symptoms, such as hallucinations and delusions).

The visual analogue scale is sensitive to drug effects and often used to measure psychomimetic effects. It typically consists of a 100 mm long line with two subjective states on the extremes (for example 'drowsy' and 'alert' or 'not at all high' to 'extremely high'). To provide an integrated measure of subjective effects, multiple scales are combined in a composite scale of a certain subjective state.

Functional magnetic resonance imaging (FMRI) of functional brain connectivity networks constitutes an important tool in understanding the physiology, pathophysiology and pharmacology of the brain. Different groups have confirmed the existence of resting state connectivity networks (also referred to as intrinsic connectivity networks) and their spatial consistency in resting conditions (e.g. Biswal et al., 1995; van de Ven et al., 2004; Beckmann et al, 2005; Damoiseaux et al., 2006; Smith et al., 2009; Biswal et

al., 2010). The spatial distribution of these networks is related to functional domains (Smith et al., 2009). Changes in connectivity can be detected following administration of drugs that act on the central nervous system (Khalili-Mahani et al., 2012; Klumpers et al., 2012; Niesters et al., 2012; Cole et al., 2013).

Although FMRI of intrinsic connectivity networks is a powerful tool to study drug effects on the central nervous system, it has not been used to investigate the neuronal foundation of drug-induced psychomimetic symptoms. Here we apply resting state FMRI with four different pharmacological challenges to study common effects that are shared between functional brain connectivity and psychomimetic symptoms. Furthermore, the identified brain areas are compared to the regions associated with functional connectivity changes in psychosis, to investigate whether these drug-induced psychomimetic symptoms can serve as a model for psychosis.

Many studies on functional connectivity have focused only on the 'default mode network'. Important nodes of this network are located in the midline areas of the posterior cingulate cortex and within the medial prefrontal cortex (Raichle et al., 2001; Holt et al., 2011). The default mode network is characterised by a decrease in tonic resting state activity during the execution of tasks, and is attributed a large evolutionary significance. Within this default mode network, the posterior cingulate cortex serves an adaptive function and regulates incoming information from internal and external environments, whereas the medial prefrontal cortex plays a role in social cognition and emotional processing (Broyd et al., 2009).

Several studies have shown altered connectivity of the default mode network in patients with schizophrenia, related to positive symptoms (Bluhm et al., 2007; Garrity et al., 2007; Whitfield-Gabrieli et al., 2009; van Lutterveld et al., 2013; Orliac et al., 2013; Tang et al., 2013). Following the assumed relationship between drug-elicited psychomimetic symptoms and the symptoms of psychosis and schizophrenia, we hypothesised that the connectivity within (structures associated with) the default mode network would be related to the psychomimetic effects. Of the structures within the default mode

network, the posterior cingulate cortex in particular was expected to show a relation (Bluhm et al., 2007; Broyd et al., 2009; Holt et al., 2011; van Lutterveld et al., 2013).

Material and methods

Data sets

The current analysis used data previously collected in three studies within the same centre. Khalili-Mahani et al. (2012) compared the effect of pseudo-steady state levels of ethanol (~600 mg/L during 2.5 hours) and morphine (~80 nmol/L during 2.5 hours) in 12 healthy male volunteers. Klumpers et al. (2012) studied the effect of three subsequent doses of THC (2 mg, 6 mg and 6 mg with 90 minute intervals) in 12 healthy volunteers. Niesters et al. (2012) compared the effect of an increasing concentration of S(+)-ketamine (20 mg/70 kg/hr during one hour followed by 40 mg/70 kg/hr for another hour) in 12 healthy male volunteers. All studies were placebo controlled and included repeated measurements of resting state FMRI, visual analogue scales for psychedelic effects (Bowdle et al., 1998) and visual analogue scales for alertness, calmness and mood as (Bond and Lader, 1974). From these visual analogue scales, three profile scores ('perception', 'relaxation' and 'dysphoria') were calculated to describe the subjective psychomimetic effects (Kleinloog et al., 2013).

Imaging protocol

All resting state images from the three studies were collected on the same 3T Philips Achieva scanner with the same sequence parameters (whole brain volume obtained with a gradient echo planar with: repetition time -TR- 2180ms; echo time -TE- 30ms; 80 degrees flip angle; 220 volumes; 3.44 mm isotropic voxels; and a matrix size of 64 x 64 x 38). A T1-weighted high-resolution structural image was acquired at the start of each session and

used for registration purposes. Lighting was dimmed and subjects were instructed to lie still with their eyes open, think of nothing in particular and to stay awake.

Software

All imaging data was pre-processed and analysed in FSL version 5.0.4 (Oxford Centre for Functional Magnetic Resonance Imaging of the Brain -FMRIB- Software Library; Jenkinson et al., 2012; http://fsl.fmrib.ox.ac.uk), which includes tools for brain extraction (Smith, 2002), motion correction (Jenkinson et al., 2002), linear registration (Jenkinson et al., 2001, 2002), non-linear registration (Andersson et al., 2007a,b), dual regression (Beckmann et al., 2009; Fillippini et al., 2009) and permutation testing (Anderson and Robinson, 2001; Bullmore et al., 1999).

Pre-processing

Imaging data was pre-processed as previously described by Filippini et al. (2009). For spatial smoothing a Gaussian kernel of full-width at half maximum (FWHM) of 6 mm and a high-pass temporal filter of 150 s (0.007 Hz) were used to reduce noise. Resting state images were registered to the individual's T1-weighted high-resolution structural scan. This structural scan was registered to standard space (MNI-152; Montreal Neurological Institute, nonlinear 6th generation) using a 12 degree of freedom linear search followed by non-linear registration with a warp resolution of 10 mm. Registration parameters were combined to put the resting state FMRI scans in MNI standard space, interpolated at a resolution of 2 x 2 x 2 mm.

Dual regression

Dual regression (Beckmann et al., 2009; Fillippini et al., 2009; Khalili-Mahani et al., 2012, 2013; Cole et al., 2013) was used with the ten 'template' connectivity networks described by Smith et al. (2009) to derive the spatial and

temporal definition of each template network within each scan session. The ten networks are 'medial visual network', 'occipital visual network', 'lateral visual network', 'default mode network', 'cerebellum network', 'sensorimotor network', 'auditory network', 'executive control network', 'right frontoparietal network' and 'left frontoparietal network'.

Dual regression consists of two consecutive linear regression models. In the first step the ten template networks (and ten additional 'nuisance' networks described by Smith et al., 2009 to control for artefacts) are used in a spatial regression model to extract the session-specific mean time course of each network. These twenty individualised template time courses are then used in a temporal regression model to describe the time course for each voxel as a linear, weighted combination of these template time courses. This results in three-dimensional maps (one per network) containing the regression coefficient (the relative contribution) of each voxel to that specific network.

Subject-level Analysis

Following dual regression, a voxel-wise comparison throughout the entire brain was performed for the ten template networks per subject to determine the linear relation between the score on the subjective measure (clusters perception, relaxation and dysphoria) and the voxel-wise connectivity with each network. The FSL function 'randomise' was used to estimate the slope of the linear relation (relative change in subjective effect versus relative change in connectivity) for each voxel.

Group-level Analysis

The resulting estimates were analysed per network and subjective effect. For the group-level analysis, a one sample group-mean test with Monte Carlo permutation testing of 10,000 random sign-flips of individual estimates was used (as implemented in randomise, see Nichols and Holmes,

2002). A corrected p-value smaller than 0.005 (corrected for 10 networks of interest), following threshold-free cluster enhancement (Smith and Nichols, 2009), was considered statistically significant.

Extracting information on structural location and function

The location of each identified cluster was described using the Harvard-Oxford cortical and subcortical structural atlases (Desikan et al., 2006).

Results

Data sets

Data from a total of 36 healthy male volunteers were included in the analysis. For each treatment (ethanol, morphine, THC and ketamine), data from 12 subjects were available for evaluation. The subjects who received ethanol and morphine were the same (both were included in the data set by Khalili-Mahani et al., 2012). Due to differences in study designs, the study days for ketamine treatment included five scan sessions, the study days for ethanol and morphine included seven scan sessions and the study days for THC included eight scan sessions. Data from one scan session of one subject receiving ketamine was missing. The total number of scan sessions used for evaluation was therefore 323.

Subjective effects

An overview of the responses on the visual analogue scales is presented in Table 1. The items within the cluster relaxation are bi-directional (two different subjective states at the extremes). Subjects were instructed that a normal resting condition would be somewhere half-way between the two extreme subjective states, in line with the original publication of the scales

(Norris, 1971), which means the theoretical zero-point for these scales is at 50 mm. In contrast, the items within the clusters perception and dysphoria are uni-directional (one subjective state, ranging from 'not at all' to 'extremely'), resulting in a theoretical zero-point of 0 mm. Administration of ketamine resulted in the largest change in all clusters of subjective effects. The induced changes that were seen were smallest for ethanol, followed by morphine and substantially larger for THC.

Cluster of correlation

The connectivity between one cluster (MNI coordinates maximum (6, -28, 46); size 456 mm^3; primarily posterior cingulate and precentral gyrus) and the sensorimotor network correlated significantly with the subjective effects of perception across all treatments (Figure 1). As shown in the upper panel of Figure 1, the spatial location of the cluster remained constrained to the posterior cingulate gyrus and the precentral gyrus, also when a more liberal *p*-value of 0.05 was used as threshold as shown in the panel below. For the subjective effects of relaxation and dysphoria, no clusters with significant correlation were identified. Figure 2 presents an overview of the average correlation between the sensorimotor network connectivity and subjective effects within the significant cluster, divided by treatment. The figure also includes the non-significant relationships with relaxation and dysphoria, to illustrate the specificity of the association for perception.

Discussion

A positive correlation, consistent for different drugs, was found between changes in functional connectivity and subjective psychomimetic effects. The connectivity between a cluster located in the posterior cingulate cortex and the precentral gyrus and the sensorimotor network corresponded to changes in perception. It was hypothesised that the subjective effects would

be associated with changes in connectivity within the default mode network. Although the posterior cingulate cortex has a role in the default mode network (Garrity et al., 2007; Whitfield-Gabrieli et al., 2009; van Lutterveld et al., 2013; Orliac et al., 2013; Tang et al., 2013), the relation between perception and the posterior cingulate cortex in the current analysis was seen within the sensorimotor network. This might be explained, because the current analysis examined ten different networks at the same time, including inter-network connectivity, whereas the previous studies specifically examined the 'default mode network' and did not look at other connectivity networks.

