Acute and chronic effects of cannabinoids on human brain: gene-environment interactions related to psychiatric disorders

Albert Batalla Cases

Acute and chronic effects of cannabinoids on human brain: gene-environment interactions related to psychiatric disorders

Thesis

submitted in order to obtain the degree of doctor at the University of Barcelona

Barcelona, July 2014

PhD candidate:	Albert Batalla Cases
Supervisor:	Dr. Rocío Martín-Santos Laffon
	Department of Psychiatry and Clinical Psychobiology, Institut Clínic de Neurociències, Hospital Clínic de Barcelona
Line of research:	Biological bases of psychiatric disorders and nuclear psychiatry

Cover design: Lluis Carretero
Printed by: Off page - www.offpage.nl

Framework

This thesis is presented by article publication, in accordance with the procedures indicated by University of Barcelona. The present work includes, in the following order: an abstract of the thesis in English and Spanish, a general introduction, the aims and hypotheses, the five original articles preceded by a brief summary, a general discussion, a conclusion, an extended summary of the thesis in Catalan and a list of references.

The five articles included in the present thesis (✓) are enclosed within a research project focused on the effects of cannaboinoids in brain structure and function through the study of early-onset chronic cannabis users. The present work has been conducted in collaboration with recognised international institutions, such as the King's College London and the University of Melbourne, including a four-month traineeship in the neuroimaging lab of the last institution, and has been supported by the following grants: *Ministerio de Sanidad y Consumo* (Plan Nacional Sobre Drogas; PNSD: PI101/2006 and PNSD: PI041731/2011, Martin-Santos R, PI) and *DIUE de la Generalitat de Catalunya* (Suport a les activitats dels Grups de Recerca; SGR 1435/2009 and SGR 1411/2014).

The aforementioned research project has lead to several publications in the last years:

» Title: Genetic effects on functional connectivity brain networks related to reward in chronic cannabis users. Authors: Batalla A, Blanco-Hinojo L, Crippa JA, Navinés R, Nogué S, Harrinson BJ, Torrens M, Pujol J, Martin-Santos R. *In preparation*.

(✓) » Title: Epistatic influence of COMT and DAT1 gene variations on hippocampal volume in chronic cannabis users: a gene-gene-environment interaction. Authors: Batalla A, Lorenzetti V, Yücel M, Soriano-Mas C, Bhattacharyya S, Torrens M, Crippa JA, Martín-Santos R. *Under review*.

» Title: Catechol O-methyltransferase *Val158Met* genotype and neural mechanisms related to response inhibition in chronic cannabis users.

Authors: Martín-Santos R*, Batalla A*, Fagundo AB*, Blanco-Hinojo L, Soriano-Mas C, López-Solà M, Navinés R, Torrens M, de la Torre R, Crippa JA, Bhattacharyya S, Harrison BJ, Pujol J, Farre M. *Under review*. *Authors contributed equally.

» Title: Functional connectivity alterations in brain networks relevant to self-awareness in chronic cannabis users. Authors: Pujol J, Blanco-Hinojo L, Batalla A, López-Solà M, Harrison BJ, Soriano-Mas C, Crippa JA, Fagundo B, Deus J, de la Torre R, Nogué S, Farré M, Torrens M, Martín-Santos R. *Journal of Psychiatric Research 2014; 51:68-78*.

(✓) » Title: Neuroimaging Studies of Acute Effects of THC and CBD in humans and animals: a Systematic Review. Authors: Batalla A, Crippa JA, Busatto GF, Guimarães FS, Zuardi AW, Valverde O, Atakan Z, McGuire PK, Bhattacharyya S, Martín-Santos R. *Current Pharmaceutical Design 2014; 20(13):2168-85*.

(✓) » Title: Screening for substance use disorders in first-episode psychosis: Implications for readmission. Authors: Batalla A, Garcia-Rizo C, Castellvi P, Fernandez-Egea E, Yücel M, Parellada E, Kirkpatrick B, Martín-Santos R, Bernardo M. *Schizophrenia Research 2013; 146(1-3):125-131*.

