CANNABIS

· BEYOND GOOD AND EVIL ·

HOW GENETIC AND EPIDEMIOLOGICAL FACTORS SHAPE
THE RELATIONSHIP BETWEEN CANNABIS AND PSYCHOSIS

COLOFON

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Cannabis Beyond Good and Evil
How genetic and epidemiological factors shape the relationship between cannabis and psychosis.

Cannabis voorbij goed en kwaad
Hoe genetische en epidemiologische factoren de relatie tussen cannabis en psychose vormgeven.
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op
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door

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te Haarlem

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PROLOGUE

• CANNABIS BEYOND GOOD AND EVIL •

Prologue

Cannabis Beyond Good and Evil

In his late work “Beyond Good and Evil” Friedrich Nietzsche (Nietzsche, 1868) strives to overcome the traditional morality and values of the masses in a philosophical approach of morality that confronts the perspectival nature of knowledge, beyond Good and Evil.

In 1753, the Swedish Botanist Carl Linnaeus introduced the name Cannabis Sativa for a plant that had already been part of human culture since more than 4,000 years. Probably originating in central Asia, throughout the centuries cannabis has evoked extreme receptions in various cultures in different parts of the world. In the early Indo-Iranian (Aryan) culture (2,000 BC), in the medieval Arabic world but also on the African continent, cannabis played an important role in religious rituals and medical traditions. However, in the western world, particularly the United States, marijuana was demonized and prohibited in the 1930s but also idealized in the hippie subculture of the 1960s (Booth, 2003). Currently, cannabis extracts are used in modern western medicine to treat chronic pain and appetite loss. Paradoxically, cannabis is prohibited in most countries, particularly due to its alleged role as a cause of psychosis. Even within the boundaries of the debate on the role of cannabis as a causative agent of psychosis, idealization and demonization takes place. Public debate on cannabis tends to polarize towards the question of legalization. As polarization creates political power, pro- and anti-cannabis advocates tend to use scientific evidence opportunistically in the context of political debate. However, cannabis use and potential associated hazards are primarily a matter of public health and should be treated as such.

The causal nature of the association between cannabis use and psychosis in the general population is heavily debated. A leading argument in this debate is that the large majority of cannabis users do not develop psychosis. However, the use of cannabis has repeatedly been associated with an increased risk of developing a psychotic disorder (Moore et al., 2007). Particularly if used by vulnerable individuals in large quantities or at young age, when brain development is in full motion, this association seems to become more robust. Given the dramatic lack of opportunities for prevention of psychotic disorders, this is a extremely meaningful finding. Simultaneously, as discussed in this thesis, particular cannabis compounds (Cannabidiol), could prove to be a valuable antipsychotic agent, providing an opportunity to treat the same psychosis this cannabis plant might have partially caused.

The current thesis is the product of a quest for factors that shape this association and therefore an exploration of alternative positions. Cannabis most probably has the potential do good as well as harm (Murray et al., 2007). It would be in the interest of public mental health to leave behind traditional

polarized positions and move towards a strategy that optimizes the good and prevents potentially related harm, beyond Good and Evil.

Reference List

Booth,M., 2003. Cannabis, Transworld Publishers, London.

Moore,T.H., Zammit,S., Lingford-Hughes,A., Barnes,T.R., Jones,P.B., Burke,M. and Lewis,G., 2007. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet. 370, 319-328.

Murray,R.M., Morrison,P.D., Henquet,C. and Di,F.M., 2007. Cannabis, the mind and society: the hash realities. Nat Rev Neurosci. 8, 885-895.

Nietzsche,F., 1868. Voorbij goed en kwaad De Arbeiderspers, Amsterdam.

CHAPTER I

• GENERAL INTRODUCTION •

Introduction

Cannabis

With an estimated 170 million users, cannabis is the most frequently used illicit drug. In Asia (particularly China) and Africa the prevalence of cannabis use has increased rapidly over the last years (United Nations Office on Drugs and Crime, 2012). Recent national surveys in some western countries suggest that the prevalence of cannabis use is stabilizing, but large variations exist among regions and user groups. In Europe, the number of regular heavy users has increased by 20% between 2004 and 2007 (European Monitoring Centre for Drugs and Drugs Addiction (EMCDDA), 2009). Moreover, several reports show that cannabis use is largely confined to young people. In the United States, the estimated lifetime prevalence of cannabis use among persons aged 12 years or older is 46% (Substance Abuse and Mental Health Services Administration (SAMHSA), 2007). In the Netherlands, prevalence levels range from 26% to 32% among 15- to 16-year-old school students (European Monitoring Centre for Drugs and Drugs Addiction (EMCDDA), 2009).

The main psychoactive constituents of the cannabis plant are Δ9-tetrahydrocannabinol (THC) and Cannabidiol (CBD), however, these are only two of the approximately 70 phytocannabinoids that are found in the Cannabis sativa plant (Mechoulam et al., 2007). These phytocannabinoids are thought to interact with the human Endocannabinoid System which consists of endocannabinoids and at least five receptors of which CB1r and CB2r are best described (Brown, 2007;Pertwee, 2008).
Cannabis use is associated with a wide range of adverse effects in several areas, varying from traffic accidents, poor social and educational performance, to pulmonary disease (Asbridge et al., 2005;Macleod et al., 2004;Taylor et al., 2002). In the last decade, increased attention has been given to the psychiatric effects of cannabis use, especially the role of cannabis use as a risk factor for schizophrenia.

Schizophrenia

Schizophrenia is a highly invalidating disorder (van Os and Kapur, 2009), with a lifetime prevalence of 0.3 – 0.6% (McGrath et al., 2008). In the World Health Report 2001 (World Health Organization, 2001), schizophrenia is listed as the 8th leading cause of disability-adjusted life years (DALYs) worldwide in the age group 15-44 years. The symptoms of schizophrenia include hallucinations, delusions, psychomotor poverty, depression and anxiety (van Os and Kapur, 2009).

Genetics of schizophrenia
Data from family, twin, and adoption studies unequivocally demonstrate the involvement of genetic factors in the transmission of vulnerability to schizophrenia. Extensive research in the last decade has provided strong molecular evidence that common genetic variants (with a modest effect on disease status) in combination with rare variants (with high impact on disease risk), play a major role in the genetic architecture of schizophrenia (Gejman et al., 2011).

Environmental risk factors
Simultaneously, a number of non-genetic, environmental risk factors have been identified and replicated in the last decade. Perinatal complications (Cannon et al., 2002), migration (Cantor-Graae and Selten, 2005), urbanicity (Krabbendam and van, 2005), paternal age (Malaspina et al., 2001;Vreeker et al., 2013) and cannabis use (Moore et al., 2007;Zammit et al., 2002) seem to have impact on neural development and are consistently associated with schizophrenia.

The relationship between cannabis and psychosis
Cannabis use is implicated as a risk factor for psychotic illness in different lines of aetiological research. First, several studies show that cannabis use decreases the age of schizophrenia onset (Myles et al., 2012;Large et al., 2011). Second, cannabis use was found to increase the amount and severity of psychotic exacerbations in schizophrenia patients (Grech et al., 2005). Third, a growing body of literature relates cannabis use to decrease in brain volume in schizophrenia patients (Rapp et al., 2012). Finally, from an epidemiological perspective, cannabis use is consistently associated with psychotic symptoms in the general population (Schubart et al., 2010a) and a higher rate of psychotic disorders in numerous studies (Zammit et al., 2002;van Os et al., 2002;Manrique-Garcia et al., 2012). However, data on the causal role of cannabis in the aetiology of schizophrenia is not uniform and conflicting results have also been published (Macleod et al., 2004;Moore et al., 2007;DeLisi, 2008;Minozzi et al., 2010).

Our understanding of the role of the individual risk factors and their interplay in this disease process is however limited. Although in the last decades a variety of hypotheses have been put forward (van Os and Kapur, 2009), to date no explanatory model exists that integrates the associated risk factors and known biological processes.

A leading argument in the discussion on the alleged causal role of cannabis in causing psychosis is that the majority of cannabis users do not develop schizophrenia and that the explained variation in the occurrence of schizophrenia due to cannabis use, is low.

Therefore it is plausible that the association between cannabis use and schizophrenia depends on individual vulnerability that might be shaped by genetic and non-genetic (epidemiological) determinants.

Goal of this thesis
The objective of this study is to contribute to the identification of epidemiological and genetic factors that moderate the influence of cannabis on the risk to develop psychosis. Understanding the complex interplay between cannabis, environmental variables, genes and psychosis could lead to a better understanding of the aetiology of psychotic disorders, such as schizophrenia, and could provide new leads for molecular studies with the ultimate goal of improved detection, prevention and treatment.

Outline
This thesis addresses two main topics. The first part discusses epidemiological aspects of the relationship between cannabis use and psychiatric symptoms. The second part focuses on genetic aspects of this relationship. For a visual depiction of the outline of this thesis, see figure 1.

The first study (**chapter 2**) focuses on effects of cannabis use on mental illness, regardless of diagnosis. The measure of mental health in this study is a history of psychiatric hospitalization. The main question is if dosage and age at first use of cannabis are associated with the chance of having a history of psychiatric hospitalizations.

The second study (**chapter 3**) investigates whether cannabis starting age and level of exposure (dose) are associated with specific profiles of subclinical psychiatric (psychotic) symptoms in the general population.

Chapter 4 is a literature review on the antipsychotic potential of cannabidiol. Cannabidiol is one of the 70 phytocannabinoids, detectable in the cannabis plant. Although cannabis use is associated with an increased risk of developing psychosis, the cannabis constituent cannabidiol may have antipsychotic properties. A number of studies have tried to explain the role of the endocannabinoid system in the development of psychosis and how cannabidiol might impact on this relationship. We review animal, human experimental, imaging, epidemiological and finally clinical studies that investigated the antipsychotic properties of cannabidiol.

In **chapter 5**, we tested the role of cannabidiol content in the association between cannabis use and psychiatric symptoms in our own sample. Different types of cannabis (i.e. marijuana, hashish) have distinctive proportions of THC and cannabidiol. Since average concentrations of THC and cannabidiol in the most popular types of cannabis sold on the Dutch market are annually measured, we were able to quantify exposure to THC and cannabidiol. We

assessed the association between cannabidiol content and sub-clinical psychiatric symptoms in a subset of 1,877 participants.

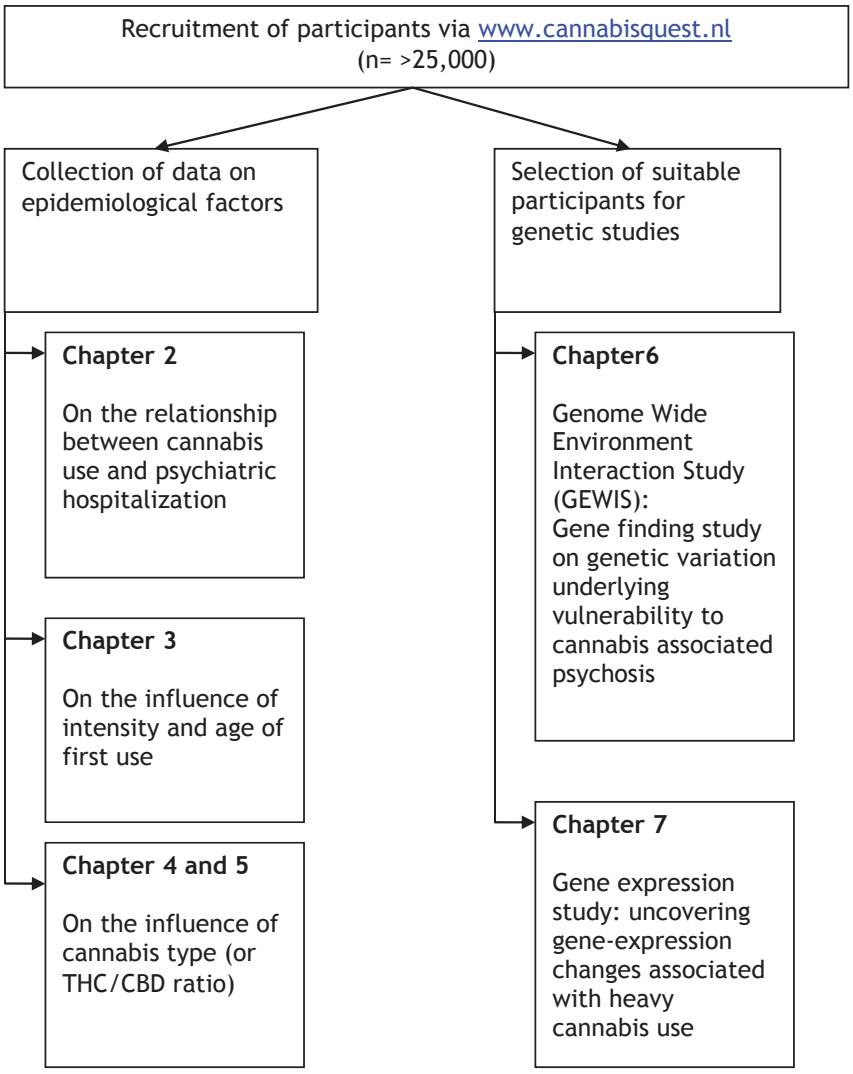
In **part II** of this thesis, we describe our efforts to understand the biological mechanisms that underlie the relationship between cannabis use and schizophrenia.

Chapter 6 describes a gene finding study designed to identify genetic polymorphisms that moderate the risk of experiencing subclinical psychiatric symptoms in heavy cannabis users. In 1,262 participants, we performed a Genome Wide Environment Interaction Study (GWEIS) that aims to imply information on cannabis use as a risk factor of psychosis in a genome wide gene finding approach.

In **chapter 7** we used genome wide gene expression profiling to gain further insight into molecular mechanisms driving the effects that are associated with cannabis use. In 100 healthy controls and 90 heavy cannabis users, we measured genome-wide expression levels from whole blood samples.

Finally, **in part III**, **chapter 8** provides a summary and general discussion of the main findings of this thesis and **chapter 9** is a summary of this thesis in Dutch.

Table 1. Olutline of this thesis



Reference List

Asbridge,M., Poulin,C. and Donato,A., 2005. Motor vehicle collision risk and driving under the influence of cannabis: evidence from adolescents in Atlantic Canada. Accid Anal Prev. 37, 1025-1034.

Brown,A.J., 2007. Novel cannabinoid receptors. Br J Pharmacol. 152, 567-575.

Cannon,M., Jones,P.B. and Murray,R.M., 2002. Obstetric complications and schizophrenia: historical and meta-analytic review. Am J Psychiatry. 159, 1080-1092.

Cantor-Graae,E. and Selten,J.P., 2005. Schizophrenia and migration: a meta-analysis and review. Am J Psychiatry. 162, 12-24.

DeLisi,L.E., 2008. The effect of cannabis on the brain: can it cause brain anomalies that lead to increased risk for schizophrenia? Curr Opin Psychiatry. 21, 140-150.

European Monitoring Centre for Drugs and Drugs Addiction (EMCDDA). Annual Report 2008: the State of the Drugs Problem in the European Union and Norway. Lisbon: EMCDDA . 2009.

Gejman,P.V., Sanders,A.R. and Kendler,K.S., 2011. Genetics of schizophrenia: new findings and challenges. Annu Rev Genomics Hum Genet. 12, 121-144.

Grech,A., van Os,J., Jones,P.B., Lewis,S.W. and Murray,R.M., 2005. Cannabis use and outcome of recent onset psychosis. Eur Psychiatry. 20, 349-353.

Krabbendam,L. and van,O.J., 2005. Schizophrenia and urbanicity: a major environmental influence--conditional on genetic risk. Schizophr Bull. 31, 795-799.

Large,M., Sharma,S., Compton,M.T., Slade,T. and Nielssen,O., 2011. Cannabis use and earlier onset of psychosis: a systematic meta-analysis. Arch Gen Psychiatry. 68, 555-561.

Macleod,J., Oakes,R., Copello,A., Crome,I., Egger,M., Hickman,M., Oppenkowski,T., Stokes-Lampard,H. and Davey,S.G., 2004. Psychological and social sequelae of cannabis and other illicit drug use by young people: a systematic review of longitudinal, general population studies. Lancet. 363, 1579-1588.

Malaspina,D., Harlap,S., Fennig,S., Heiman,D., Nahon,D., Feldman,D. and Susser,E.S., 2001. Advancing paternal age and the risk of schizophrenia. Arch Gen Psychiatry. 58, 361-367.

Manrique-Garcia,E., Zammit,S., Dalman,C., Hemmingsson,T., Andreasson,S. and Allebeck,P., 2012. Cannabis, schizophrenia and other non-affective psychoses: 35 years of follow-up of a population-based cohort. Psychol Med. 42, 1321-1328.

McGrath,J., Saha,S., Chant,D. and Welham,J., 2008. Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiol Rev. 30, 67-76.

Mechoulam,R., Peters,M., Murillo-Rodriguez,E. and Hanus,L.O., 2007. Cannabidiol--recent advances. Chem Biodivers. 4, 1678-1692.

Minozzi,S., Davoli,M., Bargagli,A.M., Amato,L., Vecchi,S. and Perucci,C.A., 2010. An overview of systematic reviews on cannabis and psychosis: discussing apparently conflicting results. Drug Alcohol Rev. 29, 304-317.

Moore,T.H., Zammit,S., Lingford-Hughes,A., Barnes,T.R., Jones,P.B., Burke,M. and Lewis,G., 2007. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet. 370, 319-328.

Myles,N., Newall,H., Nielssen,O. and Large,M., 2012. The association between cannabis use and earlier age at onset of schizophrenia and other psychoses: meta-analysis of possible confounding factors. Curr Pharm Des.

Pertwee,R.G., 2008. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. Br J Pharmacol. 153, 199-215.

Rapp,C., Bugra,H., Riecher-Rossler,A., Tamagni,C. and Borgwardt,S., 2012. Effects of cannabis use on human brain structure in psychosis: a systematic review combining in vivo structural neuroimaging and post mortem studies. Curr Pharm Des. 18, 5070-5080.

Schubart,C.D., van Gastel,W.A., Breetvelt,E.J., Beetz,S.L., Ophoff,R.A., Sommer,I.E., Kahn,R.S. and Boks,M.P. Cannabis use at young age is associated with psychotic experiences. Psychological Medicine . 2010. Oct 7. 1-10.

Substance Abuse and Mental Health Services Administration (SAMHSA). Results from the 2007 National survey on drug use and health. Maryland, Rockville. 2007. Office of Applied Studies (OAS), DEPARTMENT OF HEALTH & HUMAN SERVICES.

Taylor,D.R., Fergusson,D.M., Milne,B.J., Horwood,L.J., Moffitt,T.E., Sears,M.R. and Poulton,R., 2002. A longitudinal study of the effects of tobacco and cannabis exposure on lung function in young adults. Addiction. 97, 1055-1061.

United Nations Office on Drugs and Crime. World Drug Report 2012. 2012. Vienna, United Nations Publications.

van Os,J., Bak,M., Hanssen,M., Bijl,R.V., de,G.R. and Verdoux,H., 2002. Cannabis use and psychosis: a longitudinal population-based study. Am J Epidemiol. 156, 319-327.

van Os,J. and Kapur,S., 2009. Schizophrenia. Lancet. 374, 635-645.

Vreeker,A., Schubart,C.D., van Gastel,W.A., Kahn,R.S. and Boks,M.P., 2013. Advanced paternal age and vulnerability to psychotic-like experiences in the offspring. Schizophr Res. 143, 74-76.

World Health Organization. World Health report 2001, Mental Health: New Understanding, New Hope. 2001. Geneva, World Health Organization.
Ref Type: Report

Zammit,S., Allebeck,P., Andreasson,S., Lundberg,I. and Lewis,G., 2002. Self reported cannabis use as a risk factor for schizophrenia in Swedish conscripts of 1969: historical cohort study. BMJ. 325, 1199.

PART I

EPIDEMIOLOGICAL FACTORS

PART I: EPIDEMIOLOGICAL FACTORS
CHAPTER II

• ASSOCIATION BETWEEN CANNABIS AND PSYCHIATRIC HOSPITALIZATION •

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ABSTRACT

Objectives
To investigate the relationship between cannabis use and mental health.
Methods
A cross-sectional analysis in a sample of 17,698 individuals with a mean age of 22 years (SD: 4.2). Participants provided information on the amount and initial age of cannabis use and history of psychiatric hospitalizations through a web-based questionnaire. To quantify THC exposure we operationalised cannabis use as the amount of money spent on cannabis per week over the last month. The odds ratio of having a history of psychiatric hospitalizations was the primary outcome measure.
Results
We found a dose-response relationship between the amount of cannabis use and the odds for psychiatric hospitalisation. Adjusted odds ratios for hospitalization increased with the amount of cannabis consumed from 1.6 (95% CI: 1.1 – 2.3) in incidental users to 6.2 (95% CI: 4.3 – 8.9) in heavy users (>€25/week). Our data suggested that concomitant drug use was an intermediate factor. Exposure to cannabis before the age of 12 years was found to carry a 4.8 (95% CI: 2.9 – 7.8) times increased odds for past psychiatric hospitalizations.
Conclusion
We conclude that early and heavy uses of cannabis are each and independently associated with poor mental health in its users.

INTRODUCTION

Cannabis is the most frequently used illicit substance in most parts of the world, the number of cannabis users worldwide is currently estimated to be more than 165 million (United Nations Office on Drugs and Crime, 2009). In Asia (particularly China) and Africa the prevalence of cannabis use has increased rapidly over the last few years(United Nations Office on Drugs and Crime, 2009). Recent national surveys in some western countries suggest that the prevalence of cannabis use is stabilizing, but large variations exist among regions and user groups. In Europe for example, the number of regular heavy users has increased by 20% between 2004 and 2007 (European Monitoring Centre for Drugs and Drugs Addiction (EMCDDA), 2009). Moreover, several reports show that cannabis use is largely concentrated in young people. In the United States, the estimated lifetime prevalence of cannabis use among persons aged 12 years or older is 46% (Substance Abuse and Mental Health Services Administration (SAMHSA), 2007). In the Netherlands prevalence levels range from 26% to 32% among 15- to 16-year-old school students (European Monitoring Centre for Drugs and Drugs Addiction (EMCDDA), 2009). Several reports suggest that the age of first cannabis use in the population is decreasing (European Monitoring Centre for Drugs and Drugs Addiction (EMCDDA), 2009;Johnston et al., 2009;ESPAD, 2007).

Cannabis use is associated with a wide range of adverse effects in several areas, varying from traffic accidents, poor social and educational performance, to pulmonary disease (Asbridge et al., 2005;Macleod et al., 2004;Taylor et al., 2002). In the last decade, increased attention has been given to the psychiatric effects of cannabis use. Especially the role of cannabis use as a risk factor for psychotic disorders has recently been highlighted. It is now clear that cannabis use is associated with psychotic disorders (Moore et al., 2007;Pedersen, 2008;Satyanarayana, 2009;Skinner et al., 2010;Schubart et al., 2010a) and although the debate on causality is ongoing, an argument can be made that the public should be warned of this danger (Nordentoft and Hjorthoj, 2007). In addition to psychosis, associations between cannabis use and affective illness (Bovasso, 2001;Pedersen, 2008;Degenhardt et al., 2003), impairment of cognitive function (Ranganathan and D'Souza, 2006;D'Souza et al., 2005) and cannabis addiction (Degenhardt et al., 2008;Gillespie et al., 2009) have been reported. Less is known about the mental health burden associated with cannabis at a population level such as hospitalization rates of its users. Psychiatric hospitalization is a key indicator of the individual, social and economic burden of mental illness. Not only is psychiatric hospitalization associated with severe psychopathology, but also with recidivism (Klinkenberg and Calsyn, 1998), occupational disability (Kessler and Frank, 1997) and enduring psychiatric illness (Hoge et al., 2005). Moreover, psychiatric hospitalization is costly. The direct costs of mental health services in the US sum up to a total of more than $70.0 billion of which 27% is due to the costs of inpatient care (U.S.Department of Health and Human Services., 1999;Mark T et al., 1998). Likewise, in the United Kingdom the costs of inpatient care constitute 80% of NHS total expenditure on adult mental health services (NHS

Mental Health Network, 2009). In England alone the costs of acute adult inpatient mental health care were more than £600 millions in 2008/2009 (Mental Health Strategies, 2009).
To investigate the burden of diseases associated with cannabis use, we studied the odds of psychiatric hospitalization in relation to the amount and the initial age of cannabis use in a sample of 18,000, mainly adolescent, individuals.

MATERIAL AND METHODS

Study Population

Between June 2006 and February 2009, every month approximately 670 visitors filled out an internet-based questionnaire, resulting in more than 21,000 participants at the time of writing. Broad media attention for the project was generated and cooperation was sought with colleges and universities that were willing to advertise for this study on their intranet. Especially adolescents between 18 and 26 years of age were targeted in this advertisement campaign. In addition, commercial advertisement products (i.e. banners and text links) were used on websites that were popular among young people. The chance to win an Apple iPod ™ or a Nintendo Wii ™, was used as an incentive (Aadahl and Jorgensen, 2003). To increase the homogeneity of the sample we selected participants between the age of 10 and 60 years of age.
This study was approved by the medical ethical commission of the University Medical Centre Utrecht and all participants gave online informed consent.

Assessments

Subjects provided their name, age, educational level, ethnicity, psychiatric medical history and contact details. Each participant was asked; “Have you ever been admitted to a psychiatric ward?”. Participants could answer this question with yes or no. The assessment included two verification questions to protect for random answers. Submitting data anonymously was not possible. Participants provided their information through a project website (www.cannabisquest.nl). Web-based questionnaires are found to be reliable and useful for epidemiologic research purposes in settings in which internet access is high (Ekman et al., 2006). In 2008, 91% of the Dutch had internet access available at their homes (CBS Statistics Netherlands, 2009), among young people this number is estimated to be 99% (IVO: Addiction Institute et al., 2009).

Cannabis Measures

The amount in euros (€) spent on cannabis per week in the last month, was assessed as a proxy measure of exposure to Δ^9-tetrahydrocannabinol (THC). THC-concentration and market value of cannabis are correlated (0.365 ($p < 0.001$) in marijuana and 0.719 ($p < 0.001$) in hashish) (Niesink et al., 2009a). Therefore, the amount of money spent on cannabis provides a superior measure of exposure compared to frequency assessments of cannabis use . THC exposure was categorized into five groups: 1) cannabis naïve persons who indicated never to have used cannabis; 2) subjects using cannabis incidentally

or spending less than 3 euros per week; 3) individuals spending between 3-10 euros per week on cannabis; 4) subjects spending between 10-25 euros per week; and 5) a group of subjects spending more than 25 euros per week on cannabis. All data (except for the first two groups) applied to the last month or longer.
For reference, prices range from €4.30 for one gram of imported marijuana with an average THC percentage of 5.5% to €15, - per gram of Dutch hashish with an average THC concentration of 33.3% (29). The initial age of cannabis use was also categorized in five sub groups; 1) participants who started to use before the age of 12 years; 2) between 12 and 15 years; 3) between 15 and 18 years; 4) between the age of 18 to 20 years; and 5) after their 20th birthday. Finally, to add a further measure distinguishing between incidental and regular cannabis users, participants were asked how many times they had used cannabis in their lives, with a minimum score of zero and a maximum score of more than ten times.

Statistical Analysis
Odds ratios with 95% confidence intervals for having a psychiatric hospitalization in the medical history were calculated per range of cannabis use by means of logistic regression analysis. Two analyses were conducted: 1) estimating the crude association between cannabis use and psychiatric hospitalization, and 2) estimating this association after adjustment for age, gender and level of education. The cannabis naïve group was the reference group. To estimate the relation between cannabis use and hospitalization for the different categories of age of initial use, again logistic regression analysis was applied. The group with a modal initial age of use (15 -18 years) was chosen as the reference group. Moreover, as an overall test to analyze the proportion of hospitalizations among participants who had and who had not used cannabis more than ten times in their lives, we calculated the crude odds ratio, with 95% confidence intervals, for having used cannabis more or less frequently than ten times. To assess the sensitivity of our results to selection bias we estimated the impact of a hypothetical decrease in the number of hospitalized heavy users on the adjusted odds ratios in the heavy users group. Randomly, a predefined fraction of the hospitalized heavy users was excluded and the association between cannabis use and hospitalization was estimated in the remaining subjects. This procedure was repeated 1,000 times for each predefined fraction and odds ratios and their confidence intervals were pooled using Rubin's rule (Rubin, 1987).
To investigate the influence of concomitant drug use on the association between cannabis use and psychiatric hospitalization, the first 13,000 participants were asked to fill in a number of additional questionnaires on various topics. A sub sample of 816 participants completed the additional questionnaires on concomitant drug use. This sub sample comprised a representative selection in terms of cannabis use, gender (46% male), age (mean age 22 yrs), psychotic symptoms (Schubart et al., 2010a) and educational level. In this sub sample lifetime drug use was assessed using a

digital version of the drug use section of the Composite International Diagnostic Interview (CIDI) (Robins et al., 1988). We calculated the adjusted odds ratios described above in this subsample and adjusted for lifetime concomitant drug use. Data were analyzed using R for Windows, version 2.9.1 (Development Core Team (2005), 2005).

RESULTS

The project website was launched in the Netherlands in June 2006 and had attracted more than 117,000 unique visitors by February 2009. After filtering for falsely answered verification questions from the original number of 21,838 participants (19% of 117,000), a total of 17,698 (81% of 21,838) subjects (11,856 (67%) users and 5,842 (33%) cannabis naïves) were included in the analyses. The highest educational attainment is secondary schools in 50.4% of the sample, 8.3% have an academic diploma, 34.3% have a non-academic post-secondary school diploma and 0.1% have no educational diploma. Age, gender and numbers of hospitalizations of the sample are presented in Table 1.

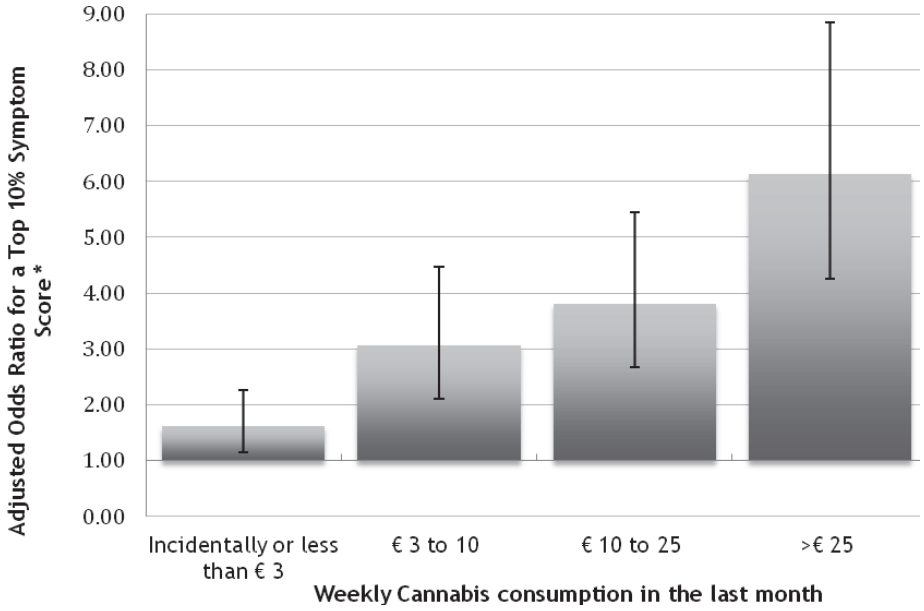
TABLE 1. Subject Characteristics.

	N	Gender (% male)	Mean Age	SD Age	Psychiatric hospitalization in history (%)
Total Group	17,698	51.0	21.6	4.2	2.6
Non-Users	5,842	32.9	21.0	4.2	1.1
Users	11,856	57.0	22.0	4.3	3.4

Participants who had used cannabis at least ten times in their lives had been hospitalized significantly more often than the less frequent users and cannabis naïve participants (odds ratio = 3.2 (95% CI 2.6 – 4.0)) . Table 2 and figure 1, show the dose-response relationship found between the amount of euros spent on cannabis use and the odds of having a prior psychiatric hospitalization. Analysis of age of initial use showed that starting cannabis at a young age was associated with higher odds of having had one or more psychiatric hospitalizations (figure 2 and table 2).

In a sub group (n=816), not shown in the table, information on concomitant drug use was available. The odds ratio for hospitalization in the group that used more than €25 weekly was 8.8 (95%CI 2.2 – 35.5) before and 4.1 (95% CI 0.5 – 34.9) after adjusting for concomitant drug use in this group. The odds ratio for hospitalization in participants that started before the age of 12 years was 2.7 (95% CI 0.3 – 34.2), after adjustment for concomitant use. The adjusted odds ratio for hospitalization associated with lifetime use of other drugs than cannabis was 2.6 (95% CI 0.3 – 21.2) in the model for amount of use. In the model for age of initial use, the odds ratio for hospitalization associated with concomitant drug use was 1.6 (95% CI 0.5 – 5.6).

Figure 1. Adjusted odds ratios for hospitalization per category of weekly amount of use with the cannabis naïve group as reference (total N= 17,698)

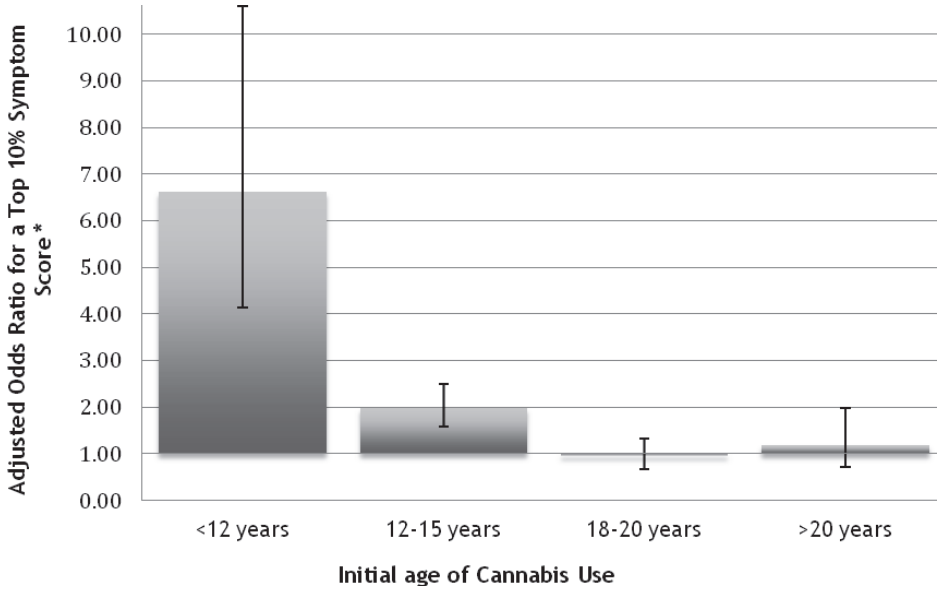


*Adjusted for age, gender and level of education.

Analysis of sensitivity to selection bias

Selection bias could have led to an overestimation of the association between cannabis use and the risk for psychiatric hospitalization. For example, heavy cannabis users with a history of psychiatric hospitalization might be more willing to answer a web-based questionnaire. To quantitatively assess the sensitivity of the results to such selection bias (i.e., to assess the impact of potential overrepresentation of hospitalized heavy users in our study population), we estimated how a decrease in the number of hospitalized heavy users would affect the adjusted odds ratio in the heavy users group. These analyses indicate that the odds ratio would remain significant until 70% of heavy users that have been hospitalized would be excluded from the calculations. Thus, only an overrepresentation by 330% of hospitalized heavy users could have biased a situation of no relation between cannabis use and hospitalization into the observed association. The adjusted odds ratios for several hypothetical steps can be found in table 3.

Figure 2. Adjusted odds ratios for hospitalization per category of initial age of cannabis use with the modal starting age category (15-18 years) as reference group (total N= 11,856)



* adjusted for age, gender, level of education and the weekly amount of use.

DISCUSSION

This study in almost 18,000 subjects presents a dose-dependent association between cannabis use and the odds of having been admitted to a psychiatric hospital. Heavy users were six-times more frequently admitted compared to cannabis naïve subjects. Independently, age at onset of cannabis use was also correlated to a history of psychiatric hospitalizations. Subjects who started using cannabis before the age of 12 years have an almost five-fold increased odds for hospitalization compared to participants that started at a modal age (15-18 years). The association of cannabis use with a history of psychiatric inpatient treatment is a clear indication of association of cannabis use with mental illness. Although many reports have been published on specific outcome measures associated with cannabis use (Hall, 2009), to our knowledge this is the first study to directly address the association between cannabis use and psychiatric hospitalization. Strengths of the study are the unequivocal measure of mental illness, the anonymous setting of the assessment, the large sample size and clear measure of cannabis exposure.

Table 2. Odds Ratios for Hospitalization. Crude and adjusted analyses with 95% confidence intervals.

	UNIVARIATE ANALYSIS ODDS RATIO	*95%CI*		*ADJUSTED ODDS RATIO*	*95 %CI*	
		lower	*upper*		*lower*	*upper*
Cannabis use per week						
Cannabis naïve**	**1.00**[b]	-	-	**1.00** **	-	-
Incidentally or less than € 3 per week	**1.63**	1.16	2.29	**1.61** *	1.14	2.27
€ 3 - 9 per week	**3.12**	2.19	4.44	**3.07** *	2.11	4.48
€ 9 - 25 per week	**4.36**	3.16	6.00	**3.81** *	2.67	5.44
> € 25 per week	**7.56**	5.54	10.32	**6.15** *	4.27	8.85
Age of initial Cannabis						
<12 years	**8.25**	5.28	12.90	**4.76** ***	2.93	7.76
12 - 15 years	**2.12**	1.69	2.66	**1.70** ***	1.34	2.15
15 - 18 years **	**1.00** **	-	-	**1.00** **	-	-
18 - 20 years	**0.98**	0.70	1.37	**1.07** ***	0.75	1.51
>20 years	**1.67**	1.06	2.64	**1.45** ***	0.86	2.43

* adjusted for age, gender and level of education.
** reference category.
*** adjusted for age, gender, level of education and the weekly amount of use.

An internet based approach could potentially lead to reduction of questionnaire validity since environmental factors such as lightning condition, background noise or the presence of distractions can not be standardized. However, since the distribution of these influencing factors throughout the sample is most probably independent of the outcome measure (hospitalization) and the exposure measure (cannabis use), the impact of potential inaccuracy due to lack of controlling for environmental factors can not systematically err the reported associations. A second possible concern on web-based questionnaires is that it might induce false positive answers. Several studies showed however, that the absence of an experimenter and the non-clinical environment of a web-based assessment may decrease the participants tendency to give socially desirable answers (Joinson, 1999;Buchanan and Smith, 1999). Recent reviews evaluate web-based questionnaires to be an overall valid method in epidemiological research (Meyerson and Tryon, 2003;Gosling et al., 2004;Balter et al., 2005;Ekman et al., 2006). Moreover, a growing number of papers presented good validity of instruments used in a variety dimensions of psychopathology such as cannabis addiction (Khazaal et al., 2008), smoking (Graham and Papandonatos, 2008) and other substance abuse (Spijkerman et al., 2009) and for a variety of well validated and broadly used instruments

such as the General Health Questionnaire (Vallejo et al., 2007), Symptoms Check-List-90-Revised (Vallejo et al., 2007), the Kessler psychological distress scale (Donker et al., 2009;Vallejo et al., 2007), the Centers for Epidemiological Studies Depression scale (Houston et al., 2001;Lin et al., 2007;Cuijpers et al., 2008), the Edinburgh Depression Scale (Spek et al., 2008), the Obsessive Compulsive Inventory (Coles et al., 2007), the Obsessive beliefs Questionnaire-44 (Coles et al., 2007) and several questionnaires assessing personality traits and disorders (Pettit, 2002;Buchanan and Smith, 1999).

Table 3. Selection Bias Analysis. Hypothetical adjusted odds ratios for psychiatric hospitalization, if different proportions of previously hospitalized heavy users (>€25/week) are excluded from the analysis.

PROPORTION OF EXCLUDED HOSPITALIZED, HEAVY USERS	ADJUSTED ODDS RATIO *	SE	95%CI	
			Lower	Upper
0	6.14	0.19	4.27	8.85
0.1	5.43	0.19	3.73	7.92
0.2	4.80	0.20	3.26	7.09
0.3	4.12	0.21	2.75	6.16
0.4	3.48	0.21	2.29	5.28
0.5	2.82	0.22	1.82	4.38
0.6	2.17	0.24	1.37	3.46
0.7	1.60	0.25	0.97	2.62
0.8	1.00	0.29	0.57	1.75
0.9	0.49	0.35	0.24	0.97

* adjusted for age, gender and level of education.

A final concern about web-based questionnaires may be that the found associations could be influenced by selection bias since roughly only one in five visitors of our website have completed the online questionnaire. Obviously, our study population is a selected population, which is indicated by for example the fact that over two-third of our study population uses cannabis. This does not, however, imply that the associations found in this population are biased. Importantly, such selection bias will only occur in case of a systematic over- or under representation that is related to both cannabis use and hospitalization. An example of this would be an overrepresentation of individuals that use cannabis and have a positive psychiatric medical history. Sensitivity analyses indicated that only very unrealistic scenarios (i.e., an overrepresentation of

more than 330%) would result in the absence of an association between cannabis use and psychiatric hospitalization. Selection bias as a full explanation of our findings is therefore most unlikely. In interpreting the results of this study, a number of other potential limitations should be considered. Since the current study is retrospective, it theoretically is more susceptible to recall- or report bias. However, since the presence or absence of psychiatric hospitalization is a clear and unequivocal outcome measure, we feel that the influences of recall bias are minimal. Self reported age and amount of illicit substance use have been found to be reliable for epidemiological applications (Johnson and Mott, 2001a). Another potential limitation is the availability of information on the use of drugs other than cannabis in a subsample of subjects only. After adjusting for concomitant drug use, the odds ratio for amount of use decreased to 4.1 (95%CI 0.5 – 34.9) in the group using more than €25 weekly. One could argue therefore that concomitant drug use can be seen as a confounder in the current association, however concomitant drug use by itself was not associated significantly with hospitalisation either in our model and a trend towards association remained. Given the limited sized sample in this sub analysis, failure to reach significance may well be a power problem. An alternative explanation could be that concomitant drug use is an intermediate factor in the association between the amount of cannabis use and a history of psychiatric hospitalization. The impact of concomitant drug use was less clear for the odds ratios of young age of onset. With an average age of around 22 years, this sample is drawn from the population sub group that uses cannabis most. The fact that our participants are relatively young compared to the average population on a psychiatric ward underlines the strength of the reported association, but caution is warranted generalizing these findings to other age groups.

The association between young initial age of use and hospitalisation could point to an increased sensitivity for the effects of cannabis at young age.

Alternatively one could argue that the association is a reflection of the effects of cumulative exposure, underlining the dose response association with amount of cannabis use.

Finally, we are not aware of data describing the educational level of individuals in this age group with comparable educational level categories. Therefore, and given our cooperation with colleges and universities, it is possible that the educational level of our study group is not representative for the general population. As a consequence the generalizability of the presented results may be compromised in this respect.

Foremost it is important to realize the current data cannot help to distinguish cause and effect. To address the issue of causality, a large cohort should be followed from early childhood until late adolescence. The focus of such a study should be the temporal dynamics between reliably measured drug use and mental health care consumption, problematic behavior, school reports and the need of support from child, family, and school social workers.

The current results are clinically meaningful irrespective of the question of causality since they suggest that early or heavy cannabis use are clearly

associated with the development of major psychiatric illness (requiring intensive in-patient treatment). The strength of the current results is the clear dose-dependent association between cannabis use and psychiatric hospitalization in a large sample of, mainly adolescent, individuals. While our results suggest that moderate use after the age of 18 is not associated with increased odds of severe mental illness, we show that both heavy cannabis use and initial use of cannabis before the age of 15 years are each strongly and independently associated with psychiatric hospitalization. Although the underlying causal pathway remains unclear, early and heavy use of cannabis are each associated with poor mental health in its users and thus with significant medical and economic costs for society.

Reference List

Aadahl,M. and Jorgensen,T., 2003. The effect of conducting a lottery on questionnaire response rates: a randomised controlled trial. Eur J Epidemiol. 18, 941-944.

Asbridge,M., Poulin,C. and Donato,A., 2005. Motor vehicle collision risk and driving under the influence of cannabis: evidence from adolescents in Atlantic Canada. Accid Anal Prev. 37, 1025-1034.

Balter,K.A., Balter,O., Fondell,E. and Lagerros,Y.T., 2005. Web-based and mailed questionnaires: a comparison of response rates and compliance. Epidemiology. 16, 577-579.

Bovasso,G.B., 2001. Cannabis abuse as a risk factor for depressive symptoms. Am J Psychiatry. 158, 2033-2037.

Buchanan,T. and Smith,J.L., 1999. Using the Internet for psychological research: personality testing on the World Wide Web. Br J Psychol. 90 (Pt 1), 125-144.

CBS Statistics Netherlands. Persbericht: Mediaproducten steeds meer via internet. PB08-071. 10-28-2009.

Coles,M.E., Cook,L.M. and Blake,T.R., 2007. Assessing obsessive compulsive symptoms and cognitions on the internet: evidence for the comparability of paper and Internet administration. Behav Res Ther. 45, 2232-2240.

Cuijpers,P., Boluijt,P. and van,S.A., 2008. Screening of depression in adolescents through the Internet : sensitivity and specificity of two screening questionnaires. Eur Child Adolesc Psychiatry. 17, 32-38.

D'Souza,D.C., bi-Saab,W.M., Madonick,S., Forselius-Bielen,K., Doersch,A., Braley,G., Gueorguieva,R., Cooper,T.B. and Krystal,J.H., 2005. Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. Biol Psychiatry. 57, 594-608.

Degenhardt,L., Chiu,W.T., Sampson,N., Kessler,R.C., Anthony,J.C., Angermeyer,M., Bruffaerts,R., de,G.G., Gureje,O., Huang,Y., Karam,A., Kostyuchenko,S., Lepine,J.P., Mora,M.E., Neumark,Y., Ormel,J.H., Pinto-Meza,A., Posada-Villa,J., Stein,D.J., Takeshima,T. and Wells,J.E., 2008. Toward a global view of alcohol, tobacco, cannabis, and cocaine use: findings from the WHO World Mental Health Surveys. PLoS Med. 5, e141.

Degenhardt,L., Hall,W. and Lynskey,M., 2003. Exploring the association between cannabis use and depression. Addiction. 98, 1493-1504.

Development Core Team (2005). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. 2005.

Donker,T., van,S.A., Marks,I. and Cuijpers,P., 2009. Brief self-rated screening for depression on the Internet. J Affect Disord. 122, 253-259.

Ekman,A., Dickman,P.W., Klint,A., Weiderpass,E. and Litton,J.E., 2006. Feasibility of using web-based questionnaires in large population-based epidemiological studies. Eur J Epidemiol. 21, 103-111.

ESPAD. Alcohol and Drug Use Among European 17-18 Year Old Students. Barbro Andersson, Björn Hibell, François Beck, Marie Choquet, Anna Kokkevi, Anastasios Fotiou, Sabrina Molinaro, Januzs Sieroslawski, and Marcis Trapencieris. Data from the ESPAD Project. 2007.

European Monitoring Centre for Drugs and Drugs Addiction (EMCDDA). Annual Report 2008: the State of the Drugs Problem in the European Union and Norway. Lisbon: EMCDDA . 2009.

Gillespie,N.A., Neale,M.C. and Kendler,K.S., 2009. Pathways to cannabis abuse: a multi-stage model from cannabis availability, cannabis initiation and progression to abuse. Addiction. 104, 430-438.

Gosling,S.D., Vazire,S., Srivastava,S. and John,O.P., 2004. Should we trust web-based studies? A comparative analysis of six preconceptions about internet questionnaires. Am Psychol. 59, 93-104.

Graham,A.L. and Papandonatos,G.D., 2008. Reliability of internet- versus telephone-administered questionnaires in a diverse sample of smokers. J Med Internet Res. 10, e8.

Hall,W., 2009. The adverse health effects of cannabis use: What are they, and what are their implications for policy? Int J Drug Policy.

Hoge,C.W., Toboni,H.E., Messer,S.C., Bell,N., Amoroso,P. and Orman,D.T., 2005. The occupational burden of mental disorders in theU.S. military: psychiatric hospitalizations, involuntary separations, and disability. Am J Psychiatry. 162, 585-591.