The cingulate cortex plays a role in the integration and processing of sensory, motor, cognitive and emotional information (Bush et al., 2000). The identified cluster is located in the area of the cingulate cortex that is functionally described as the visuospatial evaluative region and which is involved in the evaluation of the relevance of visual and auditory stimuli (Vogt et al., 1992). The main functional role of the precentral gyrus is in motor function, but it has also been associated with auditory hallucinations (Diederen et al., 2010; Jardri et al., 2011). It is therefore not surprising that these areas are related to psychomimetic changes in perception, which are the result of disruptions in the evaluation of external stimuli.

The current analysis explored the changes in drug-induced psychomimetic effects shared by four different pharmacological challenges. Administration of ketamine was previously associated with increased activation in the precentral gyrus (Daumann et al., 2010). There is also a relation between the identified brain area and psycho-active drugs that were not included in the current analysis. Salomon et al. (2012) describe a relation between life-time ecstasy exposure and functional connectivity in the precentral gyrus. The use of cocaine (Kübler et al., 2005) and administration of psilocybin (Gouzoulis-Mayfrank et al., 1999) has been associated with decreased activation of both the posterior cingulate cortex and the precentral gyrus during cognitive tasks. The strength of the current study is the combination of drugs with different mechanisms, which results in an outcome that is unrelated to the direct mechanism of action and based on

changes that are shared by these drugs. A weakness in this approach is that the absence of drug-induced changes in a specific area for one of the drugs would reduce the chance of identifying this change in this combined analysis of all drugs. Of the four drugs used in this analysis, ketamine is likely to have the strongest psychomimetic effects.

For the subjective effects of relaxation and dysphoria, no clusters were identified that had a consistent significant relation with changes in connectivity. This can in part be explained by the different characteristics of the included drugs, which had various pharmacological activities and effect profiles. Any shared effects on the cluster relaxation, which includes feelings of sedation, may be resulting from different mechanisms of central nervous system depression. This may also be the case for dysphoria, which describes a range of poorly specified negative emotional states, although it is still conceivable that drug-induced negative mood effects share common neuronal networks. Such changes may not have been detected in our study, because the effect of dysphoria occurred in only a limited subset of subjects (Kleinloog et al., 2013), which may have prevented the detection of significant clusters for the whole group. These hypotheses are consistent with the distributions presented in Figure 2.

The analytic approach used in the current analysis places a strong emphasis on the template connectivity networks described by Smith et al. (2009). The use of this standardised set of template networks improves the interpretation of the findings and makes the results more easily comparable with other studies. On the other hand, the use of the dual regression approach allows for individual differences in exact spatial and temporal definitions of the connectivity networks. It would have been too rigid to directly apply the template networks to the data (without dual regression). Although the template connectivity networks are based on a model-free approach, the use of this standard set of networks introduces a framework for the intrinsic functional architecture of the brain. The measurement of resting state functional connectivity has been shown to be highly consistent over repeated measurements (Shehzad et al., 2009; Zuo et al., 2010) without

intervention. Changes seen following a pharmacological challenge can therefore be attributed to the administered drug.

To accommodate the use of repeated measurements in the analysis, the different measures of connectivity within each subject (for a given treatment) were related to the subjective effects. This has the additional benefit that individual differences in 'anchor' points on the visual analogue scale (the range between feeling 'not at all high' to 'extremely high' can be different for different subjects) are accounted for. In other words, the relative changes in connectivity are related to the relative changes in subjective effect. However, the use of linear regression to compare functional connectivity and subjective effects assumes a linear relation, which is not necessarily the case.

As mentioned in the introduction, the psychomimetic symptoms that are elicited by the different challenge agents were hypothesised to be related to symptoms of psychosis and schizophrenia. The identified cluster is part of the areas within the cingulate cortex and the precentral gyrus that were previously associated with the positive symptoms of schizophrenia (Silbersweig et al. 1995; Choi et al., 2005; Garrity et al., 2007). Olney et al. (1999) propose a glutamate receptor hypofunction model of schizophrenia, which describes the role of specific neurons within the posterior cingulate in the occurrence of psychotic symptoms. Hyperstimulation of these neurons, for example by administration of a drug (i.e. ketamine), was associated with a limited and reversible psychomimetic reaction (Olney et al., 1999). There are similarities between drug-induced psychomimetic symptoms and psychosis, but also differences (Gouzoulis-Mayfrank et al., 1998). Models using psychoactive drugs to mimic symptoms of psychosis do not model the full spectrum of psychosis and in particular do not model the complex syndrome of schizophrenia. The four drugs (ethanol, morphine, THC and ketamine) included in this analysis all have a different mechanism of action. The psychoactive effects of these drugs are clearly not identical, although there are similarities between them. The rationale for grouping these specific drugs is rather pragmatic, as they had already been used in

resting state FMRI studies in our centre and the data of these studies was available. The measurement of psychomimetic symptoms with the visual analogue scales used in the current analysis is not validated to measure psychotic symptoms in psychiatric illness and it is possible that drug-induced psychomimetic symptoms have a different neurophysiological origin than symptoms of psychosis. Nevertheless, the current findings indicate that drug-induced psychomimetic effects and positive symptoms of psychosis share an underlying relation with functional connectivity in networks that involve the posterior cingulate and precentral gyrus.

Conclusions

The intrinsic functional architecture of the brain can be described using connectivity networks. Within these networks, a consistent relation was found between drug-induced changes in functional connectivity in the posterior cingulate cortex and the precentral gyrus and drug-induced changes in subjective psychomimetic effects following pharmacologic challenges with four psychoactive drugs (ethanol, morphine, THC and ketamine). Previous studies in patients with schizophrenia have shown a relation between functional connectivity and the positive symptoms of schizophrenia. Our findings provides further support for the use of pharmacological challenges with psychomimetic drugs as model for psychosis and for the use of resting state FMRI as a method to study the effects of drugs and diseases in the brain.

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TABLE 1 Overview of subjective effects as measured using visual analogue scales (mean ± SD)

Treatment	Perception	Relaxation	Dysphoria
Ethanol	7.6 (11.6)	59.5 (11.4)	0.6 (0.9)
Morphine	9.1 (8.9)	63.3 (11.8)	0.9 (1.3)
THC	33.1 (24.4)	64.9 (14.1)	1.0 (1.9)
Ketamine	74.1 (32.3)	67.6 (25.2)	16.2 (20.6)
Placebo	2.4 (3.7)	45.2 (20.9)	1.0 (2.1)

FIGURE 1 Overview of significant cluster within MNI-152 standard space (white area: sensorimotor connectivity network; cluster is shown in grey within network). *For colour figure see inside of back cover.*

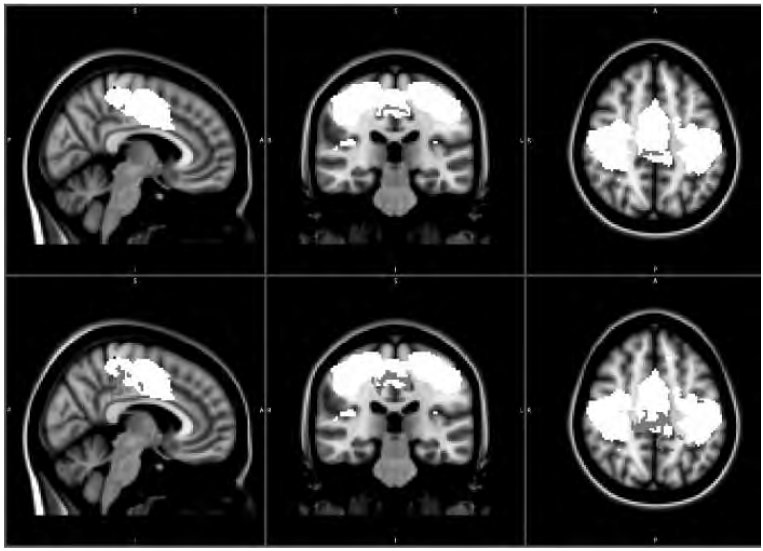
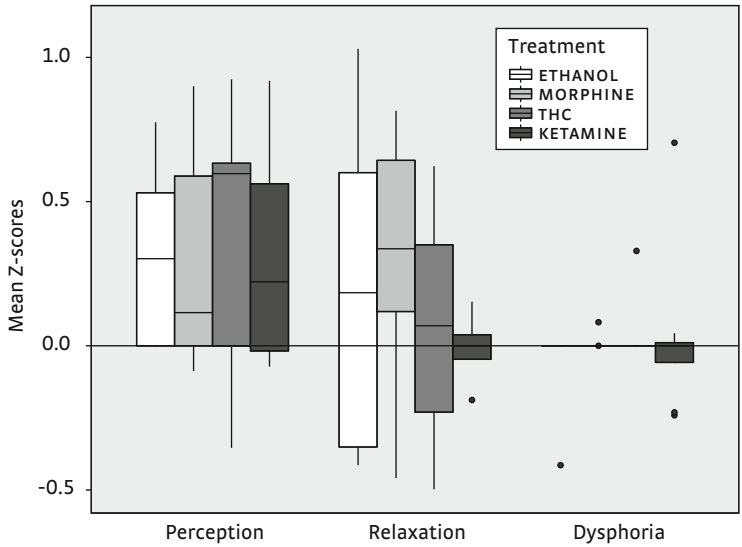


FIGURE 2 Average correlation of connectivity between the sensorimotor network and subjective effects within the identified cluster, divided by treatment. *For colour figure see inside of back cover.*



CHAPTER 8

Discussion

Schizophrenia is a severe psychiatric disorder, resulting from a complex derangement of the central nervous system (CNS). The prevalence of schizophrenia is estimated to be between 0.4% and 1.7% (Seeman, 2002). Although it has long been considered a single syndrome, the possibility of multiple underlying pathological entities is gaining more and more support (Silviera et al., 2012). The symptoms of schizophrenia are often subdivided into positive symptoms (i.e. hallucinations and delusions), negative symptoms (i.e. loss of motivation, flattened affect, social withdrawal) and cognitive impairment. The most often used diagnostic systems (Diagnostic and Statistical Manual of Mental Disorders-DSM-IV, recently replaced by the DSM-5; and International Classification of Diseases-ICD-10) are based on symptomatology and do not reflect underlying pathophysiology (van Os and Kapur, 2009).