(✓) » Title: Modulation of brain structure by catechol-O-methyltransferase *Val(158) Met* polymorphism in chronic cannabis users. Authors: Batalla A, Soriano-Mas C, Lopez-Sola M, Torrens M, Crippa JA, Bhattacharyya S, Blanco-Hinojo L, Fagundo AB, Harrison BJ, Nogué S, de la Torre R, Farré M, Pujol J, Martín-Santos R. *Addiction Biology 2013; [Epub ahead of print]*.

(✓) » Title: Structural and functional imaging studies in chronic cannabis users: a systematic review of adolescent and adult findings. Authors: Batalla A, Bhattacharyya S, Yücel M, Fusar-Poli P, Crippa JA, Nogué S, Torrens M, Pujol J, Farré M, Martín-Santos R. *PLoS One 2013; 8(2):e55821*.

» Title: Acute Effects of a Single, Oral dose of d9-tetrahydrocannabinol (THC) and Cannabidiol (CBD) Administration in Healthy Volunteers. Authors: Martín-Santos R, Crippa JA, Batalla A, Bhattacharyya S, Atakan Z, Borgwardt S, Allen P, Seal M, Langohr K, Farré M, Zuardi AW, McGuire PK. *Current Pharmaceutical Design 2012; 18(32):4966-4979*.

In addition, this research project has also lead to several presentations in national and international congresses, including two travel grant awards: Travel grant for oral communication in the 22nd European Psychiatric Association (EPA) Congress, Munich, March 2014; and travel grant for poster presentation in the ECNP Workshop on Neuropsychopharmacology for Young Scientists in Europe, Nice, March 2013.

Voor jou

Table of contents

Abstract

Abstract - English

Introduction

Cannabis use has been associated to acute and chronic mental health problems and worsened outcome of established psychiatric disorders. Disturbances of the endocannabinoid system may be responsible for long-lasting effects, such as neuropsychological deficits and morphological brain alterations. As not all the exposed individuals are equally affected, proneness to cannabis-induced impairment may rely on key factors such as age of onset, cannabis use parameters and genetic background.

Aims

The aim of the present thesis is to expand current knowledge of acute and chronic effects of cannabinoids, while assessing gene-environment interactions that are relevant for psychiatric disorders. This is achieved by investigating the consequences of drug use in first-episode psychosis and subsequently by studying the influence of dopamine-regulating genes on brain structure of early-onset chronic cannabis users and non-using controls, based on the following hypothesis:

- Cannabis use in first-episode psychosis would be associated with worse outcome regarding readmission rates, either measured by a screening drug scale or urinalysis (**Chapter 3**).
- Acute and chronic cannabis use would be associated with alterations on brain function and structure in key areas relevant for psychiatric disorders, and these alterations would be present in adolescent users (**Chapters 4 and 5**).
- Early-onset chronic cannabis users would show morphological brain alterations compared to non-using controls, and variation in the dopamine-regulating genes would result in diverse liability to experience cannabis-related brain impairment (**Chapters 6 and 7**).

Methods

We assessed a cohort of 58 first-episode psychosis patients consecutively admitted to the inpatient unit of a general hospital. The main outcome was the time until first readmission. All subjects were interviewed using the Structured Clinical Interview for DSM-IV Axis I Disorders, clinician version (SCID-I), the Spanish version of the Positive and Negative Syndrome Scale (PANSS) and the Dartmouth Assessment of Lifestyle Inventory (DALI) scale, which focuses on detecting substance use disorders in people with severe mental illness. The subjects also underwent blood and urine sampling for drug use within 48 hours after admission. The Kaplan-Meier estimator was applied to estimate the survival curves, using time to readmission as a dependent outcome. Multivariate analysis was also performed. Validity parameters were calculated and related to future readmissions, as well as ROC curves. Analyses were performed using SPSS version 19 (**Chapter 3**).