Houston,T.K., Cooper,L.A., Vu,H.T., Kahn,J., Toser,J. and Ford,D.E., 2001. Screening the public for depression through the Internet. Psychiatr Serv. 52, 362-367.

IVO: Addiction Institute, Rooij van,T., Meerkerk,G., Schoenmakers,T., Eijnden van den,R. and Mheen van de,D. Monitor internet en jongeren: Ontwikkelingen in het internetgebruik van Nederlandse Jongeren. 2009.

Johnson,T.P. and Mott,J.A., 2001. The reliability of self-reported age of onset of tobacco, alcohol and illicit drug use. Addiction. 96, 1187-1198.

Johnston,L.D., O'Malley,P.M., Bachman,J.G. and Schulenberg,J.E. Monitoring the Future national results on adolescent drug use: Overview of key findings, 2008. National Results on Adolescent Drug Use. National Institute on Drug Abuse , 73. 2009.

Joinson,A., 1999. Social desirability, anonymity, and Internet-based questionnaires. Behav Res Methods Instrum Comput. 31, 433-438.

Kessler,R.C. and Frank,R.G., 1997. The impact of psychiatric disorders on work loss days. Psychol Med. 27, 861-873.

Khazaal,Y., Chatton,A., Cochand,S. and Zullino,D., 2008. Quality of web-based information on cannabis addiction. J Drug Educ. 38, 97-107.

Klinkenberg,W.D. and Calsyn,R.J., 1998. Predictors of psychiatric hospitalization: a multivariate analysis. Adm Policy Ment Health. 25, 403-410.

Lin,C.C., Bai,Y.M., Liu,C.Y., Hsiao,M.C., Chen,J.Y., Tsai,S.J., Ouyang,W.C., Wu,C.H. and Li,Y.C., 2007. Web-based tools can be used reliably to detect patients with major depressive disorder and subsyndromal depressive symptoms. BMC Psychiatry. 7, 12.

Macleod,J., Oakes,R., Copello,A., Crome,I., Egger,M., Hickman,M., Oppenkowski,T., Stokes-Lampard,H. and Davey,S.G., 2004. Psychological and social sequelae of cannabis and other illicit drug use by young people: a systematic review of longitudinal, general population studies. Lancet. 363, 1579-1588.

Mark T, McKusick D, King E, Harwood H and Genuardi J. National expenditures for mental health, alcohol, and other substance abuse treatment. Rockville, MD Substance Abuse and Mental Health Services Administration. 1998.

Mental Health Strategies. The 2008/09 National Survey of Investment in Mental Health Services. 2009.

Meyerson,P. and Tryon,W.W., 2003. Validating internet research: a test of the psychometric equivalence of internet and in-person samples. Behav Res Methods Instrum Comput. 35, 614-620.

Moore,T.H., Zammit,S., Lingford-Hughes,A., Barnes,T.R., Jones,P.B., Burke,M. and Lewis,G., 2007. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet. 370, 319-328.

NHS Mental Health Network. Information about mental health. 2009. NHS Confederation.

Niesink,R., Rigter,S., Hoek,J. and den Boer,N. THC-concentraties in wiet, nederwiet en hasj in Nederlands coffeeshops (2008-2009). 12-1-2009. Utrecht, The Netherlands, Trimbos Institute.

Nordentoft,M. and Hjorthoj,C., 2007. Cannabis use and risk of psychosis in later life. Lancet. 370, 293-294.

Pedersen,W., 2008. Does cannabis use lead to depression and suicidal behaviours? A population-based longitudinal study. Acta Psychiatr Scand. 118, 395-403.

Pettit,F.A., 2002. A comparison of World-Wide Web and paper-and-pencil personality questionnaires. Behav Res Methods Instrum Comput. 34, 50-54.

Ranganathan,M. and D'Souza,D.C., 2006. The acute effects of cannabinoids on memory in humans: a review. Psychopharmacology (Berl). 188, 425-444.

Robins,L.N., Wing,J., Wittchen,H.U., Helzer,J.E., Babor,T.F., Burke,J., Farmer,A., Jablenski,A., Pickens,R., Regier,D.A. and ., 1988. The Composite International Diagnostic Interview. An epidemiologic Instrument suitable for use in conjunction with different diagnostic systems and in different cultures. Arch Gen Psychiatry. 45, 1069-1077.

Rubin,D.B., 1987. Multiple Imputation for Nonresponse in Surveys, 1st ed. edn. John Wiley & Sons.

Satyanarayana,V., 2009. Mental health consequences of cannabis use. Acta Psychiatr Scand. 119, 411-412.

Schubart,C.D., van Gastel,W.A., Breetvelt,E.J., Beetz,S.L., Ophoff,R.A., Sommer,I.E., Kahn,R.S. and Boks,M.P. Cannabis use at young age is associated with psychotic experiences. Psychological Medicine . 2010.

Skinner,R., Conlon,L., Gibbons,D. and McDonald,C., 2010. Cannabis use and non-clinical dimensions of psychosis in university students presenting to primary care. Acta Psychiatr Scand.

Spek,V., Nyklicek,I., Cuijpers,P. and Pop,V., 2008. Internet administration of the Edinburgh Depression Scale. J Affect Disord. 106, 301-305.

Spijkerman,R., Knibbe,R., Knoops,K., van de,M.D. and van den,E.R., 2009. The utility of online panel surveys versus computer-assisted interviews in obtaining substance-use prevalence estimates in the Netherlands. Addiction. [Epub ahead of print].

Substance Abuse and Mental Health Services Administration (SAMHSA). Results from the 2007 National survey on drug use and health. Maryland, Rockville. 2007. Office of Applied Studies (OAS), DEPARTMENT OF HEALTH & HUMAN SERVICES.

Taylor,D.R., Fergusson,D.M., Milne,B.J., Horwood,L.J., Moffitt,T.E., Sears,M.R. and Poulton,R., 2002. A longitudinal study of the effects of tobacco and cannabis exposure on lung function in young adults. Addiction. 97, 1055-1061.

U.S.Department of Health and Human Services. Mental Health: A Report of the Surgeon General. 1999. Rockville, MD U. S. Department of Health and Human Services Substance Abuse and Mental Health Services Administration Center for Mental Health Services National Institutes of Health National Institute of Mental Health.

United Nations Office on Drugs and Crime. World Drug Report. 2009. Vienna, United Nations Publications.

Vallejo,M.A., Jordan,C.M., Diaz,M.I., Comeche,M.I. and Ortega,J., 2007. Psychological assessment via the internet: a reliability and validity study of online (vs paper-and-pencil) versions of the General Health Questionnaire-28 (GHQ-28) and the Symptoms Check-List-90-Revised (SCL-90-R). J Med Internet Res. 9, e2.

PART I: EPIDEMIOLOGICAL FACTORS

CHAPTER III

• CANNABIS USE AT YOUNG AGE IS ASSOCIATED WITH PSYCHOTIC EXPERIENCES •

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ABSTRACT

Background
Cannabis use is associated with psychosis and a range of subclinical psychiatric symptoms. The strength of this association depends on dosage and age at first use. The current study investigates whether level of cannabis exposure and starting age are associated with specific profiles of subclinical symptoms.
Methods
We collected cross-sectional data from a young adult population sample by administering an online version of the Community Assessment of Psychic Experiences (CAPE). Cannabis exposure was quantified as the amount of euros spent on cannabis per week and the age of initial cannabis use. The primary outcome measure was the odds ratio to belong to the highest 10% of scores on the total CAPE and the positive-, negative- and depressive symptom dimensions.
Results
In 17,698 adolescents (mean age 21.6 SD 4.2), cannabis use at age 12 or younger was strongly associated with a top 10% score on psychotic experiences (OR: 3.1, 95%CI 2.1 – 4.3) and to a lesser degree with negative symptoms (OR: 1.7, 95%CI 1.1 – 2.5). The odds ratio of heavy users (>€25/week) for negative symptoms was 3.4 (95%CI 2.9 – 4.1), for psychotic experiences 3.0 (95%CI 2.4 – 3.6), and for depressive symptoms 2.8 (95%CI 2.3 – 3.3).
Conclusions
Early start of cannabis use is strongly associated with subclinical psychotic symptoms and to a lesser degree with negative symptoms, while smoking high amounts of cannabis is associated with increased levels of all three symptom dimensions: psychotic, negative and depressive. These results support the hypothesis that the impact of cannabis use is age specific.

INTRODUCTION

Cannabis is the most widely used illicit substance in the world. The number of users is increasing and is estimated to range from 142.6-190.3 million worldwide, with the highest prevalence in young people (Johnson and Mott, 2001b). Although in the U.S. and Canada the overall lifetime prevalence of cannabis use is around 46%, in 18- to 24-year-olds the prevalence is 70% (Adlaf et al., 2005). A recent U.S. national survey (Johnston et al., 2009) showed that the lifetime prevalence among 13-year-old children is as high as 15%. In Europe, on average one in three adolescents between 15–24 years has ever used cannabis (European Monitoring Centre for Drugs and Drugs Addiction (EMCDDA), 2009). Extensive use of cannabis by young individuals has lead to concerns regarding potential impact on population mental health. Numerous large longitudinal studies observed an independent effect of cannabis on the development of psychotic disorders, for review see (Moore et al., 2007). However, the impact of cannabis use is not restricted to clinically manifest psychotic disorders. In the general population, cannabis use is dose dependently associated with subclinical psychiatric symptoms such as psychotic experiences and negative symptoms (Arseneault et al., 2002;Verdoux et al., 2003;Stefanis et al., 2004;Miettunen et al., 2008;Konings et al., 2008;Hides et al., 2009). Three of these studies report that these associations are stronger in younger subjects (Stefanis et al., 2004;Arseneault et al., 2002;Fergusson et al., 2003). A dose dependent relationship between the amount of cannabis exposure and subclinical symptoms suggests that the level of exposure to tetrahydrocannabinol (THC), the main psychoactive component of cannabis (Mechoulam et al., 1970b), determines this relationship. The association between age of initial cannabis use and subclinical symptoms is less straightforward. One possible explanation is that individuals who are prone to psychotic experiences are more inclined to smoke cannabis at an early age. However there is also evidence suggesting that there is a window of vulnerability to cannabis exposure that explains the increased association between early use and psychiatric symptoms (Stefanis et al., 2004;Arseneault et al., 2002;Fergusson et al., 2003). Animal studies for instance show that exposure to THC during critical periods of brain maturation, such as early puberty, impacts on the development of several neurotransmitter systems (Trezza et al., 2008), suggesting that THC interferes with crucial processes in brain development. It is possible that the pathophysiological mechanisms underlying the associations with amount of use and the association with age of first use are distinct. If first exposure to cannabis early in life interferes with specific developmental processes, this may be reflected in a specific profile of subclinical psychiatric symptoms. A more detailed study of the association between cannabis use and subclinical psychiatric experiences may therefore reveal how these different aspects of cannabis use impact on subclinical psychiatric experiences.

Since several studies show that a high score on self-reported psychotic symptoms predict an increased risk of a psychotic disorder later in life (Wiles et al., 2006;Hanssen et al., 2005;Poulton et al., 2000;Chapman et al.,

1994;Yung et al., 2009), it is particularly interesting to study the relationship between cannabis use and high scores of these subclinical psychiatric experiences. We here report a study on the association between the amount of cannabis use and the age of initial cannabis use and top 10% scores in three symptom dimensions of self reported psychiatric experiences in a large population sample.

METHODS

Participants

Participants were recruited using a project website mainly targeting Dutch speaking adolescents and young adults (18-25 years). Recruitment strategies included cooperation with more than 100 colleges, universities and youth centres that were willing to advertise for this study on their intranet and the use of online commercial advertisement products (i.e. banners and text links). The chance to win an Apple iPod ™ or a Nintendo Wii ™ was used as an incentive. Participants answered questions regarding their cannabis use, filled out the Community Assessment of Psychic Experiences (CAPE)(Konings et al., 2006) questionnaire and provided their age, educational level and contact details. Submitting data anonymously was not possible. Every month approximately 670 visitors filled out our web based questionnaires between June 2006 and February 2009. This resulted in 21,838 participants. The assessment included two verification questions to protect against random answers. Participants that failed to correctly fill out the verification questions were excluded. To increase the homogeneity of the sample participants that indicated to be younger than 10 years or older than 60 years of age were excluded. After exclusion of these individuals, 17,698 participants remained (81% of 21,838). This study was approved by the UMC Utrecht medical ethical commission and all participants gave online informed consent.

Assessments

As a measure of subclinical psychiatric experiences, the CAPE questionnaire was used. The CAPE is a 42-item, self rating instrument and has a three-factor structure of 20 questions in the positive symptom dimension (delusional thinking, verbal- and visual hallucinations), 14 in the negative and 8 in the depressive dimension. It measures frequency as well as distress associated with these experiences. The questionnaire has discriminative validity for the different symptom dimensions in individuals from the general population (Konings et al., 2006;Hanssen et al., 2003;Stefanis et al., 2002)(http://www.cape42.homestead.com/). The primary outcome measure was the odds ratio to belong to the highest 10% of total- and dimensional scores (positive, negative and depressive). Web-based questionnaires are reliable for epidemiologic research purposes, especially in settings in which internet access is high (Ekman et al., 2006), as is the case in The Netherlands where 99% of all adolescents use the internet on a daily basis (CBS Statistics Netherlands, 2009).

Cannabis measures
In the Netherlands, THC-concentration and cannabis market value are highly correlated in marijuana ($r=0.365$, $p < 0.001$) and in hashish ($r=0.719$, $p < 0.001$)(Trimbos, 2009). Therefore, we assessed the amount of euros (€) spent on cannabis per week in the last month, as a proxy measure of exposure to THC. For reference, prices range from €4.30 for one gram of imported marijuana with an average THC percentage of 5.5% to €15, - per gram of Dutch hashish with an average THC concentration of 33.3% (Niesink et al., 2009b). Participants were asked how many euros equivalent of cannabis they use per week and to choose one of the following classes; 1) cannabis naïve individuals who indicated never to have used cannabis; 2) participants using cannabis incidentally or spending less than 3 euros per week; 3) individuals spending between 3-10 euros per week on cannabis; 4) participants spending between 10-25 euros per week; and 5) individuals spending more than 25 euros per week on cannabis. All categories (except for the first two groups) applied to the last month or longer. The initial age of cannabis use was categorized by asking participants which of the following five subgroups describes their cannabis use history; 1) participants who started to use before the age of 12 years; 2) first cannabis use between 12 and 15 years; 3) between 15 and 18 years; 4) between the age of 18 to 20 years; and 5) individuals that started to use after their 20th birthday.

Concomitant drug use
As part of another ongoing study, the first 13,000 participants were asked to fill out a number of additional questionnaires on various topics such as concomitant drug use. A sub sample of 816 participants completed a digital version of the drug use section of the Composite International Diagnostic Interview (CIDI) (Robins *et al.*, 1988 408). This sub sample did not differ significantly from the total sample in terms of cannabis use, CAPE score, age, sex and educational level.

Statistical analysis
Firstly, we analyzed the relation between the weekly amount of money spent on cannabis and having a top 10% score on the different symptom dimensions. Odds Ratios (ORs) and their 95% confidence intervals (95% CI) for the amount of cannabis use were calculated using logistic regression, with a dichotomized score on the total CAPE and the three dimensions of the CAPE as the dependent variable and THC exposure categories as the independent variables. Cannabis naïve individuals were used as the reference group. Corrected ORs and their 95%CI were calculated with additional adjustment for age, gender and level of education. Secondly, in the subgroup that used cannabis, ORs and their 95% CI for initial age of cannabis use were calculated using logistic regression, with a dichotomized score on the total CAPE and the three dimensions of the CAPE as the dependent variable and age categories as the independent variables. The age category of modal initial age (15 -18 years) was used as reference group to assess the risks of early use (i.e. before the age of

12 years) compared to a more common starting age of cannabis use. Corrected ORs and their 95%CI were calculated with additional adjustment for age, gender and level of education.
To assess the sensitivity of our results to selection bias, we performed two additional analyses. We estimated the impact of a hypothetical decrease in the number of heavy users (>25€/week) with total CAPE score in the top10% of the distribution. The same calculation was performed considering a hypothetical decrease of individuals with a top 10% CAPE score that started to use cannabis at or before the age of 12. Randomly, a predefined fraction of the heavy or young users was excluded and the association between cannabis use and a top 10% CAPE score was estimated in the remaining participants. This procedure was repeated 1,000 times for each predefined fraction and odds ratios and their confidence intervals were pooled using Rubin's rule (Rubin, 1987). Additional analyses were performed to assess the influence of lifetime concomitant drug use using the logistic regression model as described before with an extra indicator for concomitant use. Data were analyzed using R for Windows, version 2.9.1 (Development Core Team (2005), 2005).

RESULTS

A total of 17,698 subjects participated in our study. The mean age in our sample was 21 years (SD:4.2) and 51% was male. The educational level of the sample was comparable to the Dutch population in this age group (CBS Statistics Netherlands, 2008). No educational diploma had been attained by 0.1% of the sample, secondary school was the highest educational attainment in 50.4%, 34.3% had a non-academic post-secondary school diploma and 8.3% had an academic diploma. Table 1 presents further characteristics of the sample.

Table 1. Participants characteristics

	Total Group	Non-Users	Users
Number of participants	17,698	5,842	11,856
Gender (% male)	51	32.9	57
Mean age (SD)	21.6 (4.2)	21.0 (3.8)	22.0 (4.3)
Total cape score mean (SD)	101.3 (30,1)	99.1 (27.2)	102.4 (31.4)
Positive dimension mean (SD)	38.4 (12.7)	37.3 (11.3)	38.9 (13.3)
Negative dimension mean (SD)	39.0 (14.1)	37.8 (12.9)	39.6 (14.6)
Depressive dimension mean (SD)	23.9 (8.7)	24.0 (8.1)	23.9 (8.9)

Initial age of cannabis use

Individuals who started to use cannabis before the age of 12 years, had an adjusted odds ratio of 3.1 (95%CI 2.1 – 4.3) for the highest 10% of scores on psychotic experiences compared to participants with a modal starting age (15-18 years). Starting to use between the age of 12 and 15 years resulted in an adjusted odds ratio of 1.2 (95%CI 1.0 – 1.3). Initial age of cannabis use after 18 years was not associated with an increased score on psychotic experiences. An

with using cannabis before the age of 12 (OR: 1.7 (95%CI 1.1 – 2.5)) and also before the age of 15 (OR: 1.1 (95%CI 1.0 – 1.3)). Using cannabis for the first time after the age of 18 years was not associated with an increased OR for the negative symptom dimension. In contrast, depressive symptoms were not associated with a young initial age of cannabis use. However, individuals who started after the age of 20 years experienced more depressive symptoms than the reference group (OR: 1.4, 95%CI: 1.0 – 1.8)). Figure 1 depicts adjusted odds ratios for five categories of initial age of cannabis use and a psychotic experiences score in the top 10% in the three symptom dimensions. Table 2 shows all adjusted odds ratios and their 95% confidence intervals for top 10% scores on the total CAPE and its three symptom dimensions.

Quantity of weekly cannabis use

Analyzing the odds ratios associated with quantity of use, we found that the odds ratio for a top 10% score on psychotic experiences increases with the amount of cannabis that subjects indicate to use weekly. Odds ratios for a top 10% score on psychotic experiences range from 1.7 (96%CI 1.1 – 2.1) in users consuming €3 to €9 weekly to 3.0 (95%CI 2.4 – 3.6) in heavy users (>€25). Likewise, quantity of use was associated to negative symptoms with adjusted odds ratios ranging from 1.3 (95%CI 1.1 – 1.6) in participants who used between €3 and €9 per week to 3.4 (95%CI 2.9 – 4.1) in individuals who consume a weekly equivalent of more than €25. Computation of the adjusted odds ratios for a top 10% score on depressive symptoms produced an odds ratio of 1.3 (95%CI 1.1 - 1.5) in participants that used a weekly cannabis equivalent of €3 to €9 euros. Spending more than 25 euros per week on cannabis was associated with an adjusted odds ratio of 2.8 (95%CI 2.3 – 3.3) in this symptom dimension. Cannabis naïve subjects were used as the reference group in these analyses. All odds ratios are listed in table 2. Figure 2 depicts the adjusted odds ratios per category of weekly amount of use for a top 10% score on each of the three symptom dimensions.

Concomitant drug use

In the subsample in which information on concomitant drug use was available (N=816, not shown in tables), we performed an additional logistic regression analysis to assess the impact of lifetime use of other drugs than cannabis on the presented associations. In the group that used more than €25 worth of cannabis weekly, the odds ratio for a top 10% total CAPE score was 14.35 (95% CI 3.3 – 61.6) after adjustment for concomitant drug use. In this model, the odds ratio for a top10% CAPE score associated with concomitant drug use was 3.1 (95%CI 0.8 – 12.7). The odds ratio for a top 10% total CAPE score in participants who started before the age of 12 years was 2.3 (95%CI 0.6 – 8.7) after adjustment for concomitant use.

Table 2. Full-model odds ratios with 95% confidence interval boundaries for the top 10% scores on the three symptom dimensions and the total scores of psychiatric experiences. Significant OR's are bold.

Amount of €/week OR for a top10% total CAPE score	**Corrected OR***	**Lower 95% CI**	**Upper 95% CI**
Cannabis naïve (N=5842) **	1.00	-	-
0 to 3 € (N=6,432)	0.96	0.82	1.13
3 to 9 € (N=1,814)	**1.46**	**1.21**	**1.76**
9 to 25 € (N=2,106)	**2.00**	**1.68**	**2.38**
>25 € (N=1,504)	**3.54**	**2.94**	**4.26**
Amount of €/week OR for top10% positive dimension score	**Corrected OR***	**Lower 95% CI**	**Upper 95% CI**
Cannabis naïve (N=5842) **	1.00	-	-
0 to 3 € (N=6,432)	0.98	0.84	1.15
3 to 9 € (N=1,814)	**1.72**	**1.44**	**2.06**
9 to 25 € (N=2,106)	**1.96**	**1.65**	**2.33**
>25 € (N=1,504)	**2.95**	**2.44**	**3.56**
Amount of €/week OR for top10% negative dimension score	**Corrected OR***	**Lower 95% CI**	**Upper 95% CI**
Cannabis naïve (N=5842) **	1.00	-	-
0 to 3 € (N=6,432)	0.95	0.81	1.11
3 to 9 € (N=1,814)	**1.34**	**1.11**	**1.62**
9 to 25 € (N=2,106)	**2.05**	**1.74**	**2.42**
>25 € (N=1,504)	**3.43**	**2.87**	**4.1**
Amount of €/week OR for top10% depressive dimension score	**Corrected OR***	**Lower 95% CI**	**Upper 95% CI**
Cannabis naïve (N=5842) **	1.00	-	-
0 to 3 € (N=6,432)	1.01	0.87	1.16
3 to 9 € (N=1,814)	**1.26**	**1.05**	**1.52**
9 to 25 € (N=2,106)	**1.63**	**1.37**	**1.94**
>25 € (N=1,504)	**2.75**	**2.28**	**3.32**

Table 2. Continued

Initial age OR for a top10% total CAPE score	Corrected OR**	Lower 95% CI	Upper 95% CI
>20 (N=545)	1.18	0.90	1.55
18-20 (N=1,909)	0.94	0.78	1.13
15-18 (N=5,722) **	1.00	-	-
12-15 (N=3,426)	**1.16**	**1.01**	**1.32**
<12 (N=154)	**1.82**	**1.23**	**2.70**
Initial age OR for a top10% positive dimension score	**Corrected OR****	**Lower 95% CI**	**Upper 95% CI**
>20 (N=545)	1.06	0.76	1.48
18-20 (N=1,909)	0.84	0.69	1.01
15-18 (N=5,722) **	1.00	-	-
12-15 (N=3,426)	**1.15**	**1.01**	**1.31**
<12 (N=154)	**3.05**	**2.14**	**4.34**
Initial age OR for top10% negative dimension score	**Corrected OR****	**Lower 95% CI**	**Upper 95% CI**
>20 (N=545)	1.22	0.89	1.66
18-20 (N=1,909)	1.02	0.85	1.22
15-18 (N=5,722) **	1.00	-	-
12-15 (N=3,426)	**1.14**	**1.00**	**1.30**
<12 (N=154)	**1.66**	**1.13**	**2.45**
Initial age OR for top10% depressive dimension score	**Corrected OR****	**Lower 95% CI**	**Upper 95% CI**
>20 (N=545)	**1.35**	**1.01**	**1.80**
18-20 (N=1,909)	0.95	0.79	1.14
15-18 (N=5,722) ***	1.00	-	-
12-15 (N=3,426)	1.04	0.91	1.20
<12 (N=154)	1.24	0.80	1.94

* Adjusted for age, gender, level of education and of onset age of cannabis consumption in the total study population.
** Adjusted for age, gender, level of education and of onset age of cannabis consumption in the cannabis users.
*** Reference group in logistic regression analysis.

In the model for age of initial use, the odds ratio associated with the presence or absence of concomitant drug use was 0.9 (95%CI 0.4 – 2.0). A wide confidence interval and strong collinearity between concomitant drug use and an early initial age of cannabis use (r>0.8), indicate a weak statistical model.

Analysis of sensitivity to selection bias

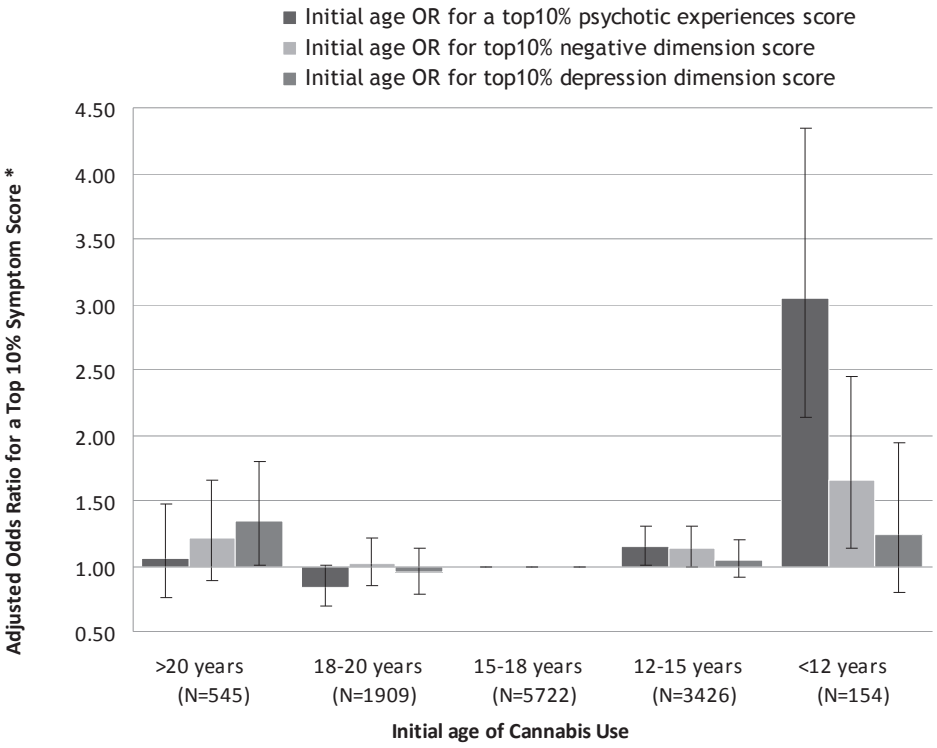
It is conceivable that subjects experiencing psychiatric symptoms were more likely to participate in our study. If such selection was simultaneously skewed towards those that started to use cannabis before the age of 12 years or use more than 25€ per week, selection bias could have influenced the results. To quantitatively assess the sensitivity of the current design to such selection bias, we calculated the impact of a decrease in the number of participants with a high total score on psychiatric experiences (total CAPE) and i) a history of initial cannabis use before the age of twelve years or ii) having used a cannabis equivalent of more than €25 during the last month. These analyses indicate that the odds ratio would remain significant until 20% of participants with a high score on psychiatric experiences who also started to use cannabis before the age of 12 years are excluded from the analysis. Exclusion of 63% of participants with a high score on psychiatric experiences and heavy use (>€25/week) over the last month would render the association nonsignificant. The adjusted odds ratios for several hypothetical steps can be found in table 3.

Table 3. Selection Bias Analysis, showing hypothetical adjusted odds ratios after exclusion of different proportions of participants with a total CAPE score in the top 10% of the distribution and 1) initial age of use before the age of 12 years or 2) heavy use (>€25/week) of cannabis.

proportion of excluded participants	adjusted odds ratio for onset age <12 *	95% CI		adjusted odds ratio for use >25€/ week *	95% CI	
		lower	upper		lower	upper
0	1.82	1.23	2.70	3.54	2.94	4.26
0.1	1.64	1.09	2.46	3.16	2.61	3.83
0.2	1.45	0.95	2.21	2.79	2.29	3.40
0.3	1.27	0.82	1.98	2.43	1.98	2.98
0.4	1.09	0.68	1.74	2.07	1.67	2.56
0.5	-	-	-	1.70	1.36	2.13
0.6	-	-	-	1.35	1.06	1.71

* adjusted for age, gender and level of education.

Figure 1. Subclinical psychiatric symptoms and initial age of cannabis usewith the modal starting age category (15-18 years) as reference group (Total N=11,856).

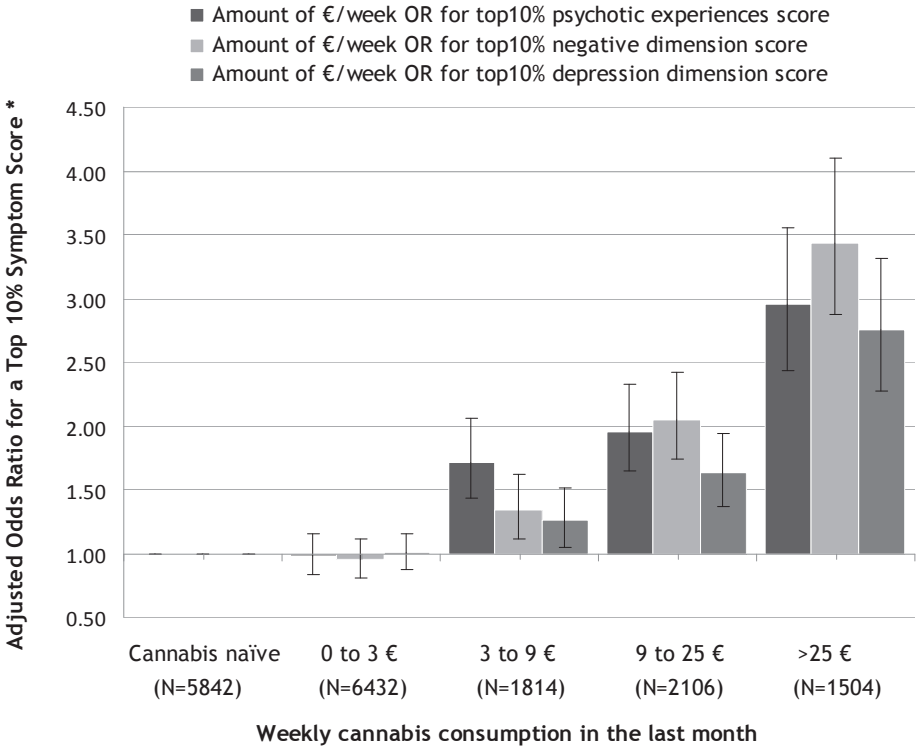


* Adjusted for age, gender, educational level and amount of cannabis use.

DISCUSSION

We investigated the association between initial age and amount of cannabis use and psychiatric experiences in three symptom dimensions (positive, negative and depressive) in a sample of over 17,500 participants with a mean age of 21 years. We found that young initial age of cannabis use is strongly associated with current psychotic experiences. Although young cannabis users also had significantly increased odds ratios of experiencing more negative symptoms, the odds ratio for psychotic experiences was almost twice as high. Depressive symptoms were not associated with early onset of cannabis use. We also found that the amount of cannabis use is equally strongly related to positive-, negative- and depressive symptoms. Finally, our results show that moderate cannabis use and onset of cannabis use after the age of 18 years, did not increase the odds for having subclinical psychiatric experiences.

Figure 2. Subclinical psychiatric symptoms and weekly amount of use during the last month or longer with the cannabis naïve group as reference (Total N=17,698).



* Adjusted for age, gender and educational level.

Initial age of cannabis use

An age-related association between cannabis use and subclinical symptoms was described before. However, from these studies it is not possible to identify the most vulnerable age group (Stefanis et al., 2004;Arseneault et al., 2002;Fergusson et al., 2003). As these studies were cross-sectional too, they also do not allow causal inference. Therefore it is possible that this association reflects an increased propensity of young people with psychotic experiences to commence cannabis use. Another alternative explanation of these findings could be higher cumulative exposure to cannabis of early users, this hypothesis assumes that subjects that started at a young age continued to use cannabis in a certain pattern until present date, however detailed information on the pattern of use from onset to current use was not available. The disproportional level of psychotic symptoms among young cannabis users, compared to the more balanced profile of psychiatric symptoms that is associated with current quantity of cannabis use, is not easily explained by reverse causality or higher cumulative exposure. However, given the cross-sectional nature of the data, do not allow such causal inference.

An alternative hypothesis is that increased vulnerability to THC during critical phases of brain maturation, as in early puberty, is reflected in a specific association between psychotic experiences and a young initial age of THC exposure. Such a window of vulnerability in early puberty is supported by a recent cohort study that showed that early cannabis use is a risk-modifying factor for psychosis-related outcomes in young adults (McGrath et al., 2010) and by experimental studies of the endocannabinoid system (ECN). The ECN plays an important role in brain organization during prenatal development and early puberty (Chevaleyre et al., 2006). Exposure to high levels of exo cannabinoids, such as THC, can disrupt neuronal signalling and might interfere with the activity of the endocannabinoid system during stages of high neuronal plasticity (Trezza et al., 2008;Lewis, 1997). In animal models, exposure to cannabinoids during critical periods of brain maturation has a profound influence on the development of GABA-ergic- (Garcia-Gil et al., 1999), glutamatergic- (Suarez et al., 2004), serotonergic- (Molina-Holgado et al., 1997) and the catecholaminergic system (Fernandez-Ruiz et al., 2000;Garcia-Gil et al., 1997;Hernandez et al., 2000). In agreement with such an impact of THC exposure early in life on the development of neurotransmitter systems, a number of papers report a dramatic effect of THC exposure in early puberty on various cognitive measures in animals (Schneider and Koch, 2003;Cha et al., 2006;O'Shea et al., 2004;Quinn et al., 2008).
We also noticed the relatively high symptom scores among individuals that started to use cannabis after the age of 20 years.

Quantity of weekly cannabis use
The second main finding of our study is that the amount of weekly cannabis use is equally associated with positive-, negative- and depressive symptoms (figure 2). In subjects who use cannabis excessively (>€25 per week) the odds ratio for increased negative symptoms is 3.4 (95%CI 2.9 – 4.1), for psychotic experiences the odds ratio is 3.0 (95%CI 2.4 – 3.6) and for a top 10% score on depressive symptoms the odds ratio is 2.8 (95%CI: 2.3 – 3.3). These odds ratios are similar to those reported for the association between the amount of cannabis use and developing a psychotic disorder (Moore et al., 2007). An association of cannabis use with depression was also found before (Patton et al., 2002;Moore et al., 2007) but not in two previous studies utilizing the CAPE (Stefanis et al., 2004;Verdoux et al., 2003).
Three previous studies reported that the association between cannabis use and psychiatric symptoms is stronger in younger subjects (Stefanis et al., 2004;Arseneault et al., 2002;Fergusson et al., 2003). However, the current study is the first to explicitly examine associations with specific symptom profiles. Due to the large sample size we are able to directly compare groups with different initial ages of cannabis use, including a group that started before the age of 12 years. Other strengths of the current study are the informative measure of THC exposure (€/week), use of a single well validated instrument (CAPE) in all subjects and an anonymous setting which potentially increases the questionnaire sensitivity (Joinson, 1999;Buchanan and Smith, 1999). By

choosing a top10%-cape score as primary outcome, a stringent measure was selected in order to increase relevancy. Individuals with particularly high scores on self-reported psychotic symptoms have a higher risk to develop a psychotic disorder later in life (Wiles et al., 2006;Hanssen et al., 2005;Poulton et al., 2000;Chapman et al., 1994;Yung et al., 2009), by choosing a top10% cut off, we intended to maximize the informational value of the study.

Web-based questionnaire
The increased availability of internet access and the development of better web-based tools have improved the possibilities to acquire information on psychiatric symptoms via the internet such that they are considered a valid additional method in epidemiological research (Meyerson and Tryon, 2003;Balter et al., 2005;Gosling et al., 2004;Ekman et al., 2006). Over the last years, numerous internet based assessments have been validated that measure a variety of psychiatric phenotypes ranging from cannabis abuse to depression (Houston et al., 2001;Vallejo et al., 2007;Khazaal et al., 2008;Graham et al., 2006;Graham and Papandonatos, 2008;Coles et al., 2007;Lin et al., 2007;Donker et al., 2009;Cuijpers et al., 2008;Spek et al., 2008). On a more critical note the use of web-based assessments could potentially have lead to instrument inaccuracy or to information bias. However, the distribution of this potential inaccuracy is most likely independent of cannabis use (exposure measure), and psychiatric experiences (outcome measure) and is therefore unlikely to have systematically influenced the reported associations. A second potential concern is the possibility of selection bias due to the online subject recruiting strategy. However, as described in the sensitivity analysis our results are fairly robust against selection bias. Even in the unlikely event that selection has lead to a 20 percent increase in participants with early cannabis use and high symptoms score the results would remain significant.
A potential limitation is the limited availability of information on concomitant drug use. However, analysis of these data shows that after adjusting for concomitant drug use, the odds ratio for psychotic experiences increased to 14.4 (95%CI 3.3 – 61.6) in the group that started before the age of 12 years. Therefore, these adjusted odds ratios do not weaken the associations reported earlier.
Finally, it is important to notice that the association presented here are based on current (last month) and not cumulative cannabis use. It is not known what proportion of users have a longer history of cannabis use, implicating that we cannot disentangle acute intoxication from long term effects.

Despite the fact that the informational value of the current dataset is limited by the retrospective and cross sectional design precluding any inference on causality, this study shows that heavy current cannabis use is associated with a different symptom profile than early cannabis use. This finding converges with epidemiological and animal studies and supports the hypothesis that there is a window of increased vulnerability of the maturing brain to the effects of exo cannabinoids such as THC, during early puberty. Given the developmental

nature of psychotic disorders (van Os and Kapur, 2009) further studies are warranted to examine the influence of cannabis on brain development.

Reference List

Adlaf,E.M., Begin,P. and Sawka,E. Canadian Addiction Survey (CAS):A national survey of Canadians' use of alcohol and other drugs: Prevalence of use and related harms: Detailed report. 2005. Ottawa: Canadian Centre on Substance Abuse.

Arseneault,L., Cannon,M., Poulton,R., Murray,R., Caspi,A. and Moffitt,T.E., 2002. Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. BMJ. 325, 1212-1213.

Balter,K.A., Balter,O., Fondell,E. and Lagerros,Y.T., 2005. Web-based and mailed questionnaires: a comparison of response rates and compliance. Epidemiology. 16, 577-579.

Buchanan,T. and Smith,J.L., 1999. Using the Internet for psychological research: personality testing on the World Wide Web. Br J Psychol. 90 (Pt 1), 125-144.

CBS Statistics Netherlands. Bijna evenveel hoogopgeleide als laagopgeleide Nederlanders. Webmagazine CBS Statistics Netherlands artikel 2436. 4-14-2008.

CBS Statistics Netherlands. Persbericht: Mediaproducten steeds meer via internet. PB08-071. 10-28-2009.

Cha,Y.M., White,A.M., Kuhn,C.M., Wilson,W.A. and Swartzwelder,H.S., 2006. Differential effects of delta9-THC on learning in adolescent and adult rats. Pharmacol Biochem Behav. 83, 448-455.

Chapman,L.J., Chapman,J.P., Kwapil,T.R., Eckblad,M. and Zinser,M.C., 1994. Putatively psychosis-prone subjects 10 years later. J Abnorm Psychol. 103, 171-183.

Chevaleyre,V., Takahashi,K.A. and Castillo,P.E., 2006. Endocannabinoid-mediated synaptic plasticity in the CNS. Annu Rev Neurosci. 29, 37-76.

Coles,M.E., Cook,L.M. and Blake,T.R., 2007. Assessing obsessive compulsive symptoms and cognitions on the internet: evidence for the comparability of paper and Internet administration. Behav Res Ther. 45, 2232-2240.

Cuijpers,P., Boluijt,P. and van,S.A., 2008. Screening of depression in adolescents through the Internet : sensitivity and specificity of two screening questionnaires. Eur Child Adolesc Psychiatry. 17, 32-38.

Development Core Team (2005). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. 2005.

Donker,T., van,S.A., Marks,I. and Cuijpers,P., 2009. Brief self-rated screening for depression on the Internet. J Affect Disord. 122, 253-259.

Ekman,A., Dickman,P.W., Klint,A., Weiderpass,E. and Litton,J.E., 2006. Feasibility of using web-based questionnaires in large population-based epidemiological studies. Eur J Epidemiol. 21, 103-111.

European Monitoring Centre for Drugs and Drugs Addiction (EMCDDA). Annual Report 2008: the State of the Drugs Problem in the European Union and Norway. Lisbon: EMCDDA . 2009.

Fergusson,D.M., Horwood,L.J. and Swain-Campbell,N.R., 2003. Cannabis dependence and psychotic symptoms in young people. Psychol Med. 33, 15-21.

Fernandez-Ruiz,J., Berrendero,F., Hernandez,M.L. and Ramos,J.A., 2000. The endogenous cannabinoid system and brain development. Trends Neurosci. 23, 14-20.

Garcia-Gil,L., de,M.R., Munoz,R.M., Cebeira,M., Villanua,M.A., Ramos,J.A. and Fernandez-Ruiz,J.J., 1997. Perinatal delta(9)-tetrahydrocannabinol exposure alters the responsiveness of hypothalamic dopaminergic neurons to dopamine-acting drugs in adult rats. Neurotoxicol Teratol. 19, 477-487.

Garcia-Gil,L., de,M.R., Romero,J., Perez,A., Ramos,J.A. and Fernandez-Ruiz,J.J., 1999. Perinatal delta9-tetrahydrocannabinol exposure augmented the magnitude of motor inhibition caused by GABA(B), but not GABA(A), receptor agonists in adult rats. Neurotoxicol Teratol. 21, 277-283.

Gosling,S.D., Vazire,S., Srivastava,S. and John,O.P., 2004. Should we trust web-based studies? A comparative analysis of six preconceptions about internet questionnaires. Am Psychol. 59, 93-104.

Graham,A.L. and Papandonatos,G.D., 2008. Reliability of internet- versus telephone-administered questionnaires in a diverse sample of smokers. J Med Internet Res. 10, e8.

Graham,A.L., Papandonatos,G.D., Bock,B.C., Cobb,N.K., Baskin-Sommers,A., Niaura,R. and Abrams,D.B., 2006. Internet- vs. telephone-administered questionnaires in a randomized trial of smoking cessation. Nicotine Tob Res. 8 Suppl 1, S49-S57.

Hanssen,M., Bak,M., Bijl,R., Vollebergh,W. and van,O.J., 2005. The incidence and outcome of subclinical psychotic experiences in the general population. Br J Clin Psychol. 44, 181-191.

Hanssen,M., Peeters,F., Krabbendam,L., Radstake,S., Verdoux,H. and van,O.J., 2003. How psychotic are individuals with non-psychotic disorders? Soc Psychiatry Psychiatr Epidemiol. 38, 149-154.

Hernandez,M., Berrendero,F., Suarez,I., Garcia-Gil,L., Cebeira,M., Mackie,K., Ramos,J.A. and Fernandez-Ruiz,J., 2000. Cannabinoid CB(1) receptors colocalize with tyrosine hydroxylase in cultured fetal mesencephalic neurons and their activation increases the levels of this enzyme. Brain Res. 857, 56-65.

Hides,L., Lubman,D.I., Buckby,J., Yuen,H.P., Cosgrave,E., Baker,K. and Yung,A.R., 2009. The association between early cannabis use and psychotic-like experiences in a community adolescent sample. Schizophr Res. 112, 130-135.

Houston,T.K., Cooper,L.A., Vu,H.T., Kahn,J., Toser,J. and Ford,D.E., 2001. Screening the public for depression through the Internet. Psychiatr Serv. 52, 362-367.

Johnson,T.P. and Mott,J.A., 2001. The reliability of self-reported age of onset of tobacco, alcohol and illicit drug use. Addiction. 96, 1187-1198.

Johnston,L.D., O'Malley,P.M., Bachman,J.G. and Schulenberg,J.E. Monitoring the Future national results on adolescent drug use: Overview of key findings, 2008. National Results on Adolescent Drug Use. National Institute on Drug Abuse , 73. 2009.

Joinson,A., 1999. Social desirability, anonymity, and Internet-based questionnaires. Behav Res Methods Instrum Comput. 31, 433-438.

Khazaal,Y., Chatton,A., Cochand,S. and Zullino,D., 2008. Quality of web-based information on cannabis addiction. J Drug Educ. 38, 97-107.

Konings,M., Bak,M., Hanssen,M., van,O.J. and Krabbendam,L., 2006. Validity and reliability of the CAPE: a self-report instrument for the measurement of psychotic experiences in the general population. Acta Psychiatr Scand. 114, 55-61.

Konings,M., Henquet,C., Maharajh,H.D., Hutchinson,G. and van,O.J., 2008. Early exposure to cannabis and risk for psychosis in young adolescents in Trinidad. Acta Psychiatr Scand.

Lewis,D.A., 1997. Development of the prefrontal cortex during adolescence: insights into vulnerable neural circuits in schizophrenia. Neuropsychopharmacology. 16, 385-398.

Lin,C.C., Bai,Y.M., Liu,C.Y., Hsiao,M.C., Chen,J.Y., Tsai,S.J., Ouyang,W.C., Wu,C.H. and Li,Y.C., 2007. Web-based tools can be used reliably to detect patients with major depressive disorder and subsyndromal depressive symptoms. BMC Psychiatry. 7, 12.

McGrath,J., Welham,J., Scott,J., Varghese,D., Degenhardt,L., Hayatbakhsh,M.R., Alati,R., Williams,G.M., Bor,W. and Najman,J.M., 2010. Association Between Cannabis Use and Psychosis-Related Outcomes Using Sibling Pair Analysis in a Cohort of Young Adults. Arch Gen Psychiatry.

Mechoulam,R., Shani,A., Edery,H. and Grunfeld,Y., 1970. Chemical basis of hashish activity. Science. 169, 611-612.

Meyerson,P. and Tryon,W.W., 2003. Validating internet research: a test of the psychometric equivalence of internet and in-person samples. Behav Res Methods Instrum Comput. 35, 614-620.

Miettunen,J., Tormanen,S., Murray,G.K., Jones,P.B., Maki,P., Ebeling,H., Moilanen,I., Taanila,A., Heinimaa,M., Joukamaa,M. and Veijola,J., 2008. Association of cannabis use with prodromal symptoms of psychosis in adolescence. Br J Psychiatry. 192, 470-471.

Molina-Holgado,F., Alvarez,F.J., Gonzalez,I., Antonio,M.T. and Leret,M.L., 1997. Maternal exposure to delta 9-tetrahydrocannabinol (delta 9-THC) alters indolamine levels and turnover in adult male and female rat brain regions. Brain Res Bull. 43, 173-178.

Moore,T.H., Zammit,S., Lingford-Hughes,A., Barnes,T.R., Jones,P.B., Burke,M. and Lewis,G., 2007. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet. 370, 319-328.

Niesink,R., Rigter,S., Hoek,J. and den Boer,N. THC-concentraties in wiet, nederwiet en hasj in Nederlands coffeeshops (2008-2009). 12-1-2009. Utrecht, The Netherlands, Trimbos Institute.

O'Shea,M., Singh,M.E., McGregor,I.S. and Mallet,P.E., 2004. Chronic cannabinoid exposure produces lasting memory impairment and increased anxiety in adolescent but not adult rats. J Psychopharmacol. 18, 502-508.

Patton,G.C., Coffey,C., Carlin,J.B., Degenhardt,L., Lynskey,M. and Hall,W., 2002. Cannabis use and mental health in young people: cohort study. BMJ. 325, 1195-1198.