Beyond the dopamine hypothesis of schizophrenia

As discussed in **Chapter 1**, the dopaminergic system has traditionally been attributed a dominant role in the pathophysiology of schizophrenia, mainly because of the large role of dopamine antagonism in the mechanism of action of known antipsychotic drugs. Amphetamine is a drug that reduces the reuptake of dopamine from the synaptic cleft, which results in increased levels of dopamine. The similarity between positive psychotic symptoms and psychomimetic symptoms induced by a pharmacological challenge with amphetamine provided further support for the dopamine hypothesis of psychosis (Lehmann and Ban, 1997; Featherstone et al., 2007).

For all currently registered antipsychotics, clinical effectiveness is related to the occupancy of the dopamine receptor (Farde et al., 1988; de Visser et al., 2001; Seeman, 2002; Agid et al., 2007). However, the mechanism of action of neither antipsychotics, nor amphetamine, is strictly dopaminergic. For example, most antipsychotic drugs, including chlorpromazine and reserpine (which formed the basis for the dopamine hypothesis), also affect the serotonergic system (Lehman and Ban, 1997; Meltzer, 1999; Seeman, 2002).

Amphetamines not only increase dopamine levels, but also levels of serotonin and noradrenaline (Freudenmann and Spitzer, 2004; Kalivas, 2007).

The role of pharmacological challenges in drug development

Pharmacological challenges with psycho-active drugs can be used as a model for psychosis and antipsychotic drug action (Gouzoulis-Mayfrank et al., 1998b) and should thus play an important role in antipsychotic drug development. The pharmacological challenge models provide information that is impossible to obtain by means of animal models for psychosis (Gouzoulis-Mayfrank et al., 1998b). Many different pharmacological challenges, affecting different neurotransmitter systems are available. Both the similarities and differences between these challenges have aided in the discovery of novel potential targets in drug development (Kalivas, 2007). There is a lot of debate about the suitability of each model and on the question which challenge model resembles psychosis 'the best'.

Of the pharmacological challenges that affect the dopaminergic system, the amphetamine challenge is most often used. There is a strong similarity between amphetamine-induced psychomimetic symptoms and the positive and negative symptoms of psychosis (Bramness et al., 2012). Positive psychomimetic symptoms induced by amphetamine can be reversed by currently registered antipsychotics, which all include a dopaminergic mechanism of action.

Several of the classical pharmacological challenges used as model for psychosis and antipsychotic drug action affect the serotonergic systems. These include challenges with mescaline, lysergic acid diethylamide -LSD- and psilocybin (Gouzoulis-Mayfrank et al., 1998b). The drug-induced changes in perception and predominant hallucinations resulted in the use of these challenges as a model for psychosis. Serotonergic challenges have been effective in identifying the antipsychotic properties of chlorpromazine (Thuillier, 1980) and reserpine (Ban et al., 2010).

The development of psychomimetic symptoms following administration of phencyclidine (PCP) and its analogue ketamine form the basis of the glutamatergic challenge model of psychosis. As shown in **Chapter 5**, ketamine induces clear, dose-dependent psychomimetic effects in healthy volunteers that show similarities to psychosis. There is a clear concentration range in which psychomimetic symptoms are present and side-effects are limited. The dose-dependent psychomimetic effects were fairly consistent between subjects, with limited inter-subject variability. Several studies have found a reduction in psychomimetic symptoms by clozapine (Malhotra et al., 1997) and lamotrogine (Anand et al., 2000; Deakin et al., 2008), but not lorazepam (Krystal et al., 1998). Results for haloperidol have been inconsistent (Lahti et al., 1995; Krystal et al., 1999). Further exploration of the potential to detect antipsychotic drug action is needed.

Drugs with other mechanisms of action, such as the cannabinoid agonist Δ^9-tetrahydrocannabinol (THC), have also been employed as models for psychosis and antipsychotic drug action. D'Souza et al. (2004) were the first to measure the psychomimetic effects of THC in healthy volunteers as a model for psychosis. They later replicated the findings in a group of patients with schizophrenia (D'Souza et al., 2005). The THC-induced psychomimetic symptoms can be (partly) reduced by haloperidol (D'Souza et al., 2008; Liem-Moolenaar et al., 2010) and olanzapine (**Chapter 2**). Compared to the ketamine model of psychosis, the THC model shows larger inter-subject variation and there are more subjects who do not show measurable psychomimetic effects (non-responders).

Interaction between different neurotransmitter systems

The different neurotransmitter systems that are related to psychosis, schizophrenia, and antipsychotic drug action are strongly interrelated. For example, the release of dopamine into the prefrontal cortex or amygdala stimulates the glutamatergic projections between these areas, as well as glutamatergic projections to the nucleus accumbens and ventral tegmental

area (Kalivas, 2007). These projections are associated with the neuroadaptive changes following the use of psychostimulants that result in increasing drug-seeking behaviour (Kalivas, 2007). The administration of ketamine, leads to a tonic activation of glutamatergic receptors on GABAergic, serotonergic and noradrenergic neurons, resulting in an inhibition of the activity of major excitatory pathways (Olney et al., 1999). As discussed in **Chapter 4**, the endocannabinoid system provides a regulatory feedback for the serotonergic system.

Within the glutamatergic receptor hypofunction model of schizophrenia, postulated by Olney et al. (1999), many other neurotransmitter systems are implicated as intrinsic components of the glutamatergic network. Interestingly, the dopamine receptor is not included in this network, although it is hypothesised that dopamine D_2 receptors may regulate the release of glutamate at NMDA receptors. A genetic aberration in the dopaminergic system, within the pathophysiology of schizophrenia could lead to hyperinhibition of glutamate release, resulting in a hypoglutamatergic state (Olney et al., 1999). As treatment with dopamine antagonists would normalise the release of glutamate, and thus normalise the glutamatergic state, the Olney model is not incompatible with current pharmacological treatment of schizophrenia.

The current adaptation of the dopamine hypothesis regards dopamine dysregulation as the final common pathway to psychosis and the result of multiple interacting 'hits' (Howes and Kapur, 2009). This hypothesis implies that antipsychotic drugs are not targeting the primary, pathophysiological abnormality of schizophrenia and are acting downstream. Paradoxically, the blocking of presynaptic dopamine D_2 receptors within a downstream context would result in a compensatory increase in dopamine synthesis, leading to relapses when patients stop their medication (Howes and Kapur, 2009).

It is interesting to note the different role of dopamine in these two hypotheses. In the model described by Olney et al., dopamine dysregulation is upstream of the main pathophysiological disturbance (and

therefore contributing to the disturbance), whereas Howes and Kapur place the dopamine dysregulation downstream (and therefore as the result of the disturbance). Regardless of which hypothesis is true, it is clear that although dopamine has a clear role in the pathophysiology of schizophrenia, it is not the only neurotransmitter involved. Therefore, non-dopaminergic mechanisms of action of potential novel drugs and potential models to test antipsychotic drug action are indispensable.

Requirements for pharmacological challenges

Pharmacologic challenges can be very useful tools in drug development. For optimal results, these challenges should meet certain requirements, laid out by van Gerven (2005). The two pharmacological challenges that were studied in this thesis for use as a model of psychosis and antipsychotic actions meet these requirements.

First requirement: the model should resemble disease

First of all, if the challenge test is used as a model for disease, it should resemble the disease. Symptoms evoked by a challenge should be similar to the symptoms of the disease and treatment that is effective for the disease should influence the symptoms evoked by the challenge. The symptoms evoked by THC (**Chapter 2**) and ketamine (**Chapter 5**), as well as the symptoms of the other pharmacological challenges described earlier, show unmistakable similarities with the symptoms of psychosis (Gouzoulis-Mayfrank et al., 1998b). For an example of psychomimetic symptoms following a pharmacological challenge, a documentary from early experiments with LSD is recommended (Bercel, 1955).

One of the arguments against the use of drug-induced psychomimetic effects as model for psychosis is that subjects maintain insight into the drug-induced changes and believe these symptoms are drug-induced (Hollister, 1962; Gouzoulis-Mayfrank et al., 1998b). However, when people

are unaware that they have ingested psychomimetic drugs like LSD, their clinical impression cannot easily be distinguished from an acute psychotic episode in the context of schizophrenia (Fishman, 1983). It should be noted that clinical trials with a drug like LSD cannot ethically be performed without informing the participating subjects. The observations described by Fishman (1983) were based on experiments by the United States' Central Intelligence Agency (CIA), which is less restrained by ethical conduct. In a study by Hoffer et al. (1956), verbatim recordings of patients with acute schizophrenia and subjects who had taken LSD were presented to psychiatrists, who could not distinguish the two groups.

Another argument against the use of psychomimetic drugs as a model for psychosis is the difference in symptom presentation, for example in the relative frequency of visual versus acoustic hallucinations (Hollister, 1962). This might be explained by over- or underreporting of specific hallucinations (Fishman, 1983). Furthermore, a pharmacological challenge is not required to fully model the disease, but can also be used to model specific aspects of a disease (van Gerven, 2005). Neither one of the challenge models is assumed to be a complete model for the complex disorder of (chronic) schizophrenia.

The fact that antipsychotic medication is able to reduce the drug-induced psychomimetic symptoms (**Chapter 2**) provides further support for the resemblance of the challenge model to psychosis. Another documentary on early LSD experiments shows the effect of a (non-registered) drug on the psychomimetic symptoms induced by a pharmacological challenge with LSD (Fabing, 1954).