In addition, we conducted two systematic literature reviews from four different databases (EMBASE, Medline, PubMed, LILACS) following a comprehensive search strategy and pre-determined protocol in accordance with PRISMA guidelines. **Chapter 4** included 43 neuroimaging studies of experimental administration of cannabinoids involving animals naïve to cannabinoids and naïve/occasional cannabis users. **Chapter 5** considered 45 neuroimaging studies involving chronic cannabis users with a matched control group.

Finally, we performed a case-control neuroimaging study in male Caucasians, 30 early-onset chronic cannabis users and 29 age-, education- and intelligence-matched non-using controls. All subjects were assessed by a structured interview (PRISM) to exclude any lifetime axis-I disorder according to DSM-IV. Catechol-O-methyltransferase (COMT *Val*^{158}*Met*, rs4680) and dopamine transporter (DAT1 VNTR) genotyping were performed. MRI data was analysed by VBM (**Chapter 6**) and manual tracing of the hippocampus via well-validated methods (**Chapter 7**).

Results

Cannabis was the most common drug found in the first-episode psychosis cohort, either in urinalysis (38%) or self-reported (50%). Both the DALI cannabis/cocaine subscale (p=0.002) and urinalysis for cannabis (p=0.02) were associated with increased readmission risk in survival curves, mainly the first five years of follow-up. The DALI cannabis/cocaine subscale at baseline was a significant predictor of readmission over the study period [HR = 4.5; 95% CI = 1.1 to 18.7; p=0.036] after controlling for potential confounders (gender, age, duration of untreated psychosis and PANSS positive subscale) (**Chapter 3**).

The studies included in **Chapter 4** showed that acute administration of cannabinoids modulate resting state activity and alter neural activity during performance of several cognitive tasks in areas related with reward and psychiatric disorders. In contrast to animal studies, the few neurochemical studies performed in humans showed inconsistencies regarding the increased dopaminergic activity that might be related to THC-induced psychosis. **Chapter 5** indicated that chronic cannabis use is associated with alterations in brain function and structure especially in medial temporal regions both in adults and adolescents, and that the amount of exposure may be related to its harmful effect.

In the case-control study, chronic cannabis users showed morphologic brain alterations in the areas highlighted in **Chapters 4 and 5**, which were differently influenced by the COMT and DAT1 genotypes depending on whether or not the individual had been regularly exposed to cannabis. In particular, the COMT genotype influenced the volume in two out of four regions studied by VBM (**Chapter 6**). Variation in the COMT genotype affected the bilateral ventral caudate nucleus in both groups in an opposite direction. That is, more copies of the *val* allele led to lesser volume in chronic cannabis users and more volume in controls. The opposite pattern was found in the left amygdala. **Chapter 7** expanded these results by showing that COMT and DAT1 genes interacted with each other moderating individual differences in the hippocampal volume. The association between these functional genotypes and hippocampal volumes suggested a linear relationship with dopamine availability in

controls, which was not observed in chronic cannabis users. Hippocampal volumes were smaller in chronic cannabis users compared to controls, and the magnitude of volumetric reduction was associated with lifetime cannabis exposure.

Conclusion

Together, these results provide support for endocannabinoid involvement in the outcome of psychiatric disorders, as well as in the control of different cognitive functions, dopamine release and brain volume, with alterations derived from chronic use appearing soon. Findings also provide evidence that dopamine-regulating genes may play a particular role in the sensitivity to the effects of cannabis on brain morphology, providing further insights into the mechanisms of cannabis-related brain impairment and genetic vulnerability.

Abstract - Spanish

Introducción

El uso de cannabis se ha asociado a la aparición de problemas de salud mental tanto agudos como crónicos, así como a una peor evolución de los trastornos psiquiátricos ya establecidos. Alteraciones del sistema endocannabinoide endógeno podrían ser las responsables de los efectos a largo plazo, tales como déficits neuropsicológicos y alteraciones en la morfología cerebral. Dado que no todos los sujetos expuestos a cannabis se ven igualmente afectados, la propensión al daño inducido por cannabis podría estar relacionada con factores clave como la edad de inicio, parámetros de consumo y vulnerabilidad genética.