Poulton,R., Caspi,A., Moffitt,T.E., Cannon,M., Murray,R. and Harrington,H., 2000. Children's self-reported psychotic symptoms and adult schizophreniform disorder: a 15-year longitudinal study. Arch Gen Psychiatry. 57, 1053-1058.

Quinn,H.R., Matsumoto,I., Callaghan,P.D., Long,L.E., Arnold,J.C., Gunasekaran,N., Thompson,M.R., Dawson,B., Mallet,P.E., Kashem,M.A., Matsuda-Matsumoto,H., Iwazaki,T. and McGregor,I.S., 2008. Adolescent rats find repeated Delta(9)-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. Neuropsychopharmacology. 33, 1113-1126.

Rubin,D.B., 1987. Multiple Imputation for Nonresponse in Surveys, 1st ed. edn. John Wiley & Sons.

Schneider,M. and Koch,M., 2003. Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory, and the performance in a progressive ratio task in adult rats. Neuropsychopharmacology. 28, 1760-1769.

Spek,V., Nyklicek,I., Cuijpers,P. and Pop,V., 2008. Internet administration of the Edinburgh Depression Scale. J Affect Disord. 106, 301-305.

Stefanis,N.C., Delespaul,P., Henquet,C., Bakoula,C., Stefanis,C.N. and van,O.J., 2004. Early adolescent cannabis exposure and positive and negative dimensions of psychosis. Addiction. 99, 1333-1341.

Stefanis,N.C., Hanssen,M., Smirnis,N.K., Avramopoulos,D.A., Evdokimidis,I.K., Stefanis,C.N., Verdoux,H. and van,O.J., 2002. Evidence that three dimensions of psychosis have a distribution in the general population. Psychol Med. 32, 347-358.

Suarez,I., Bodega,G., Fernandez-Ruiz,J., Ramos,J.A., Rubio,M. and Fernandez,B., 2004. Down-regulation of the AMPA glutamate receptor subunits GluR1 and GluR2/3 in the rat cerebellum following pre- and perinatal delta9-tetrahydrocannabinol exposure. Cerebellum. 3, 66-74.

Trezza,V., Cuomo,V. and Vanderschuren,L.J., 2008. Cannabis and the developing brain: insights from behavior. Eur J Pharmacol. 585, 441-452.

Trimbos,T.N.I.o.M.H.a.A. THC-concentraties in wiet, nederwiet en hasj in Nederlands coffeeshops (2008-2009). 1-1-2009.

Vallejo,M.A., Jordan,C.M., Diaz,M.I., Comeche,M.I. and Ortega,J., 2007. Psychological assessment via the internet: a reliability and validity study of online (vs paper-and-pencil) versions of the General Health Questionnaire-28 (GHQ-28) and the Symptoms Check-List-90-Revised (SCL-90-R). J Med Internet Res. 9, e2.

van Os,J. and Kapur,S., 2009. Schizophrenia. Lancet. 374, 635-645.

Verdoux,H., Sorbara,F., Gindre,C., Swendsen,J.D. and van,O.J., 2003. Cannabis use and dimensions of psychosis in a nonclinical population of female subjects. Schizophr Res. 59, 77-84.

Wiles,N.J., Zammit,S., Bebbington,P., Singleton,N., Meltzer,H. and Lewis,G., 2006. Self-reported psychotic symptoms in the general population: results from the longitudinal study of the British National Psychiatric Morbidity Survey. Br J Psychiatry. 188, 519-526.

Yung,A.R., Nelson,B., Baker,K., Buckby,J.A., Baksheev,G. and Cosgrave,E.M., 2009. Psychotic-like experiences in a community sample of adolescents: implications for the continuum model of psychosis and prediction of schizophrenia. Aust N Z J Psychiatry. 43, 118-128.

PART I: EPIDEMIOLOGICAL FACTORS

CHAPTER IV

• CANNABIDIOL AS A POTENTIAL TREATMENT FOR PSYCHOSIS •

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ABSTRACT

Although cannabis use is associated with an increased risk of developing psychosis, the cannabis constituent cannabidiol (CBD) may have antipsychotic properties. This targeted review aims to provide an overview of currently available animal-, human experimental-, imaging-, epidemiological- and clinical studies that investigated the antipsychotic properties of CBD. We performed a search for English articles using Medline and EMBASE. Studies were selected if they described experiments with psychosis models, psychotic symptoms or psychotic disorders as outcome measure and involved CBD as intervention. The results indicate that CBD impacts differently on the endocannabinoid system than Δ^9-tetrahydrocannabinol (THC). Evidence from several research domains suggests that CBD shows potential for antipsychotic treatment.

INTRODUCTION

Rationale and Background

Schizophrenia is a highly invalidating disorder (van Os and Kapur, 2009), with a lifetime prevalence of 0.3 – 0.6% (McGrath et al., 2008). However, since the introduction of new generation atypical antipsychotics in the 1990's, few clinically meaningful new treatment options for schizophrenia have emerged. Although cannabis use is associated with an increased risk of developing schizophrenia, the cannabis constituent cannabidiol (CBD) may have antipsychotic properties. Cannabidiol (CBD) is one of the 70 phytocannabinoids (Mechoulam et al., 2007) that can be found in the Cannabis sativa plant. CBD was first isolated in 1940 (Adams, 1940) and its molecule structure was described in the 1960s (Mechoulam et al., 1970a). In 1974 CBD was reported to interfere with the psychomimetic actions of Δ^9-tetrahydrocannabinol (THC) (Karniol et al., 1974) and is since then considered as a potential antipsychotic agent.

This review aims to provide an overview of currently available animal-, human experimental-, imaging-, epidemiological- and finally clinical studies that investigated the antipsychotic properties of CBD. To assess the evidence on the use of cannabidiol in the treatment of psychotic disorders, we performed a search for English and non-English articles using Medline and EMBASE. Search items included: "cannabidiol and treatment", "cannabidiol and psychosis" and "cannabidiol and schizophrenia". Each citation was evaluated by reading title and abstract and determining relevance and eligibility. Studies were selected if they described experiments with psychosis models, psychotic symptoms or psychotic disorders as outcome measure and involved CBD as intervention. Additional studies were identified by searching reference lists of previously identified studies. Studies on other agonists or antagonists of cannabinoid receptors were not selected. This paper first provides a brief overview of the endocannabinoid system (ECS) and a concise description of the role of the ECS in the neuropathology of psychotic disorders. Next we will review the studies that investigated the antipsychotic properties of CBD and discuss the implications for future studies.

Endocannabinoid System

CBD is one of the phytocannabinoids that interacts with the human ECS. The main constituents of the ECS are the two well described receptors, CB1r and CB2r, and a number of endocannabinoids which play a central role in ECS functioning (Pertwee, 2008).

Five endogenous cannabinoids have been identified (Devane et al., 1992) that bind to CB1 or CB2 receptors. The two ligands that are best described are 2-arachidonoylglycerol (2-AG), a full agonist of CB1r (Mechoulam et al., 1970a;Pertwee, 2008) and anandamide (N-arachidonoylethanolamine or AEA), a partial agonist of CB1r (Howlett, 2002a;Howlett et al., 2004). These endogenous cannabinoids, or endocannabinoids, are retrograde signaling, polyunsaturated fatty acid derivates. Availability and actions of cannabinoids are controlled by enzymes involved with synthesis and degradation, such as

fatty acid amide hydrolase (FAAH) and monoglycerol lipase (MGL) (Ueda et al., 2011). Endocannabinoids are thought to act as retrograde synaptic messengers; after neurotransmitters such as glutamate and γ-aminobutyric acid (GABA) induce a postsynaptic increase of intracellular calcium, they are released postsynaptically and inhibit the presynaptic release of these neurotransmitters (Pertwee, 2008). CB1 receptors thus inhibit release of excitatory and inhibitory neurotransmitters such as acetylcholine, noradrenaline, GABA, glutamate and dopamine (Freund et al., 2003). The ECS has a role in several physiological processes such as memory (Hampson and Deadwyler, 1999), appetite (Di Marzo et al., 2001) and stress responses (Hill et al., 2010).

The two cannabinoid receptors are very different. The CB1 receptor is the most prominent G-coupled endocannabinoid receptor in the central nervous system (CNS) (Marco et al., 2011b). It is a transmembrane receptor that convert extracellular stimuli into downstream intracellular signaling pathways such as downregulation of cAMP following inhibition of adenylyl cyclase, activation of MAP kinase and inhibition of voltage-gated Ca^{2+} channels (Howlett, 2002a;Howlett et al., 2004). Further downstream effects of these signaling pathways are complex and numerous and are beyond the scope of this paper, for review see Howlett 2010 (Howlett et al., 2010). CB1 receptors are found in the central and peripheral nervous system, but also in other organs such as digestive system tissue and the respiratory tract. Expression of CB1r is particularly abundant in nerve terminals in the cerebellum, hippocampus, basal ganglia and frontal cortex but is also prevalent in the basolateral amygdala, hypothalamus and midbrain (Mailleux et al., 1992;Glass et al., 1997;Herkenham et al., 1991). The expression of CB1r is not limited to neurons (Marco et al., 2011b) but is also observed on glia cells (Sanchez et al., 1998;Waksman et al., 1999;Walter et al., 2003). In contrast to CB1, the expression of CB2 receptors is most prominent in the immune system (Munro et al., 1993;Galiegue et al., 1995) where they act as immunomodulators (Onaivi et al., 2006). Recent data demonstrate however that CB2 receptors are also expressed in the CNS, most prominently on microglia, the immune cells of the brain (Van Sickle et al., 2005;Gong et al., 2006;Onaivi et al., 2006;Garcia-Gutierrez and Manzanares, 2011).

Recently, other receptors besides CB1r and CB2r, were found to be involved in endocannabinoid signaling. Two of these orphan G protein-coupled receptors are GPR119 (mainly expressed in the digestive tract) and GPR55 (CNS and bone). Moreover vanilloid type 1 (TRPV1) ion channels are also activated by endogenous cannabinoids (in CNS) (Henstridge et al., 2011;Brown, 2007;Starowicz et al., 2008;Balenga et al., 2011).

Besides endocannabinoids a number of non-endogenous compounds also interact with the ECS. These exocannabinoids include the phytocannabinoids (such as CBD and THC) and synthetic cannabinoids (such as the CB1R antagonist Rimonabant). Probably, these exocannabinoids target CB1 and CB2 receptors in a less selective manner than endocannabinoids but the effect is similar to endocannabinoids, namely attenuating neurotransmitter release. THC is a partial CB1r and CB2r agonist, but with less affinity than AEA. In interaction

with inhibitory or excitatory neurotransmitters, THC exerts a mixed inhibitory-excitatory effect on neuronal activity in different brain areas (Pertwee, 2008).

Endocannabinoid System and psychotic disorders
Two main lines of evidence suggest that the ECS is involved in the neuropathology of psychotic disorders, firstly from studies of cannabinoids and secondly from studies on their receptors.

The role of cannabinoids in psychotic disorders
A series of studies exploring the role of endogenous cannabinoids in the neurobiology of schizophrenia revealed that levels of endocannabinoids are markedly increased in the cerebrospinal fluid (CSF) and peripheral blood of schizophrenia patients. Furthermore, the increased AEA is reversed by antipsychotic therapy (Giuffrida et al., 2004;De Marchi et al., 2003;Leweke et al., 2007a). Moreover, schizophrenia patients with higher levels of AEA were less likely to develop a psychotic episode in the following 42 weeks. The authors suggest that the rise in AEA could represent reactive inhibitory feedback to over-activation of dopamine D2 receptors. Furthermore, Leweke and colleagues found that schizophrenia patients that regularly use cannabis have significantly lower AEA levels than schizophrenia patients that do not use cannabis. These findings lead to the hypothesis that cannabis use causes downregulation of AEA signaling in schizophrenia patients which may in turn facilitate psychosis (Giuffrida et al., 2004;Leweke et al., 2012a).
Besides the role of endocannabinoids in the neurobiology of psychotic disorders, a large number of studies address the role of exocannabinoids in the development of psychotic disorders. Exposure to THC can cause acute transient psychotic symptoms in healthy individuals and schizophrenia patients (D'Souza et al., 2005;Yücel et al., 2008). This effect might be related to dopamine release in the striatum in humans following THC exposure (Bossong et al., 2008;Bhattacharyya et al., 2012a) and in the prefrontal cortex in several animal models (Chen et al., 1990;Diana et al., 1998;Verrico et al., 2004). However, it is still debated if this is indeed a dopamine mediated pathway since negative studies have also been presented (Barkus et al., 2011;Kuepper et al., 2010;D'Souza et al., 2008;Stokes et al., 2009).
Although the association between cannabis and psychosis is part of an ongoing debate (Macleod et al., 2004;Arseneault et al., 2002;Minozzi et al., 2010), a long term effect of cannabinoids is suggested by studies implying cannabis as a risk factor for psychotic disorders. Numerous epidemiological studies on Psychotic Like Experiences (PLE's) (Schubart et al., 2010c;van Gastel et al., 2012;Arseneault et al., 2004) but also psychotic disorders (Moore et al., 2007), several imaging studies (Rais et al., 2008;Rapp et al., 2012;Yücel et al., 2008) and gene-environment studies (Caspi et al., 2005;Di Forti et al., 2012;Henquet et al., 2006;van Winkel R., 2011) contribute to this notion. In addition, the course of disease is significantly worsened in schizophrenia patients that regularly use cannabis (Linszen et al., 1994;Faber et al., 2012;Grech et al., 2005).

The role of cannabinoid receptors in psychotic disorders

The second line of evidence that links the ECS with psychotic illness comes from studies into the role of the CB1 and CB2 receptors in schizophrenia. A series of studies investigated changes in the expression of cannabinoid receptors associated with schizophrenia. Dean et al found an increase in cannabinoid-1 receptors in the dorsolateral prefrontal cortex in schizophrenia patients compared with healthy controls (independent of cannabis use) and an increase in the density of cannabinoid-1 receptors in the caudate-putamen in response to cannabis use, independent of diagnosis (Dean et al., 2001). In contrast, in a post-mortem study investigating the relationship between CB1r signaling and altered GABA neurotransmission in schizophrenia, Eggan et al found that in the dorsolateral pre-frontal cortex levels of CB1R mRNA were significantly lower in subjects with schizophrenia. Since impaired cognitive functioning in schizophrenia is associated with reduced GABA neurotransmission, the authors hypothesize that reduced CB1r mRNA and protein levels in schizophrenia patients represent a compensatory mechanism to increase GABA transmission in order to normalize working memory function (Eggan et al., 2008).

A different group examined the distribution and density of CB1 receptors in the left anterior cingulate cortex (ACC) taken postmortem from patients with schizophrenia and matched controls. A significant increase in CB1 receptors was found in the schizophrenia group as compared to the control group, suggesting that changes in the endogenous cannabinoid system in the ACC may be involved in the pathology of schizophrenia (Zavitsanou et al., 2004). Moreover, Newell et al demonstrated an increase in CB1 receptor density in the superficial layers of the posterior cingulate cortex in schizophrenia (independent of cannabis use) (Newell et al., 2006). Finally, a postmortem study in brain tissue of subjects with schizophrenia revealed that antipsychotic treatment induces down-regulation of CB1 receptors in the prefrontal cortex. The authors suggest that this response could represent an adaptive mechanism that reduces the endocannabinoid-mediated suppression of GABA release to normalize cognitive dysfunctions (Urigüen et al., 2009).

Although the available data are ambiguous (Dean et al., 2001;Eggan et al., 2008), and studies are scarce, they suggest that psychotic illness impacts on the functional expression of cannabinoid receptors.

A different approach that implicates a role for the CB receptors in the development of psychosis comes from a series of studies investigating the impact of polymorphisms of the CNR1 gene, coding for the CB1 receptor, on the risk to develop psychotic illness.

Ujike et al found that the presence of AAT-repeat microsatellite in the CNR1 gene is significantly associated with schizophrenia, particularly the hebephrenic subtype. This finding was corroborated in other independent samples (Ujike et al., 2002;Chavarria-Siles et al., 2008;Martinez-Gras et al., 2006). However, negative findings have also been reported on this AAT triplet (Tsai et al., 2000). Recently, a SNP in CNR1 (rs12720071) was found to

moderate the impact of cannabis use on white matter volumes and cognitive impairment, also suggesting gene-environment interaction (Ho et al., 2011). The CB2 receptor is also related to several biological processes that are involved in schizophrenia. A recent genetic association study in two independent populations suggested an increased risk of schizophrenia for people with a single nucleotide polymorphism (SNP) leading to low CB2 receptor function (Ishiguro et al., 2010). Furthermore, de Marchi and colleagues reported that CB2R mRNA levels were diminished in peripheral blood mononuclear cells in patients with schizophrenia after treatment with olanzapine (De Marchi et al., 2003). The authors argue that, since CB2R expression is subject to downregulation in activated macrophages and leukocytes (Klein et al., 2001), the decrease of CB2R mRNA levels could be a consequence of reduced activity of blood leukocytes.
A study in CB2R knock-out mouse revealed that deletion of CB2r might decrease motor activity in the open field test. Moreover, this study suggests that lacking CB2r was associated with cognitive impairment, an enhanced acute response to cocaine, increased PPI deficit and mood-related alterations. The authors conclude that in their study, deletion of CB2r is associated with schizophrenia like behaviors and point out an opportunity for pharmacologic intervention by manipulating CB2r (Ortega-Alvaro et al., 2011).

Cannabidiol and the Endocannabinoid System
Although cannabidiol has very low affinity for CB1 and CB2 receptors, Pertwee and colleagues found that CBD is capable of altering CB1R/CB2R function at relatively low concentrations by antagonizing CB1/CB2 receptor agonists such as AEA, 2-AG and THC (Thomas et al., 2007;Pertwee, 2008). CBD is therefore able to interfere with the impact of THC on the ECS, providing a biological basis for the notion that the THC/CBD ratio in cannabis products might moderate the risk of cannabis associated adverse effects. Moreover, CBD reduces the cellular uptake of AEA (Bisogno et al., 2001;Leweke et al., 2012a). Given the above mentioned hypothesis on the role of AEA in counteracting dopamine D2 receptor overactivity, CBD may have antipsychotic capacities by counteracting D2 overactivity by increasing synaptic AEA.

Cannabidiol as an antipsychotic agent
Evidence from animal studies
The first animal studies investigating the effect of cannabidiol in translational psychosis phenotypes, focused on the differential impact of THC and CBD on a number of these behavioral phenotypes. In 1970, Mechoulam reported that CBD did not induce a range of behavioral changes that was associated with THC exposure in rhesus monkeys (Mechoulam et al., 1970a), a finding that was later corroborated in a rat model (Fernandes et al., 1974).
Since the dopamine transmission system is thought to play a key role in psychosis (Howes and Kapur, 2009;Fusar-Poli and Meyer-Lindenberg, 2012a;Fusar-Poli and Meyer-Lindenberg, 2012b), several dopamine based animal models of psychosis were proposed as a mean to study the

pathophysiology of psychosis in animals. Examples of such models are apomorphine, cocaine or amphetamine induced stereotypic behavior and hyperlocomotion. Additionally, glutamate N-methyl-d-aspartate (NMDA) antagonists such as ketamine, PCP or MK-801, are used for glutamate based psychosis models (Lipska and Weinberger, 2000). In several murine psychosis model it was demonstrated THC and CBD exposure had different effects on hyperlocomotion, catalepsy, reduced startle response and increased prepulse inhibition (PPI). Evidence was found that CBD does not only exert very different effects than THC, but is capable of reversing psychosis phenotypes. CBD reversed THC induced reduction of social interaction in rats (Malone et al., 2009) and apomorphine induced sniffing, biting and stereotyped behavior (Zuardi et al., 1991). CBD (5 mg/kg) also attenuated dexamphetamine-induced hyperlocomotion in mice (Long et al., 2010). Furthermore, CBD (30-60 mg/kg) was comparable to clozapine, and superior to haloperidol in attenuating ketamine induced hyperlocomotion in mice (Moreira and Guimaraes, 2005). This correcting effect of CBD on a glutamate hypofunction was later corroborated in a similar study investigating MK-801 induced hyperactivity, deficits in prepulse inhibition and social withdrawal (Gururajan et al., 2011). MK-801 (Dizocilpine) is a non-competitive antagonist of the glutamate receptor N-methyl-d-aspartate (NMDA). In a sensory gating model CBD (5 mg/kg) has a similar efficacy as clozapine in reversing MK-801 induced prolonged PPI (Long et al., 2006). This study demonstrated that this effect of CBD is probably mediated through the vanilloid type 1 receptor (TRPV1). The vanilloid receptor is a nonselective cation channel that is has been studied extensively for involvement in nociception (Cui et al., 2006;Huang et al., 2002). It was already known that CBD is capable of binding to TRPV1 (Bisogno et al., 2001) and that endocannabinoids also activate TRPV1 (Brown, 2007). A rapidly increasing number of studies suggest intensive interplay between vanilloid and endocannabinoid systems in several behavioral functions (Umathe et al., 2012;Fogaca et al., 2012).

A different approach to evaluate the psychopharmacological profile of CBD is to compare the effect of CBD on c-fos mediated immunoreactivity to that of clozapine and haloperidol. Alteration in expression of the c-fos gene is viewed as an immediate-early marker for recent neuronal activity (Day et al., 2008). *c-fos* expression is increased in several brain regions in reaction to typical and atypical antipsychotics (Dragunow et al., 1995). Moreover, typical and atypical antipsychotics produce different activation patterns (Robertson and Fibiger, 1992). Guimaraes et al compared *c-fos* expression in the nucleus accumbens and the dorsal striatum in reaction to haloperidol, clozapine and CBD (120 mg/kg). Haloperidol induced *c-fos* expression in the nucleus accumbens (limbic region) and in the dorsal striatum (motor region). In contrast CBD and clozapine only induced activation in the nucleus accumbens. The similarity in activation patterns between CBD and clozapine is an argument for the possible relatedness in mechanism of action between atypical antipsychotics and CBD (Zuardi et al., 1991;Guimaraes et al., 2004).

Evidence from human experimental models

One of the first studies comparing the psychomimetic effects of THC and CBD in humans was performed by Perez-Reyes et al in 1973. The investigators showed that compared to THC and cannabinol, CBD did not produce any psychological or physiological effects described as feeling "high" (Perez-Reyes et al., 1973). Karniol et al showed in 1974, that simultaneous exposure to CBD, blocks THC induced effects on pulse rate, time production tasks and psychological reactions as anxiety or panic (Karniol et al., 1974). Zuardi et al in 1982, was the first to demonstrate that CBD is capable of reducing THC induced effects, particularly anxiety (Zuardi et al., 1982;Crippa et al., 2009). In a more recent study, Hallak et al found a non-significant trend of CBD to reduce ketamine-induced depersonalization in healthy subjects (Hallak et al., 2011). In a study investigating the effect of CBD on THC induced behavioral measures such as euphoria and psychomotor impairment, Dalton et al found that although pretreatment with CBD did not alter THC induced effects, simultaneous exposure to CBD did (Dalton et al., 1976).

Sensorimotor gating of startle response provides a further valuable and validated model of psychosis (Braff et al., 2001). In contrast to the animal studies described above, one study reported that CBD did not alter THC induced subjective reports, measures of cognitive task performance, EEG (electroencephalography) and ERP (event-related potential) in humans (Ilan et al., 2005). In parallel, CBD failed to demonstrate a reversal of Δ9-THC-induced P300 reduction in humans (Roser et al., 2008). However, one possible explanation for these contrasting finding is provided by Stadelman et al who argue that variation in CNR1 genotypes might differentially alter the sensitivity to the acute effects of cannabinoids on P300 generation in healthy subjects (Stadelmann et al., 2011).

A therapeutic effect of CBD is also suggested by a study comparing the effects of THC and CBD in an evoked mismatch negativity (MMN) model. MMN is an auditory ERP that represents a measure of automatic context-dependent information processing and auditory sensory memory. A meta-analysis showed that MMN deficits are a robust feature in chronic schizophrenia and indicate abnormalities in automatic context-dependent auditory information processing and auditory sensory memory (Umbricht and Krljes, 2005). Significantly greater MMN amplitude values at central electrodes were found under cannabis extract, but not with pure THC in 22 healthy subjects. These greater MMN amplitudes may imply higher cortical activation and cognitive performance related to the positive effects of CBD (at doses of 5.4mg/kg) (Juckel et al., 2007). A final, well studied experimental model for psychosis is binocular depth inversion (Schneider et al., 2002). Leweke et al investigated the capability of CBD to attenuate effects of the synthetic THC like CB1 receptor agonist nabilone on binocular depth inversion in 9 healthy subjects. They found that CBD (200 mg) clearly reversed nabilone induced effects (Leweke et al., 2000b).

Evidence from imaging studies
Studies investigating cannabis related changes in brain tissue composition provide markedly divergent results (Yücel et al., 2008;Matochik et al., 2005). Demirakca provided evidence for the idea that the THC/CBD ratio plays an explanatory role for these contrasting results. They found an inverse correlation between the THC/CBD ratio in hair samples of cannabis users and hippocampal volume, possibly explaining previous divergence in cannabis associated patterns of brain tissue composition (Demirakca et al., 2011).
THC and CBD effects on cerebral activation were also studied with fMRI in relation to their potency to induce psychotic symptoms. In one study, three healthy volunteers underwent CBD pretreatment before THC admission, which successfully blocked the emergence of psychotic symptoms measured by the Positive and Negative Syndrome scale (PANSS) (Bhattacharyya et al., 2009;Kay et al., 1987). In a later study, the same group showed that THC and CBD have opposite effects on regional brain activation during tasks of verbal memory, response inhibition, sensory processing, and emotional processing (Fusar-Poli et al., 2010b;Fusar-Poli et al., 2009), without significant effects on behavioral performance (Bhattacharyya et al., 2010). These differential activation patterns were detected in several brain areas, the authors therefore suggest that this is a reflection of the fact that the differences in action of THC and CBD are not specific for a particular brain area. Furthermore, the authors note that the widely distributed brain regions where THC and CBD had opposite effects is consistent with the distribution of CB1 receptors in the brain (Elphick and Egertova, 2001).
Borgwardt et al reported that the effects of THC in 15 healthy subjects on brain activation during a response inhibition task attenuates the engagement of brain regions that mediate response inhibition and that CBD modulated function in the left lateral temporal cortex and insula, regions not usually implicated in response inhibition (Borgwardt et al., 2008).
Recently Bhattacharyya et al. investigated the effects of THC and CBD on attentional salience processing and found that THC induced psychotic symptoms (measured by the PANSS) which was related to striatal activation. Moreover, they found that CBD had opposite effects to THC on activation of the striatum, prefrontal cortex and medial temporal cortex (Bhattacharyya et al., 2012b).
Finally, Winton-Brown and colleagues used fMRI to show that THC and CBD modulate brain function differently in areas that are involved with the processing of auditory and visual stimuli and relate to induced psychotic symptoms according to the PANSS. THC and CBD have opposite effects on activation of the right posterior superior temporal gyrus, which is the right-sided homolog of Wernicke's area. Moreover, they showed that activation of these areas was clearly associated with the experience of psychotic symptoms.

Evidence from epidemiological studies
Numerous studies show that psychotic outcomes are associated with cannabis use in a dose-dependent fashion (Moore et al., 2007;Stefanis et al., 2004;van

Gastel et al., 2012;Skinner et al., 2010). The strength of this association might be influenced by cannabis potency, which can be defined in terms of the concentrations of THC and, inversely, CBD (Potter et al., 2008). In 1982, Rotanburg et al described a cohort with a relatively high (30%) percentage of psychotic symptoms that could be attributed to the use of cannabis variants with relatively low concentrations of cannabidiol (Rottanburg et al., 1982b). In an effort to assess the influence of different CBD concentrations in different cannabis products on the association between cannabis use and psychosis, Di Forti et al compared cannabis use habits of 280 first episode psychosis patients with healthy cannabis users and found that patients with psychosis used higher-potency cannabis (with high concentrations THC and low concentrations CBD), for longer duration and with greater frequency (Di Forti et al., 2009). In a more direct approach, Morgan and colleagues showed that cannabis users (n=120) who have a higher CBD content in hair samples, have fewer psychometric psychotic experiences (Morgan and Curran, 2008), a result that was later replicated with a similar design in a different sample (Morgan et al., 2011). In a study investigating cannabis use associated cognitive performance, Morgan et al, found a clear, significant effect of CBD in attenuating THC induced deficits in memory performance (Morgan et al., 2010). Recently, we demonstrated in 1,877 subjects that habitual use of cannabis with relatively high concentrations of CBD is associated with the experience of fewer psychotic experiences than the use of low CBD cannabis types (Schubart et al., 2011b).

Clinical studies

Zuardi and colleagues published several reports on the therapeutic use of CBD monotherapy in patients with psychotic symptoms. In a case report, successful treatment with 1200mg/day CBD was described in a 19 year old woman with schizophrenia (Zuardi et al., 1995). In a short report, therapy of 3 treatment resistant schizophrenia patients with escalating doses up to 1280mg/day of CBD was described, of whom only one patient showed mild symptom improvement. The authors speculate that a low initial CBD dose and the treatment resistance in these patients, might explain this negative finding. A pilot study investigating the effects of CBD in six patients with Parkinson's Disease and psychotic symptoms, demonstrated a significant improvement of psychotic symptoms, without worsening motor functioning or cognition (Zuardi et al., 2009). In another pilot study in patients with acute manic episodes, there was no evidence of a benefit of CBD, suggesting that the efficacy is confined to non-affective psychosis.

Finally, Leweke et al reported the first double-blind controlled clinical trial in 42 acute paranoid schizophrenia or schizophreniform disorder patients comparing CBD with amisulpride in treatment during four-weeks. They found that the therapeutic effect of CBD in reducing psychotic symptoms, measured with the Positive and Negative Syndrome scale (PANSS) was similar to amisulpride. However, CBD treatment was accompanied with significantly less extrapyramidal side effects, prolactine increase and weight gain than amisulpride (Leweke et al., 2012a). Moreover, the authors report an

association between higher AEA levels and clinical improvement within subjects treated with CBD. Since CBD has the capability to inhibit FAAH and, as mentioned above, FAAH activity reduces AEA concentrations, the authors suggest that inhibition of FAAH activity by CBD might be a functionally relevant component of its antipsychotic properties (Leweke et al., 2012a).

Tolerability
Extensive in vivo and in vitro reports of CBD administration across a wide range of concentrations, did not detect important side or toxic effects, in addition, the acute administration of this cannabinoid by different routes did not induce any significant toxic effect in humans (Bergamaschi et al., 2011). With a median Lethal Dose (LD_{50}) of 212mg/Kg in rhesus monkeys, CBD has a low toxicity (Rosenkrantz et al., 1981). Bergamaschi and colleagues demonstrated that CBD is well tolerable up to doses of 1500mg/day. Some studies investigated mutagenic or teratogenic effects and describe no such events (Matsuyama and Fu, 1981;Bergamaschi et al., 2011;Dalterio et al., 1984).

Conclusion
In summary, evidence from several study domains suggests that CBD has some potential as an antipsychotic treatment.
Animal studies show that CBD is capable of reversing various THC induced psychosis like behaviors in dopaminergic but also glutamatergic animal models of psychosis. In addition, these studies found that the vanilloid (TRPV1) receptor is likely to play an important role in CBD action and some provided evidence for the notion that CBD has a neuropharmacological profile that is similar to atypical antipsychotics.
Human studies using experimental models of psychosis found higher cortical activation and cognitive performance related to CBD. Moreover, a study using a model of binocular depth inversion found that CBD is capable of reversing psychosis like effects of the CB1r agonist Nabilone. Finally, several studies suggest that CBD is capable of reducing THC induced psychological effects, particularly anxiety.

Imaging studies provided various clues on a potential antipsychotic effect of CBD. A volumetric MRI study found CBD to have a protective effect on cannabis use associated hippocampus volume loss. A functional imaging study showed that pretreatment with CBD is capable of preventing THC induced psychotic symptoms. Moreover, a series of studies by Bhattacharyya and colleagues suggests that THC and CBD have opposite effects on regional brain activation in various areas, during psychosis associated tasks.
Several epidemiological studies investigated differences in effects of cannabis type that contain different concentrations of CBD. Cannabis types containing more CBD consistently cause less psychotic like experiences in the general population.
A series of relatively small clinical studies in different patient subcategories, published by Zuardi and colleagues overall suggests that CBD might have

antipsychotic properties. Currently, the first and only clinical trial (n=42) comparing CBD to amisulpride clearly reports that CBD is capable of reducing psychotic symptoms similar to amisulpride but with significantly less side effects.
Given the high tolerability and superior cost-effectiveness, CBD may prove to be an attractive alternative to current antipsychotic treatment, possibly in specific subgroups of patients. However, since much remains unknown regarding the clinical properties of CBD, further studies are warranted about what the use can be of this compound. Illuminating pharmacological pathways through which CBD reduces the experience of psychotic symptoms could also lead to the design of new synthetic agents that act through the endocannabinoid system in ameliorating psychotic symptoms.

Reference List

Adams,R., 1940. MARIHUANA. Science. 92, 115-119.

Arseneault,L., Cannon,M., Poulton,R., Murray,R., Caspi,A. and Moffitt,T.E., 2002. Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. BMJ. 325, 1212-1213.

Arseneault,L., Cannon,M., Witton,J. and Murray,R.M., 2004. Causal association between cannabis and psychosis: examination of the evidence. Br J Psychiatry. 184, 110-117.

Balenga,N.A., Henstridge,C.M., Kargl,J. and Waldhoer,M., 2011. Pharmacology, signaling and physiological relevance of the G protein-coupled receptor 55. Adv Pharmacol. 62, 251-277.

Barkus,E., Morrison,P.D., Vuletic,D., Dickson,J.C., Ell,P.J., Pilowsky,L.S., Brenneisen,R., Holt,D.W., Powell,J., Kapur,S. and Murray,R.M., 2011. Does intravenous Delta9-tetrahydrocannabinol increase dopamine release? A SPET study. J Psychopharmacol. 25, 1462-1468.

Bergamaschi,M.M., Queiroz,R.H., Zuardi,A.W. and Crippa,J.A., 2011. Safety and side effects of cannabidiol, a Cannabis sativa constituent. Curr Drug Saf. 6, 237-249.

Bhattacharyya,S., Atakan,Z., Martin-Santos,R., Crippa,J.A., Kambeitz,J., Prata,D., Williams,S., Brammer,M., Collier,D.A. and McGuire,P.K., 2012a. Preliminary report of biological basis of sensitivity to the effects of cannabis on psychosis: AKT1 and DAT1 genotype modulates the effects of delta-9-tetrahydrocannabinol on midbrain and striatal function. Mol Psychiatry.

Bhattacharyya,S., Crippa,J.A., Allen,P., Martin-Santos,R., Borgwardt,S., Fusar-Poli,P., Rubia,K., Kambeitz,J., O'Carroll,C., Seal,M.L., Giampietro,V., Brammer,M., Zuardi,A.W., Atakan,Z. and McGuire,P.K., 2012b. Induction of psychosis by Delta9-tetrahydrocannabinol reflects modulation of prefrontal and striatal function during attentional salience processing. Arch Gen Psychiatry. 69, 27-36.

Bhattacharyya,S., Fusar-Poli,P., Borgwardt,S., Martin-Santos,R., Nosarti,C., O'Carroll,C., Allen,P., Seal,M.L., Fletcher,P.C., Crippa,J.A., Giampietro,V., Mechelli,A., Atakan,Z. and McGuire,P., 2009. Modulation of mediotemporal and ventrostriatal function in humans by Delta9-tetrahydrocannabinol: a neural basis for the effects of Cannabis sativa on learning and psychosis. Arch Gen Psychiatry. 66, 442-451.

Bhattacharyya,S., Morrison,P.D., Fusar-Poli,P., Martin-Santos,R., Borgwardt,S., Winton-Brown,T., Nosarti,C., O' Carroll,C.M., Seal,M., Allen,P., Mehta,M.A., Stone,J.M., Tunstall,N., Giampietro,V., Kapur,S., Murray,R.M., Zuardi,A.W., Crippa,J.A., Atakan,Z. and McGuire,P.K., 2010. Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. Neuropsychopharmacology. 35, 764-774.

Bisogno,T., Hanus,L., De,P.L., Tchilibon,S., Ponde,D.E., Brandi,I., Moriello,A.S., Davis,J.B., Mechoulam,R. and Di,M., V, 2001. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br J Pharmacol. 134, 845-852.

Borgwardt,S.J., Allen,P., Bhattacharyya,S., Fusar-Poli,P., Crippa,J.A., Seal,M.L., Fraccaro,V., Atakan,Z., Martin-Santos,R., O'Carroll,C., Rubia,K. and McGuire,P.K., 2008. Neural basis of Delta-9-tetrahydrocannabinol and cannabidiol: effects during response inhibition. Biol Psychiatry. 64, 966-973.

Bossong,M.G., van Berckel,B.N., Boellaard,R., Zuurman,L., Schuit,R.C., Windhorst,A.D., van Gerven,J.M., Ramsey,N.F., Lammertsma,A.A. and Kahn,R.S., 2008. Delta9-Tetrahydrocannabinol Induces Dopamine Release in the Human Striatum. Neuropsychopharmacology.

Braff,D.L., Geyer,M.A., Light,G.A., Sprock,J., Perry,W., Cadenhead,K.S. and Swerdlow,N.R., 2001. Impact of prepulse characteristics on the detection of sensorimotor gating deficits in schizophrenia. Schizophr Res. 49, 171-178.

Brown,A.J., 2007. Novel cannabinoid receptors. Br J Pharmacol. 152, 567-575.

Caspi,A., Moffitt,T.E., Cannon,M., McClay,J., Murray,R., Harrington,H., Taylor,A., Arseneault,L., Williams,B., Braithwaite,A., Poulton,R. and Craig,I.W., 2005. Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. Biol Psychiatry. 57, 1117-1127.

Chavarria-Siles,I., Contreras-Rojas,J., Hare,E., Walss-Bass,C., Quezada,P., Dassori,A., Contreras,S., Medina,R., Ramirez,M., Salazar,R., Raventos,H. and Escamilla,M.A., 2008. Cannabinoid receptor 1 gene (CNR1) and susceptibility to a quantitative phenotype for hebephrenic schizophrenia. Am J Med Genet B Neuropsychiatr Genet. 147, 279-284.

Chen,J., Paredes,W., Lowinson,J.H. and Gardner,E.L., 1990. Delta 9-tetrahydrocannabinol enhances presynaptic dopamine efflux in medial prefrontal cortex. Eur J Pharmacol. 190, 259-262.

Crippa,J.A., Zuardi,A.W., Martin-Santos,R., Bhattacharyya,S., Atakan,Z., McGuire,P. and Fusar-Poli,P., 2009. Cannabis and anxiety: a critical review of the evidence. Hum Psychopharmacol. 24, 515-523.

Cui,M., Honore,P., Zhong,C., Gauvin,D., Mikusa,J., Hernandez,G., Chandran,P., Gomtsyan,A., Brown,B., Bayburt,E.K., Marsh,K., Bianchi,B., McDonald,H., Niforatos,W., Neelands,T.R., Moreland,R.B., Decker,M.W., Lee,C.H., Sullivan,J.P. and Faltynek,C.R., 2006. TRPV1 receptors in the CNS play a key role in broad-spectrum analgesia of TRPV1 antagonists. J Neurosci. 26, 9385-9393.

D'Souza,D.C., bi-Saab,W.M., Madonick,S., Forselius-Bielen,K., Doersch,A., Braley,G., Gueorguieva,R., Cooper,T.B. and Krystal,J.H., 2005. Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. Biol Psychiatry. 57, 594-608.

D'Souza,D.C., Braley,G., Blaise,R., Vendetti,M., Oliver,S., Pittman,B., Ranganathan,M., Bhakta,S., Zimolo,Z., Cooper,T. and Perry,E., 2008. Effects of haloperidol on the behavioral, subjective, cognitive, motor, and neuroendocrine effects of Delta-9-tetrahydrocannabinol in humans. Psychopharmacology (Berl). 198, 587-603.

Dalterio,S., Steger,R., Mayfield,D. and Bartke,A., 1984. Early cannabinoid exposure influences neuroendocrine and reproductive functions in mice: II. Postnatal effects. Pharmacol Biochem Behav. 20, 115-123.

Dalton,W.S., Martz,R., Lemberger,L., Rodda,B.E. and Forney,R.B., 1976. Influence of cannabidiol on delta-9-tetrahydrocannabinol effects. Clin Pharmacol Ther. 19, 300-309.

Day,H.E., Kryskow,E.M., Nyhuis,T.J., Herlihy,L. and Campeau,S., 2008. Conditioned fear inhibits c-fos mRNA expression in the central extended amygdala. Brain Res. 1229, 137-146.

De Marchi,N., De,P.L., Orlando,P., Daniele,F., Fezza,F. and Di,M., V, 2003. Endocannabinoid signalling in the blood of patients with schizophrenia. Lipids Health Dis. 2, 5.

Dean,B., Sundram,S., Bradbury,R., Scarr,E. and Copolov,D., 2001. Studies on [3H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. Neuroscience. 103, 9-15.

Demirakca,T., Sartorius,A., Ende,G., Meyer,N., Welzel,H., Skopp,G., Mann,K. and Hermann,D., 2011. Diminished gray matter in the hippocampus of cannabis users: possible protective effects of cannabidiol. Drug Alcohol Depend. 114, 242-245.

Devane,W.A., Hanus,L., Breuer,A., Pertwee,R.G., Stevenson,L.A., Griffin,G., Gibson,D., Mandelbaum,A., Etinger,A. and Mechoulam,R., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science. 258, 1946-1949.

Di Forti,M., Iyegbe,C., Sallis,H., Kolliakou,A., Falcone,M.A., Paparelli,A., Sirianni,M., La,C.C., Stilo,S.A., Marques,T.R., Handley,R., Mondelli,V., Dazzan,P., Pariante,C., David,A.S., Morgan,C., Powell,J. and Murray,R.M., 2012. Confirmation that the AKT1 (rs2494732) Genotype Influences the Risk of Psychosis in Cannabis Users. Biol Psychiatry.

Di Forti,M., Morgan,C., Dazzan,P., Pariante,C., Mondelli,V., Marques,T.R., Handley,R., Luzi,S., Russo,M., Paparelli,A., Butt,A., Stilo,S.A., Wiffen,B., Powell,J. and Murray,R.M., 2009. High-potency cannabis and the risk of psychosis. Br J Psychiatry. 195, 488-491.

Di Marzo,V., Goparaju,S.K., Wang,L., Liu,J., Batkai,S., Jarai,Z., Fezza,F., Miura,G.I., Palmiter,R.D., Sugiura,T. and Kunos,G., 2001. Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature. 410, 822-825.

Diana,M., Melis,M. and Gessa,G.L., 1998. Increase in meso-prefrontal dopaminergic activity after stimulation of CB1 receptors by cannabinoids. Eur J Neurosci. 10, 2825-2830.

Dragunow,M., Butterworth,N., Waldvogel,H., Faull,R.L. and Nicholson,L.F., 1995. Prolonged expression of Fos-related antigens, Jun B and TrkB in dopamine-denervated striatal neurons. Brain Res Mol Brain Res. 30, 393-396.

Eggan,S.M., Hashimoto,T. and Lewis,D.A., 2008. Reduced cortical cannabinoid 1 receptor messenger RNA and protein expression in schizophrenia. Arch Gen Psychiatry. 65, 772-784.

Elphick,M.R. and Egertova,M., 2001. The neurobiology and evolution of cannabinoid signalling. Philos Trans R Soc Lond B Biol Sci. 356, 381-408.

Faber,G., Smid,H.G., Van Gool,A.R., Wunderink,L., van den Bosch,R.J. and Wiersma,D., 2012. Continued cannabis use and outcome in first-episode psychosis: data from a randomized, open-label, controlled trial. J Clin Psychiatry. 73, 632-638.

Fernandes,M., Schabarek,A., Coper,H. and Hill,R., 1974. Modification of delta9-THC-actions by cannabinol and cannabidiol in the rat. Psychopharmacologia. 38, 329-338.

Fogaca,M.V., Aguiar,D.C., Moreira,F.A. and Guimaraes,F.S., 2012. The endocannabinoid and endovanilloid systems interact in the rat prelimbic medial prefrontal cortex to control anxiety-like behavior. Neuropharmacology. 63, 202-210.

Freund,T.F., Katona,I. and Piomelli,D., 2003. Role of endogenous cannabinoids in synaptic signaling. Physiol Rev. 83, 1017-1066.

Fusar-Poli,P., Allen,P., Bhattacharyya,S., Crippa,J.A., Mechelli,A., Borgwardt,S., Martin-Santos,R., Seal,M.L., O'Carrol,C., Atakan,Z., Zuardi,A.W. and McGuire,P., 2010. Modulation of effective connectivity during emotional processing by Delta 9-tetrahydrocannabinol and cannabidiol. Int J Neuropsychopharmacol. 13, 421-432.

Fusar-Poli,P., Crippa,J.A., Bhattacharyya,S., Borgwardt,S.J., Allen,P., Martin-Santos,R., Seal,M., Surguladze,S.A., O'Carrol,C., Atakan,Z., Zuardi,A.W. and McGuire,P.K., 2009. Distinct effects of {delta}9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. Arch Gen Psychiatry. 66, 95-105.

Fusar-Poli,P. and Meyer-Lindenberg,A., 2012a. Striatal Presynaptic Dopamine in Schizophrenia, Part I: Meta-analysis of Dopamine Active Transporter (DAT) Density. Schizophr Bull.

Fusar-Poli,P. and Meyer-Lindenberg,A., 2012b. Striatal Presynaptic Dopamine in Schizophrenia, Part II: Meta-Analysis of [18F/11C]-DOPA PET Studies. Schizophr Bull.

Galiegue,S., Mary,S., Marchand,J., Dussossoy,D., Carriere,D., Carayon,P., Bouaboula,M., Shire,D., Le,F.G. and Casellas,P., 1995. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem. 232, 54-61.

Garcia-Gutierrez,M.S. and Manzanares,J., 2011. Overexpression of CB2 cannabinoid receptors decreased vulnerability to anxiety and impaired anxiolytic action of alprazolam in mice. J Psychopharmacol. 25, 111-120.

Giuffrida,A., Leweke,F.M., Gerth,C.W., Schreiber,D., Koethe,D., Faulhaber,J., Klosterkotter,J. and Piomelli,D., 2004. Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. Neuropsychopharmacology. 29, 2108-2114.

Glass,M., Dragunow,M. and Faull,R.L., 1997. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neuroscience. 77, 299-318.

Gong,J.P., Onaivi,E.S., Ishiguro,H., Liu,Q.R., Tagliaferro,P.A., Brusco,A. and Uhl,G.R., 2006. Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. Brain Res. 1071, 10-23.

Grech,A., van Os,J., Jones,P.B., Lewis,S.W. and Murray,R.M., 2005. Cannabis use and outcome of recent onset psychosis. Eur Psychiatry. 20, 349-353.

Guimaraes,V.M., Zuardi,A.W., Del Bel,E.A. and Guimaraes,F.S., 2004. Cannabidiol increases Fos expression in the nucleus accumbens but not in the dorsal striatum. Life Sci. 75, 633-638.

Gururajan,A., Taylor,D.A. and Malone,D.T., 2011. Effect of cannabidiol in a MK-801-rodent model of aspects of schizophrenia. Behav Brain Res. 222, 299-308.

Hallak,J.E., Dursun,S.M., Bosi,D.C., de Macedo,L.R., hado-de-Sousa,J.P., Abrao,J., Crippa,J.A., McGuire,P., Krystal,J.H., Baker,G.B. and Zuardi,A.W., 2011. The interplay of cannabinoid and NMDA glutamate receptor systems in humans: preliminary evidence of interactive effects of cannabidiol and ketamine in healthy human subjects. Prog Neuropsychopharmacol Biol Psychiatry. 35, 198-202.

Hampson,R.E. and Deadwyler,S.A., 1999. Cannabinoids, hippocampal function and memory. Life Sci. 65, 715-723.

Henquet,C., Rosa,A., Krabbendam,L., Papiol,S., Fananas,L., Drukker,M., Ramaekers,J.G. and van,O.J., 2006. An experimental study of catechol-o-methyltransferase Val158Met moderation of delta-9-tetrahydrocannabinol-induced effects on psychosis and cognition. Neuropsychopharmacology. 31, 2748-2757.