A further argument in favour of the resemblance between drug-induced psychomimetic symptoms and psychosis is described in **Chapter 7**. This imaging study describes a specific brain area where changes in functional connectivity can be related to drug-induced psychomimetic effects for several psycho-active drugs with a different mechanism of action. The same area has previously been shown to be affected in patients with schizophrenia, where the changes in functional connectivity could be related to the severity of positive symptoms (i.e. hallucinations and delusions).

Second requirement: known mechanism of action

The second requirement for a challenge test is that the drug should have a known pharmacologic mechanism of action (van Gerven, 2005). Information about the pharmacologic mechanism of action will provide insight into the properties of the target system of the challenge. For all drugs previously described, the mechanism of action is known and there is a relation between the drug target and schizophrenia. For both THC and ketamine pharmacokinetic models are available to describe the relation between administered dose and drug concentrations in plasma (Sigtermans et al., 2009; Heuberger et al., unpublished data). For THC, the relation between the pharmacokinetics and the subjective effect has been described in **Chapter 4**. It is good to note that both THC and ketamine have active metabolites (11-hydroxy-THC and S(+)-norketamine respectively), which complicate the challenge model as it is difficult to separate the effect of the parent compound and the active metabolite. The pharmacokinetics of these metabolites are included in the models (Sigtermans et al., 2009; **Chapter 4**). Further research into the relative contribution of the active metabolites would improve the challenge models.

Third requirement: quantifiable effect

The evoked effect should be quantifiable (van Gerven, 2005). In this thesis, much emphasis was placed on this aspect of challenge test development. To assess a drug-induced effect, repeated measures are essential to estimate the profile (time course) of effect. The outcome measure for effect should have a relation to the target of the challenge. Cholden (1956) further specified this criterion specifically for challenge tests for schizophrenia: "this tool must allow the subject of an experiment to communicate subjective data to the investigator". The fact that informed subjects maintain insight during a pharmacological challenge (see 'First requirement') significantly improves their willingness to perform neuropsychological tests and describe their

state of mind.
Within **Chapter 2** and **Chapter 5**, several outcome measures were used to quantify the psychomimetic effects of the pharmacological challenges in addition to a number of measures for 'general' central nervous system and systemic effects.

As primary outcome measure, the clinical Positive and Negative Syndrome Scale (PANSS; Kay et al., 1987) was used. This scale uses a structured clinical interview to longitudinally assess different symptoms of schizophrenia. For the PANSS to be used in the current setting, small changes were made regarding the timing of symptoms ('in the last two weeks' was replaced by 'since the last interview' or 'this morning'). These changes were discussed with and accepted by the owners of the instrument (PANSS Institute LLC, New York). The PANSS is considered the 'golden standard' for the measurement of symptoms of psychosis and schizophrenia in clinical trials.

As a self-report measure of subjective effects, the visual analogue scale (VAS) was used. The typical VAS is a 100 mm long line with two extreme subjective states on each side (e.g. 'alert' and 'drowsy' or 'not at all high' and 'extremely high'). It is very easy to use and measurement only takes one or two minutes, which means the VAS can be measured repeatedly throughout a study day. **Chapter 3** describes how three different profiles of subjective effects ('perception', 'relaxation' and 'dysphoria') can describe the subjective effects of THC. In **Chapter 4**, these profiles are used to relate the change in subjective effects induced by THC to the drug concentrations in plasma. Psychosis, and psychomimetic symptoms, constitutes a complex phenomenon, with shifts of normal aspects of consciousness and behaviour to abnormal extremes and the occurrence of novel subjective phenomena that do not normally occur in healthy, unmedicated subjects. Some of the often used VAS have limitations: the absence of psychedelic effects under normal conditions result in a score of 0 without much variation during placebo and there appears to be a threshold in effect. **Chapter 6** describes the development and evaluation of a new set of VAS for psychedelic effects. These scales

were 'two-sided' (two different subjective states on both sides of the line). During the evaluation of these scales, they were not found to be better than the scales that were previously used and are therefore not recommended for use in clinical trials.

Chapter 5 also describes the use of pre-pulse inhibition (PPI), as a more objective measure of psychomimetic symptoms. PPI measures the inhibition of the startle reflex, an involuntary contraction of the skeletal and facial muscles that follows a sudden, relatively intense stimulus. The startle reflex is present in different species and PPI is therefore used as a translational model (Braff et al., 2001). Deficits in PPI have been reported in patients with schizophrenia and people with a schizotypical personality (Braff et al., 2001). Antipsychotic drugs have been shown to reverse changes in PPI (Hamm et al., 2001). The effect of ketamine on PPI was not as robust as the effect on the PANSS and more difficult to translate to clinical effects. A pharmacologic challenge with psilocybin showed an increase in PPI, rather than a decrease as would be expected (Gouzoulis-Mayfrank et al., 1998a).

In **Chapter 7**, resting state functional magnetic resonance imaging (RS-FMRI) is used to map functional connectivity between different brain areas. This technique can be applied to patients with schizophrenia and to subjects during a pharmacological challenge. **Chapter 7** also describes how these changes in functional connectivity can be related to other outcome measures, such as changes in subjective effects.

Fourth requirement: dose-effect relation

The effect evoked by the challenge should have a relation to the dose or concentration of the pharmacologic agent used for the challenge (van Gerven, 2005). This relation should be quantifiable and reproducible (Gijsman et al., 2004). In **Chapter 2**, three subsequent doses of THC are administered and after each dose the same outcome measures were applied. For most outcome measures, the effect showed a clear dose-response relation, although for other effects only a dose-related increase in effect was seen for the first

two doses. **Chapter 5** uses two different target concentrations (and an additional two target concentrations in the first part of the study) of ketamine. Again, on most outcome measures, a clear dose-dependent response was seen. In **Chapter 4**, a pharmacokinetic / pharmacodynamic model is developed to describe the relation between THC concentrations in plasma and the subjective effects. This model allows for a more complex application of the dose-response requirement.

Fifth requirement: safe and tolerable

Last but not least, the challenge needs to be safe and tolerable (van Gerven, 2005; Gijsman et al., 2004). The THC-challenge model (**Chapter 2**) has been shown to be safe and tolerable in the doses used. During the first part of the ketamine study (**Chapter 5**), concentrations that were no longer tolerable were reached. The use of different target concentrations in the (original and adapted) study design resulted in useful information about the effect profile for different concentrations of ketamine. The psychomimetic effects were present at lower concentrations than the adverse effects.

It should be noted that these pharmacological challenges should be administered in a controlled setting and the dose of the psychomimetic drug should be carefully selected. Repeated, prolonged exposure to higher doses of psychomimetic substances can result in changes in neural plasticity (Olney et al., 1999).

Future directions

Before the different pharmacological challenge models can be used as psychosis models in drug development, more attention on their sensitivity to detect antipsychotic effects is required. In particular, a prospective study with a novel compound, preferably with a non-dopaminergic mechanism of action, and the subsequent clinical effects of this compounds would provide additional validity to these models.

Conclusion

This thesis describes the use of pharmacological challenges with psychomimetic drugs as a model for psychosis. It shows that the THC model is sensitive to the effects of antipsychotic drugs and provides insight into the dose-related effects of the ketamine challenge model. Different outcome measures, such as the VAS and RS-FMRI, and their ability to quantify drug-induced psychomimetic effects, have been explored in more detail.

Well-controlled circumstances and repeated measurement of drug concentrations and effect, are especially important in the study design when pharmacological challenges are used in drug development. Pharmacological challenges are useful tools. The psychomimetic effects that are induced by psycho-active drugs may not fully represent the complexity of (chronic) schizophrenia, but it was demonstrated that they can be used as a model for psychosis and schizophrenia. These drug-induced psychomimetic effects can be reduced by current antipsychotic drugs.

When pharmacological challenges are incorporated in early phase clinical studies for novel treatments for schizophrenia, they might provide valuable information on the effectiveness of a drug. They could provide input for go / no-go decisions and a rationale for starting doses for studies in patients with schizophrenia, that cannot be obtained through animal models.

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Summary

This thesis describes the role that pharmacological 'challenge'-models could have in the development of new drugs for psychosis and schizophrenia. A pharmacological challenge consists of the administration of a pharmacologically active compound, such as a drug, with the purpose of influencing an underlying biological system. Subsequently, the changes on certain outcome measures (this could be hormones, but also neuropsychological tests) are observed. The relation between these outcome measures and the amount of administered drug (or the changes in drug concentrations in plasma) provides information on the function of the underlying system.

Chapter 1: Introduction

The first drug that was used for the treatment of psychosis is chlopromazine (market under the name 'Largactil' in Europe and 'Thorazine' in the United States). The effectiveness of this drug in treating psychosis was first investigated in 1952 in a Parisian mental asylum. The discovery of this drug is sometimes referred to as 'the French revolution of 1952', because of the unmistakable change it brought about in psychiatry. It was also the beginning of psychopharmacology.

In the late 1960s the dopamine hypothesis of schizophrenia was formed. This hypothesis described schizophrenia as the result of a disturbance of the neurotransmitter dopamine. It was formulated when it became known that the new drugs for the treatment of psychosis blocked the dopamine receptors in the brain and that the administration of amphetamine (which causes an increase in dopamine levels) induces symptoms that resemble psychosis.

The development of clozapine (marketed as Leponex) in 1966 led to a new change in the treatment of psychosis. Clozapine acts on many other receptors (such as serotonin) and has a relatively low affinity for dopamine receptors. This led to the development of many 'atypical' antipsychotics. Clozapine remains the only drug with a proven superior efficacy in the treatment of psychosis, but it is also associated with rare but severe side-effects (i.e. agranulocytosis).

Even though dopamine is not the only neurotransmitter involved in schizophrenia, dopamine does have a major role. All currently available drugs have a dopaminergic mechanism of action (to some extent), which is related to their antipsychotic potencies. Available animal models of psychosis have mainly been validated with dopamine antagonists. In this way, positive effects of dopaminergic antipsychotics in dopaminergic animal models have become self-comfirmatory. This thwarts the development of drugs with other mechanisms of action.