Objetivos

El objetivo de la presente tesis es ampliar el conocimiento actual sobre los efectos agudos y crónicos de los cannabinoides a través del estudio de interacciones gen-ambiente que son de interés para los trastornos psiquiátricos. Para ello nos hemos propuesto estudiar las consecuencias del uso de sustancias de abuso en primeros episodios psicóticos, y posteriormente evaluar la influencia de los genes reguladores de la dopamina en la estructura cerebral de consumidores crónicos de cannabis de inicio temprano y controles no consumidores, en base a las siguientes hipótesis:

- El uso de cannabis en primeros episodios psicóticos se asociaría a una peor evolución en relación a la tasas de reingreso, ya sea medido mediante una escala de cribado o mediante análisis de orina (**Capítulo 3**).
- El uso agudo y crónico de cannabis se asociaría con alteraciones en la estructura y función cerebrales en regiones clave relacionadas con trastornos psiquiátricos, y estas alteraciones estarían presentes en población adolescente (**Capítulos 4 y 5**).
- Los consumidores de cannabis de inicio temprano presentarían alteraciones en la estructura cerebral comparados con controles no consumidores, y la variación en

los genes reguladores de la dopamina resultaría en distinta probabilidad de presentar daño cerebral relacionado con el uso de cannabis (**Capítulos 6 y 7**).

Métodos

Estudiamos una cohorte de 58 primeros episodios psicóticos ingresados consecutivamente en la unidad de hospitalización de un hospital general. Todos los pacientes fueron evaluados mediante la entrevista semiestructurada para el diagnóstico de trastornos mentales (SCID-I), la escala PANSS y la escala DALI, la cual se centra en la detección del uso de sustancias de abuso en población afecta de patología mental severa. La principal medida de resultado fue el tiempo hasta que el paciente era reingresado por primera vez. A los participantes también se les recogieron muestras de sangre y orina para la detección de sustancias de abuso dentro de las primeras 48 horas tras el ingreso. Se usó Kaplan-Meier para estimar las curvas de supervivencia, utilizando el tiempo hasta el reingreso como variable dependiente. También se realizó un análisis multivariante. Los parámetros de validez y curvas ROC se calcularon y relacionaron con futuros reingresos. Los análisis se realizaron con el programa SPSS versión 19 (**Capítulo 3**).

Además, llevamos a cabo dos revisiones sistemáticas de la literatura a partir de cuatro bases de datos (EMBASE, Medline, PubMed, LILACS) siguiendo una estrategia de búsqueda exhaustiva y un protocolo predefinido según las directrices recogidas en la guías PRISMA. En el **Capítulo 4** se incluyeron 43 estudios de neuroimagen basados en la administración experimental de cannabinoides en animales no tratados previamente y consumidores puntuales/ocasionales de cannabis. El **Capítulo 5** incluyó 45 estudios de neuroimagen en consumidores crónicos de cannabis y un grupo control emparejado.

Por último, realizamos un estudio caso-control de neuroimagen en hombres caucásicos, 30 consumidores crónicos de cannabis de inicio temprano y 29 controles no consumidores emparejados en edad, educación e inteligencia. Todos los participantes fueron evaluados mediante una entrevista estructurada (PRISM) para

excluir cualquier trastorno psiquiátrico del eje-I según el DSM-IV. Se genotiparon la catecol-O-metiltransferasa (COMT *Val*^{158}*Met*, rs4680) y el transportador de la dopamina (DAT1 VNTR). Los datos de imagen se analizaron mediante VBM (**Capítulo 6**) y el trazado manual del hipocampo siguiendo una metodología validada (**Capítulo 7**).

Resultados

El cannabis fue la sustancia de abuso más común en la cohorte de primeros episodios psicóticos, tanto en los análisis de orina (38%) como informado por los propios pacientes (50%). Tanto la subescala DALI cannabis/cocaína (p=0.002) como la positividad en orina para cannabis se asociaron a un mayor riesgo de reingreso en las curvas de supervivencia, sobretodo durante los primeros cinco años de seguimiento. La subescala DALI cannabis/cocaína se mantuvo como predictor significativo de reingreso durante el periodo de estudio [HR = 4.5; 95% CI = 1.1 to 18.7; p=0.036] tras controlar por posibles factores de confusión (genero, edad, duración de psicosis sin tratar y subescala positiva de la PANSS) (**Capítulo 3**).