Henstridge,C.M., Balenga,N.A., Kargl,J., Andradas,C., Brown,A.J., Irving,A., Sanchez,C. and Waldhoer,M., 2011. Minireview: recent developments in the physiology and pathology of the lysophosphatidylinositol-sensitive receptor GPR55. Mol Endocrinol. 25, 1835-1848.

Herkenham,M., Groen,B.G., Lynn,A.B., De Costa,B.R. and Richfield,E.K., 1991. Neuronal localization of cannabinoid receptors and second messengers in mutant mouse cerebellum. Brain Res. 552, 301-310.

Hill,M.N., Patel,S., Campolongo,P., Tasker,J.G., Wotjak,C.T. and Bains,J.S., 2010. Functional interactions between stress and the endocannabinoid system: from synaptic signaling to behavioral output. J Neurosci. 30, 14980-14986.

Ho,B.C., Wassink,T.H., Ziebell,S. and Andreasen,N.C., 2011. Cannabinoid receptor 1 gene polymorphisms and marijuana misuse interactions on white matter and cognitive deficits in schizophrenia. Schizophr Res. 128, 66-75.

Howes,O.D. and Kapur,S., 2009. The dopamine hypothesis of schizophrenia: version III--the final common pathway. Schizophr Bull. 35, 549-562.

Howlett,A.C., 2002. The cannabinoid receptors. Prostaglandins Other Lipid Mediat. 68-69, 619-631.

Howlett,A.C., Blume,L.C. and Dalton,G.D., 2010. CB(1) cannabinoid receptors and their associated proteins. Curr Med Chem. 17, 1382-1393.

Howlett,A.C., Breivogel,C.S., Childers,S.R., Deadwyler,S.A., Hampson,R.E. and Porrino,L.J., 2004. Cannabinoid physiology and pharmacology: 30 years of progress. Neuropharmacology. 47 Suppl 1, 345-358.

Huang,S.M., Bisogno,T., Trevisani,M., Al-Hayani,A., De,P.L., Fezza,F., Tognetto,M., Petros,T.J., Krey,J.F., Chu,C.J., Miller,J.D., Davies,S.N., Geppetti,P., Walker,J.M. and Di,M., V, 2002. An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. Proc Natl Acad Sci U S A. 99, 8400-8405.

Ilan,A.B., Gevins,A., Coleman,M., Elsohly,M.A. and de,W.H., 2005. Neurophysiological and subjective profile of marijuana with varying concentrations of cannabinoids. Behav Pharmacol. 16, 487-496.

Ishiguro,H., Horiuchi,Y., Ishikawa,M., Koga,M., Imai,K., Suzuki,Y., Morikawa,M., Inada,T., Watanabe,Y., Takahashi,M., Someya,T., Ujike,H., Iwata,N., Ozaki,N., Onaivi,E.S., Kunugi,H., Sasaki,T., Itokawa,M., Arai,M., Niizato,K., Iritani,S., Naka,I., Ohashi,J., Kakita,A., Takahashi,H., Nawa,H. and Arinami,T., 2010. Brain cannabinoid CB2 receptor in schizophrenia. Biol Psychiatry. 67, 974-982.

Juckel,G., Roser,P., Nadulski,T., Stadelmann,A.M. and Gallinat,J., 2007. Acute effects of Delta9-tetrahydrocannabinol and standardized cannabis extract on the auditory evoked mismatch negativity. Schizophr Res. 97, 109-117.

99Karniol,I.G., Shirakawa,I., Kasinski,N., Pfeferman,A. and Carlini,E.A., 1974. Cannabidiol interferes with the effects of delta 9 - tetrahydrocannabinol in man. Eur J Pharmacol. 28, 172-177.

Kay,S.R., Fiszbein,A. and Opler,L.A., 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull. 13, 261-276.

Klein,T.W., Newton,C.A. and Friedman,H., 2001. Cannabinoids and the immune system. Pain Res Manag. 6, 95-101.

Kuepper,R., Morrison,P.D., van,O.J., Murray,R.M., Kenis,G. and Henquet,C., 2010. Does dopamine mediate the psychosis-inducing effects of cannabis? A review and integration of findings across disciplines. Schizophr Res. 121, 107-117.

Leweke,F.M., Giuffrida,A., Koethe,D., Schreiber,D., Nolden,B.M., Kranaster,L., Neatby,M.A., Schneider,M., Gerth,C.W., Hellmich,M., Klosterkotter,J. and Piomelli,D., 2007. Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: impact of cannabis use. Schizophr Res. 94, 29-36.

Leweke,F.M., Piomelli,D., Pahlisch,F., Muhl,D., Gerth,C.W., Hoyer,C., Klosterkotter,J., Hellmich,M. and Koethe,D., 2012. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. Transl Psychiatry. 2, e94.

Leweke,F.M., Schneider,U., Radwan,M., Schmidt,E. and Emrich,H.M., 2000. Different effects of nabilone and cannabidiol on binocular depth inversion in Man. Pharmacol Biochem Behav. 66, 175-181.

Linszen,D.H., Dingemans,P.M. and Lenior,M.E., 1994. Cannabis abuse and the course of recent-onset schizophrenic disorders. Arch Gen Psychiatry. 51, 273-279.

Lipska,B.K. and Weinberger,D.R., 2000. To model a psychiatric disorder in animals: schizophrenia as a reality test. Neuropsychopharmacology. 23, 223-239.

Long,L.E., Malone,D.T. and Taylor,D.A., 2006. Cannabidiol reverses MK-801-induced disruption of prepulse inhibition in mice. Neuropsychopharmacology. 31, 795-803.

Macleod,J., Oakes,R., Copello,A., Crome,I., Egger,M., Hickman,M., Oppenkowski,T., Stokes-Lampard,H. and Davey,S.G., 2004. Psychological and social sequelae of cannabis and other illicit drug use by young people: a systematic review of longitudinal, general population studies. Lancet. 363, 1579-1588.

Mailleux,P., Parmentier,M. and Vanderhaeghen,J.J., 1992. Distribution of cannabinoid receptor messenger RNA in the human brain: an in situ hybridization histochemistry with oligonucleotides. Neurosci Lett. 143, 200-204.

Malone,D.T., Jongejan,D. and Taylor,D.A., 2009. Cannabidiol reverses the reduction in social interaction produced by low dose Delta(9)-tetrahydrocannabinol in rats. Pharmacol Biochem Behav. 93, 91-96.

Marco,E.M., Garcia-Gutierrez,M.S., Bermudez-Silva,F.J., Moreira,F.A., Guimaraes,F., Manzanares,J. and Viveros,M.P., 2011. Endocannabinoid system and psychiatry: in search of a neurobiological basis for detrimental and potential therapeutic effects. Front Behav Neurosci. 5, 63.

Martinez-Gras,I., Hoenicka,J., Ponce,G., Rodriguez-Jimenez,R., Jimenez-Arriero,M.A., Perez-Hernandez,E., Ampuero,I., Ramos-Atance,J.A., Palomo,T. and Rubio,G., 2006. (AAT)n repeat in the cannabinoid receptor gene, CNR1: association with schizophrenia in a Spanish population. Eur Arch Psychiatry Clin Neurosci. 256, 437-441.

Matochik,J.A., Eldreth,D.A., Cadet,J.L. and Bolla,K.I., 2005. Altered brain tissue composition in heavy marijuana users. Drug Alcohol Depend. 77, 23-30.

Matsuyama,S.S. and Fu,T.K., 1981. In vivo cytogenetic effects of cannabinoids. J Clin Psychopharmacol. 1, 135-140.

McGrath,J., Saha,S., Chant,D. and Welham,J., 2008. Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiol Rev. 30, 67-76.

Mechoulam,R., Peters,M., Murillo-Rodriguez,E. and Hanus,L.O., 2007. Cannabidiol--recent advances. Chem Biodivers. 4, 1678-1692.

Mechoulam,R., Shani,A., Edery,H. and Grunfeld,Y., 1970. Chemical basis of hashish activity. Science. 169, 611-612.

Minozzi,S., Davoli,M., Bargagli,A.M., Amato,L., Vecchi,S. and Perucci,C.A., 2010. An overview of systematic reviews on cannabis and psychosis: discussing apparently conflicting results. Drug Alcohol Rev. 29, 304-317.

Moore,T.H., Zammit,S., Lingford-Hughes,A., Barnes,T.R., Jones,P.B., Burke,M. and Lewis,G., 2007. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet. 370, 319-328.

Moreira,F.A. and Guimaraes,F.S., 2005. Cannabidiol inhibits the hyperlocomotion induced by psychotomimetic drugs in mice. Eur J Pharmacol. 512, 199-205.

Morgan,C.J. and Curran,H.V., 2008. Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. Br J Psychiatry. 192, 306-307.

Morgan,C.J., Gardener,C., Schafer,G., Swan,S., Demarchi,C., Freeman,T.P., Warrington,P., Rupasinghe,I., Ramoutar,A., Tan,N., Wingham,G., Lewis,S. and Curran,H.V., 2011. Sub-chronic impact of cannabinoids in street cannabis on cognition, psychotic-like symptoms and psychological well-being. Psychol Med. 1-10.

Morgan,C.J., Schafer,G., Freeman,T.P. and Curran,H.V., 2010. Impact of cannabidiol on the acute memory and psychotomimetic effects of smoked cannabis: naturalistic study: naturalistic study [corrected]. Br J Psychiatry. 197, 285-290.

Munro,S., Thomas,K.L. and bu-Shaar,M., 1993. Molecular characterization of a peripheral receptor for cannabinoids. Nature. 365, 61-65.

Newell,K.A., Deng,C. and Huang,X.F., 2006. Increased cannabinoid receptor density in the posterior cingulate cortex in schizophrenia. Exp Brain Res. 172, 556-560.

Onaivi,E.S., Ishiguro,H., Gong,J.P., Patel,S., Perchuk,A., Meozzi,P.A., Myers,L., Mora,Z., Tagliaferro,P., Gardner,E., Brusco,A., Akinshola,B.E., Liu,Q.R., Hope,B., Iwasaki,S., Arinami,T., Teasenfitz,L. and Uhl,G.R., 2006. Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. Ann N Y Acad Sci. 1074, 514-536.

Ortega-Alvaro,A., racil-Fernandez,A., Garcia-Gutierrez,M.S., Navarrete,F. and Manzanares,J., 2011. Deletion of CB2 cannabinoid receptor induces schizophrenia-related behaviors in mice. Neuropsychopharmacology. 36, 1489-1504.

Perez-Reyes,M., Timmons,M.C., Davis,K.H. and Wall,E.M., 1973. A comparison of the pharmacological activity in man of intravenously administered delta9-tetrahydrocannabinol, cannabinol, and cannabidiol. Experientia. 29, 1368-1369.

Pertwee,R.G., 2008. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. Br J Pharmacol. 153, 199-215.

Potter,D.J., Clark,P. and Brown,M.B., 2008. Potency of delta 9-THC and other cannabinoids in cannabis in England in 2005: implications for psychoactivity and pharmacology. J Forensic Sci. 53, 90-94.

Rais,M., Cahn,W., Van,H.N., Schnack,H., Caspers,E., Hulshoff,P.H. and Kahn,R., 2008. Excessive brain volume loss over time in cannabis-using first-episode schizophrenia patients. Am J Psychiatry. 165, 490-496.

Rapp,C., Bugra,H., Riecher-Rossler,A., Tamagni,C. and Borgwardt,S., 2012. Effects of cannabis use on human brain structure in psychosis: a systematic review combining in vivo structural neuroimaging and post mortem studies. Curr Pharm Des. 18, 5070-5080.

Robertson,G.S. and Fibiger,H.C., 1992. Neuroleptics increase c-fos expression in the forebrain: contrasting effects of haloperidol and clozapine. Neuroscience. 46, 315-328.

Rosenkrantz,H., Fleischman,R.W. and Grant,R.J., 1981. Toxicity of short-term administration of cannabinoids to rhesus monkeys. Toxicol Appl Pharmacol. 58, 118-131.

Roser,P., Juckel,G., Rentzsch,J., Nadulski,T., Gallinat,J. and Stadelmann,A.M., 2008. Effects of acute oral Delta9-tetrahydrocannabinol and standardized cannabis extract on the auditory P300 event-related potential in healthy volunteers. Eur Neuropsychopharmacol. 18, 569-577.

Rottanburg,D., Robins,A.H., Ben-Arie,O., Teggin,A. and Elk,R., 1982. Cannabis-associated psychosis with hypomanic features. Lancet. 2, 1364-1366.

Sanchez,C., Galve-Roperh,I., Canova,C., Brachet,P. and Guzman,M., 1998. Delta9-tetrahydrocannabinol induces apoptosis in C6 glioma cells. FEBS Lett. 436, 6-10.

Schneider,U., Borsutzky,M., Seifert,J., Leweke,F.M., Huber,T.J., Rollnik,J.D. and Emrich,H.M., 2002. Reduced binocular depth inversion in schizophrenic patients. Schizophr Res. 53, 101-108.

Schubart,C.D., Sommer,I.E., van Gastel,W.A., Goetgebuer,R.L., Kahn,R.S. and Boks,M.P., 2011. Cannabis with high cannabidiol content is associated with fewer psychotic experiences. Schizophr Res. 130, 216-221.

Schubart,C.D., van Gastel,W.A., Breetvelt,E.J., Beetz,S.L., Ophoff,R.A., Sommer,I.E., Kahn,R.S. and Boks,M.P., 2010. Cannabis use at a young age is associated with psychotic experiences. Psychol Med. 1-10.

Skinner,R., Conlon,L., Gibbons,D. and McDonald,C., 2010. Cannabis use and non-clinical dimensions of psychosis in university students presenting to primary care. Acta Psychiatr Scand.

Stadelmann,A.M., Juckel,G., Arning,L., Gallinat,J., Epplen,J.T. and Roser,P., 2011. Association between a cannabinoid receptor gene (CNR1) polymorphism and cannabinoid-induced alterations of the auditory event-related P300 potential. Neurosci Lett. 496, 60-64.

Starowicz,K., Cristino,L. and Di,M., V, 2008. TRPV1 receptors in the central nervous system: potential for previously unforeseen therapeutic applications. Curr Pharm Des. 14, 42-54.

Stefanis,N.C., Delespaul,P., Henquet,C., Bakoula,C., Stefanis,C.N. and van,O.J., 2004. Early adolescent cannabis exposure and positive and negative dimensions of psychosis. Addiction. 99, 1333-1341.

Stokes,P.R., Mehta,M.A., Curran,H.V., Breen,G. and Grasby,P.M., 2009. Can recreational doses of THC produce significant dopamine release in the human striatum? Neuroimage. 48, 186-190.

Thomas,A., Baillie,G.L., Phillips,A.M., Razdan,R.K., Ross,R.A. and Pertwee,R.G., 2007. Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. Br J Pharmacol. 150, 613-623.

Tsai,S.J., Wang,Y.C. and Hong,C.J., 2000. Association study of a cannabinoid receptor gene (CNR1) polymorphism and schizophrenia. Psychiatr Genet. 10, 149-151.

Ueda,N., Tsuboi,K., Uyama,T. and Ohnishi,T., 2011. Biosynthesis and degradation of the endocannabinoid 2-arachidonoylglycerol. Biofactors. 37, 1-7.

Ujike,H., Takaki,M., Nakata,K., Tanaka,Y., Takeda,T., Kodama,M., Fujiwara,Y., Sakai,A. and Kuroda,S., 2002. CNR1, central cannabinoid receptor gene, associated with susceptibility to hebephrenic schizophrenia. Mol Psychiatry. 7, 515-518.

Umathe,S.N., Manna,S.S. and Jain,N.S., 2012. Endocannabinoid analogues exacerbate marble-burying behavior in mice via TRPV1 receptor. Neuropharmacology. 62, 2024-2033.

Umbricht,D. and Krljes,S., 2005. Mismatch negativity in schizophrenia: a meta-analysis. Schizophr Res. 76, 1-23.

Urigüen,L., Garcia-Fuster,M.J., Callado,L.F., Morentin,B., La,H.R., Casado,V., Lluis,C., Franco,R., Garcia-Sevilla,J.A. and Meana,J.J., 2009. Immunodensity and mRNA expression of A2A adenosine, D2 dopamine, and CB1 cannabinoid receptors in postmortem frontal cortex of subjects with schizophrenia: effect of antipsychotic treatment. Psychopharmacology (Berl). 206, 313-324.

van Gastel,W.A., Wigman,J.T., Monshouwer,K., Kahn,R.S., van,O.J., Boks,M.P. and Vollebergh,W.A., 2012. Cannabis use and subclinical positive psychotic experiences in early adolescence: findings from a Dutch survey. Addiction. 107, 381-387.

van Os,J. and Kapur,S., 2009. Schizophrenia. Lancet. 374, 635-645.

Van Sickle,M.D., Duncan,M., Kingsley,P.J., Mouihate,A., Urbani,P., Mackie,K., Stella,N., Makriyannis,A., Piomelli,D., Davison,J.S., Marnett,L.J., Di,M., V, Pittman,Q.J., Patel,K.D. and Sharkey,K.A., 2005. Identification and functional characterization of brainstem cannabinoid CB2 receptors. Science. 310, 329-332.

van Winkel R., 2011. Family-based analysis of genetic variation underlying psychosis-inducing effects of cannabis: sibling analysis and proband follow-up. Arch Gen Psychiatry. 68, 148-157.

Verrico,C.D., Jentsch,J.D., Roth,R.H. and Taylor,J.R., 2004. Repeated, intermittent delta(9)-tetrahydrocannabinol administration to rats impairs acquisition and performance of a test of visuospatial divided attention. Neuropsychopharmacology. 29, 522-529.

Waksman,Y., Olson,J.M., Carlisle,S.J. and Cabral,G.A., 1999. The central cannabinoid receptor (CB1) mediates inhibition of nitric oxide production by rat microglial cells. J Pharmacol Exp Ther. 288, 1357-1366.

Walter,L., Franklin,A., Witting,A., Wade,C., Xie,Y., Kunos,G., Mackie,K. and Stella,N., 2003. Nonpsychotropic cannabinoid receptors regulate microglial cell migration. J Neurosci. 23, 1398-1405.

Yücel,M., Solowij,N., Respondek,C., Whittle,S., Fornito,A., Pantelis,C. and Lubman,D.I., 2008. Regional brain abnormalities associated with long-term heavy cannabis use. Arch Gen Psychiatry. 65, 694-701.

Zavitsanou,K., Garrick,T. and Huang,X.F., 2004. Selective antagonist [3H]SR141716A binding to cannabinoid CB1 receptors is increased in the anterior cingulate cortex in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 28, 355-360.

Zuardi,A.W., Crippa,J.A., Hallak,J.E., Pinto,J.P., Chagas,M.H., Rodrigues,G.G., Dursun,S.M. and Tumas,V., 2009. Cannabidiol for the treatment of psychosis in Parkinson's disease. J Psychopharmacol. 23, 979-983.

Zuardi,A.W., Morais,S.L., Guimaraes,F.S. and Mechoulam,R., 1995. Antipsychotic effect of cannabidiol. J Clin Psychiatry. 56, 485-486.

Zuardi,A.W., Rodrigues,J.A. and Cunha,J.M., 1991. Effects of cannabidiol in animal models predictive of antipsychotic activity. Psychopharmacology (Berl). 104, 260-264.

Zuardi,A.W., Shirakawa,I., Finkelfarb,E. and Karniol,I.G., 1982. Action of cannabidiol on the anxiety and other effects produced by delta 9-THC in normal subjects. Psychopharmacology (Berl). 76, 245-250.

PART I: EPIDEMIOLOGICAL FACTORS
CHAPTER V

• CANNABIS WITH HIGH CANNABIDIOL CONTENT IS ASSOCIATED WITH FEWER PSYCHOTIC EXPERIENCES •

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ABSTRACT

Objective
Cannabis is associated with psychotic outcomes in numerous studies, an effect that is commonly attributed to Δ^9-tetrahydrocannabinol (Δ 9-THC). An increasing number of authors identify cannabidiol, another component of the cannabis plant, as an antipsychotic agent. The objective of the current study is to investigate the role of cannabidiol content in the association between cannabis use and psychiatric symptoms in a large non-clinical population of cannabis users.

Methods
In a web-based cross-sectional study we obtained detailed information about cannabis use and subclinical psychiatric experiences using the Community Assessment of Psychic Experiences (CAPE). Different types of cannabis (i.e. marijuana, hashish etc) have distinctive proportions of Δ 9-THC and cannabidiol. Since average concentrations of Δ 9-THC and cannabidiol in the most popular types of cannabis sold on the Dutch market are annually measured, we were able to quantify exposure to Δ 9-THC and cannabidiol.

Results
We included 1877 subjects (mean age 23, SD 6.0) who used the same type of cannabis in the majority of the occasions (in >60% of occasions). We found a significant inverse relationship ($F(1,1877)$: 14.577, $p<0.001$) between cannabidiol content and self-reported positive symptoms, but not with negative symptoms or depression. The estimated effect size of cannabidiol content was small.

Conclusion
Although the observed effects are subtle, using high cannabidiol content cannabis was associated with significantly lower degrees of psychotic symptoms providing further support for the antipsychotic potential of cannabidiol.

INTRODUCTION

The association between cannabis use and psychotic outcomes is reported consistently, (Schubart et al., 2010a;Arseneault et al., 2004;Moore et al., 2007;Skinner et al., 2010) although the causality of this association is difficult to assess and still under debate (Macleod et al., 2004;DeLisi, 2008). However, as cannabis is the most widely used illicit drug in the world (United Nations Office on Drugs and Crime, 2012), clarifying mental health sequelae of cannabis use is of great importance. A possible explanation of the heterogeneous nature of the findings in this field, is that exposure to cannabis is not uniformly defined and that several factors might influence biological exposure to cannabis. A number of effect modifying factors have already been identified. Cannabis associated risk of psychotic outcomes is increased in individuals who use high amounts of cannabis (Moore et al., 2007) and in subjects who start to use cannabis early in life (McGrath et al., 2010;Schubart et al., 2010a;Konings et al., 2008). Commonly, Δ^9-tetrahydrocannabinol (Δ 9-THC) is denoted as the main psychoactive ingredient of cannabis products such as marijuana and hashish (Mechoulam et al., 1970b) and the concentration or content of Δ 9-THC is traditionally considered as the main measure of cannabis potency (McLaren et al., 2008). However, cannabis plants contain more than 70 different cannabinoids that are also found in the cannabis products on the market (Elsohly and Slade, 2005). Cannabidiol is one of these cannabinoids and a number of studies suggest that cannabidiol has antipsychotic properties and could therefore modify the mental health sequelae of cannabis use. (Morgan and Curran, 2008;Leweke et al., 2000a;Zuardi et al., 2006b;Zuardi et al., 1995). Di Forti et al. found that the use of cannabis containing a high Δ 9-THC- and a low cannabidiol (CBD) concentration was retrospectively associated with a higher risk of a first psychotic episode (Di Forti et al., 2009). Similarly, a number of authors hypothesize that cannabidiol possibly antagonizes the effects of Δ 9-THC (Smith, 2005b;McLaren et al., 2008); i.e. that it has protective properties against psychosis.

Concentrations of cannabidiol and Δ 9-THC differ greatly between various types of cannabis products such as marijuana (weed) and hashish (resin), year and by place of origin (Potter et al., 2008;Trimbos, 2008;Trimbos, 2009;Mehmedic et al., 2010). For instance, in 2008 marijuana produced in The Netherlands contained virtually no cannabidiol and had a mean Δ 9-THC concentration of 16%, whereas hashish imported from countries as Nepal, Afghanistan or Morocco, contained a similar concentration of Δ 9-THC (17%) but also contained 9% of cannabidiol (Trimbos, 2009). Given the hypothesized antipsychotic potential of cannabidiol, the variation in concentrations of cannabidiol could be reflected in a moderation of the association between cannabis use and psychotic symptoms. Further exploring the role of cannabidiol in the association between cannabis and psychosis symptoms could be of value in the debate on the impact of cannabis on population mental health. Moreover, evidence on the associated risks of particular cannabis products could improve the quality of psychoeducation on the risks of cannabis use.

This study aims to investigate the influence of the Δ 9-THC/ cannabidiol ratio in different cannabis products on the association between cannabis use and psychiatric symptoms in a large non-clinical population of young adult cannabis users.

METHODS

Participants

Participants were recruited using a project website launched in 2008 (*www.cannabisquest.nl*). Individuals were directed to the website via different media; advertisements distributed on more than 100 different collaborating colleges and universities using intranet, posters and flyers. The chance to win an Apple iPod or a Nintendo Wii was used as an incentive. The website targeted mainly Dutch speaking young adults and adolescents (18-25 years). Besides personal information as age, educational level and contact details, all participants filled out the Community Assessment of Psychic Experiences (CAPE)(Stefanis et al., 2002;Konings et al., 2006) and the Cannabis Use Inventory (CUI) (described below). Only subjects who indicated to use cannabis were included in the analyses. Participants who indicated having an inconsistent pattern of cannabis use (<60% consistent preference) or were not aware of the type of cannabis they used, were excluded from the analyses. Verification questions were used to protect against random answers and internet bots that run automated tasks. To increase the homogeneity of the sample participants who indicated to be younger than 10 years or older than 60 years of age were excluded.

This study was approved by the UMC Utrecht medical ethical commission and all participants gave online informed consent.

Assessment of psychiatric symptoms

The CAPE is a 42-item, self-rating instrument and includes three dimensions: positive symptoms, negative- and depressive symptoms with discriminative validity in individuals from the general population (Stefanis et al., 2002;Konings et al., 2006).

Cannabis quantity measure

To assess detailed information on cannabis use the Cannabis Use Inventory (CUI) questionnaire was developed. The CUI offers a retrospective comprehensive inventory of life time cannabis exposure. Participants are asked to indicate at which age they started to use cannabis and in which frequency. Thereafter subjects are asked to indicate if, and if so at what age, their consumption frequency had changed significantly and how long this period lasted. In total, participants can indicate five different periods of distinct cannabis use frequency, covering the period since first use until present day. Based on information from the CUI, the population sample was arbitrarily divided in nine categories on quantity of cannabis use in the last year: 1) Once a year or less, 2) Over once a year but not monthly, 3) Once a month, 4) Weekly for 0 to 5 euros a week, 5) Weekly for 5 to 10 euros a week, 6) Weekly

for 10 to 25 euros a week, 7) Weekly for 25 to 50 euros a week, 8) Weekly for 50 to 100 euros a week, 9) Weekly for more than 100 euros a week. Outliers in amount of use (more than two standard deviation from the mean equalling) were excluded from analysis.

Annual reports on Δ 9-THC and cannabidiol content
The Netherlands Institute of Mental Health and Addiction (Trimbos Institute) is a Dutch centre of expertise and conducts research on mental health, mental resilience and addiction. Since 1999, the Trimbos Institute annually visits a random selection of 50 Dutch Coffeeshops (establishments were the distribution of small quantities of cannabis for personal use is legal under Dutch law), for reference, approximately 700 Coffeeshops existed in The Netherlands in 2007 (Trimbos, 2008). The researchers measures the concentrations of Δ 9-THC, cannabidiol and cannabinol in the following five cannabis products: i) Dutch marijuana, ii) imported marijuana, iii) Dutch hashish, iv) imported hashish and v) the strongest marijuana sold in the Coffeeshop (Trimbos, 2009). Since all subjects in the current analysis had indicated the type of cannabis product they commonly use, the average measurements mentioned in the annual Trimbos reports, were used as a by proxy estimate of exposure to these cannabinoids.

Cannabis Type
Following the categorization of the annual Trimbos Institute measurements as described above, we asked participants which of the following types of cannabis they usually consumed; 1) Dutch marijuana, 2) imported marijuana, 3) Dutch hashish, 4) imported hashish, 5) the strongest type in my Coffeeshop. To increase the validity of our classification, participants could also give the following answers 6) hashish of unknown origin, 7) marijuana of unknown origin, 8) the most popular type in my Coffeeshop, 9) variation between two types 10) different every time, and finally 11) unknown. Only those participants were selected for further analysis who indicated using one of the cannabis types that are represented in the annual Trimbos Institute report (types 1,2,3,4 and 5). Finally, participants were asked how often they used the selected type of cannabis. We excluded subjects if they indicated using the selected type in less than 60% of the occasions where they used cannabis. Combining information from the Trimbos Institute annual reports on cannabinoid concentrations and the individual cannabis use patterns in our dataset, we were able to estimate the exposure to Δ 9-THC and cannabidiol. A Δ 9-THC /cannabidiol ratio was calculated for each participant.
As a result of different THC and cannabidiol concentrations in the five cannabis products in the years 2008 and 2009, ten (5x2) levels THC/cannabidiol concentrations were calculated within the sample (THC/cannabidiol concentrations: 2.0, 3.6, 8.8, 16.0, 24.8, 29.6, 45.8, 55.3, 75.0, 81.5).
To avoid analyses of small groups and in order to conserve power we applied a median split to dichotomize the THC/ cannabidiol ratio given that these cannabidiol concentrations broadly fall into two categories (high and low THC/

cannabidiol ratio). Median spit was at 55.3 effectively defining a high cannabidiol content group and a low cannabidiol group that were used in the final analyses.

Statistical Analysis
Firstly, analysis of Co-Variance (ANCOVA) was used to investigate the association between the degree of cannabidiol content (high/low) and the total CAPE score, adjusting for age, sex and initial age and amount of cannabis use. Secondly, a MANCOVA was used to analyze the impact of cannabidiol content (dichotomized THC/ cannabidiol ratio) on the association between cannabis use and the three CAPE symptom dimensions (positive, negative and depressive) jointly as outcome measures, likewise adjusting for age, sex and initial age and amount of cannabis use. Additionally a four linear regression analyses were performed using the total cape score, positive-, negative and depressive symptoms as dependent variables and the undichotomized THC/CBD ratio as main independent variable adjusting for age, gender, age at first use and amount of cannabis use.

RESULTS
Sample characteristics
We included a total of 1,877 participants selected from an initial number of 11,465 subjects that filled out the online questionnaires in the period from April 2008 until March 2010. The reasons for exclusion were; not using cannabis (32%), not knowing exactly which type of cannabis was used (31%), incorrect answers to verification questions (16%), an inconsistent pattern of cannabis use (2.8%) and miscellaneous reasons (1.4%). The mean age of the participants was 23 years (SD: 6) of which 34% was female. Three percent of the sample had no educational diploma, 43% had a secondary school diploma as highest academic achievement, 38% had a non-academic post-secondary diploma and 10% had a University degree. The mean cape score in this sample was 111.0 (SD: 31.7) which is higher than in a sample of non-clinical, cannabis naïve young adults as described elsewhere (Schubart et al., 2010a).

Cannabis use preferences
The majority of the sample preferred “Dutch marijuana” (69%),”Imported Hashish” was second (18%) followed by the “ strongest marijuana available” (8%) and ”Dutch Hashish” (3%). After the median split, the low cannabidiol content cannabis use group (n=663) is composed of 595 users who prefer “Dutch marijuana” in 2009 (THC: 15.0%, CBD: 0.2%) and 65 subjects who preferred the “strongest marijuana available” in 2009 (THC: 16.3%, CBD: 0.2%). The high cannabidiol content group (n=1214) consisted of 707 subjects who preferred “Dutch marijuana” in 2008 (THC: 16.6%, CBD: 0.3%), 25 subjects who prefer “ Imported marijuana” (THC: 6.4%, CBD: 0.4%), 57 subjects who use “ Dutch Hashish” (THC: 26.6%, CBD: 0.9%), 345 individuals that consistently used “imported hashish” (THC: 17.9%, CBD: 8.8%) and 80 subjects that preferred “strongest marijuana available” in 2008 (THC: 16.6%, CBD: 0.4%). As

shown in table 1, the average percentage of preference of a single type of cannabis and the amount of cannabis used was comparable between the two groups. Cannabis preference was correlated with age and gender, but post-hoc analysis revealed no significant differences between the user groups.

Table 1. Characteristics of the cannabidiol content groups and results of the analyses.

Group characteristics	High CBD	Low CBD		
N	1214	663		
Mean Age (SD)	23.1 (4.4)	24.3 (7.0)		
% male	67%	64%		
CBD/THC ratio's	2.0, 3.6, 8.8, 16.0, 24.8, 29.6, 45.8, 55.3	75.0, 81.5		
Cannabis type loyalty*	85%	86%		
Median cannabis use category	1x/month	weekly <€5		
Outcome**			**F**	**p-value**
Mean CAPE Total (SD)	109.8 (31.5)	112.8 (33.6)	5.182	0.023
Mean Positive (SD)	39.7 (12.8)	41.7 (14.5)	14.577	<0.001
Mean Negative (SD)	45.5 (15.5)	45.9 (16.0)	0.366	0.545
Mean Depressive (SD)	24.6 (8.9)	25.1 (9.1)	1.971	0.161

* Loyalty is defined as the mean percentage of the cases in which the participants choose their cannabis type of preference.
**All analyses were adjusted for age, gender, amount of cannabis use and age of first cannabis use.

Cannabis use and CAPE scores

After adjusting for age, gender, amount of cannabis use and age of first use, cannabidiol content had a significant effect on total CAPE score. Subjects who use cannabis with high cannabidiol content had significantly lower total CAPE scores than low cannabidiol content cannabis users (F(1,1877):5.182, p:0.023) albeit with a small effect size (Partial Eta^2: 0.003). Figure 1 shows the estimated marginal means of total cape scores in subjects that use high or low cannabidiol containing cannabis types. A multiple analysis of covariance (MANCOVA) showed a positive and significant relationship between the quantity of cannabis use in the last year and the outcome on all three dimension of the CAPE, (Pillai's trace F: 3.303, p: 0.001).

The age at onset of cannabis use was not associated with the total CAPE (F(1,1877): 0.578, p:0.447) or with the CAPE sub scores (Pillai's trace F: 0.004, p:0.055) most likely due to the absence of an effect on the negative and depressive symptoms (between group effect, negative (F(1,1877): 0,142, p:0.706), depressive(F(1,1877): 0.014, p:0,905)) in the presence of an effect on positive symptoms only (F(1,1877): 4.809, p: 0.028) consistent with our previous findings (Schubart et al., 2010a).

CBD content and CAPE scores
A multiple analysis of covariance (MANCOVA) with the three CAPE sub scores (positive, negative an depressive symptoms) as outcomes, showed a significant effect of cannabidiol content (Pillai's trace F:5.691, p=0.001). Participants who indicated using cannabis types with high cannabidiol content reported significantly less positive symptoms (F(1,1877): 14.577, p<:0.001) again with small effect size (Partial Eta^2:0.008). Figure 2 shows the estimated marginal means of the relationship between cannabis exposure and CAPE outcome for the high and low cannabidiol content groups. The associations of cannabidiol content with negative and depressive symptoms were not significant (negative (F(1,1877))= 0.366, p:0.545) and depressive (F(1,1877): 1.971, p:0.161). See table 1. The amount of use was independently associated with the total CAPE score (Pillai's trace F: 0.048, p: 0.000) and between group effect (F:8.853, p<0.001). Finally linear regression analyses showed that the THC/CBD ratio of preferred types of cannabis, after adjustment for age, gender, age at first use and frequency of use, is only associated with positive symptoms (ß:0.052, p<0.001) as opposed to negative symptoms (ß:0.004,p:0.850), depressive symptoms (ß: 0.031,p:0.178) and the total CAPE score (ß:0.031,p:0.155).

DISCUSSION
In a large cross-sectional sample of cannabis users from the general population, we investigated the association between cannabidiol content of preferred cannabis types and self-reported positive-, negative- and depressive psychiatric experiences. A subtle but significant association between using a cannabis product with low cannabidiol content and high levels of psychotic symptoms was observed. In contrast, low cannabidiol content was not associated with differences in negative or depressive symptoms. Our findings support earlier reports that attribute a role to cannabidiol in modifying the impact of Δ 9-THC on the risk of various psychotic outcomes.

The relationship between CBD and psychotic symptoms
The hypothesis that cannabidiol impacts on the effect of Δ 9-THC was firstly postulated in 1982 by Rottanburg et al. (Rottanburg et al., 1982a) who found an increased prevalence of psychotic disorders among users of cannabis with high Δ 9-THC content and lack of cannabidiol. Zuardi et al. observed that cannabidiol, co-administered with Δ 9-THC, significantly reduced the psychotomimetic symptoms induced by Δ 9-THC (Rottanburg et al., 1982c). A later study, using binocular depth inversion as a psychosis model, reported cannabidiol to attenuate the effects of a synthetic Δ 9-THC cannabinoid, Nabilone, suggesting cannabidiol has antipsychotic properties (Leweke et al., 2000a). Moreover, recently a series of papers was published on distinct effects of CBD and THC on various measures of brain function, generating an explanatory hypothesis for the phenomenological differences associated with these two cannabinoids (Bhattacharyya et al., 2010;Fusar-Poli et al., 2010a). The findings of the current study also concur with the results of Di Forti et al. who report an association between the use of cannabis with high THC and low

cannabidiol content and a higher risk of developing a first psychotic episode (Di Forti et al., 2009) and a previous study in a small non-clinical population that showed that in hair samples of 140 individuals, the measured cannabidiol /Δ 9-THC ratio was associated with the report of schizophrenia-like symptoms (Morgan and Curran, 2008), with low ratio's predicting high levels of symptoms.

Figure 1. Mean total CAPE score and cannabis exposure per cannabidiol content group (n=1,877).

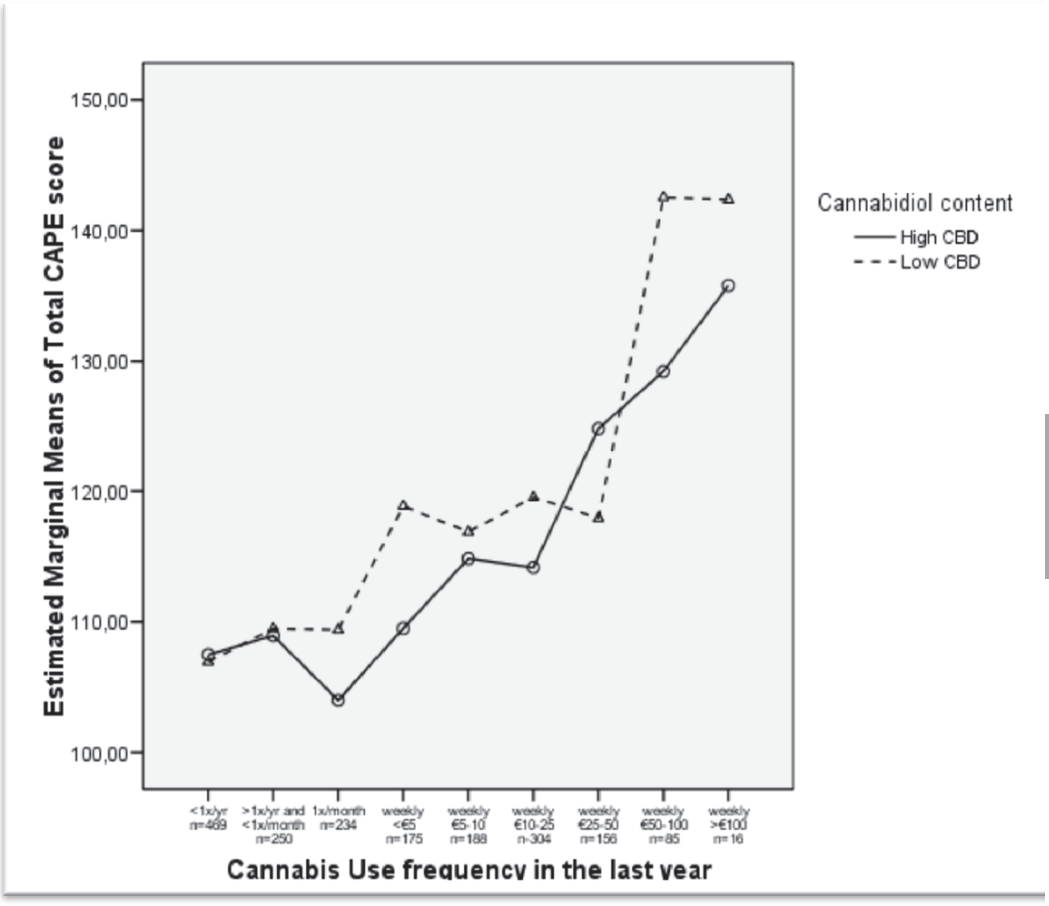
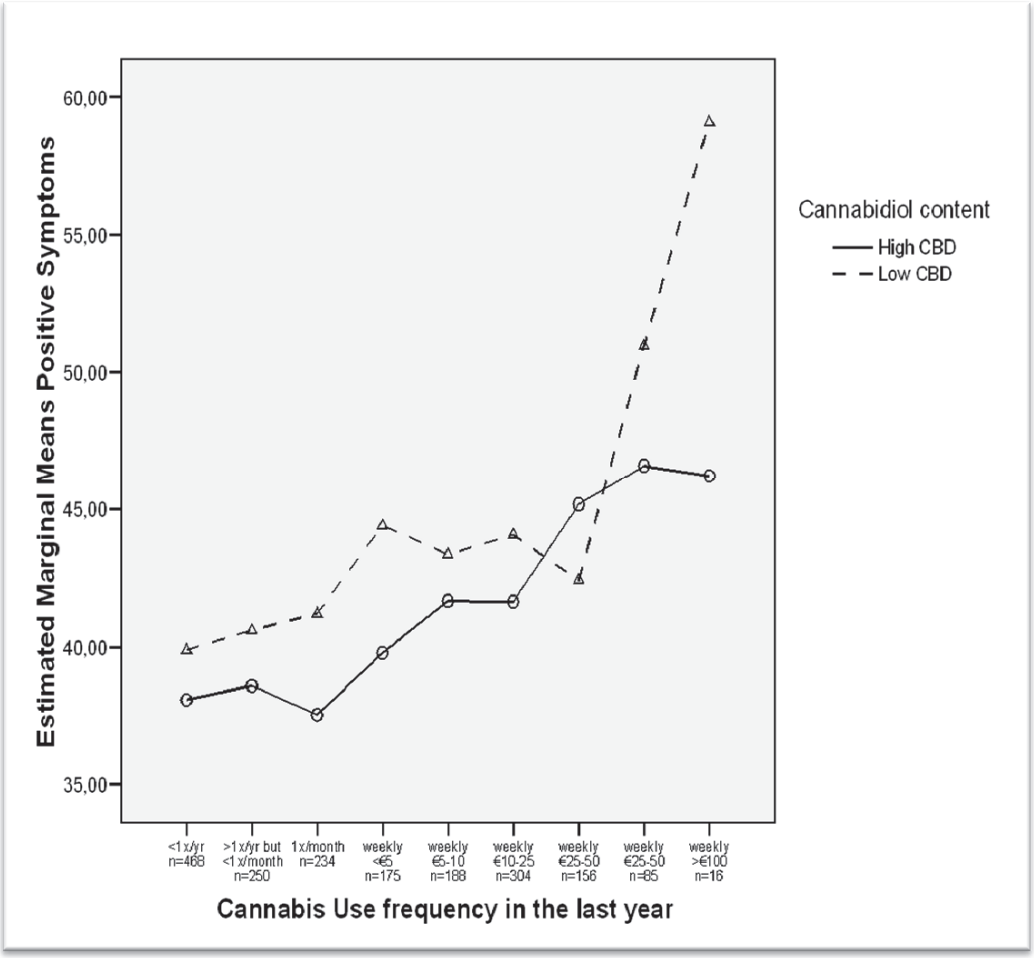


Figure 2. Mean score on positive symptoms and cannabis exposure per cannabidiol content group (n=1,877).



Strengths and limitations

Although the observed effect of CBD on the level of positive symptoms is significant, the effect sizes in our study are small compared to earlier studies investigating the antipsychotic potential of cannabidiol. A possible explanation of this small effect size might be that the dosage of cannabidiol in cannabis products intended for smoking is much lower than in the purified oral form that is used in treatment studies. The highest median cannabidiol concentration measured in cannabis samples, was 8.8% (Trimbos, 2008) . Estimating that a joint contains 1 gram of hashish, this would equal 88 mg of cannabidiol, which is 10 times less than applied in earlier treatment studies (Zuardi et al., 2006a;Leweke et al., 2000a). Moreover, the therapeutic properties of cannabidiol are most probably further reduced by the burning process that

occurs when cannabis is smoked. Furthermore, the cannabis products used by the subjects in our study are not actually the same samples in which cannabinoid concentrations were measured. Although this clearly constitutes a measurement bias, the fact that a small albeit significant effect was still found, further underlines the potential antipsychotic properties of CBD. Since the design of the current study is cross-sectional, causal inference on the reported association is not possible. An alternative explanation for the current findings therefore, is that individuals in the general population who experience psychotic symptoms are more likely to prefer cannabis products with lower cannabidiol content. Although this may not be the most intuitive explanation, it cannot be ruled out. A prospective, longitudinal design, accounting for baseline psychotic symptoms and genetic (family) risk of psychosis, could thoroughly assess the temporal dynamics between psychotic symptoms and exposure to THC and cannabidiol. Another potential limitation of the current study is that all data was collected using the internet. The increased availability of internet access and the development of better web-based tools have improved the possibilities to acquire information on psychiatric symptoms via the internet. Web-based tools are therefore considered a valid additional method in epidemiological research (Meyerson and Tryon, 2003;Balter et al., 2005;Gosling et al., 2004;Ekman et al., 2006). Nevertheless, the use of web-based assessments can potentially lead to inaccuracy. The distribution of this potential inaccuracy however, is most likely independent of the type of cannabis used (exposure measure) and is therefore unlikely to have systematically influenced the reported associations. Moreover, the sample is comprised of cannabis users only and therefore is a selected sample by definition. We also only included subjects with consistent and explicit preferences of cannabis use. However, by adjusting our statistical model for age, gender and cannabis use, the risk of confounding due to selection within the sample is minimized. Furthermore, a median split was applied to avoid separate analyses of groups with small numbers of subjects. The use of a median split has potential disadvantages, since all values on each side of the median are collapsed, information is lost, However since the distribution of the CBD ratio's is not normal and the small group sizes lead to reduced power, a median split is a necessary mean to preserve power. Finally, concomitant use of other drugs than cannabis was not measured and could potentially have influenced our results.

Conclusion

To our knowledge this is the first large study to investigate the influence of cannabidiol content on the presence of psychiatric symptoms in cannabis users from the general population. The key finding of the current study is that cannabidiol mitigates the psychotic symptoms associated with cannabis use with a small, albeit significant, effect. Given the low dose of cannabidiol in joints as compared to oral administration in treatment studies, a larger effect could not be expected. The current data add to our knowledge of cannabidiol as a potential antipsychotic agent, suggesting that cannabis types with high

cannabidiol content are significantly less strongly associated with psychotic symptoms.

Reference List

Arseneault,L., Cannon,M., Witton,J. and Murray,R.M., 2004. Causal association between cannabis and psychosis: examination of the evidence. Br J Psychiatry. 184, 110-117.

Balter,K.A., Balter,O., Fondell,E. and Lagerros,Y.T., 2005. Web-based and mailed questionnaires: a comparison of response rates and compliance. Epidemiology. 16, 577-579.

Bhattacharyya,S., Morrison,P.D., Fusar-Poli,P., Martin-Santos,R., Borgwardt,S., Winton-Brown,T., Nosarti,C., O' Carroll,C.M., Seal,M., Allen,P., Mehta,M.A., Stone,J.M., Tunstall,N., Giampietro,V., Kapur,S., Murray,R.M., Zuardi,A.W., Crippa,J.A., Atakan,Z. and McGuire,P.K., 2010. Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. Neuropsychopharmacology. 35, 764-774.

DeLisi,L.E., 2008. The effect of cannabis on the brain: can it cause brain anomalies that lead to increased risk for schizophrenia? Curr Opin Psychiatry. 21, 140-150.

Di Forti,M., Morgan,C., Dazzan,P., Pariante,C., Mondelli,V., Marques,T.R., Handley,R., Luzi,S., Russo,M., Paparelli,A., Butt,A., Stilo,S.A., Wiffen,B., Powell,J. and Murray,R.M., 2009. High-potency cannabis and the risk of psychosis. Br J Psychiatry. 195, 488-491.

Ekman,A., Dickman,P.W., Klint,A., Weiderpass,E. and Litton,J.E., 2006. Feasibility of using web-based questionnaires in large population-based epidemiological studies. Eur J Epidemiol. 21, 103-111.