The use of pharmacological challenge models could constitute a solution. There are different compounds, with different mechanisms of action that can induce psychomimetic effects (resembling psychosis). These compounds are (also) often used as recreative drugs, like amphetamine (dopaminergic), LSD (serotonergic), ketamine (glutamatergic) and THC (cannabinoid). These compounds can be used to induce psychomimetic symptoms in healthy volunteers. These drug-induced symptoms could constitute a model for psychosis.

Chapter 2: Does olanzapine inhibit the psychomimetic effects of THC?

In this chapter, THC (the active ingredient of cannabis) is used to induce psychomimetic symptoms in healthy volunteers. Previous studies with this model have shown that THC can be used to achieve this effect, and that it can be reversed by the 'classic' antipsychotic haloperidol. The most important aim of this study was to investigate whether the 'atypical' antipsychotic olanzapine was also able to reduce these psychomimetic effects. As a positive control for the histaminergic effects of olanzapine (i.e. somnolence) diphenhydramine was added to the study design.

Forty-nine healthy male volunteers participated in this study. The study consisted of five study days that were similar, except for the combination of drugs that were administered. These combinations were: (1) only THC, (2) THC preceded by 10 mg of olanzapine, (3) THC preceded by two doses of

15 mg diphenhydramine, (4) only 10 mg of olanzapine, and (5) only placebo. The study was blinded and placebo-controlled, which means that nobody was aware of the order of treatments during the execution of the study and that a placebo was used if a certain treatment was not given. Baseline measurements were performed in the morning, after which the oral medication (olanzapine, diphenhydramine or placebo) was administered. In the afternoon three balloons filled with THC or placebo (subsequently 2 mg, 4 mg and 6 mg with a 90 minute interval) were administered. In the afternoon many outcome measures were tested again to measure the effect. The most important outcome measure was the PANSS, an interview that is being used to quantify the severity of symptoms in patients with schizophrenia.

As expected, administration of THC resulted in a significant increase on the positive subscale of the PANSS (a measure for hallucinations and delusions) of 20.6%. If this was preceded by administration of olanzapine, THC only increased the positive PANSS by 13.7%. This was a statistically significant reduction in psychomimetic symptoms ($p < 0.001$). This reduction is slightly smaller than previously found for haloperidol. The study confirmed that THC can be used as a model for psychosis and that this model is sensitive for the effects of antipsychotic drugs with (partly) different mechanisms of action.

Chapter 3: Profiling the subjective effects of THC using VAS-scales.

The study described in Chapter 2 also showed that measuring psychomimetic effects is complicated by non-linear responses, multiple dimensions and non-responders. Subjective drug effects are often measured with different visual analogue scales (VAS), which typically consist of a 10 cm line, with a subjective feeling on both sides (for example 'alert' and 'drowsy'). Other more unsual subjective effects are measured on unipolar VAS-scales, where the intensity is rated from absent (0 mm) to extreme (100 mm). A subject is asked repeatedly throughout a study day to indicate his or her feelings on

these lines. During the analysis described in this chapter, the underlying structure in the reaction on two often-used sets of VAS-scales after administration of THC was examined.

To this end, the data from ten studies, performed at CHDR, were used. In all these studies, THC was administered to healthy volunteers (217 in total). There were 29 different VAS-scales: 16 describing mood, alertness and calmness and 13 describing psychedelic effects. Different statistical methods were employed to discover underlying patterns in the response on these VAS-scales. Each method has its own advantages and this analysis focussed on the similarities between these methods.

This resulted in three effect clusters that describe the subjective effect of THC: 'perception', 'relaxation' and 'dysphoria'. The cluster 'perception' includes the 'high' feeling of THC, changes in time perception and the control of one's own thoughts. 'Relaxation' describes the mental relaxation that is experienced after administration of THC. The cluster 'dysphoria' consists of more negative reactions that occurred in a small subset of subjects. This includes the hearing of voices, suspicion or delusions. These symptoms were, when present, of mild severity. These VAS-scales probably did not include all subjective effects of THC, and may for instance have missed feelings like hunger or craving, or aspects of anxiety and panic. Still, the composite scales encompass a range of distinguishable THC-effects which cover most feelings associated with psychotic-like states. These clustered scales can thus be used to investigate factors that are associated with psychomimetic propensity, or effects of antipsychotic drugs.

Chapter 4: The influence of personality on the sensitivity to subjective effects of THC.

Using the three subjective effect clusters identified in the previous chapter, the sensitivity of mild cannabis users to THC was investigated. This chapter once again used data collected in previous studies. Data of seven previous studies, with administration of THC by inhalation, were combined.

Previously a pharmacokinetic model, describing the course of THC-concentrations in plasma, was made using these data. This model was extended by describing the concentrations of the active metabolite (11-OH-THC). Also, a mathematical model was developed to describe the relationship between THC plasma concentrations and the three clusters of subjective effects.
This mathematical model can be used to estimate individual parameters that describe the sensitivity to THC for each subject. This measure of sensitivity was then compared to scores on different subscales of a personality questionnaire (the TCI) using multiple linear regression. It was found that the cluster 'perception' is influenced by the personality trait 'harm avoidance' and the cluster 'dysphoria' by 'self-transcendence'. According to the underlying psychobiological model of personality the personality trait 'harm avoidance' is regulated by the neurotransmitter serotonin. Given the effects of serotonergic drugs like LSD and psilocybin (the active compound of magical mushrooms), it is not surprising that this trait is related to changes in 'perception'. The endocannabinoid system regulates and fine-tunes the action of several neurotransmitter systems, including serotonin. Higher scores on 'self-transcendence' are associated with schizotypy and it is therefore not surprising that people with higher scores are more sensitive to the dysphoric effects of THC. These results provide insight into the neuropharmacological systems underlying personality traits.

Chapter 5: Optimizing the glutamatergic challenge model of psychosis, using S(+)-ketamine to induce psychomimetic symptoms in healthy volunteers.

Ketamine is an NMDA-receptor anatagonist that is mainly used in anaesthesiology. Since the drug has psychomimetic side-effects at lower concentrations, ketamine is also used as a psychosis model. The studies describing this model use different doses of ketamine, only measure the

effect on a single timepoint (at different times relative to administration) and do not measure ketamine concentrations in plasma. Therefore, this study further explored the ketamine model of psychosis, with the aim of describing the relations between ketamine concentrations in plasma, and the psychomimetic effects on one hand and the undesirable effects (that affect tolerability) on the other hand.

This study consisted of three study days, during which a constant S(+)-ketamine concentration in the plasma was obtained (a high target concentration, a low target concentration and placebo respectively). This was accomplished by calculating an infusion scheme using a previously described pharmacokinetic model. In the first part of the study the target ketamine concentrations were 360 ng/mL, 180 ng/mL and 0 ng/mL. The high concentration in particular led to many side-effects and the study was temporarily halted to revisit the target concentrations. In the first part of study six male subjects were dosed. After the temporary halt, the study was continued with target concentrations of 240 ng/mL, 120 ng/mL and 0 ng/mL. This revised infusion regimen also incorporated the accumulation of the active metabolite norketamine, which has roughly a third of the activity of the parent compound. Thirty subjects (15 males and 15 females) participated in this second part of the study. As was done in Chapter 2, many different measurements were performed before, during and after the infusion and the PANSS was again selected as main outcome measure.

A dose-dependent, robust increase in psychomimetic symptoms was seen on all subscales of the PANSS. The positive PANSS was increased by 43.7% for the 120 ng/mL concentration and by 70.5% for the 240 ng/mL concentration relative to placebo. Most other outcome measures also showed a dose-dependent increase in effect. Based on the occurrence of side-effects (and thus tolerability) a ketamine concentration of about 200 ng/mL is advised for the use of ketamine as a psychosis model. The concentration-range is sufficient to induce psychomimetic symptoms and provides enough possibility to reduce the effects with antipsychotic drugs.

Chapter 6: Development and evaluation of a new VAS for psychedelic effects.

As was already discussed in Chapter 3, VAS-scales are often used to quantify subjective feelings. However, there are several limitations to these VAS-scales. For example, psychedelic effects are absent under normal conditions, which leads to a score of 0 (without much variation) during placebo treatment. Furthermore, there appears to be a 'threshold' in psychedelic effects. For these reasons, a new set of VAS-scales has been developed to measure psychedelic effects. It was decided not to measure one effect per scale (ranging from 'not at all' to 'extremely'), but to measure two opposing subjective effects (e.g. from 'I feel sad' to 'I feel happy').

This new set of VAS-scales was measured during the study described in Chapter 5, together with the original set and several comparable questionnaires. During the evaluation, it was first examined which items responded to the ketamine challenge. Thereafter all items that responded were analysed for underlying patterns of effect, which resulted in two subscales. These subscales were not superior in the discrimination between the different target concentrations of ketamine. Furthermore, these subscales were more focussed on mental and physical sedation and not as much on the typical psychomimetic effects, which the scales were intended to measure. It is therefore not recommended to use these new VAS-scales in clinical trials.

Chapter 7: Subjective effects of ethanol, morphine, tetrahydrocannabinol and ketamine following a pharmacological challenge are related to functional brain connectivity.

To investigate which areas of the brain are involved in the origin of psychomimetic effects of a pharmacological challenge, the data of previous studies with a pharmacological challenge with ethanol (alcohol), morphine, THC and ketamine were compared. In all these studies, changes over time were measured using an MR-scanner during rest. These measurements in

the MR-scanner can be used to describe 'functional connectivity', which is a measure for the synchronicity of activation or deactivation of different brain areas. All these studies also measured the subjective effects using VAS-scales, following the pharmacological challenges.

For each drug, data of 12 healthy, male participants were available. The changes in functional connectivity were related to the changes in the three subjective clusters described in Chapter 3 ('perception', 'relaxation' and 'dysphoria'). The analysis resulted in a cluster, located in the posterior cingulate gyrus and the precentral gyrus, with a statistically significant relation between changes in functional connectivity and changes in subjective 'perception'. This area of the brain was previously found to show a relation between functional connectivity and positive psychotic symptoms (e.g. hallucinations and delusions) in patients with schizophrenia.