Los estudios incluidos en el **Capítulo 4** mostraron que la administración aguda de cannabinoides es capaz de modular la actividad del cerebro en reposo y alterar la actividad neural durante la ejecución de diversas tareas cognitivas en regiones cerebrales relacionas con el circuito de recompensa y trastornos mentales. En contraste con los estudios en animales, los escasos estudios neuroquímicos realizados en humanos presentaron inconsistencias en relación al incremento de la actividad dopaminérgica que podría estar relacionada con la psicosis inducida por THC. Del **Capítulo 5** se desprende que el uso crónico de cannabis está asociado con alteraciones en la función y estructura cerebral, especialmente en regiones temporales mediales tanto en adultos como en adolescentes, y que la cantidad de exposición a cannabis puede estar relacionada con su efecto perjudicial.

En el estudio caso-control, los consumidores crónicos de cannabis de inicio temprano presentaron alteraciones morfológicas en las regiones señaladas en los

Capítulos 4 y 5, las cuales fueron influenciadas de forma distinta por los genotipos COMT y DAT1 dependiendo si el sujeto había estado expuesto regularmente a cannabis. En particular, el genotipo de la COMT moduló el volumen de dos de las cuatro regiones exploradas mediante VBM (**Capítulo 6**). La variación del genotipo de la COMT afectó al núcleo caudado ventral bilateral en ambos grupos en una dirección opuesta. Es decir, más copias del alelo *val* se relacionaron con un menor volumen en los consumidores crónicos de cannabis pero un mayor volumen en los controles. El patrón opuesto se halló en la amígdala izquierda. El **Capítulo 7** amplió estos resultados al mostrar que los genes COMT y DAT1 interactuaron entre sí para moderar diferencias individuales en el volumen del hipocampo. La asociación entre estos polimorfismos funcionales y los volúmenes del hipocampo sugirieron una relación lineal con la disponibilidad de dopamina en los controles que no se observó en los consumidores crónicos de cannabis. Los volúmenes del hipocampo fueron menores en los consumidores crónicos de cannabis en comparación con los controles, y la magnitud de la reducción volumétrica se asoció con la exposición de cannabis a lo largo de la vida.

Conclusión

En su conjunto, estos resultados dan soporte a la participación del sistema endocannabinoide en el curso de los trastornos mentales, así como en el control de distintas funciones cognitivas, modulación de dopamina y volumen cerebral, apareciendo alteraciones derivadas de su uso crónico de forma temprana. Los resultados también demuestran que los genes reguladores de la dopamina pueden desempeñar un papel particular en la sensibilidad a los efectos del cannabis en la morfología cerebral, proporcionando nuevos conocimientos sobre los mecanismos subyacentes al daño cerebral inducido por cannabis y sobre aspectos de vulnerabilidad genética.

1

Introduction

Introduction

Given its status as the most commonly used illicit drug worldwide, there is growing interest in the potential effects of cannabis on mental health. Cannabis main psychoactive constituent, Δ9-tetrahydrocannabinol (THC), may lead in some individuals to a range of acute and chronic mental health problems, such as dependence, mood, anxiety and psychotic disorders, as well as to neuropsychological deficits. Moreover, cannabis use may worsen the outcome of established mental disorders. THC exerts its effects through the endocannabinoid system, which plays an important role in neurodevelopment and regulating the neuronal activity of other neurotransmitter systems. Disturbances of the endocannabinoid system by exogenous cannabinoids may be responsible for long-lasting effects, such as psychiatric disorders and brain impairment. As not all the exposed individuals are affected, proneness to cannabis-induced impairment may rely on key factors such as age of onset and cannabis use parameters (e.g., quantity, frequency, duration), as well as aspects related to individual's genetic background.