Elsohly,M.A. and Slade,D., 2005. Chemical constituents of marijuana: the complex mixture of natural cannabinoids. Life Sci. 78, 539-548.

Fusar-Poli,P., Allen,P., Bhattacharyya,S., Crippa,J.A., Mechelli,A., Borgwardt,S., Martin-Santos,R., Seal,M.L., O'Carrol,C., Atakan,Z., Zuardi,A.W. and McGuire,P., 2010. Modulation of effective connectivity during emotional processing by Delta 9-tetrahydrocannabinol and cannabidiol. Int J Neuropsychopharmacol. 13, 421-432.

Gosling,S.D., Vazire,S., Srivastava,S. and John,O.P., 2004. Should we trust web-based studies? A comparative analysis of six preconceptions about internet questionnaires. Am Psychol. 59, 93-104.

Konings,M., Bak,M., Hanssen,M., van,O.J. and Krabbendam,L., 2006. Validity and reliability of the CAPE: a self-report instrument for the measurement of psychotic experiences in the general population. Acta Psychiatr Scand. 114, 55-61.

Konings,M., Henquet,C., Maharajh,H.D., Hutchinson,G. and van,O.J., 2008. Early exposure to cannabis and risk for psychosis in young adolescents in Trinidad. Acta Psychiatr Scand.

Leweke,F.M., Schneider,U., Radwan,M., Schmidt,E. and Emrich,H.M., 2000. Different effects of nabilone and cannabidiol on binocular depth inversion in Man. Pharmacol Biochem Behav. 66, 175-181.

Macleod,J., Oakes,R., Copello,A., Crome,I., Egger,M., Hickman,M., Oppenkowski,T., Stokes-Lampard,H. and Davey,S.G., 2004. Psychological and social sequelae of cannabis and other illicit drug use by young people: a systematic review of longitudinal, general population studies. Lancet. 363, 1579-1588.

McGrath,J., Welham,J., Scott,J., Varghese,D., Degenhardt,L., Hayatbakhsh,M.R., Alati,R., Williams,G.M., Bor,W. and Najman,J.M., 2010. Association Between Cannabis Use and Psychosis-Related Outcomes Using Sibling Pair Analysis in a Cohort of Young Adults. Arch Gen Psychiatry.

McLaren,J., Swift,W., Dillon,P. and Allsop,S., 2008. Cannabis potency and contamination: a review of the literature. Addiction. 103, 1100-1109.

Mechoulam,R., Shani,A., Edery,H. and Grunfeld,Y., 1970. Chemical basis of hashish activity. Science. 169, 611-612.

Mehmedic,Z., Chandra,S., Slade,D., Denham,H., Foster,S., Patel,A.S., Ross,S.A., Khan,I.A. and Elsohly,M.A., 2010. Potency Trends of Delta(9)-THC and Other Cannabinoids in Confiscated Cannabis Preparations from 1993 to 2008*. J Forensic Sci.

Meyerson,P. and Tryon,W.W., 2003. Validating internet research: a test of the psychometric equivalence of internet and in-person samples. Behav Res Methods Instrum Comput. 35, 614-620.

Moore,T.H., Zammit,S., Lingford-Hughes,A., Barnes,T.R., Jones,P.B., Burke,M. and Lewis,G., 2007. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet. 370, 319-328.

Morgan,C.J. and Curran,H.V., 2008. Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. Br J Psychiatry. 192, 306-307.

Potter,D.J., Clark,P. and Brown,M.B., 2008. Potency of delta 9-THC and other cannabinoids in cannabis in England in 2005: implications for psychoactivity and pharmacology. J Forensic Sci. 53, 90-94.

Rottanburg,D., Robins,A.H., Ben-Arie,O., Teggin,A. and Elk,R., 1982a. Cannabis-associated psychosis with hypomanic features. Lancet. 2, 1364-1366.

Rottanburg,D., Robins,A.H., Ben-Arie,O., Teggin,A. and Elk,R., 1982b. Cannabis-associated psychosis with hypomanic features. Lancet. 2, 1364-1366.

Schubart,C.D., van Gastel,W.A., Breetvelt,E.J., Beetz,S.L., Ophoff,R.A., Sommer,I.E., Kahn,R.S. and Boks,M.P. Cannabis use at young age is associated with psychotic experiences. Psychol Med. 2011 Jun;41(6):1301-10.

Skinner,R., Conlon,L., Gibbons,D. and McDonald,C., 2010. Cannabis use and non-clinical dimensions of psychosis in university students presenting to primary care. Acta Psychiatr Scand.

Smith,N., 2005. High potency cannabis: the forgotten variable. Addiction. 100, 1558-1560.

Stefanis,N.C., Hanssen,M., Smirnis,N.K., Avramopoulos,D.A., Evdokimidis,I.K., Stefanis,C.N., Verdoux,H. and van,O.J., 2002. Evidence that three dimensions of psychosis have a distribution in the general population. Psychol Med. 32, 347-358.

Trimbos,T.N.I.o.M.H.a.A. THC-concentraties in wiet, nederwiet en hasj in Nederlands coffeeshops (2007-2008). 1-1-2008.

Trimbos,T.N.I.o.M.H.a.A. THC-concentraties in wiet, nederwiet en hasj in Nederlands coffeeshops (2008-2009). 1-1-2009.

United Nations Office on Drugs and Crime. World Drug Report 2012. 2012. Vienna, United Nations Publications.

Zuardi,A.W., Crippa,J.A., Hallak,J.E., Moreira,F.A. and Guimaraes,F.S., 2006a. Cannabidiol, a Cannabis sativa constituent, as an antipsychotic drug. Braz J Med Biol Res. 39, 421-429.

Zuardi,A.W., Hallak,J.E., Dursun,S.M., Morais,S.L., Sanches,R.F., Musty,R.E. and Crippa,J.A., 2006b. Cannabidiol monotherapy for treatment-resistant schizophrenia. J Psychopharmacol. 20, 683-686.

Zuardi,A.W., Morais,S.L., Guimaraes,F.S. and Mechoulam,R., 1995. Antipsychotic effect of cannabidiol. J Clin Psychiatry. 56, 485-486.

PART II

GENETIC FACTORS

PART II: GENETIC FACTORS
CHAPTER VI

• A POLYMORPHISM (RS 7958311) IN THE P2X7 RECEPTOR GENE IS A CANDIDATE TO MODERATE THE EFFECT OF CANNABIS ON PSYCHOSIS RISK •

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ABSTRACT

Introduction
Cannabis is a known risk factor for psychosis but the majority of cannabis users do not develop a psychotic illness as schizophrenia. Therefore it is plausible that the association between cannabis use and psychosis depends on individual biological vulnerability that may be genetically determined. We conducted a Genome Wide Environment-Interaction Study (GWEIS) to identify genetic variation at the SNP level that confers psychosis vulnerability associated with cannabis use.

Methods
We included a population sample of 1262 individuals from the general population that was enriched with participants that were selected for absent or extreme cannabis exposure and extreme high or low scores on psychosis vulnerability measured by a questionnaire assessing subclinical psychotic features; the Community Assessment of Psychic Experiences (CAPE). Whole genome genotype data was generated using Illumina arrays and subsequent imputation resulting in 2,504,766 SNP's for analysis.

Results
We found that a SNP in the P2RX7 gene (rs7958311) was strongly associated with an increased risk (OR: 1.746, p: 1.10E-07) for a high CAPE score in heavy cannabis users.

Conclusions
We conclude that the P2RX7 SNP rs 7958311, is a viable candidate to partially explain the genetically determined variation in vulnerability to cannabis as a risk factor for psychosis. The P2X7 receptor closely interacts with the endocannabinoid system in several pathways that are relevant for the aetiology of psychotic disorders as schizophrenia.
Moreover, the current study is a proof of concept that combining extreme phenotypic and environmental sampling strongly increase power to detect gene environment interaction.

INTRODUCTION

Cannabis use is implicated as a risk factor for psychotic illnesses in different lines of research. From an epidemiological perspective, cannabis use is consistently associated with psychotic symptoms in the general population (Schubart et al., 2010) and a higher rate of psychotic disorders (Zammit et al., 2002;van Os et al., 2002;Manrique-Garcia et al., 2012;Moore et al., 2007). Moreover, numerous studies suggest increased vulnerability to brain volume loss due to cannabis exposure in patients suffering from psychosis (Rapp et al., 2012). Several studies show that cannabis use significantly decreases the age of the first psychotic episode (Myles et al., 2012;Power et al., 2012;Large et al., 2011) and increases the amount and severity of psychotic exacerbations in schizophrenia patients (Grech et al., 2005).

Nevertheless the majority of cannabis users do not develop psychosis and the explained variation in the occurrence of psychotic disorders as schizophrenia due to cannabis use is low. Therefore it is plausible that the association between cannabis use and psychosis depends on individual biological vulnerability that might be genetically determined.

Genetic vulnerability for cannabis as a risk factor for psychotic disorders was investigated using several strategies. Family studies reveal that psychosis risk associated with cannabis exposure is higher in first grade relatives of schizophrenia patients (van Winkel, 2011;Boydell et al., 2006;McGuire et al., 1995). Evidence for a second, more specific level of gene environment interaction is provided by various candidate gene- environment studies. Caspi et al (Caspi et al., 2005) reported that a single nucleotide polymorphism (SNP) in the cathecholamine transferase (COMT) gene, increased the risk of psychotic disorders in cannabis users. This finding was corroborated (Henquet et al., 2008) as well as refuted (Zammit et al., 2011;Wigman et al., 2011). More recently, investigators of the GROUP study showed that a SNP in the AKT1 gene increased the risk of psychosis in cannabis users (van Winkel et al., 2011). This finding was recently replicated in an independent study in schizophrenia patients (Di Forti et al., 2012;Boks, 2012).

These candidate gene studies have produced relevant hypotheses for the aetiology of schizophrenia. However, due to methodological constrains such as bias due to study- and population heterogeneity, candidate gene studies tend to overestimate effect size and are often not replicated (Ioannidis et al., 2001). Moreover, the a priori selection of candidate SNP's takes place within the boundaries of current models which decreases the potential of this methodology to discover new leads to the underlying biological pathways. In contrast, genome wide association studies provide the possibility to screen the genome without a priori selection.

We therefore conducted a study that aims to incorporate information on cannabis use as risk factor of psychosis in a genome wide approach. Using a previously described extreme sampling method (Boks et al., 2007b), we aimed to increase power to detect how genetic variation on the SNP level impacts on the cannabis associated risk to psychosis.

METHODS

We screened for suitable participants for our study using a project website (www.cannabisquest.nl) targeting Dutch speaking adolescents and young adults (18-25 years). Strategies to generate traffic on the project website included cooperation with over 100 colleges, universities and youth centres that were willing to advertise for this study on their intranet, and the use of online commercial advertisement products (i.e. banners and text links). The chance to win an Apple iPod ™ or a Nintendo Wii ™ was used as an incentive, contact details were used to inform participants about their price. Double entries were prevented by automated deletion of identical data rows and by monthly visual inspection of the data, excluding subjects with the same e-mail address or name and date of birth. Submitting data anonymously was not possible. The assessment included two verification questions to protect against random answers. Participants that failed to correctly fill out the verification questions were excluded. Averagely, every month 490 visitors filled out our web based questionnaires between June 2006 and September 2010. This resulted in 25,959 individuals for our screening.

Online assessments

Participants provided the country of birth of their grandparents, their age, educational level and contact details.

As a measure of psychometric psychosis vulnerability, an online version of the Community Assessment of Psychic Experiences (CAPE)(Konings et al., 2006) was used. The CAPE is a 42-item, self rating instrument and has a three-factor structure of 20 questions in the positive symptom dimension (delusional thinking, verbal- and visual hallucinations), 14 in the negative and 8 in the depressive dimension. The CAPE measures frequency as well as distress associated with these experiences. The questionnaire has discriminative validity for the different symptom dimensions in individuals from the general population and is considered a valid instrument for detecting individuals at ultra high risk for psychosis (Konings et al., 2006;Hanssen et al., 2003;Stefanis et al., 2002;Mossaheb et al., 2012) (http://www.cape42.homestead.com/). The use of an online version of the CAPE for this purpose was validated and is described in detail elsewhere (Vleeschouwer, submitted).

Through online questionnaires we assessed the amount in euro's (€) that individuals spent on cannabis per week. THC-concentration and market value of cannabis are highly correlated in The Netherlands (Trimbos-instituut, 2009b). We therefore used the weekly amount of euro's spent on cannabis as a proxy measure of exposure to Δ^9-tetrahydrocannabinol (THC). The THC exposure was categorized into five classes; cannabis naïve, cannabis use equivalent of less than €3 per week, €3 to €10 per week, €10 to €25 per week and more than €25 per week.

Power
The sample sizes that are required to detect gene-environment interactions generally need to be much larger than those necessary to detect genetic or environmental factors in isolation (Luan et al., 2001;Boks et al., 2007b). This is in part a consequence of the fact that genetic and environmental risks are relatively infrequent in an unselected epidemiological sample. This leads to insufficient numbers of subjects suffering from the disorder that have also been exposed to the environmental factor. Abecasis and colleagues (Abecasis et al., 2001) pointed out that selecting extremely discordant subjects from the population can substantially improve power, sometimes with as much a 20 fold increase. In the current study we exploit this effect further by using an adaptation from an extreme discordant case-control design (Abecasis et al., 2001;Purcell, 2002). We selected part of our sample not only for extremes regarding phenotypes, in this case psychometric risk for psychosis, but also regarding environmental (cannabis) exposure.
Thus we aimed to enrich our sample with individuals reporting extremely high cannabis use and those with extremely low cannabis use. Simulations have pointed out that sampling the top and bottom 10 percent of environmental exposure leads to a 70 percent reduction of the required subjects for genotyping (Boks et al., 2007a).

General inclusion criteria
Participants that filled out the online questionnaires were selected if they were of age 18 or older. To prevent population stratification, we confined to inclusion of individuals with four grandparents born in The Netherlands. Selected subjects where contacted by telephone, invalid contact details automatically lead to exclusion.

Table 1 Cannabis-psychotic symptoms quadrants of the selected sample

	Heavy users (C+)	**Cannabis Naïve (C-)**
Top 20% psychosis scores (P+)	Quadrant 1	Quadrant 3
Bottom 20% psychosis scores (P-)	Quadrant 2	Quadrant 4

Samples
To optimize the power to identify SNP's that moderate the risk of cannabis associated psychosis vulnerability, we included in three waves. First, (as described above) we prioritized a sample of participants between the age of 18 and 25 years, with extreme phenotypes and extreme exposure to cannabis. We contacted participants who belonged to the top- or bottom 20% of total CAPE scores who also where either cannabis naïve (i.e. had not used more than 6 times lifetime) or heavy cannabis use (i.e. used more that 10 euro's per week). This procedure created four CAPE-Cannabis profile quadrants, see table 1. Second, we invited an unselected sample of individuals who could have any score on the CAPE questionnaire and could fall into either category of cannabis use. Third, we included a sample of individuals who were willing to participate but refused to visit our hospital.

These subjects returned a saliva DNA sample and questionnaires by mail. The primary reason to include these individuals was the need to increase sample size but the inclusion of this sub-sample may also reduce potential selection bias.

Assessment at inclusion
Participants that visited our hospital underwent a structured clinical interview (SCID) (Spitzer et al., 1992) , to assess DSM-IV diagnoses. Concomitant drug use was assessed by using the drugs- and alcohol sections of the Composite International Diagnostic Interview (Who, 1993).

Biological material
Participants donated two 10cc blood EDTA blood tubes for DNA extraction. Moreover, urine drugs screen was performed to assess recent cannabis use. In a subgroup, a saliva sample was collected for DNA extraction.

Ethical considerations
This study was approved by the UMC Utrecht medical ethical commission and all participants gave informed consent.

Genotyping
For logistic reasons, genotype data was generated on three different array platforms; for 576 individuals on Illumina® HumanOmniExpress (733,202 SNPs), for 768 individuals on the Illumina® Human610-Quad Beadchip (620,901 SNPs) for 34 individuals on the Illumina® HumanHap550 array.

Pre-processing genotype data, QC and imputation
For each SNP platform, quality control procedures were initially performed separately using PLINK (Purcell et al., 2007). Participants were excluded based on gender errors and on >5% missing genotypes. We used linkage disequilibrium (LD) based SNP pruning to select the most informative SNPs (R^2<0.2), only for the subsequent quality control step. Datasets were merged with Hapmap Phase 3 to check ethnicity. Ethnic outliers were detected by visual inspection. After these QC procedures on subjects, we performed quality control on SNP's. All SNP's were filtered on missingness (>2%), Minor Allele Frequency (MAF) > 5% and Hardy Weinberg (p>1e-6) before merging the three datasets. We imputed the merged dataset with Hapmap2, release 24 using Beagle (Browning and Browning, 2009). SNP's with an imputation score > 0.8 and SNP's that were present originally in all of the datasets were extracted.

Other genes of interest
Based on earlier publications (van Winkel, 2011) (Di Forti et al., 2012;Boks, 2012) (Caspi et al., 2005;Henquet et al., 2008), we planned to extract genotypes of 5 additional SNP's in four genes (LRRTM1, AKT1, COMT, P2XR7) from our database.

Statistical Analysis
To calculate the effect of gene by environment interaction on CAPE scores, we performed a logistic regression, using genotype, cannabis use, a GxE interaction term and gender as predictors. Dichotomized CAPE scores (by median split) were used as the dependent variable. All genome-wide association calculations were performed using the open-source whole genome association analysis toolset PLINK (Purcell et al., 2007).

RESULTS
Online screening
The project website was launched in June 2006 and attracted more than 100,000 unique visitors, resulting in more than 25,943 participants at the time of the final inclusion. The questionnaires contained verification questions as a measure of quality control. Approximately 17% of the subjects failed to answer these questions correctly, leaving 21,435 potential participants. The mean age of this group was 21.7 years, 51% was male, the average CAPE score was 66 and 34.5% of the sample was cannabis naïve.

Subject flow
Selection of individuals with four Dutch grandparents and extreme phenotype and environmental (cannabis) exposure resulted in 4,119 potential participants for our study (for a more detailed subject flow see table 2). From a pool of 4,119 subjects, we found 1,364 participants who were successfully contacted and willing to participate in our study.

Table 2. Subject flow

Stage	Number
Online screening	25,958
After verification questions	21,435
Dutch grandparents	16,030
Cannabis use pattern	11,228
Top- / bottom 20% CAPE	4,119
Visit to hospital from the	802
Visit to hospital from the	382
Saliva samples	180
Total samples before QC *	1,364
Total samples after QC *	1,262

* QC = Genotype data Quality Control

Sample description
The mean age of the included participants was 21.0 years. The mean CAPE score in the total sample was 67. For further details on characteristics of the different subsamples, see table 3. For an overview of DSM-IV diagnosis and description of the psychiatric history of the different samples, see table 4.

Table 3 Design and sample description

Sample	N	Sex (% male)	Age, Mean (sd)	Cannabis use (%) > 3 euro/week	CAPE score (mean)
Quadrant 1 (P+, C+)	136	66.9	21.1 (2.2)	100	86
Quadrant 2 (P-, C+)	114	78.9	21.1 (2.0)	100	55
Quadrant 3 (P+, C-)	206	25.2	20.4 (1.8)	0	80
Quadrant 4 (P-, C-)	261	30.3	20.8 (2.0)	0	55
Unselected	373	54.7	21.2 (2.5)	24	66
Saliva sample	172	47.7	21.9 (5.7)	37	68

Pre-processing, quality control and imputation
Linkage disequilibrium (LD) based SNP pruning resulted in ~78k SNPs for the sets to assess heterozygosity (F<3SD), homozygosity (F>3SD) and relatedness by pairwise IBD values (pihat >0.1). Subject quality control resulted in the exclusion of 101 individuals. Four duplicates and three related sample-pairs were detected in the merged datasets (according to criteria described in methods section) and one outlier after clustering the merged dataset. Imputation resulted in 2,504,766 SNP's for analysis.

PLINK GWEIS analysis
Table 5 lists the top 10 results of the G x E logistic regression analysis for a high CAPE score as a binary trait. We found that a novel variant in the P2RX7 receptor gene (rs7958311) was ranked first of SNPs associated with an increased risk (OR: 1.746, p: 1.10E-07) for experiencing psychotic symptoms in heavy cannabis users. The identified SNP in P2RX7 is an imputed SNP, the imputation score is: 0.885.
Table 6 shows the Odds ratios (adjusted for gender) for A/G or A/A carriers compared with G/G carriers depending on cannabis use status. Figure 2 shows the Q-Q plot for the interaction in the imputed data set (2.5M snp's), λ= 0.993. Figure 3 shows a Manhattan plot of p-values for the G x E term.
Six SNP's that are in LD in the CADM2 gene (p: 1.05E-06), rank second in lowest p-values in our G x E interaction association analysis. Figure 1 shows the adjusted Odds ratio (OR) of high CAPE score for participants with P2RX7 rs 7958311 G/A or A/A compared to the G/G genotype.

Other genes of interest
Five additional pre-selected SNP's of interest were looked up in our study database. Unfortunately LRRTM1 (rs673871), AKT1 (rs2494732) and (rs1130233) were not genotyped, imputed or called in our dataset. The COMT SNP rs4680 was included but was not significantly associated with gene environment interaction (p: 0.695). A previously investigated variant in P2XR7 (rs2230912) was nominally significant (p: 0.0112).

Table 4. Psychiatric history (SCID or MINI) per sample (more than 1 diagnosis per subject)

Sample	Depression and Dysthymia	Psychotic disorder	Bipolar disorder	Anxiety disorder	Autism spectrum	Cannabis related disorder	Other substance related disorder	Other diagnoses	Lifetime Psychiatric Medication	Lifetime Psychiatric Hospitalisation
Quadrant 1 (n=136)	32 (23.5%)	4 (2.9%)	5 (3.7%)	29 (21.3%)	1 (0.7%)	121(88.9%)	5 (3.7%)	12 (8.8%)	7 (5.1%)	4 (2.9%)
Quadrant 2 (n=114)	7 (6.1%)	1 (0.9%)	1(0.9%)	4 (3.5%)	1 (0.9%)	57 (50.0%)	8 (7.0%)	9 (7.9%)	2 (1.8%)	1 (0.9%)
Quadrant 3 (n=206)	73 (35.4%)	2 (1.0%)	6 (2.9%)	49 (23.8%)	1 (1.0%)	0 (0%)	3 (1.5%)	15 (7.3%)	11 (5.3%)	1 (0.49%)
Quadrant 4 (n=261)	10 (3.8%)	0 (0%)	0 (0%)	4 (1.5%)	0 (0%)	0 (0%)	1 (0.4%)	5 (1.9%)	2 (0.8%)	0 (0%)
Unselected (n=373)	55 (14.7%)	5 (1.3%)	5 (1.3%)	27 (7.2%)	5 (1.3%)	69 (18.5%)	35 (9.4%)	61 (16.4%)	9 (2.4%)	9 (2.4%)
Saliva sample (n=172)	ND	ND	ND	ND	ND	ND	ND	ND	10 (5.8%)	3 (1.7%)

Table 5. Top 10 genome-wide G x E association results for heavy cannabis use and psychosis vulnerability.

CHR	SNP	BP	A1	MAF	OR	STAT	P	GENE
12	rs7958311	120089738	A	0.2889	1.746	5.309	1.10E-07	P2RX7
3	rs1003984	85952889	T	0.2587	1.981	4.881	1.05E-06	CADM2
3	rs1003985	85952993	A	0.217	1.981	4.881	1.05E-06	CADM2
3	rs1003986	85953053	T	0.2582	1.981	4.881	1.05E-06	CADM2
3	rs12487728	85953621	T	0.2212	1.981	4.881	1.05E-06	CADM2
3	rs9968137	85953797	G	0.2995	1.981	4.881	1.05E-06	CADM2
3	rs12488483	85954040	A	0.2212	1.981	4.881	1.05E-06	CADM2
10	rs4342983	115440504	C	0.0421	2.031	4.76	1.93E-06	CASP7
6	rs17710848	153108495	A	0.0696	2.469	4.696	2.65E-06	Not annotated
10	rs3121454	115365937	C	0.0481	2.029	4.645	3.39E-06	NRAP

Table 6. Adjusted * Odds Ratio for A/G or A/A carriers compared with G/G carriers depending on cannabis use status.

	NO CANNABIS			CANNABIS USE		
genotype	adjusted OR	95% CI	P Value	adjusted OR	95% CI	P Value
P2RX7 (GG)	1	-	-	1	-	-
P2RX7(GA)	0.622	0.47-0.83	0.001	2.347	1.54-3.60	0.000
P2RX7 (AA)	0.478	0.27-0.85	0.011	3.693	1.50 -9.11	0.005

* adjusted for gender

DISCUSSION

We performed the first Gene-Environment Wide Interaction Study (GWEIS) on the risk for psychosis and cannabis use in a sample of 1261 participants, enriched for individuals with extreme phenotypes and extreme cannabis exposure. We found that a SNP in the P2X7 receptor was associated with an increased psychosis vulnerability by cannabis use albeit with just below genome-wide significance.

P2X7

The P2RX7 gene is located on Chromosome 12 at 12q24.31 and encodes for the P2X7 receptor. The P2X7 receptor is a two-transmembrane ionotropic receptor that is activated by high concentrations of extracellular ATP as induced by for example cell damage or hypoxia. It has a high Ca^{2+} permeability and upon adequate stimulation a possibility to act as a non-selective large pore which can lead to cell death (Li et al., 2005). This receptor is present in all tissues, but is primarily expressed in immune cells, liver and whole brain, predominantly in microglia (BioGPS gene expression database).

Figure 1. Adjusted * Odds ratio (OR) of high CAPE score for participants with P2RX7 rs 7958311 G/A or A/A compared to the G/G genotype. (* adjusted for gender)

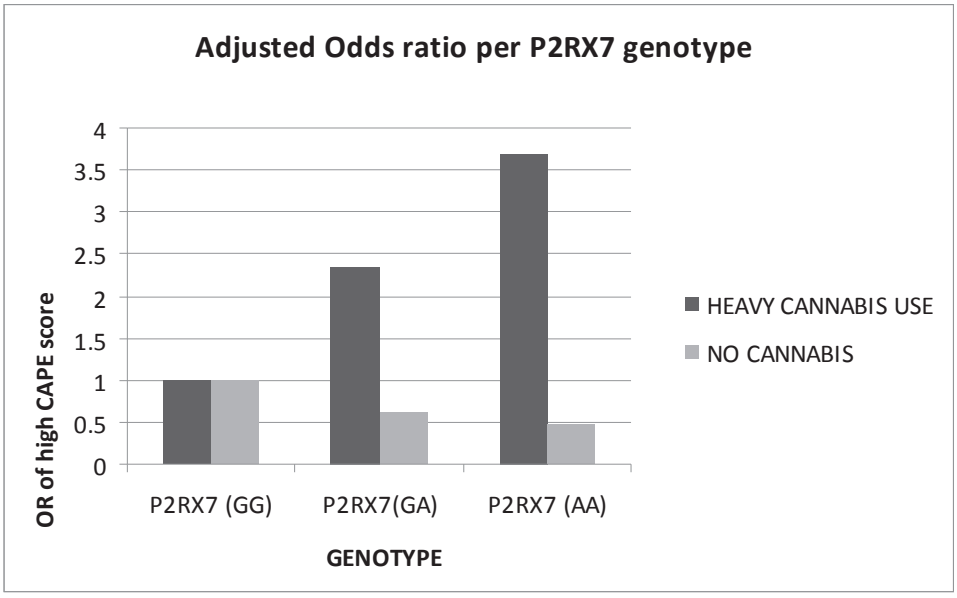


Figure 2: QQ plot for interaction term in the imputed set (2.5M) using a full model

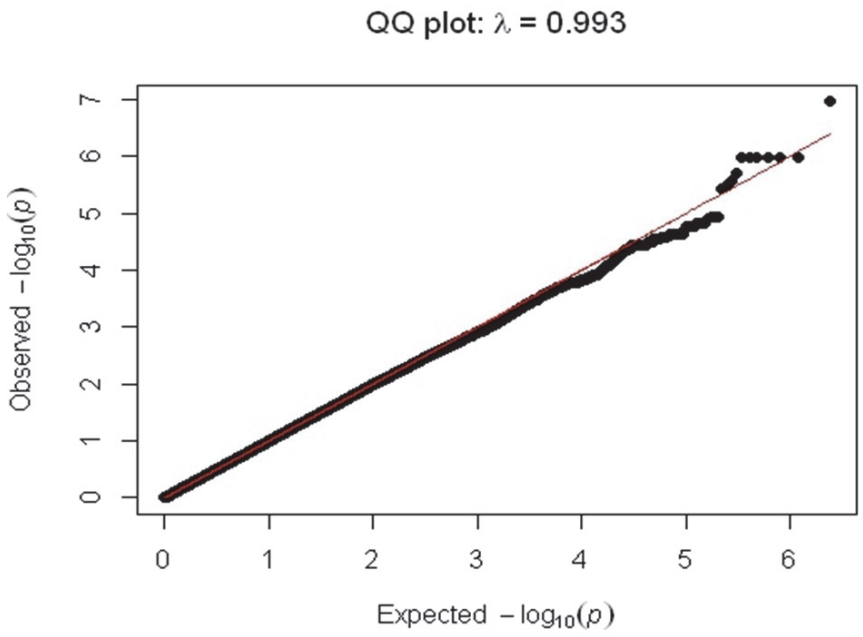
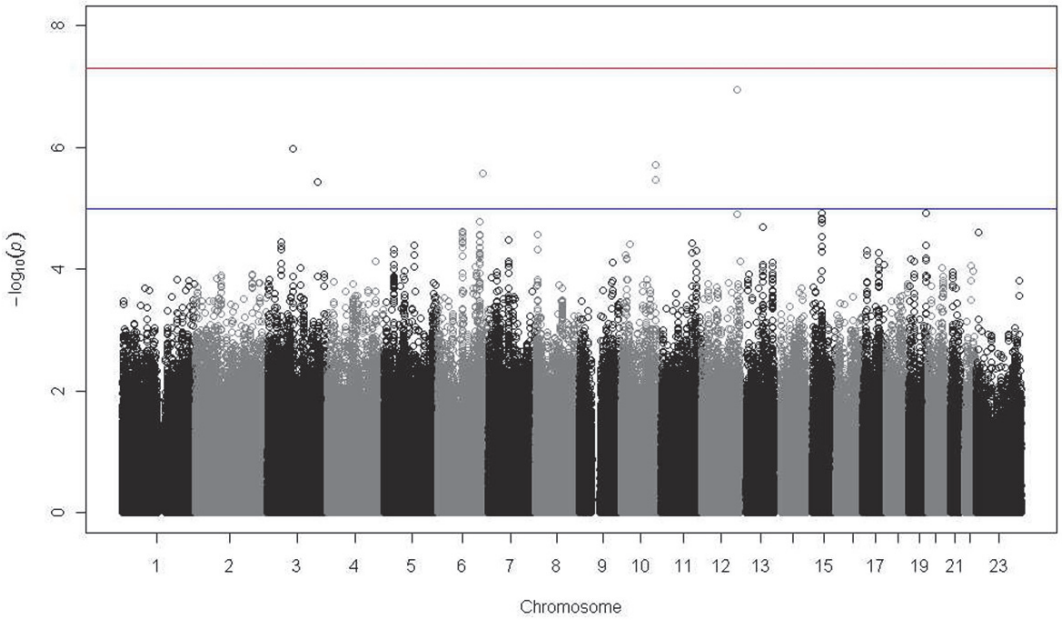


Figure 3. Manhattan plot



In the central nervous system P2X7 is also expressed on astrocytes and neurons (Sperlagh et al., 2006;Wiley et al., 2011). Many different effects have been attributed to activation of P2X7 by ATP such as secretion of TNF-a, IL-1ß and IL-18, nitric-oxide release and secretion of caspase-1, for review see Wiley 2011 (Wiley et al., 2011). Besides regulation of inflammatory reactions, the P2X7 receptor is also involved in regulation of neurotransmitter release, mainly GABA and glutamate (Sperlagh et al., 2006). The identified SNP, rs 7958311, has a minor allele frequency (MAF) of 0.289 and is a non-synonymous coding SNP (835A>G) that results in an amino acid change from Histidine to Arginine (His270>Arg) in an extracellular subunit of this transmembrane receptor. Stokes et al suggested that this SNP causes gain of function, measured as an increased P2X7-dependent ethidium uptake (195% of wild-type P2X7 response) (Stokes et al., 2010;Wiley et al., 2011), however elsewhere loss of function is also described (Sorge et al., 2012;Sun et al., 2010). Variation in the P2RX7 gene has been associated with various diseases. The most frequently described association is that of rs3751143 with increased susceptibility to tuberculosis (Xiao et al., 2010). It is suggested that mutations leading to a loss of function of the P2X7 receptor may affect macrophage function leading to decreased resistance to intracellular microorganisms, such as mycobacterium tuberculosis and toxoplasma gondii (Kapoor, 2009;Lees et al., 2010;Saunders et al., 2003). Moreover, P2X7 is implied in pain sensation (Ando and Sperlagh, 2012). The particular SNP identified in our study (rs 7958311) was associated with lower pain intensity in two independent human chronic pain cohorts (Sorge et al.,

2012). This is interesting considering the recent interest in cannabis for pain control (Lynch and Campbell, 2011).

P2RX7 and psychiatric disorders
Variation in the P2RX7 gene is also associated with an increased risk for several psychiatric disorders. The most prominent association between the P2X7 receptor and psychiatric disease is that with bipolar disorder. Since 1995, the 12q24.31 region has been associated with bipolar disorder in various genome wide linkage scans (Hayden and Nurnberger, Jr., 2006;Barden et al., 2006;Dawson et al., 1995;Degn et al., 2001;Morissette et al., 1999;Shink et al., 2005b;Shink et al., 2005a;Curtis et al., 2003) (McQuillin et al., 2009). Later candidate gene studies corroborated this finding and particularly associated one P2RX7 SNP (rs2230912) (Gln460Arg) with bipolar disorder in several independent cohorts (Soronen et al., 2011;Backlund et al., 2011;Backlund et al., 2012). However, also negative findings have been published about this association (Grigoroiu-Serbanescu et al., 2009;Green et al., 2009). The 12q24 area, in which the P2RX7 gene is located, was also implied as an area of interest in two genome wide linkage scans for unipolar depression (Abkevich et al., 2003;McGuffin et al., 2005), later several candidate gene studies demonstrated that the same SNP (rs2230912) is associated with major depression (Hejjas et al., 2009;Nagy et al., 2008;Lucae et al., 2006;Soronen et al., 2011). Likewise, the 12q24 region was implied in several genome wide linkage scans in schizophrenia (Bailer et al., 2002;Bailer et al., 2000;DeLisi et al., 2002). However, in the single candidate gene study on P2RX7 and schizophrenia, no association was found with 9 different P2RX7 SNP's (Hansen et al., 2008). Recent GWAS studies and meta-analysis also do not imply SNP's in the P2RX7 gene in the pathophysiology of schizophrenia (Ripke et al., 2011;Steinberg et al., 2011). Given the complex aetiology of schizophrenia, it is possible that the biological mechanism behind the described G x E interaction in our study is only indirectly related with genetic vulnerability to schizophrenia as such.

In a previous G x E candidate gene study on cannabis use and psychosis vulnerability, P2RX7 SNP rs2230912, was also investigated but did not show any evidence for G x E interaction (van Winkel, 2011). In the current study, this SNP was nominally significant (p: 0.0112). Given that the rs2230912 SNP shows some LD with the SNP identified in the current study (rs 7958311) (Fuller et al., 2009) (Stokes et al., 2010), this is a likely finding.

P2X7 and the endocannabinoid system
The two best described endocannabinoids are 2-arachidonoylglycerol (2-AG) and anandamide (Howlett, 2002a;Howlett et al., 2004), both agonists of the cannabinoid receptors CB1 and CB2 (Pertwee, 2006). Interestingly, the P2X7 receptor regulates 2-AG production in microglia (Witting et al., 2004) and astrocytes (Walter et al., 2004). Activation of cannabinoid receptors by 2-AG leads to attenuation of neuroinflammation (Van Sickle et al., 2005;Stella,

2004;Stella, 2009;Stella, 2010;Miller and Stella, 2008) through activation of the CB1(Stella, 2010;Marsicano et al., 2003;Shen and Thayer, 1998;Sullivan, 1999) and CB2(Klegeris et al., 2003) receptors.
It is therefore suggested that 2-AG may function as a gliotransmitter, involved in attenuating neuroinflammation (Walter et al., 2004) and that increased synthesis of 2-AG leads to an anti-inflammatory (M2) phenotype (Stella, 2010). This function may be mediated through the P2X7 receptor.

Hypotheses on P2X7 - cannabis interaction
The P2X7 receptor is thought to be involved in several key proceses in the CNS; neuroinflammation, neuroprotection, but also neurotransmission. It is, however, currently unclear how the polymorphism that we identified in this study affects these processes at the molecular level. Several studies suggest that 2-AG and other endocannabinoids have a neuroprotective (van der Stelt and Di Marzo, 2005) and even an antipsychotic function (Giuffrida et al., 2004;Leweke et al., 1999;Leweke et al., 2007b;Leweke et al., 2012b;De Marchi et al., 2003). One of our hypothesis is that the described polymorphism in the P2X7 receptor alters 2-AG production and subsequently alters the neuroprotective function of the endocannabinoid system. Since THC interferes with the endocannabinoid system functioning, this may lead to a further dysfunction of the neuroprotective mechanisms of the endocannabinoid system upon high levels of ATP exposure. The combined effect of the P2RX7 polymorphism and continuous exposure to THC could be the biological basis for the described interaction effect on psychosis vulnerability.
A second hypothesis is that both the P2X7 receptor and THC (Stella, 2010) influence neuroinflammation , (Stella, 2009;Stella, 2010). As a consequence, regulation of neuroinflammation could be altered in carriers of the P2RX7 risk genotype that also continuously use cannabis. Several lines of research point towards the notion that neuroinflammation plays a key role in the aetiology of schizophrenia, (for a recent and detailed review see Monji et al) (Monji et al., 2011).THC exposure and inadequate P2X7 function could interact in causing suboptimal orchestration of neuroinflamatory responses, leading to an increased susceptibility for psychotic disorders as schizophrenia.

CADM2
Although not genome wide significant, as shown in table 5, 6 SNP's in the CADM2 gene rank on places 2-7 in lowest p-values in our G x E interaction association analysis.
CADM2 encodes a nectin-like member of the immunoglobulin-like cell adhesion molecules (Berger et al., 2011;Beroukhim et al., 2010). Although further replication is needed, recently, CADM2 was implied as a novel Autism Spectrum Disorder risk gene in a homozygous haplotype (HH) mapping analysis in 1,402 Autism Genome Project trios (Casey et al., 2012). Given the large genetic overlap between schizophrenia and autism (Sullivan et al., 2012), further follow up is warranted.

Genome wide significance
Significance threshold levels in GWAS studies are subject of ongoing debate (Hoggart et al., 2008;Pe'er et al., 2008). In studies for gene discovery, a Genome-wide significance threshold of approximately 5 ×10 -8 has become the norm (Cichon et al., 2009;Barsh et al., 2012;Dudbridge and Gusnanto, 2008). The SNP's described in our study do not reach genome wide significance.

Other genes of interest
Previously described SNPs in LRRTM1 (rs673871), AKT1 (rs2494732) and (rs1130233) were unavailable for analysis. We therefore can not add to the previously stated hypotheses concerning these variants. The COMT SNP rs4680 showed no evidence of gene environment interaction in our data.

Limitations
The current study has strong points, limitations and innovations.
An innovative aspect of our study is the enrichment of our sample with individuals with extreme phenotypes and extreme environmental exposure. We enriched our sample not only for extreme pairs regarding phenotypes (high and low CAPE scores) but also regarding environmental exposure and selected individuals with extremely high cannabis use and those without cannabis use. Using this design, we generated more power to detect SNP's that are genome wide significantly associated with interaction.
Furthermore, our sample is enriched with late adolescents (18-25 years) and we therefore included an at risk population both for the environmental exposure as well as for the occurrence of psychosis. Finally, to avoid population stratification, we only included individuals with four Dutch born grandparents.
A number of limitations have to be considered when interpreting the presented data.
One limitation is the use of a dichotomized outcome measure (CAPE score) instead of treating the CAPE as a quantitative trait. Due to our extreme phenotype sampling method, the distribution of the CAPE scores was far from normal. Particularly when performing regression analysis with a G x E interaction term, the risk of heteroscedasticity is much higher (Voorman et al., 2011), given the increased differences in variation due to small number of subjects per cells when combining SNP and environmental exposure data. In our sample, this resulted in inflation of probability (p values) and a high lambda. Robust error correction did not resolve this issue. We therefore decided to dichotomize the dependent (CAPE score) and performed a binary trait analysis.
With an intermediate MAF of 0.289, rs 7958311 is a fairly common SNP. Given the relatively high prevalence of cannabis use and the relatively low prevalence of psychosis, it is likely that other, unknown, variables exist that also play a role in determining the risk of psychosis in cannabis users.
Another limitation is the fact that the SNP in P2RX7 is an imputed SNP (R2:0.885). As a consequence the current finding is statistical, rather than

biological, in nature. We are currently performing validation PCR assays and sequencing to confirm the finding in our samples.
The main outcome measure of this study is a high score on the CAPE questionnaire. The CAPE questionnaire is a well validated instrument to measure psychosis vulnerability, however it is an outcome measure based on self report and the majority of participants that have high scores on the CAPE questionnaire have not and will never develop a psychotic disorder. Although individuals with psychiatric problems are overrepresented in the subsamples with high CAPE scores (see table 4), a high score on the CAPE questionnaire is a proxy for psychosis vulnerability (Konings et al., 2006;Hanssen et al., 2003;Stefanis et al., 2002;Mossaheb et al., 2012) but is not the exact same. The fact that some of the data was obtained online is unlikely to be a major limitation (Vleeschouwer et al., 2013a) but a potential limitation is that we relied on self report data regarding cannabis use. However, we measured the presence of cannabis metabolites in urine in the majority of our sample and there is no clear reason to assume that the over- or underreporting is over-represented in subjects with high or low cape scores. There is therefore also no reason to assume that the distribution of inaccurate measurement of cannabis exposure is unequal and has lead to bias.

Considering the relatively high risk of false positive findings in genetic association studies (Ioannidis, 2008), replication of the current results in an independent sample is essential before attributing further etiological significance to this finding. Moreover, it is needed that the impact of the identified SNP (rs 7958311) on 2-AG production in microglia and astrocytes is clarified. To further understand the impact of THC exposure on carriers of this SNP, relevant cell functions are currently investigated under experimental conditions with and without THC.

Conclusion
We conducted the first Gene-Environment-Wide Study (GWEIS) to uncover genetic variation underlying sensitivity to cannabis as a risk factor for psychometric psychosis vulnerability. To increase the statistical power of this approach, we included individuals with extreme phenotypes and extreme environmental exposure. The current study is a proof of concept that combining extreme phenotypic and environmental sampling strongly increase power to detect G x E.
Given that the P2X7 receptor closely interacts with the endocannabinoid system in several relevant pathways, we conclude that the P2RX7 SNP rs 7958311, is a viable candidate to explain some of the sensitivity to cannabis as a risk factor for psychosis.

Reference List

Abecasis,G.R., Cookson,W.O. and Cardon,L.R., 2001. The power to detect linkage disequilibrium with quantitative traits in selected samples. Am J Hum Genet. 68, 1463-1474.

Abkevich,V., Camp,N.J., Hensel,C.H., Neff,C.D., Russell,D.L., Hughes,D.C., Plenk,A.M., Lowry,M.R., Richards,R.L., Carter,C., Frech,G.C., Stone,S., Rowe,K., Chau,C.A., Cortado,K., Hunt,A., Luce,K., O'Neil,G., Poarch,J., Potter,J., Poulsen,G.H., Saxton,H., Bernat-Sestak,M., Thompson,V., Gutin,A., Skolnick,M.H., Shattuck,D. and Cannon-Albright,L., 2003. Predisposition locus for major depression at chromosome 12q22-12q23.2. Am J Hum Genet. 73, 1271-1281.

Ando,R.D. and Sperlagh,B., 2012. The role of glutamate release mediated by extrasynaptic P2X7 receptors in animal models of neuropathic pain. Brain Res Bull.

Backlund,L., Lavebratt,C., Frisen,L., Nikamo,P., Hukic,S.D., Traskman-Bendz,L., Landen,M., Edman,G., Vawter,M.P., Osby,U. and Schalling,M., 2012. P2RX7: Expression Responds to Sleep Deprivation and Associates with Rapid Cycling in Bipolar Disorder Type 1. PLoS ONE. 7, e43057.

Backlund,L., Nikamo,P., Hukic,D.S., Ek,I.R., Traskman-Bendz,L., Landen,M., Edman,G., Schalling,M., Frisen,L. and Osby,U., 2011. Cognitive manic symptoms associated with the P2RX7 gene in bipolar disorder. Bipolar Disord. 13, 500-508.

Bailer,U., Leisch,F., Meszaros,K., Lenzinger,E., Willinger,U., Strobl,R., Gebhardt,C., Gerhard,E., Fuchs,K., Sieghart,W., Kasper,S., Hornik,K. and Aschauer,H.N., 2000. Genome scan for susceptibility loci for schizophrenia. Neuropsychobiology. 42, 175-182.

Bailer,U., Leisch,F., Meszaros,K., Lenzinger,E., Willinger,U., Strobl,R., Heiden,A., Gebhardt,C., Doge,E., Fuchs,K., Sieghart,W., Kasper,S., Hornik,K. and Aschauer,H.N., 2002. Genome scan for susceptibility loci for schizophrenia and bipolar disorder. Biol Psychiatry. 52, 40-52.

Barden,N., Harvey,M., Gagne,B., Shink,E., Tremblay,M., Raymond,C., Labbe,M., Villeneuve,A., Rochette,D., Bordeleau,L., Stadler,H., Holsboer,F. and Muller-Myhsok,B., 2006. Analysis of single nucleotide polymorphisms in genes in the chromosome 12Q24.31 region points to P2RX7 as a susceptibility gene to bipolar affective disorder. Am J Med Genet B Neuropsychiatr Genet. 141B, 374-382.

Barsh,G.S., Copenhaver,G.P., Gibson,G. and Williams,S.M., 2012. Guidelines for genome-wide association studies. PLoS Genet. 8, e1002812.

Berger,M.F., Lawrence,M.S., Demichelis,F., Drier,Y., Cibulskis,K., Sivachenko,A.Y., Sboner,A., Esgueva,R., Pflueger,D., Sougnez,C., Onofrio,R., Carter,S.L., Park,K., Habegger,L., Ambrogio,L., Fennell,T., Parkin,M., Saksena,G., Voet,D., Ramos,A.H., Pugh,T.J., Wilkinson,J., Fisher,S., Winckler,W., Mahan,S., Ardlie,K., Baldwin,J., Simons,J.W., Kitabayashi,N., MacDonald,T.Y., Kantoff,P.W., Chin,L., Gabriel,S.B., Gerstein,M.B., Golub,T.R., Meyerson,M., Tewari,A., Lander,E.S., Getz,G., Rubin,M.A. and Garraway,L.A., 2011. The genomic complexity of primary human prostate cancer. Nature. 470, 214-220.

Beroukhim,R., Mermel,C.H., Porter,D., Wei,G., Raychaudhuri,S., Donovan,J., Barretina,J., Boehm,J.S., Dobson,J., Urashima,M., Mc Henry,K.T., Pinchback,R.M., Ligon,A.H., Cho,Y.J., Haery,L., Greulich,H., Reich,M., Winckler,W., Lawrence,M.S., Weir,B.A., Tanaka,K.E., Chiang,D.Y., Bass,A.J., Loo,A., Hoffman,C., Prensner,J., Liefeld,T., Gao,Q., Yecies,D., Signoretti,S., Maher,E., Kaye,F.J., Sasaki,H., Tepper,J.E., Fletcher,J.A., Tabernero,J., Baselga,J., Tsao,M.S., Demichelis,F., Rubin,M.A., Janne,P.A., Daly,M.J., Nucera,C., Levine,R.L., Ebert,B.L., Gabriel,S., Rustgi,A.K., Antonescu,C.R., Ladanyi,M., Letai,A., Garraway,L.A., Loda,M., Beer,D.G., True,L.D., Okamoto,A., Pomeroy,S.L., Singer,S., Golub,T.R., Lander,E.S., Getz,G., Sellers,W.R. and Meyerson,M., 2010. The landscape of somatic copy-number alteration across human cancers. Nature. 463, 899-905.