Chapter 8: Discussion

Different pharmacological challenge models can be used as model for psychosis and as model for antipsychotic drug activity. These pharmacological challenge models act through different neurotransmitter systems. The psychomimetic symptoms induced by all these models can be reduced by antipsychotic drugs. It is important to note that these different neurotransmitter systems do not operate independently, but they are related through complicated interactions. Different hypotheses on the pathophysiology of schizophrenia have been formulated that unite the role of these different neurotransmitter systems. The role of dopamine in these hypotheses is different, but it is clear that other neurotransmitters play a role in the development of schizophrenia.

Professor van Gerven (my doctoral supervisor) described five requirements for pharmacological challenges during his inaugural address (latin: oratio).

First of all, a good model of a disease should resemble the symptoms of the disease. The psychomimetic symptoms that were induced by THC and

ketamine have unmistakable similarities with the symptoms of psychosis. There are also differences between the two. For example, subjects remain insight in the development of psychomimetic symptoms, whereas patients loose insight when they become psychotic. If a subject is not explained that they receive a psychomimetic drug, the presentation is similar to the clinical presentation of (acute) schizophrenia. Also, the relative frequency of acoustic and visual hallucination is different for subjects and patients, which might be explained by underreporting of hallucinations by patients. The fact the psychomimetic symptoms of a pharmacological challenges can be reduced by antipsychotic drugs is a strong argument in favour of the validity of the pharmacological challenge as model for psychosis. The finding that the brain area that shows a relation between functional connectivity and psychomimetic symptoms also shows a relation between functional connectivity and positive psychotic symptoms in patients with schizophrenia, further supports the role of pharmacological challenges as model for psychosis.

The second requirement for a pharmacological challenge is a known pharmacological mechanism of action. Both THC and ketamine have known but different mechanisms of action and pharmacokinetic models have been described. This thesis also presents a pharmacokinetic / pharmacodynamic model for the subjective effects of THC, which is an indication that these subjective effects are pharmacologically related to (endo-)cannabinoid activity. Such a PK-PD model has not yet been developed for ketamine, although Chapter 5 provides the necessary data for such a model.

The third requirement is that the evoked effect should be quantifiable. In this thesis, much emphasis was placed on this aspect of challenge test development. Psychosis is a complex condition, which includes shifts of normal aspects of consciousness and behaviour to abnormal extremes, as well as the occurrence of novel subjective experiences that do not normally occur in healthy subjects. This mix of quantitatively and qualitatively abnormal mental states creates difficulties for measurement instruments suitable for research. It is an important requirement for useful psychomimetic challenge tests that subjects remain aware of their altered state of mind.

Because subjects retain insight, it is easier for them to share their experiences with the investigator and perform different measurements to quantify the evoked effect. The PANSS appeared to be a suitable method to quantify psychomimetic effects. As this is a clinically validated scale, the evoked symptoms are easily related to the symptoms of schizophrenia. The VAS-scales are also suitable to measure subjective effects of a pharmacological challenge. This thesis further substantiates this claim by the introduction of three clusters of subjective effects, which have also been described using a pharmacokinetic / pharmacodynamic model. An attempt was made to create an improved VAS for psychedelic effects, which was unsuccessful.

The fourth requirement for a pharmacological challenge test is the presence of a dose- or concentration-dependent effect. For the effects induced by THC and ketamine a relation with drug concentration was established.
The fifth requirement is that a pharmacological challenge should be safe and tolerable. At the doses of THC used during the different studies, THC was found safe and tolerable. In the first part of the study with the ketamine model, the highest target concentration was no longer tolerable. After an adjustment of the dose, a clear range in concentrations could be identified in which ketamine administration is tolerable, while producing the desired psychomimetic effects. Therefore, both models also meet this requirement.

Conclusion

Pharmacological challenges with psychomimetic drugs may not fully represent the complexity of (chronic) schizophrenia, but constitute a useful model for psychosis and antipsychotic drug action. This thesis describes the use of THC and ketamine as models and explores different outcome measurements that can be used to quantify psychomimetic effects, in particular VAS-scales and FMRI. The phenomenological and pharmacological basis of psychomimetic challenge models were discussed. Well-controlled circumstances and repeated measurement of drug concentrations and effect greatly benefit the use of pharmacological challenges in drug development.

Samenvatting
Summary in Dutch

Dit proefschrift beschrijft de rol die farmacologische 'challenge'-modellen kunnen hebben in de ontwikkeling van nieuwe geneesmiddelen voor psychose en schizofrenie. Een farmacologische challenge bestaat uit het toedienen van een farmacologisch actief middel, zoals een medicijn, met als doel invloed uit te oefenen op een onderliggend biologisch systeem. Vervolgens wordt gekeken naar de verandering op bepaalde uitkomstmaten (dit kunnen bijvoorbeeld hormonen zijn, maar ook neuropsychologische testen). Door de verandering op deze uitkomstmaten te relateren aan de hoeveelheid toegediende stof (of de verandering in concentratie van de stof in het plasma), wordt informatie verkregen over de werking van het onderliggende systeem.

Hoofdstuk 1: Introductie

Het eerste medicijn dat werd gebruikt voor de behandeling van psychose is chloorpromazine (op de markt gebracht onder de naam Largactil). De werking van dit medicijn voor psychose werd in 1952 voor het eerst onderzocht in een Parijs gesticht. De ontdekking van dit medicijn wordt weleens beschreven als 'de Franse revolutie van 1952', omdat het een onmiskenbare verandering in de psychiatrie teweeg heeft gebracht. Het was tevens het begin van de psychofarmacologie.

Eind jaren zestig van de vorige eeuw werd de dopamine hypothese van schizofrenie opgesteld Deze hypothese houdt in dat schizofrenie het gevolg is van een verstoring van de neurotransmitter dopamine. Hij kwam tot stand toen bekend werd dat de nieuwe medicijnen voor psychose de dopamine-receptoren in de hersenen blokkeerden en dat het toedienen van amfetamine (dat juist zorgt voor een toename van de hoeveelheid dopamine) leidt tot symptomen die wat weg hebben van psychose.

De ontwikkeling van clozapine (merknaam Leponex) in 1966 leidde tot een nieuwe verandering in de behandeling van psychose. Clozapine werkt op veel meer receptoren (o.a. serotonine) en heeft eigenlijk relatief weinig affiniteit met dopamine-receptoren. Hierna volgde de ontwikkeling van

veel 'atypische' antipsychotica. Clozapine blijft overigens het enige medicijn dat een bewezen hogere effectiviteit heeft bij de behandeling van psychose, maar het gaat ook gepaard met ernstige, maar zeldzame bijwerkingen (m.n. agranulocytose).

Hoewel dopamine dus niet de enige neurotransmitter blijkt te zijn die bij schizofrenie betrokken is, speelt dopamine wel een grote rol. Alle medicijnen die momenteel beschikbaar zijn hebben dopamine als (onderdeel van) het werkingsmechanisme, wat gerelateerd is aan de antipsychotische potentie. De diermodellen die beschikbaar zijn om psychose te onderzoeken zijn voornamelijk gevalideerd met dopamine antagonisten. Hierdoor zijn positieve bevindingen van dopaminenerge antipsychotica in deze diermodellen zelf-bevestigend. Dit verhindert de ontwikkeling van medicijnen met andere werkingsmechanismen.

Het gebruik van farmacologische 'challenge' modellen zou een uitkomst kunnen bieden. Er zijn verschillende stoffen, met verschillende werkingsmechanismen, die psychomimetische ('op psychose gelijkende') effecten teweeg kunnen brengen. Dit zijn middelen die vaak (ook) als recreatieve drugs gebruikt worden, zoals amfetamine (dopamine), LSD (serotonine), ketamine (glutamine) en THC (cannabinoid). Met deze middelen kunnen psychomimetische symptomen worden opgewekt in gezonde vrijwilligers. Deze symptomen kunnen dienen als model voor psychose.

Hoofdstuk 2: Leidt olanzapine tot een afname van de psychomimetische effecten van THC?

In dit hoofdstuk wordt THC (de werkzame stof van cannabis) gebruikt om psychomimetische effecten op te wekken in gezonde vrijwilligers. Eerdere studies met dit model hebben al laten zien dat THC voor dit doel gebruikt kan worden en dat deze effecten onderdrukt kunnen worden met het 'klassieke' antipsychoticum haloperidol. Het belangrijkste doel van deze studie was dan ook om te onderzoeken of het 'atypische' antipsychoticum olanzapine eveneens in staat was om deze psychomimetische effecten te

onderdrukken. Als positieve controle voor de histaminerge effecten van olanzapine (o.a. slaperigheid) werd difenhydramine aan het studieontwerp toegevoegd.

Aan deze studie deden 49 gezonde, mannelijke vrijwilligers mee. De studie bestond uit vijf studiedagen die er hetzelfde uitzagen, maar waarop steeds een andere combinatie van middelen werd gegeven. Deze combinaties waren: (1) alleen THC, (2) THC voorafgegaan door 10 mg olanzapine, (3) THC voorafgegaan door twee doses van 15 mg difenhydramine, (4) alleen 10 mg olanzapine en (5) alleen placebo's. De studie was geblindeerd en placebo-gecontroleerd, wat wil zeggen dat tijdens de studie niemand wist wat de volgorde van de behandelingen was en dat een placebo werd gebruikt als de behandeling niet gegeven werd. In de ochtend werden nulmetingen gedaan, waarna de orale medicatie (olanzapine, difenhydramine of placebo) werd toegediend. In de middag volgden drie toedieningen met een ballon waarin THC of placebo zat (achtereenvolgens 2 mg, 4 mg en 6 mg met een interval van 90 minuten). In de middag werden opnieuw veel testen uitgevoerd om het effect te meten. De belangrijkste uitkomstmaat was de PANSS, een interview dat wordt gebruikt om de ernst van symptomen van patiënten met schizofrenie te meten.