Boks,M.P., 2012. Progress in gene environment studies. Biol Psychiatry. 72, 799-800.

Boks,M.P., Rietkerk,T., van de Beek,M.H., Sommer,I.E., de Koning,T.J. and Kahn,R.S., 2007a. Reviewing the role of the genes G72 and DAAO in glutamate neurotransmission in schizophrenia. Eur Neuropsychopharmacol.

Boks,M.P., Schipper,M., Schubart,C.D., Sommer,I.E., Kahn,R.S. and Ophoff,R.A., 2007b. Investigating gene environment interaction in complex diseases: increasing power by selective sampling for environmental exposure. Int J Epidemiol. 36, 1363-1369.

Boydell,J., van,O.J., Caspi,A., Kennedy,N., Giouroukou,E., Fearon,P., Farrell,M. and Murray,R.M., 2006. Trends in cannabis use prior to first presentation with schizophrenia, in South-East London between 1965 and 1999. Psychol Med. 36, 1441-1446.

Browning,B.L. and Browning,S.R., 2009. A unified approach to genotype imputation and haplotype-phase inference for large data sets of trios and unrelated individuals. Am J Hum Genet. 84, 210-223.

Casey,J.P., Magalhaes,T., Conroy,J.M., Regan,R., Shah,N., Anney,R., Shields,D.C., Abrahams,B.S., Almeida,J., Bacchelli,E., Bailey,A.J., Baird,G., Battaglia,A., Berney,T., Bolshakova,N., Bolton,P.F., Bourgeron,T., Brennan,S., Cali,P., Correia,C., Corsello,C., Coutanche,M., Dawson,G., de,J.M., Delorme,R., Duketis,E., Duque,F., Estes,A., Farrar,P., Fernandez,B.A., Folstein,S.E., Foley,S., Fombonne,E., Freitag,C.M., Gilbert,J., Gillberg,C., Glessner,J.T., Green,J., Guter,S.J., Hakonarson,H., Holt,R., Hughes,G., Hus,V., Igliozzi,R., Kim,C., Klauck,S.M., Kolevzon,A., Lamb,J.A., Leboyer,M., Le,C.A., Leventhal,B.L., Lord,C., Lund,S.C., Maestrini,E., Mantoulan,C.,

Marshall,C.R., McConachie,H., McDougle,C.J., McGrath,J., McMahon,W.M., Merikangas,A., Miller,J., Minopoli,F., Mirza,G.K., Munson,J., Nelson,S.F., Nygren,G., Oliveira,G., Pagnamenta,A.T., Papanikolaou,K., Parr,J.R., Parrini,B., Pickles,A., Pinto,D., Piven,J., Posey,D.J., Poustka,A., Poustka,F., Ragoussis,J., Roge,B., Rutter,M.L., Sequeira,A.F., Soorya,L., Sousa,I., Sykes,N., Stoppioni,V., Tancredi,R., Tauber,M., Thompson,A.P., Thomson,S., Tsiantis,J., van,E.H., Vincent,J.B., Volkmar,F., Vorstman,J.A., Wallace,S., Wang,K., Wassink,T.H., White,K., Wing,K., Wittemeyer,K., Yaspan,B.L., Zwaigenbaum,L., Betancur,C., Buxbaum,J.D., Cantor,R.M., Cook,E.H., Coon,H., Cuccaro,M.L., Geschwind,D.H., Haines,J.L., Hallmayer,J., Monaco,A.P., Nurnberger,J.I., Jr., Pericak-Vance,M.A., Schellenberg,G.D., Scherer,S.W., Sutcliffe,J.S., Szatmari,P., Vieland,V.J., Wijsman,E.M., Green,A., Gill,M., Gallagher,L., Vicente,A. and Ennis,S., 2012. A novel approach of homozygous haplotype sharing identifies candidate genes in autism spectrum disorder. Hum Genet. 131, 565-579.

Caspi,A., Moffitt,T.E., Cannon,M., McClay,J., Murray,R., Harrington,H., Taylor,A., Arseneault,L., Williams,B., Braithwaite,A., Poulton,R. and Craig,I.W., 2005. Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. Biol Psychiatry. 57, 1117-1127.

Cichon,S., Craddock,N., Daly,M., Faraone,S.V., Gejman,P.V., Kelsoe,J., Lehner,T., Levinson,D.F., Moran,A., Sklar,P. and Sullivan,P.F., 2009. Genomewide association studies: history, rationale, and prospects for psychiatric disorders. Am J Psychiatry. 166, 540-556.

Curtis,D., Kalsi,G., Brynjolfsson,J., McInnis,M., O'Neill,J., Smyth,C., Moloney,E., Murphy,P., McQuillin,A., Petursson,H. and Gurling,H., 2003. Genome scan of pedigrees multiply affected with bipolar disorder provides further support for the presence of a susceptibility locus on chromosome 12q23-q24, and suggests the presence of additional loci on 1p and 1q. Psychiatr Genet. 13, 77-84.

Dawson,E., Parfitt,E., Roberts,Q., Daniels,J., Lim,L., Sham,P., Nothen,M., Propping,P., Lanczik,M., Maier,W. and ., 1995. Linkage studies of bipolar disorder in the region of the Darier's disease gene on chromosome 12q23-24.1. Am J Med Genet. 60, 94-102.

De Marchi,N., De,P.L., Orlando,P., Daniele,F., Fezza,F. and Di,M., V, 2003. Endocannabinoid signalling in the blood of patients with schizophrenia. Lipids Health Dis. 2, 5.

Degn,B., Lundorf,M.D., Wang,A., Vang,M., Mors,O., Kruse,T.A. and Ewald,H., 2001. Further evidence for a bipolar risk gene on chromosome 12q24 suggested by investigation of haplotype sharing and allelic association in patients from the Faroe Islands. Mol Psychiatry. 6, 450-455.

DeLisi,L.E., Shaw,S.H., Crow,T.J., Shields,G., Smith,A.B., Larach,V.W., Wellman,N., Loftus,J., Nanthakumar,B., Razi,K., Stewart,J., Comazzi,M., Vita,A., Heffner,T. and Sherrington,R., 2002. A genome-wide scan for linkage to chromosomal regions in 382 sibling pairs with schizophrenia or schizoaffective disorder. Am J Psychiatry. 159, 803-812.

Di Forti,M., Iyegbe,C., Sallis,H., Kolliakou,A., Falcone,M.A., Paparelli,A., Sirianni,M., La,C.C., Stilo,S.A., Marques,T.R., Handley,R., Mondelli,V., Dazzan,P., Pariante,C., David,A.S., Morgan,C., Powell,J. and Murray,R.M., 2012. Confirmation that the AKT1 (rs2494732) Genotype Influences the Risk of Psychosis in Cannabis Users. Biol Psychiatry.

Dudbridge,F. and Gusnanto,A., 2008. Estimation of significance thresholds for genomewide association scans. Genet Epidemiol. 32, 227-234.

Fuller,S.J., Stokes,L., Skarratt,K.K., Gu,B.J. and Wiley,J.S., 2009. Genetics of the P2X7 receptor and human disease. Purinergic Signal. 5, 257-262.

Giuffrida,A., Leweke,F.M., Gerth,C.W., Schreiber,D., Koethe,D., Faulhaber,J., Klosterkotter,J. and Piomelli,D., 2004. Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. Neuropsychopharmacology. 29, 2108-2114.

Grech,A., van Os,J., Jones,P.B., Lewis,S.W. and Murray,R.M., 2005. Cannabis use and outcome of recent onset psychosis. Eur Psychiatry. 20, 349-353.

Green,E.K., Grozeva,D., Raybould,R., Elvidge,G., Macgregor,S., Craig,I., Farmer,A., McGuffin,P., Forty,L., Jones,L., Jones,I., O'Donovan,M.C., Owen,M.J., Kirov,G. and Craddock,N., 2009. P2RX7: A bipolar and unipolar disorder candidate susceptibility gene? Am J Med Genet B Neuropsychiatr Genet. 150B, 1063-1069.

Grigoroiu-Serbanescu,M., Herms,S., Muhleisen,T.W., Georgi,A., Diaconu,C.C., Strohmaier,J., Czerski,P., Hauser,J., Leszczynska-Rodziewicz,A., Jamra,R.A., Babadjanova,G., Tiganov,A., Krasnov,V., Kapiletti,S., Neagu,A.I., Vollmer,J., Breuer,R., Rietschel,M., Nothen,M.M., Cichon,S., Propping,P., Nothen,M.M. and Cichon,S., 2009. Variation in P2RX7 candidate gene (rs2230912) is not associated with bipolar I disorder and unipolar major depression in four European samples. Am J Med Genet B Neuropsychiatr Genet. 150B, 1017-1021.

Hansen,T., Jakobsen,K.D., Fenger,M., Nielsen,J., Krane,K., Fink-Jensen,A., Lublin,H., Ullum,H., Timm,S., Wang,A.G., Jorgensen,N.R. and Werge,T., 2008. Variation in the purinergic P2RX(7) receptor gene and schizophrenia. Schizophr Res. 104, 146-152.

Hanssen,M., Peeters,F., Krabbendam,L., Radstake,S., Verdoux,H. and van,O.J., 2003. How psychotic are individuals with non-psychotic disorders? Soc Psychiatry Psychiatr Epidemiol. 38, 149-154.

Hayden,E.P. and Nurnberger,J.I., Jr., 2006. Molecular genetics of bipolar disorder. Genes Brain Behav. 5, 85-95.

Hejjas,K., Szekely,A., Domotor,E., Halmai,Z., Balogh,G., Schilling,B., Sarosi,A., Faludi,G., Sasvari-Szekely,M. and Nemoda,Z., 2009. Association between depression and the Gln460Arg polymorphism of P2RX7 gene: a dimensional approach. Am J Med Genet B Neuropsychiatr Genet. 150B, 295-299.

Henquet,C., Rosa,A., Delespaul,P., Papiol,S., Fananas,L., van,O.J. and Myin-Germeys,I., 2008. COMT Val(158)Met moderation of cannabis-induced psychosis: a momentary assessment study of 'switching on' hallucinations in the flow of daily life. Acta Psychiatr Scand.

Hoggart,C.J., Clark,T.G., De,I.M., Whittaker,J.C. and Balding,D.J., 2008. Genome-wide significance for dense SNP and resequencing data. Genet Epidemiol. 32, 179-185.

Howlett,A.C., 2002. The cannabinoid receptors. Prostaglandins Other Lipid Mediat. 68-69, 619-631.

Howlett,A.C., Breivogel,C.S., Childers,S.R., Deadwyler,S.A., Hampson,R.E. and Porrino,L.J., 2004. Cannabinoid physiology and pharmacology: 30 years of progress. Neuropharmacology. 47 Suppl 1, 345-358.

Ioannidis,J.P., 2008. Why most discovered true associations are inflated. Epidemiology. 19, 640-648.

Ioannidis,J.P., Ntzani,E.E., Trikalinos,T.A. and Contopoulos-Ioannidis,D.G., 2001. Replication validity of genetic association studies. Nat Genet. 29, 306-309.

Kapoor,S., 2009. Systemic and psychiatric disorders associated with polymorphisms of the P2RX7 gene. Am J Med Genet B Neuropsychiatr Genet. 150B, 597-598.

Klegeris,A., Bissonnette,C.J. and McGeer,P.L., 2003. Reduction of human monocytic cell neurotoxicity and cytokine secretion by ligands of the cannabinoid-type CB2 receptor. Br J Pharmacol. 139, 775-786.

Konings,M., Bak,M., Hanssen,M., van,O.J. and Krabbendam,L., 2006. Validity and reliability of the CAPE: a self-report instrument for the measurement of psychotic experiences in the general population. Acta Psychiatr Scand. 114, 55-61.

Large,M., Sharma,S., Compton,M.T., Slade,T. and Nielssen,O., 2011. Cannabis use and earlier onset of psychosis: a systematic meta-analysis. Arch Gen Psychiatry. 68, 555-561.

Lees,M.P., Fuller,S.J., McLeod,R., Boulter,N.R., Miller,C.M., Zakrzewski,A.M., Mui,E.J., Witola,W.H., Coyne,J.J., Hargrave,A.C., Jamieson,S.E., Blackwell,J.M., Wiley,J.S. and Smith,N.C., 2010. P2X7 receptor-mediated killing of an intracellular parasite, Toxoplasma gondii, by human and murine macrophages. J Immunol. 184, 7040-7046.

Leweke,F.M., Giuffrida,A., Koethe,D., Schreiber,D., Nolden,B.M., Kranaster,L., Neatby,M.A., Schneider,M., Gerth,C.W., Hellmich,M., Klosterkotter,J. and Piomelli,D., 2007. Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: impact of cannabis use. Schizophr Res. 94, 29-36.

Leweke,F.M., Giuffrida,A., Wurster,U., Emrich,H.M. and Piomelli,D., 1999. Elevated endogenous cannabinoids in schizophrenia. Neuroreport. 10, 1665-1669.

Leweke,F.M., Piomelli,D., Pahlisch,F., Muhl,D., Gerth,C.W., Hoyer,C., Klosterkotter,J., Hellmich,M. and Koethe,D., 2012. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. Transl Psychiatry. 2, e94.

Li,Q., Luo,X. and Muallem,S., 2005. Regulation of the P2X7 receptor permeability to large molecules by extracellular Cl- and Na+. J Biol Chem. 280, 26922-26927.

Luan,J.A., Wong,M.Y., Day,N.E. and Wareham,N.J., 2001. Sample size determination for studies of gene-environment interaction. Int J Epidemiol. 30, 1035-1040.

Lucae,S., Salyakina,D., Barden,N., Harvey,M., Gagne,B., Labbe,M., Binder,E.B., Uhr,M., Paez-Pereda,M., Sillaber,I., Ising,M., Bruckl,T., Lieb,R., Holsboer,F. and Muller-Myhsok,B., 2006. P2RX7, a gene coding for a purinergic ligand-gated ion channel, is associated with major depressive disorder. Hum Mol Genet. 15, 2438-2445.

Lynch,M.E. and Campbell,F., 2011. Cannabinoids for treatment of chronic non-cancer pain; a systematic review of randomized trials. Br J Clin Pharmacol. 72, 735-744.

Manrique-Garcia,E., Zammit,S., Dalman,C., Hemmingsson,T., Andreasson,S. and Allebeck,P., 2012. Cannabis, schizophrenia and other non-affective psychoses: 35 years of follow-up of a population-based cohort. Psychol Med. 42, 1321-1328.

Marsicano,G., Goodenough,S., Monory,K., Hermann,H., Eder,M., Cannich,A., Azad,S.C., Cascio,M.G., Gutierrez,S.O., van der,S.M., Lopez-Rodriguez,M.L., Casanova,E., Schutz,G., Zieglgansberger,W., Di,M., V, Behl,C. and Lutz,B., 2003. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. Science. 302, 84-88.

McGuffin,P., Knight,J., Breen,G., Brewster,S., Boyd,P.R., Craddock,N., Gill,M., Korszun,A., Maier,W., Middleton,L., Mors,O., Owen,M.J., Perry,J., Preisig,M., Reich,T., Rice,J., Rietschel,M., Jones,L., Sham,P. and Farmer,A.E., 2005. Whole genome linkage scan of recurrent depressive disorder from the depression network study. Hum Mol Genet. 14, 3337-3345.

McGuire,P.K., Jones,P., Harvey,I., Williams,M., McGuffin,P. and Murray,R.M., 1995. Morbid risk of schizophrenia for relatives of patients with cannabis-associated psychosis. Schizophr Res. 15, 277-281.

McQuillin,A., Bass,N.J., Choudhury,K., Puri,V., Kosmin,M., Lawrence,J., Curtis,D. and Gurling,H.M., 2009. Case-control studies show that a non-conservative amino-acid change from a glutamine to arginine in the P2RX7 purinergic receptor protein is associated with both bipolar- and unipolar-affective disorders. Mol Psychiatry. 14, 614-620.

Miller,A.M. and Stella,N., 2008. CB2 receptor-mediated migration of immune cells: it can go either way. Br J Pharmacol. 153, 299-308.

Monji,A., Kato,T.A., Mizoguchi,Y., Horikawa,H., Seki,Y., Kasai,M., Yamauchi,Y., Yamada,S. and Kanba,S., 2011. Neuroinflammation in schizophrenia especially focused on the role of microglia. Prog Neuropsychopharmacol Biol Psychiatry.

Moore,T.H., Zammit,S., Lingford-Hughes,A., Barnes,T.R., Jones,P.B., Burke,M. and Lewis,G., 2007. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet. 370, 319-328.

Morissette,J., Villeneuve,A., Bordeleau,L., Rochette,D., Laberge,C., Gagne,B., Laprise,C., Bouchard,G., Plante,M., Gobeil,L., Shink,E., Weissenbach,J. and Barden,N., 1999. Genome-wide search for linkage of bipolar affective disorders in a very large pedigree derived from a homogeneous population in quebec points to a locus of major effect on chromosome 12q23-q24. Am J Med Genet. 88, 567-587.

Mossaheb,N., Becker,J., Schaefer,M.R., Klier,C.M., Schloegelhofer,M., Papageorgiou,K. and Amminger,G.P., 2012. The Community Assessment of Psychic Experience (CAPE) questionnaire as a screening-instrument in the detection of individuals at ultra-high risk for psychosis. Schizophr Res. 141, 210-214.

Myles,N., Newall,H., Nielssen,O. and Large,M., 2012. The association between cannabis use and earlier age at onset of schizophrenia and other psychoses: meta-analysis of possible confounding factors. Curr Pharm Des.

Nagy,G., Ronai,Z., Somogyi,A., Sasvari-Szekely,M., Rahman,O.A., Mate,A., Varga,T. and Nemoda,Z., 2008. P2RX7 Gln460Arg polymorphism is associated with depression among diabetic patients. Prog Neuropsychopharmacol Biol Psychiatry. 32, 1884-1888.

Pe'er,I., Yelensky,R., Altshuler,D. and Daly,M.J., 2008. Estimation of the multiple testing burden for genomewide association studies of nearly all common variants. Genet Epidemiol. 32, 381-385.

Pertwee,R.G., 2006. Cannabinoid pharmacology: the first 66 years. Br J Pharmacol. 147 Suppl 1, S163-S171.

Power,B.D., Dragovic,M., Jablensky,A. and Stefanis,N.C., 2012. Does accumulating exposure to illicit drugs bring forward the age at onset in schizophrenia? Aust N Z J Psychiatry.

Purcell,S., 2002. Variance components models for gene-environment interaction in twin analysis. Twin Res. 5, 554-571.

Purcell,S., Neale,B., Todd-Brown,K., Thomas,L., Ferreira,M.A., Bender,D., Maller,J., Sklar,P., de Bakker,P.I., Daly,M.J. and Sham,P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 81, 559-575.

Rapp,C., Bugra,H., Riecher-Rossler,A., Tamagni,C. and Borgwardt,S., 2012. Effects of cannabis use on human brain structure in psychosis: a systematic review combining in vivo structural neuroimaging and post mortem studies. Curr Pharm Des. 18, 5070-5080.

Ripke,S., Sanders,A.R., Kendler,K.S., Levinson,D.F., Sklar,P., Holmans,P.A., Lin,D.Y., Duan,J., Ophoff,R.A., Andreassen,O.A., Scolnick,E., Cichon,S., St,C.D., Corvin,A., Gurling,H., Werge,T., Rujescu,D., Blackwood,D.H., Pato,C.N., Malhotra,A.K., Purcell,S., Dudbridge,F., Neale,B.M., Rossin,L., Visscher,P.M., Posthuma,D., Ruderfer,D.M., Fanous,A., Stefansson,H., Steinberg,S., Mowry,B.J., Golimbet,V., De,H.M., Jonsson,E.G., Bitter,I., Pietilainen,O.P., Collier,D.A., Tosato,S., Agartz,I., Albus,M., Alexander,M., Amdur,R.L., Amin,F., Bass,N., Bergen,S.E., Black,D.W., Borglum,A.D., Brown,M.A., Bruggeman,R., Buccola,N.G., Byerley,W.F., Cahn,W., Cantor,R.M., Carr,V.J., Catts,S.V., Choudhury,K., Cloninger,C.R., Cormican,P., Craddock,N., Danoy,P.A., Datta,S., De,H.L., Demontis,D., Dikeos,D., Djurovic,S., Donnelly,P., Donohoe,G., Duong,L., Dwyer,S., Fink-Jensen,A., Freedman,R., Freimer,N.B., Friedl,M., Georgieva,L., Giegling,I., Gill,M., Glenthoj,B., Godard,S., Hamshere,M., Hansen,M., Hansen,T., Hartmann,A.M., Henskens,F.A., Hougaard,D.M., Hultman,C.M., Ingason,A., Jablensky,A.V., Jakobsen,K.D., Jay,M., Jurgens,G., Kahn,R.S., Keller,M.C., Kenis,G., Kenny,E., Kim,Y., Kirov,G.K., Konnerth,H., Konte,B., Krabbendam,L., Krasucki,R., Lasseter,V.K., Laurent,C., Lawrence,J., Lencz,T., Lerer,F.B., Liang,K.Y., Lichtenstein,P., Lieberman,J.A., Linszen,D.H., Lonnqvist,J., Loughland,C.M., Maclean,A.W., Maher,B.S., Maier,W., Mallet,J., Malloy,P., Mattheisen,M., Mattingsdal,M., McGhee,K.A., McGrath,J.J., McIntosh,A., McLean,D.E., McQuillin,A., Melle,I., Michie,P.T., Milanova,V., Morris,D.W., Mors,O., Mortensen,P.B., Moskvina,V., Muglia,P., Myin-Germeys,I., Nertney,D.A., Nestadt,G., Nielsen,J., Nikolov,I., Nordentoft,M., Norton,N., Nothen,M.M., O'Dushlaine,C.T., Olincy,A., Olsen,L., O'Neill,F.A., Orntoft,T.F., Owen,M.J., Pantelis,C., Papadimitriou,G., Pato,M.T., Peltonen,L., Petursson,H., Pickard,B., Pimm,J., Pulver,A.E., Puri,V., Quested,D., Quinn,E.M., Rasmussen,H.B., Rethelyi,J.M., Ribble,R., Rietschel,M., Riley,B.P., Ruggeri,M., Schall,U., Schulze,T.G., Schwab,S.G., Scott,R.J., Shi,J., Sigurdsson,E., Silverman,J.M., Spencer,C.C., Stefansson,K., Strange,A., Strengman,E., Stroup,T.S., Suvisaari,J., Terenius,L., Thirumalai,S., Thygesen,J.H., Timm,S., Toncheva,D., van den,O.E., van,O.J., van,W.R., Veldink,J., Walsh,D., Wang,A.G., Wiersma,D., Wildenauer,D.B., Williams,H.J., Williams,N.M., Wormley,B., Zammit,S., Sullivan,P.F., O'Donovan,M.C., Daly,M.J. and Gejman,P.V., 2011. Genome-wide association study identifies five new schizophrenia loci. Nat Genet. 43, 969-976.

Saunders,B.M., Fernando,S.L., Sluyter,R., Britton,W.J. and Wiley,J.S., 2003. A loss-of-function polymorphism in the human P2X7 receptor abolishes ATP-mediated killing of mycobacteria. J Immunol. 171, 5442-5446.

Schubart,C.D., van Gastel,W.A., Breetvelt,E.J., Beetz,S.L., Ophoff,R.A., Sommer,I.E., Kahn,R.S. and Boks,M.P. Cannabis use at young age is associated with psychotic experiences. Psychological Medicine . 2010. Ref Type: In Press

Shen,M. and Thayer,S.A., 1998. Cannabinoid receptor agonists protect cultured rat hippocampal neurons from excitotoxicity. Mol Pharmacol. 54, 459-462.

Shink,E., Harvey,M., Tremblay,M., Gagne,B., Belleau,P., Raymond,C., Labbe,M., Dube,M.P., Lafreniere,R.G. and Barden,N., 2005a. Analysis of microsatellite markers and single nucleotide polymorphisms in candidate genes for susceptibility to bipolar affective disorder in the chromosome 12Q24.31 region. Am J Med Genet B Neuropsychiatr Genet. 135B, 50-58.

Shink,E., Morissette,J., Sherrington,R. and Barden,N., 2005b. A genome-wide scan points to a susceptibility locus for bipolar disorder on chromosome 12. Mol Psychiatry. 10, 545-552.

Sorge,R.E., Trang,T., Dorfman,R., Smith,S.B., Beggs,S., Ritchie,J., Austin,J.S., Zaykin,D.V., Vander,M.H., Costigan,M., Herbert,T.A., Yarkoni-Abitbul,M., Tichauer,D., Livneh,J., Gershon,E., Zheng,M., Tan,K., John,S.L., Slade,G.D., Jordan,J., Woolf,C.J., Peltz,G., Maixner,W., Diatchenko,L., Seltzer,Z., Salter,M.W. and Mogil,J.S., 2012. Genetically determined P2X7 receptor pore formation regulates variability in chronic pain sensitivity. Nat Med. 18, 595-599.

Soronen,P., Mantere,O., Melartin,T., Suominen,K., Vuorilehto,M., Rytsala,H., Arvilommi,P., Holma,I., Holma,M., Jylha,P., Valtonen,H.M., Haukka,J., Isometsa,E. and Paunio,T., 2011. P2RX7 gene is associated consistently with mood disorders and predicts clinical outcome in three clinical cohorts. Am J Med Genet B Neuropsychiatr Genet. 156B, 435-447.

Sperlagh,B., Vizi,E.S., Wirkner,K. and Illes,P., 2006. P2X7 receptors in the nervous system. Prog Neurobiol. 78, 327-346.

Spitzer,R.L., Williams,J.B., Gibbon,M. and First,M.B., 1992. The Structured Clinical Interview for DSM-III-R (SCID). I: History, rationale, and description. Arch Gen Psychiatry. 49, 624-629.

Stefanis,N.C., Hanssen,M., Smirnis,N.K., Avramopoulos,D.A., Evdokimidis,I.K., Stefanis,C.N., Verdoux,H. and van,O.J., 2002. Evidence that three dimensions of psychosis have a distribution in the general population. Psychol Med. 32, 347-358.

Steinberg,S., de,J.S., Andreassen,O.A., Werge,T., Borglum,A.D., Mors,O., Mortensen,P.B., Gustafsson,O., Costas,J., Pietilainen,O.P., Demontis,D., Papiol,S., Huttenlocher,J., Mattheisen,M., Breuer,R., Vassos,E., Giegling,I., Fraser,G., Walker,N., Tuulio-Henriksson,A., Suvisaari,J., Lonnqvist,J., Paunio,T., Agartz,I., Melle,I., Djurovic,S., Strengman,E., Jurgens,G., Glenthoj,B., Terenius,L., Hougaard,D.M., Orntoft,T., Wiuf,C., Didriksen,M., Hollegaard,M.V., Nordentoft,M., van,W.R., Kenis,G., Abramova,L., Kaleda,V., Arrojo,M., Sanjuan,J., Arango,C., Sperling,S., Rossner,M., Ribolsi,M., Magni,V., Siracusano,A., Christiansen,C., Kiemeney,L.A., Veldink,J., van den,B.L., Ingason,A., Muglia,P., Murray,R., Nothen,M.M., Sigurdsson,E., Petursson,H., Thorsteinsdottir,U., Kong,A., Rubino,I.A., De,H.M., Rethelyi,J.M., Bitter,I., Jonsson,E.G., Golimbet,V., Carracedo,A., Ehrenreich,H., Craddock,N., Owen,M.J., O'Donovan,M.C., Ruggeri,M., Tosato,S., Peltonen,L., Ophoff,R.A., Collier,D.A., St,C.D., Rietschel,M., Cichon,S., Stefansson,H., Rujescu,D. and Stefansson,K., 2011. Common variants at VRK2 and TCF4 conferring risk of schizophrenia. Hum Mol Genet. 20, 4076-4081.

Stella,N., 2004. Cannabinoid signaling in glial cells. Glia. 48, 267-277.

Stella,N., 2009. Endocannabinoid signaling in microglial cells. Neuropharmacology. 56 Suppl 1, 244-253.

Stella,N., 2010. Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. Glia. 58, 1017-1030.

Stokes,L., Fuller,S.J., Sluyter,R., Skarratt,K.K., Gu,B.J. and Wiley,J.S., 2010. Two haplotypes of the P2X(7) receptor containing the Ala-348 to Thr polymorphism exhibit a gain-of-function effect and enhanced interleukin-1beta secretion. FASEB J. 24, 2916-2927.

Sullivan,J.M., 1999. Mechanisms of cannabinoid-receptor-mediated inhibition of synaptic transmission in cultured hippocampal pyramidal neurons. J Neurophysiol. 82, 1286-1294.

Sullivan,P.F., Daly,M.J. and O'Donovan,M., 2012. Genetic architectures of psychiatric disorders: the emerging picture and its implications. Nat Rev Genet. 13, 537-551.

Sun,C., Chu,J., Singh,S. and Salter,R.D., 2010. Identification and characterization of a novel variant of the human P2X(7) receptor resulting in gain of function. Purinergic Signal. 6, 31-45.

Trimbos-instituut,U. THC-concentraties in wiet, nederwiet en hasj in Nederlands coffeeshops (2007-2008). Netherlands Institute of Mental Health and Addiction. 2009.
Ref Type: Generic

van der Stelt,M. and Di Marzo,V., 2005. Cannabinoid receptors and their role in neuroprotection. Neuromolecular Med. 7, 37-50.

van Os,J., Bak,M., Hanssen,M., Bijl,R.V., de,G.R. and Verdoux,H., 2002. Cannabis use and psychosis: a longitudinal population-based study. Am J Epidemiol. 156, 319-327.

Van Sickle,M.D., Duncan,M., Kingsley,P.J., Mouihate,A., Urbani,P., Mackie,K., Stella,N., Makriyannis,A., Piomelli,D., Davison,J.S., Marnett,L.J., Di,M., V, Pittman,Q.J., Patel,K.D. and Sharkey,K.A., 2005. Identification and functional characterization of brainstem cannabinoid CB2 receptors. Science. 310, 329-332.

van Winkel,R., 2011. Family-based analysis of genetic variation underlying psychosis-inducing effects of cannabis: sibling analysis and proband follow-up. Arch Gen Psychiatry. 68, 148-157.

van Winkel,R., van Beveren,N.J. and Simons,C., 2011. AKT1 moderation of cannabis-induced cognitive alterations in psychotic disorder. Neuropsychopharmacology. 36, 2529-2537.

Vleeschouwer,M., Schubart,C.D., Henquet,C., van Gastel,W.A., Hillegers,M., Kahn,R.S., Boks,M.P. and Derks,E. Does Assessment type matter? A Measurement Invariance analysis of online and paper and pencil assessment of the Community Assessment of Psychic Experiences (CAPE). 2013.
Ref Type: Unpublished Work

Voorman,A., Lumley,T., McKnight,B. and Rice,K., 2011. Behavior of QQ-plots and genomic control in studies of gene-environment interaction. PLoS ONE. 6, e19416.

Walter,L., Dinh,T. and Stella,N., 2004. ATP induces a rapid and pronounced increase in 2-arachidonoylglycerol production by astrocytes, a response limited by monoacylglycerol lipase. J Neurosci. 24, 8068-8074.

Who. Composite International Diagnostic Interview. 1-1-1993. Geneva, World Health Organisation. Ref Type: Generic

Wigman,J.T., van,W.R., Raaijmakers,Q.A., Ormel,J., Verhulst,F.C., Reijneveld,S.A., van,O.J. and Vollebergh,W.A., 2011. Evidence for a persistent, environment-dependent and deteriorating subtype of subclinical psychotic experiences: a 6-year longitudinal general population study. Psychol Med. 41, 2317-2329.

Wiley,J.S., Sluyter,R., Gu,B.J., Stokes,L. and Fuller,S.J., 2011. The human P2X7 receptor and its role in innate immunity. Tissue Antigens. 78, 321-332.

Witting,A., Walter,L., Wacker,J., Moller,T. and Stella,N., 2004. P2X7 receptors control 2-arachidonoylglycerol production by microglial cells. Proc Natl Acad Sci U S A. 101, 3214-3219.

Xiao,J., Sun,L., Yan,H., Jiao,W., Miao,Q., Feng,W., Wu,X., Gu,Y., Jiao,A., Guo,Y., Peng,X. and Shen,A., 2010. Metaanalysis of P2X7 gene polymorphisms and tuberculosis susceptibility. FEMS Immunol Med Microbiol. 60, 165-170.

Zammit,S., Allebeck,P., Andreasson,S., Lundberg,I. and Lewis,G., 2002. Self reported cannabis use as a risk factor for schizophrenia in Swedish conscripts of 1969: historical cohort study. BMJ. 325, 1199.

Zammit,S., Owen,M.J., Evans,J., Heron,J. and Lewis,G., 2011. Cannabis, COMT and psychotic experiences. Br J Psychiatry. 199, 380-385.

PART II: GENETIC FACTORS
CHAPTER VII

• GENOME WIDE EXPRESSION PROFILING OF WHOLE BLOOD FROM HEAVY CANNABIS USERS REVEALS DIFFERENTIAL REGULATION OF LIPRIN ALPHA 2 (PPFIA2) •

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ABSTRACT

Introduction
Cannabis is the most widely used illicit drug and is associated with numerous physiological and neuropsychiatric effects. Little is known about the biological mechanisms that underlie these associations. Gene-expression profiling may provide further insight into molecular mechanisms driving the effects that are associated with cannabis use.
Methods
90 heavy cannabis users and 100 cannabis naïve participants were matched for sex, use of other drugs than cannabis, cigarette smoking, use of alcohol, use of medication, and DSM IV diagnoses. All individuals were examined using standardized structure clinical interviews (SCID, CIDI) for psychiatric diagnosis and substance use. Genome-wide RNA expression profiling was obtained with HumanHT-12 v3 arrays using Illumina's standard protocol. Gene expression data were transformed and normalized. After quality control 20,765 transcripts were available for further analysis. Expression levels were analyzed for association with cannabis status using linear regression. Cigarette smoking, use of other drugs than cannabis, age and gender were used as covariates. FDR correction for multiple testing was applied at the 0.05 level. Genome wide significant findings were validated using quantitative real-time PCR (qPCR).
Results
The expression levels of two transcripts; CX3CR1 (LogFold Change -0.42) and PPFIA2 (LogFold Change 0.17), were significantly associated with cannabis use after correction for covariates and multiple testing. The up-regulation of PPFIA2, but not CX3CR1 was validated by qPCR.
Conclusions
Changes in Liprin alpha 2 levels is a plausible mechanism by wich cannabis could influence psychiatric phenotypes. PPFIA2 is expressed in the brain and invloved in memory formation. The finding that PPFIA2 and CX3CR1 are differentially expressed in cannabis users could help us to generate new hypotheses on the etiology of adverse effects associated with cannabis use.

INTRODUCTION

With an estimated annual prevalence of 170 million users, cannabis is the most frequently used illicit drug worldwide (United Nations Office on Drugs and Crime, 2012).
Acute neuropsychological effects of cannabis are euphoria, increased awareness of sensation, dissociation, (pseudo)hallucinations and anxiety but also somatic responses as reddening of the eyes, a dry mouth and increased heart rate (Hall and Solowij, 1998). Moreover, cannabis acutely impairs all aspects of short-term memory, especially short-term episodic and working memory (Ranganathan and D'Souza, 2006). Long-term sequelae were investigated in a recent study that suggests that persistent cannabis use is associated with neuropsychological decline broadly across domains of functioning (Meier et al., 2012) and psychiatric disorders (Macleod et al., 2004;Taylor et al., 2002;Degenhardt et al., 2008;Gillespie et al., 2009;Satyanarayana, 2009;Schubart et al., 2011a). Particularly, the association with psychotic disorders is reported repeatedly (Moore et al., 2007;Schubart et al., 2010b;Pedersen, 2008;Skinner et al., 2010;Andreasson et al., 1987).

The biological mechanisms underlying these adverse effects of cannabis use are still poorly understood. The most important psychoactive constituents of the cannabis plant are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), however these are only two of the approximately 70 phytocannabinoids that are found in the Cannabis sativa plant (Mechoulam et al., 2007). These phytocannabinoids are thought to interact with the human endocannabinoid System (ECS) which consists of endocannabinoids and at least five receptors of which CB1r and CB2r are most extensively described (Brown, 2007;Pertwee, 2008;Starowicz et al., 2008). Through activation of cannabinoid receptors, cannabinoids induce the inhibition of release of excitatory and inhibitory neurotransmitters, such as acetylcholine, noradrenaline, GABA, glutamate and dopamine (Freund et al., 2003;Howlett, 2002b) resulting in either inhibition or excitation. Pharmacological imaging studies showed dopamine release in the striatum in humans following THC exposure (Bossong et al., 2008;Bhattacharyya et al., 2012a). Moreover, activation of CB2r impacts on migration of immune cells, including microglia (Miller and Stella, 2008) and THC is shown to have a modulating effect on cell-mediated and humoral immunity (Yuan et al., 2002;Jan et al., 2003;Stella, 2009;Stella, 2010;Tanasescu and Constantinescu, 2010).

Few hypothesis exist that relate these findings on the cellular level to the earlier described neuropsychological effects (Marco et al., 2011a). Gene expression studies could help to uncover a new perspective through identifying genes and biological pathways involved in the response to cannabis exposure (Lockhart and Winzeler, 2000).
Previous gene expression studies investigated the impact of THC on expression of pre-selected transcripts in several isolated models. Examples include post-mortem samples (Lehrmann et al., 2006), animal models (Parmentier-Batteur

et al., 2002;Dean et al., 2001;Corchero et al., 1997) or specific cell lines (Samson et al., 2003;Sarafian et al., 2005). A priori selection of transcripts limits the possibility to uncover new leads in understanding the biological pathways underlying cannabis associated adverse effects. To date no studies were published investigating the effects of cannabis on gene expression on whole blood associated with cannabis use. Moreover, we are not aware of studies screening for genome wide expression changes associated with cannabis use.
In this study we therefore directly compared genome wide gene expression patterns between heavy cannabis smokers and cannabis naïve participants by measuring gene expression profiles in whole blood.

METHODS

Recruitment

Participants were recruited among subjects of an ongoing study on genetic and epidemiological determinants of cannabis related psychotic experiences. Individuals for the current study were recruited in three phases. First, we screened for suitable individuals using a project website (www.cannabisquest.nl) described elsewhere in detail (Schubart et al., 2010b). In short, participants provided information on cannabis use, the experience of psychotic like experiences (PLEs), country of birth of their grandparents, age, educational level and contact details (N=25,959). Second, individuals between the age of 18 and 25 years were invited to participate in an ongoing multilevel genetics study. Further inclusion criteria were; absent or extreme cannabis exposure and high or low scores (top- or bottom 20%) on the Community Assessment of Psychic Experience questionnaire (CAPE) (Konings et al., 2006). To control population stratification, we confined to inclusion of individuals with four grandparents born in The Netherlands. Selected subjects where contacted by telephone, invalid contact details automatically lead to exclusion. Third, for the current study, we recruited among individuals that participated in our genetics study. All participants gave written informed consent and this study was approved by Medical Ethical Committee Utrecht.

Matching

In order to minimize confounding by other factors that could potentially influence gene-expression, we performed a group wise matching procedure. The current study aims to detect gene expression changes associated with cannabis use, indifferent of absence or presence of PLE's. We therefore firstly matched the samples of cannabis users and cannabis naïves for their score on the CAPE questionnaire, see also table 1. To balance the presence of other potential confounding factors in the cannabis using group, we selected from the cannabis naïve group in the following order of priority: sex, use of other drugs than cannabis, cigarette smoking, use of alcohol, use of medication, and finally DSM- IV diagnosis. See table 1 for further details.

Cannabis exposure
In the first (web-based) recruitment round, we assessed the amount in euro's (€) spent on cannabis per week. In The Netherlands THC-concentration and market value of cannabis are highly correlated (Trimbos, 2009), we therefore used the weekly amount of euro's spent on cannabis as a proxy measure of exposure to Δ^9-tetrahydrocannabinol (THC). The THC exposure was categorized into five classes; cannabis naïve, cannabis use equivalent of less than €3 per week, €3 to €10 per week, €10 to €25 per week and more than €25 per week. Cannabis use that exceeded €10 per week and absence of cannabis use were inclusion criteria.

Assessment at inclusion
Drug use was further assessed by using the drugs- and alcohol sections of the Composite International Diagnostic Interview (CIDI) (Trimbos-instituut, 2009a). Assessments took place at the University Medical Center Utrecht and also included a standardized structure clinical interviews (SCID) (Spitzer et al., 1992) to assess psychiatric diagnoses.

Biological materials
RNA from whole blood was obtained using two 10cc blood PAXgene (Qiagen ™) tubes. All participants donated a urine sample to perform drugs screen in order to verify their report on recent cannabis use. PAX-gene tubes were stored in -20C and RNA was isolated within 6 months after phlebotomy.

Laboratory measurements
mRNA was isolated and purified from whole blood using the PAXGene extraction kit (Qiagen ™) according to the manufacturer's instructions including an DNase digestion step. Total mRNA was quantified using a ribogreen assay (Invitrogen Quant-it™ Ribogreen, #R11490). RNA quality was determined using Agilent 2100 Bioanalyzer. RNA samples with an RNA integrity number (RIN) values under 5.0 were excluded (Schroeder et al., 2006). Genome-wide RNA expression profiling was obtained with a HumanHT-12 v3 Beadchip, conferring over 48,000 probes and using Illumina's standard protocol at the University of California, Los Angeles (UCLA) Illumina facility. RNA samples were prepared with the Illumina TotalPrep kit amplification and labeling protocol. 750 ng of amplified and biotinylated labeled cRNA was then used for array hybridization. BeadChips were scanned using an Illumina BeadArray reader. To prevent batch effects, the samples were randomly distributed over the different arrays.

Expression data preprocessing
BeadStudio© software version 3.2 was used to extract raw data and generate background-corrected gene expression data. Background correction was performed by subtracting the average value of negative control beads present on the array. Further pre-processing was done using the Lumi package for R. A variance stabilizing transformation (VST) and robust spline normalization (RSN) were applied to the data according to the Lumi procedure (Du et al., 2008).

Genes were then filtered based on detection values generated by BeadStudio©. Expression probes had to reach the detection *p*-value threshold <0.01 in at least one sample. Chip quality and outlier detection was performed by assessing quality statistics and plots before and after transformation and normalization.

Expression data analysis
Expression values were taken as dependent variables and tested for association with cannabis status as the independent variable using the Limma software package (Smith, 2005a). Cigarette smoking (present or abscent), the use of other drugs besides cannabis (present or abscent), age and gender were taken along as covariates. Significance threshold was set at a False-Discovery-Rate (FDR) corrected P < 0.05.

Quantitative Real Time PCR (qPCR)
For validation of the results, we analyzed the expression levels of the genome wide significant transcripts by *qPCR* (Applied Biosystems ™). Absolute quantities were obtained by running all samples in quadruplicates. First-strand cDNA was synthesized according to the manufacturer's instructions. Quantitative PCR was carried out using a TaqMan assay from Applied Biosystems. The following TaqMan gene expression assays were used: human genes for CX3CR1 (Hs00365842), CXCR3 (Hs01598433), PPFIA2 (Hs01548846), PPFIA2 (Hs01548855), PPFIA2 (Hs01548860). Ct values were normalized against GUS expression (ΔCt). Finally, the amplification profiles were analyzed using a threshold cycle (C_T) relative quantification method. Log-fold changes and significance were calculated using a non parametric delta-delta method.

cis expression Quantitative train loci (eQTL)
In the previously described study on genetic determinants of cannabis associated psychotic symptoms, we generated whole-genome Single Nucleotide polymorphisms (SNP) data of all included subjects. We performed a *cis* Expression quantitative trait loci (eQTL) analysis to identify SNP's that regulate mRNA expression levels of relevant loci. Genetic association were calculated using PLINK (Purcell et al., 2007), with a standard regression analysis, using gene expression as a quantitative trait. The p-value significance threshold was set at *0.05 /n*, (*n* being the number of SNP's to be tested).

RESULTS
Sample
Between December 2006 and December 349 individuals were included in the current gene expression study. Due to failed response verification by urine analyses, unsuccessful RNA extraction, logistic and transportation problems, the final amount of available samples was 284, 188 from cannabis naïve individuals and 96 from heavy users. Characteristics of the drop-out was representative of the entire population with respect to age gender, cannabis use and CAPE score (data not shown).

We used the 188 cannabis naïve participants to perform groupwise matching for the group of 96 heavy users in the following order: CAPE score, sex, use of other drugs than cannabis, cigarette smoking, use of alcohol, use of medication, and finally DSM IV diagnosis. The matching procedure resulted in 105 cannabis naïve participants balancing characteristics of 96 heavy users.

After exclusion of participants due to technical reasons (technical outliers, n=6) and RNA samples with RNA integrity number (RIN) (Schroeder et al., 2006) under the cut-off value of 5.0, we performed our analyses with a total of 100 cannabis naïve subjects and 90 heavy cannabis users. For sample characteristics see *table 1*.

Table 1. Sample characteristics

	Total group	Cannabis naïve	Heavy Cannabis users	p-value
n	190	100	90	
Mean age (sd)	24.0 (2.0)	23.8 (1.8)	24.3 (2.2)	0.06
Gender (%male)	59.5	39.6	81.1	<0.001
Other drugs	52 (27%)	0 (0%)	52 (58%)	<0.001
Cigarette smoking	78 (41%)	8 (8%)	70 (78%)	<0.001
Alcohol use lifetime	170 (89%)	90 (90%)	80 (89%)	0.804
Medication use recent	43 (23%)	24 (24%)	19 (21%)	0.359
DSM IV diagnosis	72 (38%)	20 (20%)	52 (58%)	<0.001
High CAPE score (%)	93 (49%)	50 (50%)	43 (48%)	0.864

Differential expression
After filtering and quality control, 20,765 probes remained (42.5%) for further analysis. Linear regression analysis yielded 2,131 probes with nominal significance. After FDR-correction at the 0.05 level, the expression of two transcripts, CX3CR1 (probe ID: ILMN_1745788) (LogFold Change -0.42) and PPFIA2 (probe ID: ILMN_1803318) (LogFold Change 0.17), were significantly associated with cannabis use. For further details, see table 2.

qPCR validation
Due to depletion of RNA samples, we were able to perform quantitative real-time PCR (q*PCR)* in only 143 participants; 65 heavy users and 78 samples from cannabis naïve participants. These participants did not deviate from the original sample with respect to the covariates. Following the comparative C_T (Applied Biosystems ^{TM}) protocol for PPFIA2 and CX3CR1, we validated the upregulation of the PPFIA2 transcript (log Fold Change 1.40, p: 0.046). However, the downregulation of CX3CR1 could not be validated (Fold Change 1.04, p: 0.516).

cis expression Quantitative train loci (eQTL)
We performed a *cis* eQTL analysis to identify Single Nucleotide polymorphisms (SNP's) in the PPFIA2 gene that regulate expression levels of PPFIA2 mRNA. From the 190 participants included in the current study, genotype data was available from 143 individuals and 262 SNP's in the PPFIA2 gene region (according to the Ensembl genome browser). Genetic association were calculated using standard association regression analysis in PLINK (Purcell et al., 2007). Given the fact that we tested 262 SNP's, the p-value significance threshold was set at (0.05/262): 1.9 e-04. None of the 262 analyzed PPFIA2 SNP's was significantly associated with the expression of the PPFIA2 gene.

DISCUSSION
In this study of genome wide gene expression in whole blood of heavy cannabis users, we found that CX3CR1 and PPFIA2 expression were significantly different from controls. After validation with qPCR, the upregulated expression of PPFIA2 in heavy cannabis users was verified.
PPFIA2 encodes for liprin-α-2, a member of the liprin-α family. Liprin-α-2 is primarily expressed in hippocampus and cerebellum (Misawa et al., 2001). Spangler et al described that liprin-a2 was localized in mossy fiber endings in the CA3 region of the hippocampus, in synapses, the axon, dendrites and cell body, suggesting a broad array of functions for liprin- α proteins in neuronal cells (Spangler et al., 2011;Spangler and Hoogenraad, 2007). Liprin-α proteins were originally described as binding partners for the common antigen-related (LAR) family of receptor protein tyrosine phosphatases (LAR-RPTPs) (Dunah et al., 2005;Pulido et al., 1995). In interaction with liprins, LAR-RPTPs moderate axon guidance (Choe et al., 2006;Hofmeyer et al., 2006;Prakash et al., 2009) and intracellular cargo transport (Miller et al., 2005). Currently, liprin-α proteins are predominantly investigated for their role key organizers of the presynaptic terminal, involved in organizing pre- and post synaptic vesicle preparation and neurotransmitter function in the synapse active zone (Patel et al., 2006;Ko et al., 2003;Jin and Garner, 2008;Owald et al., 2012;Spangler and Hoogenraad, 2007;Spangler et al., 2011;Spangler and Hoogenraad, 2007).

Since LAR-RPTPs are intensively involved in the biology of memory and learning (Kolkman et al., 2004), the suggested upregulation of PPFIA2 in cannabis users points toward a potential biological pathway underlying the cannabis associated adverse effects on memory function.

As described earlier, cannabis use is associated with impairment of all aspects of short-term, especially episodic and working, memory (Ranganathan and D'Souza, 2006) and long term decline of neuropsychological functioning in all domains (Meier et al., 2012).
A possible biological pathway underlying this association and involving LAR-RPTPs and PPFIA2, is that THC influences LTD (Long-Term-Depression) in the hippocampus. LTD is a neurophysiological process that selectively weakens synaptic transmission and influences hippocampal functioning. LTD is

associated with deleting unnecessary memory traces. Interestingly, the latter function is consistently attributed to the ECS (Di Marzo, 2011;van der Stelt and Di Marzo, 2005), moreover it has been suggested that the ECS plays an important role in LTD (Chavez et al., 2010;Izumi and Zorumski, 2012). Likewise, LAR-RPTPs have a role in the induction of LTD. Several studies found that the induction of mAChR-LTD (muscarinic acetylcholine receptor-dependent Long-Term-Depression) in the hippocampus required the activation of protein tyrosine phosphatase (PTP), and that expression was partly mediated by liprin-α – LAR binding (Dickinson et al., 2009;Wu et al., 2009). As described above, liprin-α-2 is well known for their role as binding partners LAR-RPTPs (Dunah et al., 2005;Pulido et al., 1995). Moreover, accurate binding of liprin-α to LAR-RPTP in rat hippocampal synapses is crucial for the development and maintenance of excitatory synapses (Dunah et al., 2005), and LAR phosphatase deficiency has profound impact on spatial learning (Kolkman et al., 2004).

Cannabis, PPFIA2 and psychosis

Given the alleged role of cannabis in the etiology of schizophrenia, the upregulation of PPFIA2 in cannabis users is noteworthy. Besides its role in hippocampal functioning, liprin-α2 plays a role in postsynaptic targeting of AMPA (*a*-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate) glutamate receptors (Ko et al., 2003). In the context of the glutamate hypothesis of schizophrenia, dysfunctional trafficking of AMPA subtype glutamate receptors has been associated with the development of schizophrenia (Hammond et al., 2011). As THC impacts on liprin-α2 expression, this could potentially imply the AMPA trafficking pathway as an underlying mechanism in the association between THC exposure and psychotic illness. In a recent genome wide association study (GWAS) in multiplex schizophrenia pedigrees, a polymorphism in PPFIA2 (rs12426725) was implicated as one of the SNP's that were associated with schizophrenia with a very low p values (4.2E-06) albeit not genome wide significant (Levinson et al., 2012). We did not find evidence of a genetic contribution (including rs12426725) to the liprin levels in our data.

CX3CR1(Fractalkine receptor)

Our genome wide RNA expression profiling analysis suggested that cannabis use leads to downregulation of CX3CR1 in whole blood. Validation with qPCR did not confirm this finding. However, considering the limited power of our qPCR validation analysis due to loss of 47 subjects, CX3CR1 remains a potential candidate.

This transcript codes for the CX3C chemokine receptor 1, which is a dedicated receptor for the chemokine Fractalkine (CX3L1). Fractaline is primarily expressed in neurons, whereas the fractalkine receptor is expressed on microglia (Harrison et al., 1998). Fractalkine plays a crucial role in the initiation and progression of inflammation, inducing numerous monocyte-mediated proinflammatory signals and chemotaxis of monocytes, T-lymphocytes and microglia (Maciejewski-Lenoir et al., 1999;Hughes et al.,

2002;Meucci et al., 2000;Harrison et al., 1998;Lee et al., 2006). It is reported that augmenting CX3CR1 signaling may protect against microglial neurotoxicity (Cardona et al., 2006). Moreover, fractalkine plays a role in the phosphorylation and activation of Akt (Hughes et al., 2002;Imai et al., 1997;Meucci et al., 2000) and is involved in pruning (Schafer et al., 2012). The biological implications of a possible association between cannabis use and CX3CR1 expression remain unclear. We are currently performing functional experiments on the interaction between THC and PPFIA2 and CX3CR1 regulation, hoping to further unravel the relationship between liprins, fractalkine and the ECS.

Previous studies on THC induced gene-expression

Although not in living cannabis users and not genome wide, a number of studies investigated the effect of THC exposure on gene expression. A series of studies investigated effects of THC on the density of cannabinoid receptors in post mortem brain tissue reporting contradictory findings (Dean et al., 2001;Villares, 2007;Eggan et al., 2008). Moreover, cannabis use is associated with differential gene expression in postmortem anterior prefrontal cortex tissue, particularly in calmodulin-related signaling, Golgi and endoplasmatic reticulum-related transcripts and lipid metabolism (Lehrmann et al., 2006). Besides post-mortem designs, some papers describe the effects of cannabinoids under in vitro conditions in specific cell-lines. The main finding of these studies is that THC contributes to DNA damage, inflammation, alterations in apoptosis regulation (Sarafian et al., 2005) and induces inhibition of cell cycle progression (Galanti et al., 2008).

In our study, we did not find the pathways described above to be differentially regulated. However, post-mortem studies examine specific brain areas and report on local gene expression regulation (accounting for a post-mortem interval) and the current study measured gene expression changes in peripheral blood. A further explanation for these convergent findings is that we did not, a priori, select transcripts, but performed genome wide expression analysis in order to generate new hypotheses on the underlying mechanisms of cannabis use associated adverse effects.

Strengths and limitations

A strongpoint of our study is that we exclusively included heavy cannabis users or cannabis naïve individuals to maximize the potential contrast in gene expression changes. We then intended to match optimally between the two groups for several potentially confounding factors. Further, in our regression analyses we included the matching factors as co-variables to further increase the reliability of our results.

A limiting aspect of our study is that, due to sample depletion, we could only validate our genome wide expression array results only in a limited proportion of participants. The non-replication of the differential expression of CX3CR1 is potentially due to insufficient power. A further limiting factor is the fact that we did not measure white blood cell count (WBC), introducing potential

confounding due to unequal distribution of high or low WBC in either group caused by chance or even related to cannabis use. Further, we did not draw blood from our participants at a standardized time of day, potentially introducing variation due to circadian rhythm of gene expression. Another potential limitation is the fact that the inhalation of a cannabis cigarette does not only comprise inhalation of cannabinoids, but also thousands of organic and inorganic chemical compounds. Approximate matching and statistical adjustment may not entirely adjust for this potential confounding.
Finally, we are not sure to what extent gene expression in whole blood is correlated with gene expression in the central nervous system. Several authors stated that whole genome gene expression measured in peripheral blood, and particularly in lymphocytes, could reflect the metabolism of brain cells, and may be exploited as a neural probe in studies of psychiatric disorders (Gladkevich et al., 2004;Davies et al., 2009). Given the postulated neuroimmunological role and ubiquitous expression patterns of CB1r and CB2r (Sanchez et al., 1998;Nunez et al., 2004;Cabral et al., 2008;Waksman et al., 1999;Walter et al., 2003), it is plausible that measuring cannabis use associated differences in genome wide gene expression patterns could help to further understand the neurobiological mechanisms behind the numerous neuropsychiatric effects associated with cannabis use. Moreover the transcripts implied in this study show documented expression in brain (Spangler et al., 2011).

Conclusion
This is the first study that directly investigates genome wide gene expression patterns in whole blood of heavy cannabis users. After qPCR validation, we found that the liprin alpha-2 gene (PPFIA2) is significantly up-regulated in cannabis users. Modifying Liprin alpha 2 levels is a plausible biological mechanism by wich cannabis could influence memory formation in the human cortex. Moreover liprin alpha-2 expression modification could be part of the biological mechanism underlying cannabis associated psychosis. The current findings could to generate new hypotheses on the etiology of adverse effects associated with cannabis use. Further molecular studies are warrented to follow up this new lead.

Table 2. differentially expressed transcripts

Gene	Transcript	Probe_Id	Log Fold Change	Ave. Expr.	t	p-value	adj. p-value	qPCR Fold Change	qPCR p-value
PPFIA2	ILMN_7280	ILMN_1803318	0.17	790018503033019.000	479.822.509.875.381	3,27E+08	0.04	1.4	0.05
CX3CR1	ILMN_8593	ILMN_1745788	-0.43	923673199374769.000	-477.728.777.731.576	3,59E+08	0.04	1.0	0.50

Reference List

Andreasson,S., Allebeck,P., Engstrom,A. and Rydberg,U., 1987. Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. Lancet. 2, 1483-1486.

Bhattacharyya,S., Atakan,Z., Martin-Santos,R., Crippa,J.A., Kambeitz,J., Prata,D., Williams,S., Brammer,M., Collier,D.A. and McGuire,P.K., 2012. Preliminary report of biological basis of sensitivity to the effects of cannabis on psychosis: AKT1 and DAT1 genotype modulates the effects of delta-9-tetrahydrocannabinol on midbrain and striatal function. Mol Psychiatry.

Bossong,M.G., van Berckel,B.N., Boellaard,R., Zuurman,L., Schuit,R.C., Windhorst,A.D., van Gerven,J.M., Ramsey,N.F., Lammertsma,A.A. and Kahn,R.S., 2008. Delta9-Tetrahydrocannabinol Induces Dopamine Release in the Human Striatum. Neuropsychopharmacology.

Brown,A.J., 2007. Novel cannabinoid receptors. Br J Pharmacol. 152, 567-575.

Cabral,G.A., Raborn,E.S., Griffin,L., Dennis,J. and Marciano-Cabral,F., 2008. CB2 receptors in the brain: role in central immune function. Br J Pharmacol. 153, 240-251.

Cardona,A.E., Pioro,E.P., Sasse,M.E., Kostenko,V., Cardona,S.M., Dijkstra,I.M., Huang,D., Kidd,G., Dombrowski,S., Dutta,R., Lee,J.C., Cook,D.N., Jung,S., Lira,S.A., Littman,D.R. and Ransohoff,R.M., 2006. Control of microglial neurotoxicity by the fractalkine receptor. Nat Neurosci. 9, 917-924.

Chavez,A.E., Chiu,C.Q. and Castillo,P.E., 2010. TRPV1 activation by endogenous anandamide triggers postsynaptic long-term depression in dentate gyrus. Nat Neurosci. 13, 1511-1518.

Choe,K.M., Prakash,S., Bright,A. and Clandinin,T.R., 2006. Liprin-alpha is required for photoreceptor target selection in Drosophila. Proc Natl Acad Sci U S A. 103, 11601-11606.

Corchero,J., Fuentes,J.A. and Manzanares,J., 1997. delta 9-Tetrahydrocannabinol increases proopiomelanocortin gene expression in the arcuate nucleus of the rat hypothalamus. Eur J Pharmacol. 323, 193-195.

Davies,M.N., Lawn,S., Whatley,S., Fernandes,C., Williams,R.W. and Schalkwyk,L.C., 2009. To What Extent is Blood a Reasonable Surrogate for Brain in Gene Expression Studies: Estimation from Mouse Hippocampus and Spleen. Front Neurosci. 3, 54.

Dean,B., Sundram,S., Bradbury,R., Scarr,E. and Copolov,D., 2001. Studies on [3H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. Neuroscience. 103, 9-15.

Degenhardt,L., Chiu,W.T., Sampson,N., Kessler,R.C., Anthony,J.C., Angermeyer,M., Bruffaerts,R., de,G.G., Gureje,O., Huang,Y., Karam,A., Kostyuchenko,S., Lepine,J.P., Mora,M.E., Neumark,Y., Ormel,J.H., Pinto-Meza,A., Posada-Villa,J., Stein,D.J., Takeshima,T. and Wells,J.E., 2008. Toward a global view of alcohol, tobacco, cannabis, and cocaine use: findings from the WHO World Mental Health Surveys. PLoS Med. 5, e141.

Di Marzo,V., 2011. Endocannabinoid signaling in the brain: biosynthetic mechanisms in the limelight. Nat Neurosci. 14, 9-15.

Dickinson,B.A., Jo,J., Seok,H., Son,G.H., Whitcomb,D.J., Davies,C.H., Sheng,M., Collingridge,G.L. and Cho,K., 2009. A novel mechanism of hippocampal LTD involving muscarinic receptor-triggered interactions between AMPARs, GRIP and liprin-alpha. Mol Brain. 2, 18.

Du,P., Kibbe,W.A. and Lin,S.M., 2008. lumi: a pipeline for processing Illumina microarray. Bioinformatics. 24, 1547-1548.

Dunah,A.W., Hueske,E., Wyszynski,M., Hoogenraad,C.C., Jaworski,J., Pak,D.T., Simonetta,A., Liu,G. and Sheng,M., 2005. LAR receptor protein tyrosine phosphatases in the development and maintenance of excitatory synapses. Nat Neurosci. 8, 458-467.

Eggan,S.M., Hashimoto,T. and Lewis,D.A., 2008. Reduced cortical cannabinoid 1 receptor messenger RNA and protein expression in schizophrenia. Arch Gen Psychiatry. 65, 772-784.

Freund,T.F., Katona,I. and Piomelli,D., 2003. Role of endogenous cannabinoids in synaptic signaling. Physiol Rev. 83, 1017-1066.

Galanti,G., Fisher,T., Kventsel,I., Shoham,J., Gallily,R., Mechoulam,R., Lavie,G., Amariglio,N., Rechavi,G. and Toren,A., 2008. Delta 9-tetrahydrocannabinol inhibits cell cycle progression by downregulation of E2F1 in human glioblastoma multiforme cells. Acta Oncol. 47, 1062-1070.

Gillespie,N.A., Neale,M.C. and Kendler,K.S., 2009. Pathways to cannabis abuse: a multi-stage model from cannabis availability, cannabis initiation and progression to abuse. Addiction. 104, 430-438.

Gladkevich,A., Kauffman,H.F. and Korf,J., 2004. Lymphocytes as a neural probe: potential for studying psychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry. 28, 559-576.

Hall,W. and Solowij,N., 1998. Adverse effects of cannabis. Lancet. 352, 1611-1616.

Hammond,J.C., McCullumsmith,R.E., Haroutunian,V. and Meador-Woodruff,J.H., 2011. Endosomal trafficking of AMPA receptors in frontal cortex of elderly patients with schizophrenia. Schizophr Res. 130, 260-265.

Harrison,J.K., Jiang,Y., Chen,S., Xia,Y., Maciejewski,D., McNamara,R.K., Streit,W.J., Salafranca,M.N., Adhikari,S., Thompson,D.A., Botti,P., Bacon,K.B. and Feng,L., 1998. Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. Proc Natl Acad Sci U S A. 95, 10896-10901.

Hofmeyer,K., Maurel-Zaffran,C., Sink,H. and Treisman,J.E., 2006. Liprin-alpha has LAR-independent functions in R7 photoreceptor axon targeting. Proc Natl Acad Sci U S A. 103, 11595-11600.

Howlett,A.C., 2002. The cannabinoid receptors. Prostaglandins Other Lipid Mediat. 68-69, 619-631.

Hughes,P.M., Botham,M.S., Frentzel,S., Mir,A. and Perry,V.H., 2002. Expression of fractalkine (CX3CL1) and its receptor, CX3CR1, during acute and chronic inflammation in the rodent CNS. Glia. 37, 314-327.

Imai,T., Hieshima,K., Haskell,C., Baba,M., Nagira,M., Nishimura,M., Kakizaki,M., Takagi,S., Nomiyama,H., Schall,T.J. and Yoshie,O., 1997. Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. Cell. 91, 521-530.

Izumi,Y. and Zorumski,C.F., 2012. NMDA receptors, mGluR5, and endocannabinoids are involved in a cascade leading to hippocampal long-term depression. Neuropsychopharmacology. 37, 609-617.

Jan,T.R., Farraj,A.K., Harkema,J.R. and Kaminski,N.E., 2003. Attenuation of the ovalbumin-induced allergic airway response by cannabinoid treatment in A/J mice. Toxicol Appl Pharmacol. 188, 24-35.

Jin,Y. and Garner,C.C., 2008. Molecular mechanisms of presynaptic differentiation. Annu Rev Cell Dev Biol. 24, 237-262.

Ko,J., Kim,S., Valtschanoff,J.G., Shin,H., Lee,J.R., Sheng,M., Premont,R.T., Weinberg,R.J. and Kim,E., 2003. Interaction between liprin-alpha and GIT1 is required for AMPA receptor targeting. J Neurosci. 23, 1667-1677.

Kolkman,M.J., Streijger,F., Linkels,M., Bloemen,M., Heeren,D.J., Hendriks,W.J. and Van der Zee,C.E., 2004. Mice lacking leukocyte common antigen-related (LAR) protein tyrosine phosphatase domains demonstrate spatial learning impairment in the two-trial water maze and hyperactivity in multiple behavioural tests. Behav Brain Res. 154, 171-182.

Konings,M., Bak,M., Hanssen,M., van,O.J. and Krabbendam,L., 2006. Validity and reliability of the CAPE: a self-report instrument for the measurement of psychotic experiences in the general population. Acta Psychiatr Scand. 114, 55-61.

Lee,S.J., Namkoong,S., Kim,Y.M., Kim,C.K., Lee,H., Ha,K.S., Chung,H.T., Kwon,Y.G. and Kim,Y.M., 2006. Fractalkine stimulates angiogenesis by activating the Raf-1/MEK/ERK- and PI3K/Akt/eNOS-dependent signal pathways. Am J Physiol Heart Circ Physiol. 291, H2836-H2846.

Lehrmann,E., Colantuoni,C., ep-Soboslay,A., Becker,K.G., Lowe,R., Huestis,M.A., Hyde,T.M., Kleinman,J.E. and Freed,W.J., 2006. Transcriptional changes common to human cocaine, cannabis and phencyclidine abuse. PLoS ONE. 1, e114.

Levinson,D.F., Shi,J., Wang,K., Oh,S., Riley,B., Pulver,A.E., Wildenauer,D.B., Laurent,C., Mowry,B.J., Gejman,P.V., Owen,M.J., Kendler,K.S., Nestadt,G., Schwab,S.G., Mallet,J., Nertney,D., Sanders,A.R., Williams,N.M., Wormley,B., Lasseter,V.K., Albus,M., Godard-Bauche,S., Alexander,M., Duan,J., O'Donovan,M.C., Walsh,D., O'Neill,A., Papadimitriou,G.N., Dikeos,D., Maier,W., Lerer,B., Campion,D., Cohen,D., Jay,M., Fanous,A., Eichhammer,P., Silverman,J.M., Norton,N., Zhang,N., Hakonarson,H., Gao,C., Citri,A., Hansen,M., Ripke,S., Dudbridge,F. and Holmans,P.A., 2012. Genome-Wide Association Study of Multiplex Schizophrenia Pedigrees. Am J Psychiatry. 169, 963-973.

Lockhart,D.J. and Winzeler,E.A., 2000. Genomics, gene expression and DNA arrays. Nature. 405, 827-836.

Maciejewski-Lenoir,D., Chen,S., Feng,L., Maki,R. and Bacon,K.B., 1999. Characterization of fractalkine in rat brain cells: migratory and activation signals for CX3CR-1-expressing microglia. J Immunol. 163, 1628-1635.

Macleod,J., Oakes,R., Copello,A., Crome,I., Egger,M., Hickman,M., Oppenkowski,T., Stokes-Lampard,H. and Davey,S.G., 2004. Psychological and social sequelae of cannabis and other illicit drug use by young people: a systematic review of longitudinal, general population studies. Lancet. 363, 1579-1588.

Marco,E.M., Garcia-Gutierrez,M.S., Bermudez-Silva,F.J., Moreira,F.A., Guimaraes,F., Manzanares,J. and Viveros,M.P., 2011. Endocannabinoid system and psychiatry: in search of a neurobiological basis for detrimental and potential therapeutic effects. Front Behav Neurosci. 5, 63.

Mechoulam,R., Peters,M., Murillo-Rodriguez,E. and Hanus,L.O., 2007. Cannabidiol--recent advances. Chem Biodivers. 4, 1678-1692.

Meier,M.H., Caspi,A., Ambler,A., Harrington,H., Houts,R., Keefe,R.S., McDonald,K., Ward,A., Poulton,R. and Moffitt,T.E., 2012. Persistent cannabis users show neuropsychological decline from childhood to midlife. Proc Natl Acad Sci U S A.

Meucci,O., Fatatis,A., Simen,A.A. and Miller,R.J., 2000. Expression of CX3CR1 chemokine receptors on neurons and their role in neuronal survival. Proc Natl Acad Sci U S A. 97, 8075-8080.

Miller,A.M. and Stella,N., 2008. CB2 receptor-mediated migration of immune cells: it can go either way. Br J Pharmacol. 153, 299-308.

Miller,K.E., DeProto,J., Kaufmann,N., Patel,B.N., Duckworth,A. and Van,V.D., 2005. Direct observation demonstrates that Liprin-alpha is required for trafficking of synaptic vesicles. Curr Biol. 15, 684-689.

Misawa,H., Kawasaki,Y., Mellor,J., Sweeney,N., Jo,K., Nicoll,R.A. and Bredt,D.S., 2001. Contrasting localizations of MALS/LIN-7 PDZ proteins in brain and molecular compensation in knockout mice. J Biol Chem. 276, 9264-9272.

Moore,T.H., Zammit,S., Lingford-Hughes,A., Barnes,T.R., Jones,P.B., Burke,M. and Lewis,G., 2007. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet. 370, 319-328.

Nunez,E., Benito,C., Pazos,M.R., Barbachano,A., Fajardo,O., Gonzalez,S., Tolon,R.M. and Romero,J., 2004. Cannabinoid CB2 receptors are expressed by perivascular microglial cells in the human brain: an immunohistochemical study. Synapse. 53, 208-213.

Owald,D., Khorramshahi,O., Gupta,V.K., Banovic,D., Depner,H., Fouquet,W., Wichmann,C., Mertel,S., Eimer,S., Reynolds,E., Holt,M., Aberle,H. and Sigrist,S.J., 2012. Cooperation of Syd-1 with Neurexin synchronizes pre- with postsynaptic assembly. Nat Neurosci. 15, 1219-1226.

Parmentier-Batteur,S., Jin,K., Xie,L., Mao,X.O. and Greenberg,D.A., 2002. DNA microarray analysis of cannabinoid signaling in mouse brain in vivo. Mol Pharmacol. 62, 828-835.

Patel,M.R., Lehrman,E.K., Poon,V.Y., Crump,J.G., Zhen,M., Bargmann,C.I. and Shen,K., 2006. Hierarchical assembly of presynaptic components in defined C. elegans synapses. Nat Neurosci. 9, 1488-1498.

Pedersen,W., 2008. Does cannabis use lead to depression and suicidal behaviours? A population-based longitudinal study. Acta Psychiatr Scand. 118, 395-403.

Pertwee,R.G., 2008. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. Br J Pharmacol. 153, 199-215.

Prakash,S., McLendon,H.M., Dubreuil,C.I., Ghose,A., Hwa,J., Dennehy,K.A., Tomalty,K.M., Clark,K.L., Van,V.D. and Clandinin,T.R., 2009. Complex interactions amongst N-cadherin, DLAR, and Liprin-alpha regulate Drosophila photoreceptor axon targeting. Dev Biol. 336, 10-19.

Pulido,R., Serra-Pages,C., Tang,M. and Streuli,M., 1995. The LAR/PTP delta/PTP sigma subfamily of transmembrane protein-tyrosine-phosphatases: multiple human LAR, PTP delta, and PTP sigma isoforms are expressed in a tissue-specific manner and associate with the LAR-interacting protein LIP.1. Proc Natl Acad Sci U S A. 92, 11686-11690.

Purcell,S., Neale,B., Todd-Brown,K., Thomas,L., Ferreira,M.A., Bender,D., Maller,J., Sklar,P., de Bakker,P.I., Daly,M.J. and Sham,P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 81, 559-575.

Ranganathan,M. and D'Souza,D.C., 2006. The acute effects of cannabinoids on memory in humans: a review. Psychopharmacology (Berl). 188, 425-444.

Samson,M.T., Small-Howard,A., Shimoda,L.M., Koblan-Huberson,M., Stokes,A.J. and Turner,H., 2003. Differential roles of CB1 and CB2 cannabinoid receptors in mast cells. J Immunol. 170, 4953-4962.

Sanchez,C., Galve-Roperh,I., Canova,C., Brachet,P. and Guzman,M., 1998. Delta9-tetrahydrocannabinol induces apoptosis in C6 glioma cells. FEBS Lett. 436, 6-10.

Sarafian,T., Habib,N., Mao,J.T., Tsu,I.H., Yamamoto,M.L., Hsu,E., Tashkin,D.P. and Roth,M.D., 2005. Gene expression changes in human small airway epithelial cells exposed to Delta9-tetrahydrocannabinol. Toxicol Lett. 158, 95-107.

Satyanarayana,V., 2009. Mental health consequences of cannabis use. Acta Psychiatr Scand. 119, 411-412.

Schafer,D.P., Lehrman,E.K., Kautzman,A.G., Koyama,R., Mardinly,A.R., Yamasaki,R., Ransohoff,R.M., Greenberg,M.E., Barres,B.A. and Stevens,B., 2012. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron. 74, 691-705.

Schroeder,A., Mueller,O., Stocker,S., Salowsky,R., Leiber,M., Gassmann,M., Lightfoot,S., Menzel,W., Granzow,M. and Ragg,T., 2006. The RIN: an RNA integrity number for assigning integrity values to RNA measurements. BMC Mol Biol. 7, 3.

Schubart,C.D., Boks,M.P., Breetvelt,E.J., van Gastel,W.A., Groenwold,R.H., Ophoff,R.A., Sommer,I.E. and Kahn,R.S., 2011. Association between cannabis and psychiatric hospitalization. Acta Psychiatr Scand. 123, 368-375.

Schubart,C.D., van Gastel,W.A., Breetvelt,E.J., Beetz,S.L., Ophoff,R.A., Sommer,I.E., Kahn,R.S. and Boks,M.P., 2010. Cannabis use at a young age is associated with psychotic experiences. Psychol Med. 1-10.

Skinner,R., Conlon,L., Gibbons,D. and McDonald,C., 2010. Cannabis use and non-clinical dimensions of psychosis in university students presenting to primary care. Acta Psychiatr Scand.

Smith,G.K., 2005. Limma: linear models for microarray data. . In: Gentleman R., Carey V., Dudoit S., Irizarry R. and Huber W. (Eds.), Bioinformatics and Computational Biology Solutions using R and Bioconductor., Springer, New Yotk, pp. 397-420.

Spangler,S.A. and Hoogenraad,C.C., 2007. Liprin-alpha proteins: scaffold molecules for synapse maturation. Biochem Soc Trans. 35, 1278-1282.

Spangler,S.A., Jaarsma,D., De,G.E., Wulf,P.S., Akhmanova,A. and Hoogenraad,C.C., 2011. Differential expression of liprin-alpha family proteins in the brain suggests functional diversification. J Comp Neurol. 519, 3040-3060.

Spitzer,R.L., Williams,J.B., Gibbon,M. and First,M.B., 1992. The Structured Clinical Interview for DSM-III-R (SCID). I: History, rationale, and description. Arch Gen Psychiatry. 49, 624-629.

Starowicz,K., Cristino,L. and Di,M., V, 2008. TRPV1 receptors in the central nervous system: potential for previously unforeseen therapeutic applications. Curr Pharm Des. 14, 42-54.

Stella,N., 2009. Endocannabinoid signaling in microglial cells. Neuropharmacology. 56 Suppl 1, 244-253.

Stella,N., 2010. Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. Glia. 58, 1017-1030.

Tanasescu,R. and Constantinescu,C.S., 2010. Cannabinoids and the immune system: an overview. Immunobiology. 215, 588-597.

Taylor,D.R., Fergusson,D.M., Milne,B.J., Horwood,L.J., Moffitt,T.E., Sears,M.R. and Poulton,R., 2002. A longitudinal study of the effects of tobacco and cannabis exposure on lung function in young adults. Addiction. 97, 1055-1061.

Trimbos,T.N.I.o.M.H.a.A. THC-concentraties in wiet, nederwiet en hasj in Nederlands coffeeshops (2008-2009). 1-1-2009.

Trimbos-instituut,U. THC-concentraties in wiet, nederwiet en hasj in Nederlands coffeeshops (2007-2008). Netherlands Institute of Mental Health and Addiction. 2009.

United Nations Office on Drugs and Crime. World Drug Report 2012. 2012. Vienna, United Nations Publications.

van der Stelt,M. and Di Marzo,V., 2005. Cannabinoid receptors and their role in neuroprotection. Neuromolecular Med. 7, 37-50.

Villares,J., 2007. Chronic use of marijuana decreases cannabinoid receptor binding and mRNA expression in the human brain. Neuroscience. 145, 323-334.

Waksman,Y., Olson,J.M., Carlisle,S.J. and Cabral,G.A., 1999. The central cannabinoid receptor (CB1) mediates inhibition of nitric oxide production by rat microglial cells. J Pharmacol Exp Ther. 288, 1357-1366.

Walter,L., Franklin,A., Witting,A., Wade,C., Xie,Y., Kunos,G., Mackie,K. and Stella,N., 2003. Nonpsychotropic cannabinoid receptors regulate microglial cell migration. J Neurosci. 23, 1398-1405.

Wu,L.J., Wang,Y.T. and Zhuo,M., 2009. Hook-up of GluA2, GRIP and liprin-alpha for cholinergic muscarinic receptor-dependent LTD in the hippocampus. Mol Brain. 2, 17.

Yuan,M., Kiertscher,S.M., Cheng,Q., Zoumalan,R., Tashkin,D.P. and Roth,M.D., 2002. Delta 9-Tetrahydrocannabinol regulates Th1/Th2 cytokine balance in activated human T cells. J Neuroimmunol. 133, 124-131.

PART III

SUMMARY AND DISCUSSION

PART III: SUMMARY AND DISCUSSION

CHAPTER VIII

• SUMMARY AND GENERAL DISCUSSION •

Summary

In **part I** of this thesis, epidemiological determinants that influence the association between cannabis use and the development of psychosis are investigated.

Chapter 1 provides a general introduction to this thesis.

Chapter 2 is a study that focuses on the general mental health burden of cannabis use. The objective of this study was to investigate the relationship between cannabis use and the risk of psychiatric hospitalization, regardless of diagnosis. In a cross-sectional analysis in a sample of 17,698 individuals with a mean age of 22 years, we found a dose-response relationship between the amount of cannabis use and the odds for psychiatric hospitalization. The chance of ever having had a psychiatric hospitalization increased sixfold in heavy users compared to cannabis naïve participants. Exposure to cannabis before the age of 12 years was found to carry a 4.8 times increased odds for past psychiatric hospitalizations. We conclude that, although the underlying causal pathway remains unclear, both early and heavy use of cannabis are each and independently associated with poor mental health, and thus with significant medical and economic costs for society.

Chapter 3 describes an epidemiological analysis in 17,698 participants on whether cannabis starting age and level of exposure are associated with specific profiles of psychiatric symptoms. We found that a young onset age of cannabis use was strongly associated with subclinical psychotic symptoms and to a lesser degree with negative symptoms, while smoking high amounts of cannabis was associated with increased levels of all three symptom dimensions: psychotic, negative and depressive. These results support the hypothesis that the impact of cannabis use is age-specific. Despite the fact that the informational value of the current dataset is limited by the retrospective and cross-sectional design precluding any inference on causality, this study shows that heavy current cannabis use is associated with a different symptom profile than early cannabis use. This finding converges with epidemiological and animal studies and supports the hypothesis that there is a window of increased vulnerability of the maturing brain to the effects of exo-cannabinoids such as Δ^9-tetrahydrocannabinol (THC), during early puberty.

Chapter 4 and 5 describe a further epidemiological factor that modulates the association between cannabis use and psychotic symptoms: the type of cannabis. As different types of cannabis contain different cannabinoids, it is plausible that they exert different impact on the risk of developing psychopathology. One of the cannabinoids that is of interest is cannabidiol (CBD).

Chapter 4 is a review of the literature on potential antipsychotic properties of CBD. First a brief overview is given of the endocannabinoid system (ECS) and a concise description of the role of the ECS in the neuropathology of psychotic disorders. Second, this review provides an overview of currently available animal, human experimental, imaging, epidemiological and finally clinical studies that investigated the antipsychotic properties of CBD. We performed a search for English articles using Medline and EMBASE. The results suggest that CBD impacts differently on the endocannabinoid system than THC. Evidence from several study domains suggests that CBD shows potential for antipsychotic treatment. Experimental animal and human studies show that CBD has an attenuating role in various animal psychosis models and in a number of studies has a similar pharmacological profile to atypical antipsychotics. The first clinical trials by Leweke and colleagues (Leweke et al., 2012b) are promising and underscore the need of large randomized, double blind, controlled trials comparing the effect of CBD to atypical antipsychotics. Given the high tolerability and superior cost-effectiveness, CBD may possibly be a highly attractive alternative to current antipsychotic treatment.

Chapter 5 aims to investigate the role of CBD content in the association between cannabis use and psychiatric symptoms in a large non-clinical population of cannabis users. In a web-based cross-sectional study in 1877 adolescents we obtained detailed information about cannabis use and subclinical psychotic experiences (CAPE). Different types of cannabis (i.e. marijuana, hashish etc) contain distinctive proportions of THC and CBD. Since average concentrations of THC and CBD in the most popular types of cannabis sold on the Dutch market are annually measured, we were able to quantify exposure to THC and CBD. We found a significant inverse relationship between CBD content and self-reported positive symptoms, but not with negative symptoms or depression. The estimated effect size of CBD content was small. Although the observed effects are subtle, the use of high CBD content cannabis was associated with significantly lower degrees of psychotic symptoms, providing further support for the antipsychotic potential of CBD. To our knowledge this is the first large study to investigate the influence of CBD content on the presence of psychiatric symptoms in cannabis users from the general population. Given the low dose of CBD in cannabis cigarette (“joint”) as compared to oral administration in treatment studies, a larger effect could not be expected.
As our data suggest that cannabis types with high CBD content are significantly less strongly associated with psychotic symptoms, the current data add to our knowledge of CBD as a potential antipsychotic agent.

In **part II** two studies are described that intend to unravel the underlying neurobiological mechanisms of cannabis use associated psychotic symptoms using a genetics approach.

Chapter 6 describes a Genome-Wide Environment Interaction Study (GWEIS) in a sample enriched with participants that were selected by sampling for extreme or absent cannabis exposure and extreme high or low scores of self-reported psychotic symptoms (in total 1261 subjects).
We describe that a variant in the P2X7 receptor gene (rs7958311) was, although not with genome-wide significance, associated with an increased risk for experiencing psychotic symptoms in heavy cannabis users. Since the P2X7 receptor closely interacts with the endocannabinoid system in orchestrating microglia neuroinflammation response, the P2RX7 gene is a viable candidate to partially explain the biological interaction between cannabis exposure and genetic vulnerability in the development of psychotic symptoms. Moreover, the current study is a proof of concept that combining extreme phenotypic and environmental sampling strongly increase power to detect Gene Environment Interaction. Potential hypotheses will be presented in the general discussion of this thesis.

Chapter 7 describes a study on gene expression changes that are associated with cannabis use. After a rigorous selection and matching procedure, we included 190 participants. Expression levels were analyzed for association with cannabis use status. Cigarette smoking, the use of other drugs than cannabis, age and gender were used as co-variants. Moreover, we corrected for multiple testing (FDR).The genome-wide significant findings were validated using qRT-PCR. The expression levels of two transcripts (CX3CR1 and PPFIA2) were significantly associated with cannabis use after correction for multiple testing. The up-regulation of PPFIA2 was validated by quantitative PCR. Changes in liprin-alpha 2 levels point towards a plausible mechanism by which cannabis could influence memory formation and neuronal spouting in the human cortex. The finding that PPFIA2 and CX3CR1 are differentially expressed in cannabis users could help us to generate new hypotheses on the etiology of adverse effects associated with cannabis use.

General Discussion

1. On causality

Although empirical research utterly relies on the notion of causality, the formulation of criteria for causality is not straightforward. The famous British epidemiologist Bradford Hill intended to formulate such criteria (HILL, 1965) which have been used but also passionately disputed in the history of science. He established the following list:

> *Strength of association (relative risk, odds ratio), Consistency, Specificity, Temporal relationship (temporality), Biological gradient (dose-response relationship), Plausibility (biological plausibility), Coherence, Experiment (reversibility).*

Several of these criteria concerning the link between cannabis use and psychosis have been described in this thesis; strength of association (chapter 3), dose-response relationship (chapter 3), biological plausibility (chapter 6 and 7). Others such as experiment (Bhattacharyya et al., 2012a) and consistency (Moore et al., 2007) have been suggested elsewhere. However, the notion that cannabis is indeed a causal agent of schizophrenia is still heavily debated (DeLisi, 2008;Hickman et al., 2007;Macleod, 2007;Macleod et al., 2004). Although several prospective studies and meta-analyses of these studies (Moore et al., 2007) suggest cannabis to be a risk factor, conflicting data have been reported (Minozzi et al., 2010;Faber et al., 2012). The association between cannabis use and psychosis could therefore also be the result of self-medication behavior of subjects experiencing psychotic-like or prodromal symptoms. A plausible alternative explanation of apparently conflicting findings is that there is a causal bi-directional association between psychosis and cannabis use. A recent large epidemiological study investigating the temporal dynamics of the link between cannabis and psychosis tested and corroborated this hypothesis (Griffith-Lendering et al., 2012).

Finally, as eloquently formulated by Cecile Henquet and Jim van Os, it is the level of coherence between observational, biological and experimental studies on that will finally inform the community about the validity and causality of any association. (Henquet and van, 2008).

One could conclude that causality is a fuzzy concept.

2. Window of vulnerability

One of the hypotheses that is corroborated in this thesis, is the notion of increased vulnerability to THC during critical phases of brain maturation. We found that cannabis use in early puberty

is specifically associated with (positive)psychotic symptoms in contrast to a broader range of psychiatric symptoms that is dose dependently associated with cannabis use in general.

THC impacts strongly on the functioning of the endocannabinoid system. This system plays an important role in brain organization during prenatal development and early puberty (Chevaleyre et al., 2006). Experimental studies of the endocannabinoid system show that exposure to high levels of exo-cannabinoids, such as THC, can disrupt neuronal signalling and might interfere

with the activity of the endocannabinoid system during stages of high neuronal plasticity (Trezza et al., 2008;Lewis, 1997). In animal models, exposure to cannabinoids during critical periods of brain maturation has a profound influence on the development of the GABA-ergic (Garcia-Gil et al., 1999), glutamatergic (Suarez et al., 2004), serotonergic (Molina-Holgado et al., 1997) and catecholaminergic system (Fernandez-Ruiz et al., 2000;Garcia-Gil et al., 1997;Hernandez et al., 2000). In agreement with such an impact of THC exposure early in life on the development of neurotransmitter systems, a number of papers report a dramatic effect of THC exposure in early puberty on various cognitive measures in animals (Schneider and Koch, 2003;Cha et al., 2006;O'Shea et al., 2004;Quinn et al., 2008).
As described in chapter 3, such a window of vulnerability in early puberty is also supported by a recent cohort study that showed that early cannabis use is a risk-modifying factor for psychosis-related outcomes in young adults (McGrath et al., 2010). In chapter 2 and chapter 3 we describe that young age of first cannabis use strengthens the association with several measures of psychiatric vulnerability. This finding converges with epidemiological and animal studies and supports the hypothesis that there is a window of increased vulnerability of the maturing brain to the effects of exo-cannabinoids such as THC during early puberty. Given the developmental nature of psychotic disorders (van Os and Kapur, 2009), the finding that cannabis use, as a risk factor, exerts maximal influence during early puberty creates a unique opportunity for prevention of psychotic disorders.

3. Hypotheses on how the P2X7 receptor interacts with THC in causing psychosis vulnerability
Although discussed in chapter 6, in the following paragraph I would like to elaborate further on potential explanations of the involvement of P2RX7 in the cannabis-associated risk of schizophrenia.

As described previously, G x E interaction between cannabis and psychosis has been studied previously in family studies (van Winkel, 2011;Boydell et al., 2006;McGuire et al., 1995) and candidate gene studies, generating promising but also contradicting results involving AKT-1 and COMT (Caspi et al., 2005;Henquet et al., 2009;Di Forti et al., 2012;Zammit et al., 2007;van Winkel et al., 2011). P2RX7 has not been described in this context before.
However, the P2X7 receptor is closely involved in endocannabinoid system functioning.
The P2RX7 gene is located on Chromosome 12 at 12q24.31 and encodes for the P2X7 receptor. The P2X7 receptor is a two-transmembrane ionotropic receptor activated by ATP with high Ca^{2+} permeability and upon adequate stimulation (Li et al., 2005). This receptor is present in all tissues, but is primarily expressed in immune cells, liver and whole brain, predominantly in microglia (BioGPS gene expression database). In the central nervous system P2X7 is also expressed on astrocytes and neurons (Sperlagh et al., 2006;Wiley et al., 2011). Many different effects have been attributed to activation of P2X7 by ATP such

as secretion of TNF-a, IL-1ß and IL-18, nitric-oxide release and secretion of caspase-1, for review see Wiley 2011 (Wiley et al., 2011). Besides regulation of inflammatory reactions, the P2X7 receptor is also involved in regulation of neurotransmitter release, mainly GABA and glutamate (Sperlagh et al., 2006). The relationship between P2X7 and the endocannabinoid system revolves around the finding that P2X7 plays a key role in the regulation of endocannabinoid production in microglia and astrocytes. Walter et al. described that ATP strongly increases the production of the endocannabinoid 2-Arachidonoylglycerol (2-AG) by astrocytes by activation of the P2X7 receptor. The authors propose that 2-AG may be involved in restraining the propagation of harmful neuroinflammation (Walter et al., 2004). Similarly, Witting et al. show that activation of P2X7-receptors increases 2-AG production in microglial cells. It is thought that this accumulation of 2-AG leads to an anti-inflammatory (M2) phenotype (Stella, 2010). Activation of CB2 receptors by 2-AG, for example, decreases cytotoxic agent release (Klegeris et al., 2003). On CB1 receptors, 2-AG activation leads to a decrease of glutamate release, less exitotoxicity (Marsicano et al., 2003;Shen and Thayer, 1998;Sullivan, 1999) and a decrease in cerebral blood flow, resulting in less cerebral edema (Stella, 2010). Overall, these are all anti-inflammatory effects of increased availability of 2-AG following P2X7-activation.
Simultaneously, P2X7-stimulation on itself also leads to neuroinflammation. Stimulation of P2X7-receptors on microglia leads to maturation, migration and production of cytokines (IL-1beta/IL-18) (Stella, 2010), for review see Wiley 2011 (Wiley et al., 2011).

Hypotheses
P2X7 is a receptor with many functions that is involved in neuroinflammation, neuroprotection, but also neurotransmission. The biological effect of the proposed genetic variant (rs 7958311) is unknown. It could have no effect or could imply a gain or loss in each of its functions. Since it is unclear what the consequences are of the identified polymorphism at the molecular level, hypothesizing about the mechanism behind the discovered G x E interaction remains highly speculative.

1) Neuroprotection
The first hypothesis appreciates the neuroprotective role of the endocannabinoid system. We postulate that P2X7-dysfunction and continuous THC-exposure both compromise the endocannabinoid system functioning and thereby increase psychosis vulnerability. A series of studies exploring the role of endogenous cannabinoids in the neurobiology of schizophrenia revealed that the levels of endocannabinoids (as anandamide or 2-AG) are markedly increased in the cerebrospinal fluid (CSF) and peripheral blood of schizophrenia patients compared to healthy controls. Although this association might not be causative, the idea is strengthened by the fact that such increase of the endocannabinoid anandamide is reversed by antipsychotic therapy (Giuffrida et al., 2004;Leweke et al., 1999;Leweke et al., 2012b;Leweke et al., 2007a;De

Marchi et al., 2003). It is suggested that the rise in anandamide could represent an inhibitory feedback reaction to over-activation of dopamine D2 receptors. Furthermore, Leweke and colleagues found that schizophrenia patients who regularly use cannabis have significantly lower anandamide levels than schizophrenia patients not using cannabis. These findings lead to the model that cannabis use causes downregulation of anandamide signaling in schizophrenia patients, and that this mechanism may in turn facilitate psychosis (Giuffrida et al., 2004;Leweke et al., 2007a;Leweke et al., 2012b). Assuming that the identified SNP (rs7958311) causes a gain or loss of function in the previously described function of P2X7 in production of 2-AG in microglia and astrocytes (Walter et al., 2004;Witting et al., 2004), it is possible that the neuroprotective function of the endocannabinoid system is compromised by THC exposure and simultaneously by differential regulation of 2-AG production caused by a hypofunctional P2X7-receptor. A possible mechanism could be that the increased availability of 2-AG impacts on the sensitivity of the ECS in terms of CB1r and CB2r expression.

2) Neuroinflammation

A second hypothesis is that the interaction between THC and altered P2X7 in increasing psychosis vulnerability revolves around neuroinflammation. Recent meta-analyses of genome-wide association studies of schizophrenia suggest that the pathophysiology of schizophrenia is at least partly immunological (Ripke et al., 2011;Steinberg et al., 2011). Mounting evidence from a large variety of neurobiological research methodologies corroborate the notion of neuroinflammation as a central aspect of the etiology of schizophrenia, for a recent and detailed review see Monji et al (Monji et al., 2011).
As described above, P2X7 is involved in 2-AG production. This endocannabinoid has an important role as activator of CB1 and CB2 receptors, that are part of the cascade which induces the M2 anti-inflammatory phenotype (Stella, 2010). Alteration of 2-AG levels most probably has implications for the orchestration of an immunological response to pathogens or tissue damage of any kind. Moreover, as described in detail above, P2X7 has a role as an activator of pro-inflammatory (M1 phenotype) mechanisms (Wiley et al., 2011). It is therefore possible that variation in P2X7 function impacts on its role in neuroinflammation. THC on the other hand, also has profound impact on several aspects of immunological functioning (Cabral and Staab, 2005;Stella, 2010). The severely increased availability of THC in brains of heavy cannabis users causes downregulation of the endocannabinoid system receptors CB1r and CB2r (Stella, 2009;Stella, 2010). As a consequence, regulation of neuroinflammation could be altered and a hypofunctional P2X7 receptor would then not be capable of producing adequate levels of 2-AG to regulate neuroinflammation. In this model, THC exposure and inadequate P2X7 function interact in causing suboptimal orchestration of neuroinflamatory responses, leading to an increased susceptibility for psychotic disorders.

We are currently conducting additional functional experiments to test the hypotheses postulated above.

4. Some methodological remarks

4.1 CAPE as a model for schizophrenia

In this thesis, one of the main outcome measures are scores on self-reported subclinical psychiatric symptoms measured by the Community Assessment of Psychic Experiences (CAPE) questionnaire. The CAPE measures symptoms in three dimensions; positive symptoms (psychosis), negative symptoms (deficits in emotional response and cognition) and depression symptoms. In our studies, high scores on the CAPE serve as a model for schizophrenia vulnerability, as suggested in several studies (Konings et al., 2006;Lataster et al., 2009;Stefanis et al., 2002;van Os et al., 1999;Verdoux and van, 2002). Several authors show that a high score on self-reported psychotic symptoms predicts an increased risk (odds ratio up to 10) of a psychotic disorder later in life (Wiles et al., 2006;Hanssen et al., 2005;Poulton et al., 2000;Chapman et al., 1994;Yung et al., 2009). While still contributing to our understanding of cannabis use on psychosis in general, this approach has a number of important advantages. Sampling from the population (in contrast to patient-based studies) overcomes the limitations of current categorical diagnosis of schizophrenia. Confounding factors as social and cognitive deterioration, duration of illness, treatment and severity of illness are bypassed. However, although individuals with psychiatric problems are overrepresented in the subsamples with high CAPE-scores (see chapter 6, table 4), it is an outcome-measure based on self-report and the majority of participants who have high scores on the CAPE-questionnaire have not and will never develop a psychotic disorder. When considering the results presented in this thesis, the reader must realize that a high score on the CAPE-questionnaire is merely a model for schizophrenia.