Zoals verwacht, leidde de toediening van THC tot een significante verhoging op de positieve schaal van de PANSS (een maat voor o.a. hallucinaties en waanbeelden) van 20.6%. Als eerst olanzapine was toegediend, leidde toediening van THC slechts tot een stijging van 13.7%. Dit was een statistisch significante afname van psychomimetische symptomen ($p < 0.001$). Deze afname is iets kleiner dan eerder is gevonden voor haloperidol. De studie heeft bevestigd dat THC gebruikt kan worden als model voor psychose en dat dit model gevoelig is voor de effecten van medicijnen tegen psychose met (deels) verschillende werkingsmechanismen.

Hoofdstuk 3: Profilering van de subjectieve effecten van THC met behulp van VAS-schalen

Uit de studie die wordt beschreven in Hoofdstuk 2 kwam ook naar voren dat het meten van psychomimetische symptomen wordt bemoeilijkt door een niet-lineaire respons, meerdere dimensies en non-responders. Subjectieve effecten van geneesmiddelen worden vaak gemeten met visuele analoge schalen (VAS), die meestal bestaan uit een lijn van 10 cm met aan beide kanten een subjectief gevoel (bijvoorbeeld 'alert' en 'suf'). Andere, meer ongebruikelijke subjectieve effecten worden op unipolaire VAS-schalen gemeten, waarbij de intensiteit van een effect wordt gescoord van afwezig (0 mm) tot extreem (100 mm). Een proefpersoon wordt tijdens een studiedag herhaaldelijk gevraagd om op deze lijnen aan te geven hoe hij of zij zich voelt. Tijdens de analyse die beschreven wordt in dit hoofdstuk is gekeken naar onderliggende verbanden in de reactie op twee veelgebruikte sets van VAS-schalen na toediening van THC.

Om dit te doen zijn de gegevens van tien studies, die zijn uitgevoerd op het CHDR, gebruikt. Bij al deze studies werd THC toegediend aan gezonde vrijwilligers (217 in totaal). Er waren in totaal 29 verschillende VAS-schalen: 16 die kijken naar stemming, alertheid en kalmte en 13 die kijken naar psychedelische effecten. Er zijn verschillende statistische methodes gebruikt om onderliggende verbanden in de reactie op deze VAS-schalen te ontdekken. Elke methode heeft zijn eigen voordelen en bij deze analyse is dan ook gekeken naar wat deze verschillende methodes met elkaar gemeen hadden.

Dit heeft geresulteerd in een drietal effect clusters die het subjectieve effect van THC beschrijven: 'waarneming', 'ontspanning' en 'dysforie'. Het cluster 'waarneming' omvat het 'high' gevoel van THC, veranderingen in de tijdswaarneming en het beheersen van de eigen gedachten. 'Ontspanning' bestaat uit de mentale ontspanning die ervaren wordt na toediening van THC. Het cluster 'dysforie' gaat in op de meer negatieve reacties die bij een klein deel van de proefpersonen plaatsvond. Het gaat dan om het horen van stemmen, achterdocht of waanachtige denkbeelden. Deze waren overigens

zelfs als ze aanwezig waren, mild van aard. Waarschijnlijk bevatten deze VAS-schalen niet alle subjectieve effecten van THC. Zo kunnen bijvoorbeeld honger of trek en aspecten van angst en paniek gemist zijn. Desondanks beschrijven de samengestelde schalen een scala aan THC-effecten waar de meeste psychose-achtige effecten onder vallen. Deze samengestelde schalen kunnen dan ook gebruikt worden om aspecten van psychomimetische gevoeligheid of de effecten van antipsychotica te onderzoeken.

Hoofdstuk 4: De invloed van persoonlijkheid op de gevoeligheid voor de subjectieve effecten van THC

De drie cluster van subjectieve effecten die zijn gevonden in het vorige hoofdstuk worden gebruikt om de gevoeligheid van milde cannabis gebruikers voor THC te onderzoeken. In dit hoofdstuk werd opnieuw gebruik gemaakt van gegevens die tijdens eerdere studies al verzameld zijn. Van zeven verschillende studies, waarbij THC via inhalatie werd toegediend, zijn de gegevens samengevoegd. Eerder was van deze data al een farmacokinetisch model gemaakt, dat het beloop van THC-concentraties in het bloed beschrijft na de toediening. Dit model is uitgebreid door ook de concentraties van de actieve metaboliet (11-OH-THC) te beschrijven. Daarnaast is een wiskundig model ontwikkeld om de relatie tussen THC concentraties in het plasma op de drie clusters van subjectieve effecten te beschrijven.

Uit dit wiskundige model kunnen voor elke proefpersoon parameters worden afgeleid die informatie geven over de gevoeligheid van die persoon voor THC. Deze maat voor gevoeligheid is vervolgens vergeleken met scores op verschillende subschalen van een vragenlijst voor persoonlijkheid (de TCI) met behulp van meervoudige lineaire regressie. Hieruit blijkt dat het cluster 'waarneming' beïnvloedt wordt door de persoonlijkheidseigenschap 'leedvermijdend' en het cluster 'dysforie' door 'zelftranscendent'. Volgens het onderliggende psychobiologische model van persoonlijkheid wordt de eigenschap 'leedvermijdend' geregeld door de neurotransmitter serotonine. Gezien de effecten van serotonerge middelen als LSD en psilocybine (de

werkzame stof in paddo's) is het niet verrassend dat dit een verband houdt met veranderingen van de 'waarneming'. Het endocannabinoïd systeem is betrokken bij de regulatie en afstemming van verschillende neurotransmitter systemen, waaronder serotonine. Hogere 'zelftranscendent' scores hangen samen met schizotypie en het is dan ook niet verrassend dat mensen met hogere scores gevoeliger zijn voor de dysfore effecten van THC. Deze resultaten geven meer inzicht in de neurofarmacologische systemen die aan persoonlijkheidskenmerken ten grondslag liggen.

Hoofdstuk 5: Het optimaliseren van het glutamaterge challenge model voor psychose: S(+)-ketamine voor het induceren van psychomimetische symptomen bij gezonde vrijwilligers

Ketamine is een NMDA-receptor antagonist die vooral in de anesthesie wordt gebruikt. Omdat de stof bij lagere concentraties psychomimetische bijwerkingen heeft, wordt ketamine tevens gebruikt als psychose model. De studies die dit model beschrijven, gebruiken verschillende doseringen ketamine, meten het effect maar op één tijdpunt (wisselend t.o.v. het tijdstip van doseren) en meten geen ketamine-concentraties in het bloed. In deze studie wordt het model dan ook verder uitgewerkt, met het doel om de relaties tussen de ketamine-concentratie in het bloed en de psychomimetische effecten enerzijds en ongewenste bijwerkingen (die de verdraagbaarheid beïnvloeden) anderzijds te beschrijven.

Deze studie bestond uit drie studiedagen, waarbij gedurende twee uur een constante S(+)-ketamine-concentratie in het bloed werd verkregen (respectievelijk een hoge concentratie, een lage concentratie en placebo). Dit werd gedaan door een infusieschema te berekenen aan de hand van een eerder beschreven farmacokinetisch model. In het eerste deel van de studie waren de beoogde ketamine-concentraties 360 ng/mL, 180 ng/mL en 0 ng/mL. Met name de hoge concentratie leidde tot veel bijwerkingen

bij de proefpersonen en de studie werd tijdelijk stopgezet om de beoogde concentraties te herzien. In dit eerste deel zijn 6 mannelijke proefpersonen gedoseerd. Na de tijdelijke stop werd de studie hervat met als beoogde ketamine-concentraties 240 ng/mL, 120 ng/mL en 0 ng/mL. In het infusieschema werd tevens rekening gehouden met de aanmaak van de actieve metaboliet norketamine, die ongeveer een derde van de activiteit van de moederstof heeft. Aan dit tweede deel van de studie namen 30 proefpersonen deel (15 mannen en 15 vrouwen). Net als in Hoofdstuk 2 werden veel verschillende metingen gedaan voor, tijdens en na de infusie en opnieuw was de PANSS de belangrijkste uitkomstmaat.

Er werd een dosis-afhankelijke, robuste toename in psychomimetische symptomen gezien op alle subschalen van de PANSS. De positieve PANSS steeg met 43.7% voor de 120 ng/mL concentratie en met 70.5% voor de 240 ng/mL concentratie vergeleken met placebo. Ook op de meeste andere maten werd een dosis-afhankelijk effect gezien. Aan de hand van de bijwerkingen (en dus vanuit het oogpunt van verdraagbaarheid) wordt een concentratie tot ongeveer 200 ng/mL aangeraden voor het gebruik van ketamine. Dit concentratie-bereik is ruim voldoende om psychomimetische effecten op te wekken en er is dan dus ook nog ruimte voor het verminderen van deze effecten met medicatie.

Hoofdstuk 6: Ontwikkeling en evaluatie van een nieuwe VAS-schaal voor psychedelische effecten

Zoals al gezien in Hoofdstuk 3, worden VAS-schalen veel gebruikt om subjectieve gevoelens te kwantificeren. Er zitten echter ook een aantal beperkingen aan de VAS-schalen. Zo zijn psychedelische effecten afwezig onder normale omstandigheden, wat leidt tot een score van 0 (zonder noemenswaardige variatie) tijdens placebo dagen. Daarnaast lijkt er sprake te zijn van een 'drempel' bij het meten van psychedelische effecten. Om deze redenen is een nieuwe set van VAS-schalen ontwikkeld voor het meten van psychedelische effecten. Er is hierbij gekozen om niet een effect per

VAS-schaal te meten (van 'helemaal niet' tot 'heel erg'), maar twee tegenovergestelde subjectieve effecten (zoals 'ik voel me verdrietig' tot 'ik voel me vrolijk').

Deze nieuwe set VAS-schalen is, samen met de oorspronkelijke set en een aantal vergelijkbare vragenlijsten, gemeten tijdens de studie die is beschreven in Hoofdstuk 5. Tijdens de evaluatie is eerst gekeken welke VAS-schalen reageerden op de ketamine-challenge. Daarna is binnen de VAS-schalen die reageerden gekeken of er onderliggende verbanden te ontdekken zijn, waaruit twee subschalen zijn samengesteld. Deze subschalen waren niet beter in het onderscheid maken tussen de verschillende concentraties ketamine. Bovendien richtten de subschalen zich meer op geestelijke en lichamelijke ontspanning en minder op de typische psychomimetische effecten waar de schaal voor bedoeld was. Het wordt dan ook niet aangeraden om deze nieuwe VAS-schalen te gebruiken in klinische studies.