4.2. Web-based data

We collected information on cannabis use and symptom scores in almost 26,000 individuals (mean age 21.6 years) recruited via the internet (www.cannabisquest.nl). Participants answered questions regarding their cannabis use, filled out the CAPE-questionnaire, answered questions on their psychiatric history and provided their age, educational level and contact details. With these data we performed the analyses described in part 1 of this thesis. Moreover, these data served in screening for subjects that match inclusion criteria for our genetics studies described in part two of this thesis. As described in chapter 2, the increased availability of internet access and the development of better web-based tools have improved the possibilities to acquire information on psychiatric symptoms via the internet. They are currently considered a valid additional method in epidemiological research (Meyerson and Tryon, 2003;Balter et al., 2005;Gosling et al., 2004;Ekman et al., 2006). Over the last years, numerous internet-based assessments have been validated that measure a variety of psychiatric phenotypes ranging from cannabis abuse to depression (Houston et al., 2001;Vallejo et al., 2007;Khazaal

et al., 2008;Graham et al., 2006;Graham and Papandonatos, 2008;Coles et al., 2007;Lin et al., 2007;Donker et al., 2009;Cuijpers et al., 2008;Spek et al., 2008). On a more critical note, the use of web-based assessments could potentially have lead to instrument inaccuracy or to information bias. However, as shown in our sensitivity analyses in chapters 2 and 3, this does not seem to affect the quality of our data.
We have tested measurement invariance of the online version of the CAPE-questionnaire in a sample of 23,254 participants. Our results show that for research purposes the measurement differences between the online assessed CAPE and it's paper and pencil original could be neglected without major consequences because of small effect sizes (Vleeschouwer et al., 2013).

4.3. Cannabis measures
In epidemiological analysis, the definition and operationalization of exposure variables is vital. The main psychoactive component of cannabis is THC. Concentrations of THC show great variation among different cannabis products. We therefore feel that measuring frequency of use or assessing a diagnosis of cannabis use or dependency is not the most accurate way to quantify THC exposure. In the Netherlands, THC-concentration and cannabis market value are highly correlated in marijuana ($r=0.365$, $p < 0.001$) and in hashish ($r=0.719$, $p < 0.001$)(Vleeschouwer et al., 2013). Prices range from €4.30 for one gram of imported marijuana with an average THC percentage of 5.5% to €15 per gram of Dutch hashish with an average THC concentration of 33.3% (Niesink et al., 2009c). We therefore assessed the amount of euros (€) spent on cannabis per week in the last month, as a proxy measure of exposure to THC.

4.4. Extreme sampling
In chapter 6, we describe a study that aims to incorporate information on cannabis use as risk factor for schizophrenia in a genome-wide gene finding approach. Using a previously described extreme sampling method (Boks et al., 2007b), we aimed to increase power to detect how genetic variation on the SNP level impacts on the cannabis associated risk for psychosis.
Regarding the statistical power, studies on gene-environment interactions form a special group of studies (Luan et al., 2001;Boks et al., 2007b). The sample sizes that are required to detect gene-environment interactions are much larger than those necessary to detect genetic or environmental factors in isolation. This is the consequence of the fact that risks are relatively small in an unselected epidemiological sample, leading to insufficient numbers of subjects suffering from the disorder and having been exposed to the environmental factor. Selecting subjects who suffer from schizophrenia in an attempt to overcome this problem, introduces a selection-bias that excludes those subjects who have been exposed to the environmental factor but did not develop the illness. As a consequence of these difficulties, successful Gene Environment Interaction (GEI) studies are rare. In our GWEIS study we overcome the above mentioned limitation by using an adaptation from an extreme discordant case-control design. Several authors pointed out that

selecting extremely discordant subjects from the population can substantially improve power (Abecasis et al., 2001;Purcell, 2002). We therefore enriched the sample of our study not only for extreme pairs regarding phenotypes (in this case subclinical symptoms of psychosis as measured with the CAPE) but also regarding environmental (cannabis) exposure by selecting individuals with extremely high cannabis use and those with extremely low cannabis use.

5. Conclusion

The studies presented in this thesis aimed to identify genetic and non-genetic (epidemiological) factors that shape the association between cannabis use and psychosis. We showed that the age of first use of cannabis is a determinant for the strength of the association between cannabis use and psychotic symptoms and general mental health, as is the amount of cannabis that is consumed. Moreover it is likely that the THC/CBD ratio in consumed cannabis products further shapes the relationship between cannabis and psychosis. In the second part we suggest that carriers of the "A" risk allele in a SNP of the P2RX7 gene might have an increased risk of experiencing psychosis symptoms if they are heavy cannabis users. Finally we showed that the expression of liprin-α-2 mRNA is increased in the blood of heavy cannabis users compared to cannabis-naïve individuals. The meaning of the latter two findings is still unclear. We are currently conducting additional functional analyses to test these hypotheses. In conclusion, the current thesis adds to the notion that exposure of the developing brain to THC increases the risk of developing psychosis. Several potential pathophysiological pathways are suggested, including interference with microglial function.

Reference List

Abecasis,G.R., Cookson,W.O. and Cardon,L.R., 2001. The power to detect linkage disequilibrium with quantitative traits in selected samples. Am J Hum Genet. 68, 1463-1474.

Balter,K.A., Balter,O., Fondell,E. and Lagerros,Y.T., 2005. Web-based and mailed questionnaires: a comparison of response rates and compliance. Epidemiology. 16, 577-579.

Bhattacharyya,S., Atakan,Z., Martin-Santos,R., Crippa,J.A., Kambeitz,J., Prata,D., Williams,S., Brammer,M., Collier,D.A. and McGuire,P.K., 2012. Preliminary report of biological basis of sensitivity to the effects of cannabis on psychosis: AKT1 and DAT1 genotype modulates the effects of delta-9-tetrahydrocannabinol on midbrain and striatal function. Mol Psychiatry.

Boks,M.P., Schipper,M., Schubart,C.D., Sommer,I.E., Kahn,R.S. and Ophoff,R.A., 2007. Investigating gene environment interaction in complex diseases: increasing power by selective sampling for environmental exposure. Int J Epidemiol. 36, 1363-1369.

Boydell,J., van,O.J., Caspi,A., Kennedy,N., Giouroukou,E., Fearon,P., Farrell,M. and Murray,R.M., 2006. Trends in cannabis use prior to first presentation with schizophrenia, in South-East London between 1965 and 1999. Psychol Med. 36, 1441-1446.

Cabral,G.A. and Staab,A., 2005. Effects on the immune system. Handb Exp Pharmacol. 385-423.

Caspi,A., Moffitt,T.E., Cannon,M., McClay,J., Murray,R., Harrington,H., Taylor,A., Arseneault,L., Williams,B., Braithwaite,A., Poulton,R. and Craig,I.W., 2005. Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. Biol Psychiatry. 57, 1117-1127.

Cha,Y.M., White,A.M., Kuhn,C.M., Wilson,W.A. and Swartzwelder,H.S., 2006. Differential effects of delta9-THC on learning in adolescent and adult rats. Pharmacol Biochem Behav. 83, 448-455.

Chapman,L.J., Chapman,J.P., Kwapil,T.R., Eckblad,M. and Zinser,M.C., 1994. Putatively psychosis-prone subjects 10 years later. J Abnorm Psychol. 103, 171-183.

Chevaleyre,V., Takahashi,K.A. and Castillo,P.E., 2006. Endocannabinoid-mediated synaptic plasticity in the CNS. Annu Rev Neurosci. 29, 37-76.

Coles,M.E., Cook,L.M. and Blake,T.R., 2007. Assessing obsessive compulsive symptoms and cognitions on the internet: evidence for the comparability of paper and Internet administration. Behav Res Ther. 45, 2232-2240.

Cuijpers,P., Boluijt,P. and van,S.A., 2008. Screening of depression in adolescents through the Internet : sensitivity and specificity of two screening questionnaires. Eur Child Adolesc Psychiatry. 17, 32-38.

De Marchi,N., De,P.L., Orlando,P., Daniele,F., Fezza,F. and Di,M., V, 2003. Endocannabinoid signalling in the blood of patients with schizophrenia. Lipids Health Dis. 2, 5.

DeLisi,L.E., 2008. The effect of cannabis on the brain: can it cause brain anomalies that lead to increased risk for schizophrenia? Curr Opin Psychiatry. 21, 140-150.

Di Forti,M., Iyegbe,C., Sallis,H., Kolliakou,A., Falcone,M.A., Paparelli,A., Sirianni,M., La,C.C., Stilo,S.A., Marques,T.R., Handley,R., Mondelli,V., Dazzan,P., Pariante,C., David,A.S., Morgan,C., Powell,J. and Murray,R.M., 2012. Confirmation that the AKT1 (rs2494732) Genotype Influences the Risk of Psychosis in Cannabis Users. Biol Psychiatry.

Donker,T., van,S.A., Marks,I. and Cuijpers,P., 2009. Brief self-rated screening for depression on the Internet. J Affect Disord. 122, 253-259.

Ekman,A., Dickman,P.W., Klint,A., Weiderpass,E. and Litton,J.E., 2006. Feasibility of using web-based questionnaires in large population-based epidemiological studies. Eur J Epidemiol. 21, 103-111.

Faber,G., Smid,H.G., Van Gool,A.R., Wunderink,L., van den Bosch,R.J. and Wiersma,D., 2012. Continued cannabis use and outcome in first-episode psychosis: data from a randomized, open-label, controlled trial. J Clin Psychiatry. 73, 632-638.

Fernandez-Ruiz,J., Berrendero,F., Hernandez,M.L. and Ramos,J.A., 2000. The endogenous cannabinoid system and brain development. Trends Neurosci. 23, 14-20.

Garcia-Gil,L., de,M.R., Munoz,R.M., Cebeira,M., Villanua,M.A., Ramos,J.A. and Fernandez-Ruiz,J.J., 1997. Perinatal delta(9)-tetrahydrocannabinol exposure alters the responsiveness of hypothalamic dopaminergic neurons to dopamine-acting drugs in adult rats. Neurotoxicol Teratol. 19, 477-487.

Garcia-Gil,L., de,M.R., Romero,J., Perez,A., Ramos,J.A. and Fernandez-Ruiz,J.J., 1999. Perinatal delta9-tetrahydrocannabinol exposure augmented the magnitude of motor inhibition caused by GABA(B), but not GABA(A), receptor agonists in adult rats. Neurotoxicol Teratol. 21, 277-283.

Giuffrida,A., Leweke,F.M., Gerth,C.W., Schreiber,D., Koethe,D., Faulhaber,J., Klosterkotter,J. and Piomelli,D., 2004. Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. Neuropsychopharmacology. 29, 2108-2114.

Gosling,S.D., Vazire,S., Srivastava,S. and John,O.P., 2004. Should we trust web-based studies? A comparative analysis of six preconceptions about internet questionnaires. Am Psychol. 59, 93-104.

Graham,A.L. and Papandonatos,G.D., 2008. Reliability of internet- versus telephone-administered questionnaires in a diverse sample of smokers. J Med Internet Res. 10, e8.

Graham,A.L., Papandonatos,G.D., Bock,B.C., Cobb,N.K., Baskin-Sommers,A., Niaura,R. and Abrams,D.B., 2006. Internet- vs. telephone-administered questionnaires in a randomized trial of smoking cessation. Nicotine Tob Res. 8 Suppl 1, S49-S57.

Griffith-Lendering,M.F., Wigman,J.T., Prince van,L.A., Huijbregts,S.C., Huizink,A.C., Ormel,J., Verhulst,F.C., van,O.J., Swaab,H. and Vollebergh,W.A., 2012. Cannabis use and vulnerability for psychosis in early adolescence-a TRAILS study. Addiction.

Hanssen,M., Bak,M., Bijl,R., Vollebergh,W. and van,O.J., 2005. The incidence and outcome of subclinical psychotic experiences in the general population. Br J Clin Psychol. 44, 181-191.

Henquet,C., Rosa,A., Delespaul,P., Papiol,S., Fananas,L., van,O.J. and Myin-Germeys,I., 2009. COMT ValMet moderation of cannabis-induced psychosis: a momentary assessment study of 'switching on' hallucinations in the flow of daily life. Acta Psychiatr Scand. 119, 156-160.

Henquet,C. and van Os,J., 2008. The coherence of the evidence linking cannabis with psychosis. Psychol Med. 38, 461-462.

Hernandez,M., Berrendero,F., Suarez,I., Garcia-Gil,L., Cebeira,M., Mackie,K., Ramos,J.A. and Fernandez-Ruiz,J., 2000. Cannabinoid CB(1) receptors colocalize with tyrosine hydroxylase in cultured fetal mesencephalic neurons and their activation increases the levels of this enzyme. Brain Res. 857, 56-65.

Hickman,M., Vickerman,P., Macleod,J., Kirkbride,J. and Jones,P.B., 2007. Cannabis and schizophrenia: model projections of the impact of the rise in cannabis use on historical and future trends in schizophrenia in England and Wales. Addiction. 102, 597-606.

HILL,A.B., 1965. THE ENVIRONMENT AND DISEASE: ASSOCIATION OR CAUSATION? Proc R Soc Med. 58, 295-300.

Houston,T.K., Cooper,L.A., Vu,H.T., Kahn,J., Toser,J. and Ford,D.E., 2001. Screening the public for depression through the Internet. Psychiatr Serv. 52, 362-367.

Khazaal,Y., Chatton,A., Cochand,S. and Zullino,D., 2008. Quality of web-based information on cannabis addiction. J Drug Educ. 38, 97-107.

Klegeris,A., Bissonnette,C.J. and McGeer,P.L., 2003. Reduction of human monocytic cell neurotoxicity and cytokine secretion by ligands of the cannabinoid-type CB2 receptor. Br J Pharmacol. 139, 775-786.

Konings,M., Bak,M., Hanssen,M., van,O.J. and Krabbendam,L., 2006. Validity and reliability of the CAPE: a self-report instrument for the measurement of psychotic experiences in the general population. Acta Psychiatr Scand. 114, 55-61.

Lataster,T., Myin-Germeys,I., Derom,C., Thiery,E. and van,O.J., 2009. Evidence that self-reported psychotic experiences represent the transitory developmental expression of genetic liability to psychosis in the general population. Am J Med Genet B Neuropsychiatr Genet. 150B, 1078-1084.

Leweke,F.M., Giuffrida,A., Koethe,D., Schreiber,D., Nolden,B.M., Kranaster,L., Neatby,M.A., Schneider,M., Gerth,C.W., Hellmich,M., Klosterkotter,J. and Piomelli,D., 2007. Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: impact of cannabis use. Schizophr Res. 94, 29-36.

Leweke,F.M., Giuffrida,A., Wurster,U., Emrich,H.M. and Piomelli,D., 1999. Elevated endogenous cannabinoids in schizophrenia. Neuroreport. 10, 1665-1669.

Leweke,F.M., Piomelli,D., Pahlisch,F., Muhl,D., Gerth,C.W., Hoyer,C., Klosterkotter,J., Hellmich,M. and Koethe,D., 2012. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. Transl Psychiatry. 2, e94.

Lewis,D.A., 1997. Development of the prefrontal cortex during adolescence: insights into vulnerable neural circuits in schizophrenia. Neuropsychopharmacology. 16, 385-398.

Li,Q., Luo,X. and Muallem,S., 2005. Regulation of the P2X7 receptor permeability to large molecules by extracellular Cl- and Na+. J Biol Chem. 280, 26922-26927.

Lin,C.C., Bai,Y.M., Liu,C.Y., Hsiao,M.C., Chen,J.Y., Tsai,S.J., Ouyang,W.C., Wu,C.H. and Li,Y.C., 2007. Web-based tools can be used reliably to detect patients with major depressive disorder and subsyndromal depressive symptoms. BMC Psychiatry. 7, 12.

Luan,J.A., Wong,M.Y., Day,N.E. and Wareham,N.J., 2001. Sample size determination for studies of gene-environment interaction. Int J Epidemiol. 30, 1035-1040.

Macleod,J., 2007. Cannabis use and symptom experience amongst people with mental illness: a commentary on Degenhardt et al. Psychol Med. 37, 913-916.

Macleod,J., Oakes,R., Copello,A., Crome,I., Egger,M., Hickman,M., Oppenkowski,T., Stokes-Lampard,H. and Davey,S.G., 2004. Psychological and social sequelae of cannabis and other illicit drug use by young people: a systematic review of longitudinal, general population studies. Lancet. 363, 1579-1588.

Marsicano,G., Goodenough,S., Monory,K., Hermann,H., Eder,M., Cannich,A., Azad,S.C., Cascio,M.G., Gutierrez,S.O., van der,S.M., Lopez-Rodriguez,M.L., Casanova,E., Schutz,G., Zieglgansberger,W., Di,M., V, Behl,C. and Lutz,B., 2003. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. Science. 302, 84-88.

McGrath,J., Welham,J., Scott,J., Varghese,D., Degenhardt,L., Hayatbakhsh,M.R., Alati,R., Williams,G.M., Bor,W. and Najman,J.M., 2010. Association Between Cannabis Use and Psychosis-Related Outcomes Using Sibling Pair Analysis in a Cohort of Young Adults. Arch Gen Psychiatry.

McGuire,P.K., Jones,P., Harvey,I., Williams,M., McGuffin,P. and Murray,R.M., 1995. Morbid risk of schizophrenia for relatives of patients with cannabis-associated psychosis. Schizophr Res. 15, 277-281.

Meyerson,P. and Tryon,W.W., 2003. Validating internet research: a test of the psychometric equivalence of internet and in-person samples. Behav Res Methods Instrum Comput. 35, 614-620.

Minozzi,S., Davoli,M., Bargagli,A.M., Amato,L., Vecchi,S. and Perucci,C.A., 2010. An overview of systematic reviews on cannabis and psychosis: discussing apparently conflicting results. Drug Alcohol Rev. 29, 304-317.

Molina-Holgado,F., Alvarez,F.J., Gonzalez,I., Antonio,M.T. and Leret,M.L., 1997. Maternal exposure to delta 9-tetrahydrocannabinol (delta 9-THC) alters indolamine levels and turnover in adult male and female rat brain regions. Brain Res Bull. 43, 173-178.

Monji,A., Kato,T.A., Mizoguchi,Y., Horikawa,H., Seki,Y., Kasai,M., Yamauchi,Y., Yamada,S. and Kanba,S., 2011. Neuroinflammation in schizophrenia especially focused on the role of microglia. Prog Neuropsychopharmacol Biol Psychiatry.

Moore,T.H., Zammit,S., Lingford-Hughes,A., Barnes,T.R., Jones,P.B., Burke,M. and Lewis,G., 2007. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet. 370, 319-328.

Niesink,R., Rigter,S., Hoek,J. and den Boer,N. THC-concentraties in wiet, nederwiet en hasj in Nederlands coffeeshops (2008-2009). 12-1-2009. Utrecht, The Netherlands, Trimbos Institute.
Ref Type: Generic

O'Shea,M., Singh,M.E., McGregor,I.S. and Mallet,P.E., 2004. Chronic cannabinoid exposure produces lasting memory impairment and increased anxiety in adolescent but not adult rats. J Psychopharmacol. 18, 502-508.

Poulton,R., Caspi,A., Moffitt,T.E., Cannon,M., Murray,R. and Harrington,H., 2000. Children's self-reported psychotic symptoms and adult schizophreniform disorder: a 15-year longitudinal study. Arch Gen Psychiatry. 57, 1053-1058.

Purcell,S., 2002. Variance components models for gene-environment interaction in twin analysis. Twin Res. 5, 554-571.

Quinn,H.R., Matsumoto,I., Callaghan,P.D., Long,L.E., Arnold,J.C., Gunasekaran,N., Thompson,M.R., Dawson,B., Mallet,P.E., Kashem,M.A., Matsuda-Matsumoto,H., Iwazaki,T. and McGregor,I.S., 2008. Adolescent rats find repeated Delta(9)-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. Neuropsychopharmacology. 33, 1113-1126.

Ripke,S., Sanders,A.R., Kendler,K.S., Levinson,D.F., Sklar,P., Holmans,P.A., Lin,D.Y., Duan,J., Ophoff,R.A., Andreassen,O.A., Scolnick,E., Cichon,S., St,C.D., Corvin,A., Gurling,H., Werge,T., Rujescu,D., Blackwood,D.H., Pato,C.N., Malhotra,A.K., Purcell,S., Dudbridge,F., Neale,B.M., Rossin,L., Visscher,P.M., Posthuma,D., Ruderfer,D.M., Fanous,A., Stefansson,H., Steinberg,S., Mowry,B.J., Golimbet,V., De,H.M., Jonsson,E.G., Bitter,I., Pietilainen,O.P., Collier,D.A., Tosato,S., Agartz,I., Albus,M., Alexander,M., Amdur,R.L., Amin,F., Bass,N., Bergen,S.E., Black,D.W., Borglum,A.D., Brown,M.A., Bruggeman,R., Buccola,N.G., Byerley,W.F., Cahn,W., Cantor,R.M., Carr,V.J., Catts,S.V., Choudhury,K., Cloninger,C.R., Cormican,P., Craddock,N., Danoy,P.A., Datta,S., De,H.L., Demontis,D., Dikeos,D., Djurovic,S., Donnelly,P., Donohoe,G., Duong,L., Dwyer,S., Fink-Jensen,A., Freedman,R., Freimer,N.B., Friedl,M., Georgieva,L., Giegling,I., Gill,M., Glenthoj,B., Godard,S., Hamshere,M., Hansen,M., Hansen,T., Hartmann,A.M., Henskens,F.A., Hougaard,D.M., Hultman,C.M., Ingason,A., Jablensky,A.V., Jakobsen,K.D., Jay,M., Jurgens,G., Kahn,R.S., Keller,M.C., Kenis,G., Kenny,E., Kim,Y., Kirov,G.K., Konnerth,H., Konte,B., Krabbendam,L., Krasucki,R., Lasseter,V.K., Laurent,C., Lawrence,J., Lencz,T., Lerer,F.B., Liang,K.Y., Lichtenstein,P., Lieberman,J.A., Linszen,D.H., Lonnqvist,J., Loughland,C.M., Maclean,A.W., Maher,B.S., Maier,W., Mallet,J., Malloy,P., Mattheisen,M., Mattingsdal,M., McGhee,K.A., McGrath,J.J., McIntosh,A., McLean,D.E., McQuillin,A., Melle,I., Michie,P.T., Milanova,V., Morris,D.W., Mors,O., Mortensen,P.B., Moskvina,V., Muglia,P., Myin-Germeys,I., Nertney,D.A., Nestadt,G., Nielsen,J., Nikolov,I., Nordentoft,M., Norton,N., Nothen,M.M., O'Dushlaine,C.T., Olincy,A., Olsen,L., O'Neill,F.A., Orntoft,T.F., Owen,M.J., Pantelis,C., Papadimitriou,G., Pato,M.T., Peltonen,L., Petursson,H., Pickard,B., Pimm,J., Pulver,A.E., Puri,V., Quested,D., Quinn,E.M., Rasmussen,H.B., Rethelyi,J.M., Ribble,R., Rietschel,M., Riley,B.P., Ruggeri,M., Schall,U., Schulze,T.G., Schwab,S.G., Scott,R.J., Shi,J., Sigurdsson,E., Silverman,J.M., Spencer,C.C., Stefansson,K., Strange,A., Strengman,E., Stroup,T.S.,

Suvisaari,J., Terenius,L., Thirumalai,S., Thygesen,J.H., Timm,S., Toncheva,D., van den,O.E., van,O.J., van,W.R., Veldink,J., Walsh,D., Wang,A.G., Wiersma,D., Wildenauer,D.B., Williams,H.J., Williams,N.M., Wormley,B., Zammit,S., Sullivan,P.F., O'Donovan,M.C., Daly,M.J. and Gejman,P.V., 2011. Genome-wide association study identifies five new schizophrenia loci. Nat Genet. 43, 969-976.

Schneider,M. and Koch,M., 2003. Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory, and the performance in a progressive ratio task in adult rats. Neuropsychopharmacology. 28, 1760-1769.

Shen,M. and Thayer,S.A., 1998. Cannabinoid receptor agonists protect cultured rat hippocampal neurons from excitotoxicity. Mol Pharmacol. 54, 459-462.

Spek,V., Nyklicek,I., Cuijpers,P. and Pop,V., 2008. Internet administration of the Edinburgh Depression Scale. J Affect Disord. 106, 301-305.

Sperlagh,B., Vizi,E.S., Wirkner,K. and Illes,P., 2006. P2X7 receptors in the nervous system. Prog Neurobiol. 78, 327-346.

Stefanis,N.C., Hanssen,M., Smirnis,N.K., Avramopoulos,D.A., Evdokimidis,I.K., Stefanis,C.N., Verdoux,H. and van,O.J., 2002. Evidence that three dimensions of psychosis have a distribution in the general population. Psychol Med. 32, 347-358.

Steinberg,S., de,J.S., Andreassen,O.A., Werge,T., Borglum,A.D., Mors,O., Mortensen,P.B., Gustafsson,O., Costas,J., Pietilainen,O.P., Demontis,D., Papiol,S., Huttenlocher,J., Mattheisen,M., Breuer,R., Vassos,E., Giegling,I., Fraser,G., Walker,N., Tuulio-Henriksson,A., Suvisaari,J., Lonnqvist,J., Paunio,T., Agartz,I., Melle,I., Djurovic,S., Strengman,E., Jurgens,G., Glenthoj,B., Terenius,L., Hougaard,D.M., Orntoft,T., Wiuf,C., Didriksen,M., Hollegaard,M.V., Nordentoft,M., van,W.R., Kenis,G., Abramova,L., Kaleda,V., Arrojo,M., Sanjuan,J., Arango,C., Sperling,S., Rossner,M., Ribolsi,M., Magni,V., Siracusano,A., Christiansen,C., Kiemeney,L.A., Veldink,J., van den,B.L., Ingason,A., Muglia,P., Murray,R., Nothen,M.M., Sigurdsson,E., Petursson,H., Thorsteinsdottir,U., Kong,A., Rubino,I.A., De,H.M., Rethelyi,J.M., Bitter,I., Jonsson,E.G., Golimbet,V., Carracedo,A., Ehrenreich,H., Craddock,N., Owen,M.J., O'Donovan,M.C., Ruggeri,M., Tosato,S., Peltonen,L., Ophoff,R.A., Collier,D.A., St,C.D., Rietschel,M., Cichon,S., Stefansson,H., Rujescu,D. and Stefansson,K., 2011. Common variants at VRK2 and TCF4 conferring risk of schizophrenia. Hum Mol Genet. 20, 4076-4081.

Stella,N., 2009. Endocannabinoid signaling in microglial cells. Neuropharmacology. 56 Suppl 1, 244-253.

Stella,N., 2010. Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. Glia. 58, 1017-1030.

Suarez,I., Bodega,G., Fernandez-Ruiz,J., Ramos,J.A., Rubio,M. and Fernandez,B., 2004. Down-regulation of the AMPA glutamate receptor subunits GluR1 and GluR2/3 in the rat cerebellum following pre- and perinatal delta9-tetrahydrocannabinol exposure. Cerebellum. 3, 66-74.

Sullivan,J.M., 1999. Mechanisms of cannabinoid-receptor-mediated inhibition of synaptic transmission in cultured hippocampal pyramidal neurons. J Neurophysiol. 82, 1286-1294.

Trezza,V., Cuomo,V. and Vanderschuren,L.J., 2008. Cannabis and the developing brain: insights from behavior. Eur J Pharmacol. 585, 441-452.

Vallejo,M.A., Jordan,C.M., Diaz,M.I., Comeche,M.I. and Ortega,J., 2007. Psychological assessment via the internet: a reliability and validity study of online (vs paper-and-pencil) versions of the General Health Questionnaire-28 (GHQ-28) and the Symptoms Check-List-90-Revised (SCL-90-R). J Med Internet Res. 9, e2.

van Os,J. and Kapur,S., 2009. Schizophrenia. Lancet. 374, 635-645.

van Os,J., Verdoux,H., Maurice-Tison,S., Gay,B., Liraud,F., Salamon,R. and Bourgeois,M., 1999. Self-reported psychosis-like symptoms and the continuum of psychosis. Soc Psychiatry Psychiatr Epidemiol. 34, 459-463.

van Winkel,R., 2011. Family-based analysis of genetic variation underlying psychosis-inducing effects of cannabis: sibling analysis and proband follow-up. Arch Gen Psychiatry. 68, 148-157.

van Winkel,R., van Beveren,N.J. and Simons,C., 2011. AKT1 moderation of cannabis-induced cognitive alterations in psychotic disorder. Neuropsychopharmacology. 36, 2529-2537.

Verdoux,H. and van,O.J., 2002. Psychotic symptoms in non-clinical populations and the continuum of psychosis. Schizophr Res. 54, 59-65.

Vleeschouwer,M., Schubart,C.D., Henquet,C., van Gastel,W.A., Hillegers,M., Kahn,R.S., Boks,M.P. and Derks,E. Does Assessment type matter? A Measurement Invariance analysis of online and paper and pencil assessment of the Community Assessment of Psychic Experiences (CAPE). 2013.

Walter,L., Dinh,T. and Stella,N., 2004. ATP induces a rapid and pronounced increase in 2-arachidonoylglycerol production by astrocytes, a response limited by monoacylglycerol lipase. J Neurosci. 24, 8068-8074.

Wiles,N.J., Zammit,S., Bebbington,P., Singleton,N., Meltzer,H. and Lewis,G., 2006. Self-reported psychotic symptoms in the general population: results from the longitudinal study of the British National Psychiatric Morbidity Survey. Br J Psychiatry. 188, 519-526.

Wiley,J.S., Sluyter,R., Gu,B.J., Stokes,L. and Fuller,S.J., 2011. The human P2X7 receptor and its role in innate immunity. Tissue Antigens. 78, 321-332.

Witting,A., Walter,L., Wacker,J., Moller,T. and Stella,N., 2004. P2X7 receptors control 2-arachidonoylglycerol production by microglial cells. Proc Natl Acad Sci U S A. 101, 3214-3219.

Yung,A.R., Nelson,B., Baker,K., Buckby,J.A., Baksheev,G. and Cosgrave,E.M., 2009. Psychotic-like experiences in a community sample of adolescents: implications for the continuum model of psychosis and prediction of schizophrenia. Aust N Z J Psychiatry. 43, 118-128.

Zammit,S., Spurlock,G., Williams,H., Norton,N., Williams,N., O'Donovan,M.C. and Owen,M.J., 2007. Genotype effects of CHRNA7, CNR1 and COMT in schizophrenia: interactions with tobacco and cannabis use. Br J Psychiatry. 191, 402-407.

PART III: SUMMARY AND DISCUSSION

CHAPTER IX

• NEDERLANDSE SAMENVATTING •

Achtergrond
Schizofrenie is een ernstige psychiatrische aandoening die bij ongeveer 1% van de mensen voorkomt. De ziekte kenmerkt zich door terugkerende psychoses, afvlakking van het gevoelsleven en cognitieve achteruitgang. De ziekte heeft voor veel mensen een verwoestende uitwerking en kan tot blijvende invaliditeit leiden. Tot op heden weten wij niet goed waar de ziekte door wordt veroorzaakt. Duidelijk is wel dat de ziekte door een complex samenspel van genetische en omgevingsfactoren ontstaat.

In de laatste decennia zijn er steeds meer aanwijzingen dat cannabis een risicofactor is voor het ontstaan van schizofrenie. Mensen die cannabis gebruiken hebben ongeveer tweemaal zoveel kans op het ontwikkelen van een psychotische stoornis, zoals schizofrenie, als mensen die dit niet doen (Moore et al., 2007). Het is echter nog altijd onbekend hoe cannabis dit risico verhoogt, bovendien ontwikkelt lang niet iedereen die cannabis gebruikt een psychose. Waarom de één wel en de ander niet ziek wordt is onduidelijk. Er zijn aanwijzingen dat verschillende (epidemiologische) factoren rondom het cannabisgebruik alsook genetische kwetsbaarheid hierbij een belangrijke rol spelen (van Os et al., 2010).

Doelstelling
Het doel van dit onderzoek is het identificeren van genetische en niet-genetische (eppidemiologische) factoren die de gevoeligheid bepalen voor het ontwikkelen van een psychose door het roken van cannabis. Door te ontrafelen hoe deze factoren de kans op psychose vergroten, hopen we bovendien beter te kunnen begrijpen welke effecten cannabis heeft op het brein en hoe psychose ontstaat.
Wij hopen dat deze studie uiteindelijk kan bijdragen aan vroege detectie en preventie van psychiatrische aandoeningen.

Strategie
Om factoren te identificeren die het risico op psychotische symptomen vergroten, hebben wij een website opgezet om zo grote groepen mensen vragenlijsten te kunnen laten invullen. Op de website www.cannabisquest.nl heeft een groep van bijna 26,000 deelnemers vragenlijsten ingevuld over hun cannabisgebruik, psychiatrische voorgeschiedenis en over het hebben van psychotische symptomen (gemeten met de CAPE vragenlijst (Konings et al., 2006)). De gegevens van deze mensen hebben wij gebruikt om; 1) verbanden te analyseren te analyseren tussen eigenschappen van deze mensen (epidemiologische factoren) en hun cannabis gebruik en 2) mensen te selecteren die geschikt waren voor onze genetische studie.

Samenvatting van de belangrijkste resultaten uit dit proefschrift:
Hoofdstuk 1 is een engelstalige introductie op het onderwerp van dit proefschrift. In **deel I** van dit proefschrift wordt ingegaan op de vraag welke

***epidemiologische* factoren** invloed hebben op de kans om een psychose te ontwikkelen bij cannabisgebruik.

In **hoofdstuk 2** wordt de vraag behandeld of cannabisgebruik geassocieerd is met het hebben van een (niet nader gespecificeerde) psychiatrische voorgeschiedenis met een klinische opname. Onze conclusie was dat dit inderdaad het geval is. Onder mensen die jonger dan 12 en zelfs jonger dan 15 jaar waren toen zij voor het eerst cannabis gebruikten, is de kans op opname in een psychiatrische instelling duidelijk (tot 4x) verhoogd. Ook de hoeveelheid cannabis die mensen gebruiken verhoogt dit risico tot 6x bij de zeer ernstige gebruikers.

Hoofdstuk 3 beschrijft een studie naar het verband tussen cannabisgebruik en het ervaren van psychische klachten. Uit onderzoek is gebleken dat het ervaren van psychiatrische klachten (positieve, negatieve en depressie-symptomen) het risico op een psychotische stoornis zoals schizofrenie, sterk verhoogt. Uit analyse in een groep van 17,698 proefpersonen blijkt dat mensen die zeer jong (voor hun 12de) maar ook jong (voor hun 15de) zijn begonnen met cannabisgebruik, een sterk verhoogd risico hebben om psychische klachten te ervaren. Met name het risico op psychotische klachten (wanen en hallucinaties) is sterk verhoogd bij deze groep. Ook het gebruik van veel cannabis verhoogt dit risico, waardoor het lijkt dat er sprake is van een dosis-respons relatie. Hoe meer cannabis iemand gebruikt, hoe hoger het risico.

Hoofdstuk 4 is een review waarin het mogelijke antipsychotische effect van cannabidiol wordt beschreven, wat naast Δ9-tetrahydrocannabinol (THC) een ander belangrijk bestanddeel is van de cannabisplant. Wij concluderen dat er met gebruik van zeer uiteenlopende onderzoeksmethodes, vanuit verschillende benaderingen bewijs is ontstaan voor een mogelijke antipsychotische werking van cannabidiol. Of cannabidiol ook daadwerkelijk als antipsychoticum kan worden gebruikt, moet nog blijken uit verder onderzoek. Aangezien de concentraties THC en cannabidiol sterk uiteenlopen tussen de verschillende soorten cannabis, is de hoeveelheid cannabidiol die iemand binnenkrijgt tijdens cannabisgebruik afhankelijk van het type cannabis dat gebruikt wordt.

In **hoofdstuk 5** wordt gekeken of het type cannabis (en daarmee de verhouding THC/cannabidiol) van invloed is op het risico om psychotische klachten te ontwikkelen in onze eigen onderzoekspopulatie. Uit onze studie blijkt dat de verhouding THC/cannabidiol een, weliswaar kleine, maar significante invloed heeft op het ervaren van psychotische klachten. De beschermende functie van cannabidiol wordt hierdoor bevestigd. Interessant is dat met name de buitenlandse hasjsoorten veel cannabidiol bevatten. Wiet, en met name Nederlandse wiet, bevat weinig tot geen cannabidiol.

In **deel II** van dit proefschrift wordt ingegaan op de vraag welke ***genetische factoren*** een rol spelen bij het risico om een psychose te ontwikkelen bij

cannabisgebruik. Om deze genen te identificeren, hebben wij een “genome wide environment interaction study (GWEIS)” opgezet. Deze wordt beschreven in **hoofdstuk 6.** Wij hebben in een groep van 1262 proefpersonen geanalyseerd welke genetische varianten geassocieerd zijn met de gevoeligheid om psychotische symptomen te ontwikkelen bij cannabisgebruik. Eén van de vernieuwende aspecten van het gebruikte design is dat wij proefpersonen hebben geselecteerd op twee eigenschappen. Ten eerste het zeer hoog of zeer laag scoren op het hebben van psychiatrische klachten en ten tweede op het gebruik van zeer veel of praktisch geen cannabis. Door vooral mensen te includeren met deze “extreme” profielen, konden wij de statistische bewijskracht van onze analyses sterk vergroten. Voor een uitgebreidere bespreking van deze methode zie ook (Boks et al., 2007b).
Uit de groep van 26.000 mensen die op de website vragen heeft beantwoord, zijn 1364 proefpersonen geselecteerd die aan de strenge inclusiecriteria voldeden. 1184 van deze mensen waren bereid om naar het UMC Utrecht te komen voor bloedafname. Bij deze proefpersonen is bloed afgenomen voor DNA-analyse en tevens is er bij hen een uitgebreide hoeveelheid aan klinische interviews en vragenlijsten afgenomen. Daarnaast hebben nog 178 mensen deelgenomen die niet naar het ziekenhuis wilden komen. Zij hebben de vragenlijsten per mail ingevuld en een speekselmonster met DNA naar ons opgestuurd per post. Uit deze studie blijkt dat onder cannabisgebruikers met name een variant van het gen P2RX7 een risicofactor is om hoog te scoren op psychosevragenlijsten. Het P2RX7-gen codeert voor het P2X7-eiwit dat een functie heeft in het immuunsysteem, maar ook samen blijkt te werken met het lichaamseigen cannabissysteem (het endocannabinoïd systeem). Deze ontdekking geeft aanleiding tot verschillende hypotheses die in verder onderzoek getoetst moeten worden.

Het laatste hoofdstuk, **hoofdstuk 7** gaat over genexpressie-veranderingen door cannabisgebruik. Genexpressie is de hoeveelheid DNA die daadwerkelijk wordt omgezet in messenger-RNA en uiteindelijk tot productie van een aminozuursequentie, ofwel eiwit, leidt. De mate van genexpressie is een maat voor de activiteit van een gen en is sterk afhankelijk van de functie van de cel waarin het gen tot expressie moet komen en, in mindere mate, van verschillende omgevingsfactoren die invloed hebben op het functioneren van de cel. Bij een deel van de mensen die geïncludeerd zijn (n=190), is RNA uit bloed geïsoleerd en genexpressie bepaald met behulp van micro-arrays. In deze data hebben wij geanalyseerd in hoeverre cannabisgebruik invloed heeft op genexpressie. In deze analyse wordt de activiteit van ca. 20.000 genen in het bloed van cannabisgebruikers vergeleken met de activiteit bij cannabis-naïeve proefpersonen. Uit deze analyse komt naar voren dat twee genen (CX3CR1 en PPFIA2) een significant ander activatiepatroon hebben onder cannabisgebruikers vergeleken met niet-cannabisgebruikers. Middels een aanvullende analyse (qPCR) is getracht deze ontdekking te valideren. Met deze analyse is alleen de veranderde regulatie van PPFIA2 bevestigd. PPFIA2 is een gen dat codeert voor een eiwit dat liprine-2-alpha heet. Dit eiwit is onder

andere betrokken bij het functioneren van synapsen en de geheugenfunctie. Verder onderzoek moet uitwijzen wat dit verband tussen PPFIA2 en cannabis betekent en wat dit zegt over de manier waarop cannabis invloed heeft op het functioneren van ons brein.

Conclusie
De doelstelling van dit proefschrift was om te onderzoeken welke genetische en niet-genetische factoren invloed hebben op de relatie tussen cannabis en psychose. Uit dit proefschrift blijkt dat zowel de leeftijd op en hoeveelheid van cannabisgebruik sterk geassocieerd zijn met zowel de kans dat iemand al ooit in een psychiatrische instelling is opgenomen alsook het risico op psychotische symptomen. Ook het type cannabis dat men gebruikt heeft invloed op dit risico. Tevens blijkt uit het tweede deel van dit proefschrift dat cannabis voor dragers van een variant in het P2RX7-gen mogelijk extra risicovol is en dat cannabis invloed heeft op de expressie van liprine-2-alpha. De betekenis van deze laatste twee bevindingen is nog onduidelijk. De resultaten zullen in onafhankelijke populaties moeten worden bevestigd en de achterliggende biologische mechanismen moeten met moleculair-biologische, functionele experimenten verder worden ontrafeld.

Reference List

Boks,M.P., Schipper,M., Schubart,C.D., Sommer,I.E., Kahn,R.S. and Ophoff,R.A., 2007. Investigating gene environment interaction in complex diseases: increasing power by selective sampling for environmental exposure. Int J Epidemiol. 36, 1363-1369.

Konings,M., Bak,M., Hanssen,M., van,O.J. and Krabbendam,L., 2006. Validity and reliability of the CAPE: a self-report instrument for the measurement of psychotic experiences in the general population. Acta Psychiatr Scand. 114, 55-61.

Moore,T.H., Zammit,S., Lingford-Hughes,A., Barnes,T.R., Jones,P.B., Burke,M. and Lewis,G., 2007. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet. 370, 319-328.

van Os,J., Kenis,G. and Rutten,B.P., 2010. The environment and schizophrenia. Nature. 468, 203-212.

LIST OF PUBLICATIONS

- W.A. van Gastel, J.H. MacCabe, **C.D. Schubart**, A. Vreeker, W. Tempelaar, R.S. Kahn, M.P.M. Boks. Cigarette Smoking and Cannabis Use are equally strongly associated with Psychotic-like Experiences Psychological Medicine (2013)

- Vreeker, A, **Schubart CD**, van Gastel WA, Kahn RS, Boks MPM. Advanced paternal age and vulnerability to psychotic-like experiences in the offspring. Schizophr Res. 2012 Nov 19. [Epub ahead of print]

- W.A. van Gastel, W. Tempelaar, C. Bun, **C.D. Schubart**, R.S. Kahn, C.Plevier, M. P.M. Boks. Cannabis use as an indicator of risk for mental health problems. Psychol Med. 2012 Dec 3:1-8. [Epub ahead of print]

- de Jong S, Boks MP, Fuller TF, Strengman E, Janson E, de Kovel CG, Ori AP, Vi N, Mulder F, Blom JD, Glenthøj B, **Schubart CD**, Cahn W, Kahn RS, Horvath S, Ophoff RA. A gene co-expression network in whole blood of schizophrenia patients is independent of antipsychotic-use and enriched for brain-expressed genes. PLoS One. 2012;7(6):e39498. Epub 2012 Jun 27.

- **Schubart CD**, Sommer IEC, van Gastel WA, Rogier L. Goetgebeur, Kahn RS, Boks MPM. Cannabis with high cannabidiol content is associated with fewer psychotic experiences. Schizophrenia research. 2011 Aug;130(1-3):216-21.

- **Schubart CD**, van Gastel WA, Breetvelt EJ, Beetz SL, Ophoff RA, Sommer IEC, Kahn RS, Boks MPM. Cannabis use at young age is associated with psychotic experiences. Psychol Med. 2010 Oct 7:1-10.

- **Schubart CD**, Boks MPM, Breetvelt EJ, van Gastel WA, Groenwold R, Ophoff RA, Sommer IEC, Kahn RS Association between cannabis and psychiatric hospitalization. Acta Psychiatr Scand. 2011 May;123(5):368-75.

- Boks MP, Schipper M, **Schubart CD**, Sommer IE, Kahn RS, Ophoff RA. Investigating gene environment interaction in complex diseases: increasing power by selective sampling for environmental exposure.Int J Epidemiol. 2007 Dec;36(6):1363-9.

- Wijkstra J, **Schubart CD**, Nolen WA. Treatment of unipolar psychotic depression: The use of evidence in practice guidelines. World J Biol Psychiatry. 2007 Oct 24:1-7.

- **Schubart CD**, Selten JP. (In Dutch) [Referaat: Geen bewijs voor verhoogd risico op zelfmoord door behandeling met venlafaxine.] Nederlands Tijdschrift voor Geneeskunde. Juli 2007.

- **Schubart CD**, Mturi N, Beld MG, Wertheim PM, Newton CR. Role of viruses in Kenyan children presenting with acute encephalopathy in a malaria-endemic area. Am J Trop Med Hyg. 2006 Dec;75(6):1148-50.

SUBMITTED

- **Schubart CD**, Boks MPM, Sommer IEC, Kahn RS, Fusar-Poli P. Cannabidiol as novel treatment for schizophrenia. Review. Submitted

- W.A. van Gastel, J.H. MacCabe, **C.D. Schubart**, E. van Otterdijk, W. Tempelaar, R.S. Kahn, M.P.M. Boks. Moderate Cannabis Use is a Better Indicator of Poor Mental Health in Women than in Men. Submitted

- M. Vleeschouwer, **C.D. Schubart**, C. Henquet , W. van Gastel, M. H. J. Hillegers, R.S. Kahn, dr. M.P.M. Boks, E. M. Derks. Does Assessment type matter? A Measurement Invariance analysis of online and paper and pencil assessment of the Community Assessment of Psychic Experiences (CAPE). Submitted

- C.H. Vinkers, W.A. van Gastel, **C.D. Schubart**, K.R. van Eijk, J.J. Luykx, R. van Winkel, GROUP investigators, M. Joëls, R.A. Ophoff, M.P.M. Boks. The effect of childhood maltreatment and cannabis use on adult psychotic symptoms is modified by the *COMT* Val^{158}Met polymorphism

IN PREPARATION

- W.A. van Gastel, **C.D. Schubart**, J. MacCabe, R.S. Kahn, M.P.M. Boks. Synchronicity of Change in Cannabis Use and Psychotic-Like Experiences. In preparation

- **C.D. Schubart**, S. de Jong, L. de Witte, W.A. van Gastel, J. Helthuis, B.P.C. Koeleman, R. A. Ophoff, R.S. Kahn, M.P.M. Boks. Genome wide expression profiling of whole blood from heavy cannabis users and controls reveals differential regulation of liprin alpha 2 (PPFIA2). In preparation

- **CD Schubart,** WA van Gastel, , L. de Witte, K van Eijk, B Koeleman, A Caspi , RA Ophoff, RS Kahn, MPM Boks. A polymorphism (rs 7958311) in theP2X7 receptor gene is a candidate to moderate the effect of cannabis on psychosis risk. In preparation

CURRICULUM VITAE

Curriculum Vitae

Chris Schubart was born on November 24th 1976 in Haarlem, and was raised in Spain and the Netherlands. He received his European Baccalaureate from the European School in Bergen, The Netherlands, in 1995. After a year of civilian service at the Lebenshilfe Organization, in Darmstadt, Germany, he attended the University of Amsterdam to study Medicine. As a medical student he worked in the KEMRI-Wellcome Trust laboratory in Kenya investigating the role of neurotropic viruses in the clinical presentation of cerebral malaria. Since 2004, Chris is involved in an internationally operating NGO, The WAMA foundation, that intends to improve capability of medical professionals in developing countries. Shortly after graduating from medical school in 2005, Chris started his clinical residencies in psychiatry and his PhD project under the supervision of Professor René Kahn. He was involved in several studies focussing on the role of genetic and epidemiological factors that shape the relationship between cannabis use and psychosis. He finished his residencies in clinical psychiatry in October 2012 under Dr. J. Wijkstra and is currently working as a psychiatrist at Tergooi Hospitals.

DANKWOORD

Dankwoord

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