Hoofdstuk 7: Subjectieve effecten van een farmacologische challenge met ethanol, morfine, THC en ketamine zijn gerelateerd aan functionele hersen connectiviteit

Om te onderzoeken welke hersengebieden een rol spelen bij het ontstaan van de psychomimetische effecten van een farmacologische challenge, werden de gegevens van eerdere studies met farmacologische challenges met ethanol (alcohol), morfine, THC en ketamine met elkaar vergeleken. Bij al deze studies werd gekeken naar de veranderingen over de tijd, gemeten in een MRI-scanner tijdens rust. Met deze metingen in de MRI-scanner kan gekeken worden naar 'functionele connectiviteit', ofwel de mate waarin verschillende delen van de hersenen synchroon met elkaar activeren of deactiveren. Daarnaast werden bij al deze studies de subjectieve effecten als gevolg van de farmacologisch challenge gemeten met VAS-schalen.

Per geneesmiddel waren de gegevens van steeds 12 gezonde, mannelijke proefpersonen beschikbaar. De verschillen in functionele connectiviteit

werden gerelateerd aan de verschillen op de drie subjectieve clusters die zijn beschreven in Hoofdstuk 3 ('waarneming', 'ontspanning' en 'dysforie'). Uit de analyse bleek dat een cluster, gelegen in de 'achterste gordelwinding' (latijn: gyrus cinguli posterior) en de 'voorste centrale winding' (latijn: gyrus praecentralis) een statistisch significante relatie heeft tussen verschillen in functionele connectiviteit en veranderingen in subjectieve 'waarneming'. Van ditzelfde hersengebied is in eerdere studies aangetoond dat bij patiënten met schizofrenie verschillen in functionele connectiviteit gerelateerd zijn aan positieve psychotische symptomen (zoals hallucinaties en waanbeelden).

Hoofdstuk 8: Discussie

Er zijn verschillende farmacologische challenge-modellen die gebruikt kunnen worden als model voor psychose en als model voor de werking van medicijnen tegen psychose. Deze farmacologische challenge-modellen werken via verschillende neurotransmittersystemen. Voor al deze middelen is aangetoond dat antipsychotica de opgewekte psychomimetische symptomen kunnen onderdrukken. Het is belangrijk te onderschrijven dat deze verschillende neurotransmittersystemen niet los van elkaar opereren, maar dat er sprake is van ingewikkelde interacties tussen de verschillende neurotransmittersystemen. Er zijn verschillende hypotheses over de pathofysiologie van schizofrenie die de rol van deze verschillende neurotransmittersytemen met elkaar verenigen. De rol van dopamine in deze hypotheses is wisselend, maar het is duidelijk dat ook andere neurotransmittersytemen een rol spelen bij de ontwikkeling van schizofrenie.

Professor van Gerven (mijn promotor) beschreef tijdens zijn inaugurele rede (latijn: oratio) vijf voorwaarden waaraan een farmacologisch challenge-model moet voldoen.

Allereerst dient een goed ziektemodel te lijken op de ziekteverschijnselen. De psychomimetische symptomen die worden opgewekt door THC en

ketamine hebben onmiskenbaar overeenkomsten met symptomen van psychose. Verschillen zijn er echter ook. Zo houden proefpersonen inzicht in het ontstaan van symptomen, terwijl patiënten met een psychose dit inzicht verliezen. Als proefpersonen niet uitgelegd zou worden dat zij deze geneesmiddelen toegediend krijgen is het beeld overigens vergelijkbaar met het toestandsbeeld van (acute) schizofrenie. Daarnaast is de verhouding tussen akoestische (auditieve) en visuele hallucinaties niet hetzelfde bij proefpersonen en patiënten, hetgeen mogelijk verklaard kan worden door onderrapportage van hallucinaties bij patiënten. Het feit dat de psychomimetische symptomen van een farmacologische challenge onderdrukt worden door antipsychotische medicatie is een sterk argument voor de validiteit van de farmacologische challenge als model voor psychose. Ook de bevinding dat het hersengebied dat een relatie laat zien tussen functionele connectiviteit en psychomimetische symptomen ook bij patiënten met schizofrenie een relatie vertoond tussen functionele connectiviteit en positieve psychotische symptomen ondersteunt de rol van farmacologische challenges als model voor psychose.

De tweede voorwaarde voor een farmacologische challenge is een bekend farmacologisch werkingsmechanisme. Van zowel THC als ketamine is het werkingsmechanisme bekend (al is dit wel verschillend) en er bestaan modellen voor de farmacokinetiek. In dit proefschrift wordt tevens een farmacokinetisch / farmacodynamisch model beschreven voor de subjectieve effecten van THC, wat een indicatie geeft dat deze subjectieve effecten een farmacologische relatie hebben met de activiteit van het (endo-)cannabinoid systeem.

Als derde voorwaarde geldt dat de effecten meetbaar moeten zijn. In dit proefschrift is veel aandacht besteedt aan dit aspect van challenge test ontwikkeling. Psychose is een complex ziektebeeld, waarbij normale aspecten van bewustzijn en gedrag verschuiven naar het extreme en waarbij zich nieuwe subjectieve belevenissen voordoen die normaal gesproken niet voorkomen bij gezonde mensen. Deze mengeling van kwantitatieve en kwalitatieve abnormale geestelijke toestandsbeelden bemoeilijken het

gebruik van geschikte meetinstrumenten voor onderzoek. Het is voor een psychomimetische challenge test belangrijk dat proefpersonen zich bewust blijven van hun veranderd toestandsbeeld. Doordat proefpersonen inzicht houden kunnen ze hun ervaringen namelijk makkelijker delen met de onderzoeker en meewerken aan verschillende metingen om het effect te kwantificeren. De PANSS bleek een geschikt middel om de psychomimetische effecten te meten en omdat dit een klinisch gebruikte schaal is zijn de gemeten symptomen makkelijk te relateren aan de symptomen van schizofrenie. Ook de VAS-schalen zijn geschikt om de subjectieve effecten van een farmacologische challenge te meten. In dit proefschrift wordt hier verdere invulling aan gegeven door het voorstellen van drie clusters van subjectieve effecten, waarvan tevens een farmacokinetisch / farmacodynamisch model is gemaakt. Ook is geprobeerd een verbeterde VAS voor psychedelische effecten samen te stellen, hetgeen niet gelukt is.

De vierde voorwaarde voor een farmacologische challenge-test is dat sprake moet zijn van een dosis- of concentratie-afhankelijk effect. Voor THC en ketamine is aangetoond dat de geïnduceerde effecten concentratie-afhankelijk zijn.

Als vijfde voorwaarde geldt dat een farmacologische challenge-test veilig en verdraagbaar moet zijn. Bij de doseringen van THC die gebruikt zijn tijdens de verschillende studies bleek dat THC veilig en verdraagbaar is. Bij het eerste deel van de studie met het ketamine model bleek dat de hoogst beoogde concentratie niet langer verdraagbaar was. Na een dosisaanpassing kon echter een duidelijk bereik in concentraties worden vastgesteld waarin het toedienen van ketamine verdraagbaar is, terwijl ook een gewenst psychomimetisch effect wordt bereikt. Ook aan deze voorwaarde wordt dus door beide modellen voldaan.

Conclusie

Farmacologische challenge tests met psychomimetische middelen geven geen volledig beeld van de complexiteit van (chronische) schizofrenie,

maar vormen wel een nuttig model voor psychose en antipsychotische activiteit. Dit proefschrift beschrijft het gebruik van THC en ketamine als model en verkent verschillende uitkomstmaten die gebruikt kunnen worden om psychomimetische effecten te kwantificeren (in het bijzonder VAS-schalen en FMRI). De farmacologische basis en het fenomeen van de psychomimetische challenge test als model voor psychose werden besproken. Goed gecontroleerde onderzoeksomstandigheden en het herhaaldelijk meten van geneesmiddelconcentraties en effect leiden tot een grote verbetering bij het gebruik van farmacologische challenge tests in de geneesmiddelontwikkeling.

CURRICULUM VITAE

Hendrik Daniël Kleinloog-Fernández González was born on August 7, 1983 in the city of 's-Hertogenbosch, The Netherlands. He grew up in the villages Haaften and Wijk en Aalburg together with his older brother and sister and his parents and completed his secondary eduction at the 'William of Orange College' in Waalwijk in 2001 and went on to study Medicine at Maastricht University. During his Medicine study he first came in contact with pharmacological research during a research internship at the Mario Negri Institute at Bergamo University in Italy where he studied the effect of adherence to antiretroviral therapy on the occurrence of mutations of the human immonodeficiency virus. After obtaining his medical degree (M.D.) in 2007 he started working as a psychiatric resident in the Intensive and Forensic Psychiatric Clinic of the Academic Medical Centre in Amsterdam. In 2008 he made his move to Leiden to start with an (unfinished) specialization in Psychiatry under supervision of Prof. Dr. F.G. Zitman in the Leiden University Medical Center (LUMC), which started with a rotation in social psychiatry at GGZ Leiden. After six months he commenced his PhD program at the Centre for Human Drug Research (CHDR) in Leiden, under supervision of Prof. Dr. J.M.A. van Gerven and Prof. Dr. A.C. Cohen. Whilst working as a Research Physician at CHDR, he completed a degree in clinical pharmacology in 2012. In April 2012 he joined the clinical research organisation P1vital in Oxford (United Kingdom) as Medical Director and was involved in early phase clinical trials for depressive disorders, with a large emphasis on neuroimaging studies, where he worked until 2013. Since October 2013 he works as resident on the Intensive Care Unit of the Tergooi hospital in Hilversum, after which he will commence with his specialisation in Anesthesiology under supervision of Prof. Dr. L.P.H.J. Aarts in the LUMC. Between 2004 and 2009, he was a board member of the Dutch Section of Amnesty International. Daniël met Alberto in 2005 and they got married in 2012. They currently live in Amsterdam.

LIST OF PUBLICATIONS

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