Psychiatric illnesses and effects of persistent cannabis use

Despite some evidence of decreasing trends, cannabis use in Europe remains disturbingly high, especially among young population (1) (Figure 1.1). An estimated 15.4 million young Europeans aged between 15 and 34 (11.7% of this age group) used cannabis in the last year, with 9.2 million of these aged 15-24 (14.9%). Cannabis products are generally smoked and frequently mixed with tobacco. However, there is also an increasing diversity in the types of cannabis products available. Herbal cannabis, sometimes of high potency, now plays a more important role, accompanied by the

Figure 1.1. Last year prevalence of cannabis use among young adults (15-34 years) in Europe (from European Monitoring Centre for Drugs and Drug Addiction (2013). European drug report 2013: trends and developments. Lisbon, EMCDDA).

recent appearance of synthetic cannabinoids, which mimic the effects of the naturally occurring psychoactive compounds found in cannabis (1).

The main active and most studied compounds isolated from cannabis are THC and cannabidiol (CBD) (2, 3). THC is thought to be responsible for most of its psychotropic effects. In contrast, CBD is the major non-psychomimetic constituent, and it has been found to induce anxiolytic effects (4) and even antipsychotic properties (5). However, concentrations of THC and CBD in the different preparations of cannabis have changed in the last few years, with claims of a sharp increase in the THC/CBD ratio (1), which may result in a heightened risk of psychiatric symptoms (6, 7).

Dependence

Although most of individuals tend to cease cannabis use after the initial trial with the drug (1), about one in ten users eventually becomes dependent (8). Cannabis use represents the second most commonly reported substance for clients entering specialised drug treatment (1). Average profile of first-time treatment entrants with cannabis as primary drug are 25-year old males (84%), whom started using cannabis at 16 and report daily use (47%) (1). Signs of dependence and psychiatric problems are frequently reported among those users who have repeatedly tried to discontinue cannabis use and seek for treatment (9, 10).

Cognitive deficits

During acute cannabis intoxication, several cognitive effects have been identified, including effects on learning and memory performance (11). Beyond the period of acute intoxication, accumulating evidence suggests that chronic cannabis use may cause enduring neuropsychological impairment (12, 13), with some (14, 15) but not all studies (13) showing that the effects may remain even after extended periods of abstinence. The precise mechanism underlying the association between cannabis use and neuropsychological decline has not been clearly elucidated. However, it has been

suggested that cannabis use may cause brain functional and structural changes that may result in neuropsychological impairment (16).

Mood and anxiety disorders

Panic and anxiety attacks are commonly psychiatric symptoms reported during cannabis intoxication and are often responsible for the discontinuation of the use of the drug (17). Bipolar disorder, panic disorder, social phobia, generalized anxiety disorder and obsessive-compulsive disorder have also been related to cannabis use. Probability ratios of these disorders among cannabis users seeking treatment for dependence are higher compared to age- and gender-matched control subjects (18). In addition, results from longitudinal studies point out that whereas individuals with depression would not be more prone to use cannabis during follow-up, cannabis use may modestly increase depressive symptoms (19).

Psychotic disorders

Cannabis use has consistently been associated to psychotic symptoms (20), including disabling psychotic disorders (21). After cannabis use, around 15% of users experience transitory psychotic symptoms (22) and the risk to develop later psychotic illness is roughly doubled (21). Theories indicating that cannabis use may be secondary to a pre-existing psychosis have lost support (10, 23). The contrasting conclusions of self-report and epidemiological studies raise the possibility that schizophrenia patients may derive some immediate benefits from cannabis at the expense of later, negative consequences (24).

Outcome of persistent cannabis use

Substance misuse among patients with established psychiatric diagnose, particularly those with psychotic illness, is higher than in the general population (25). Cannabis use, especially in the early stages of the psychotic illness, may alter the

course, the phenomenology and the outcome of the disease by decreasing age of onset (7) and increasing non-adherence, relapses and hospitalisations (26). Therefore, assessing for substance use disorders in the early stages of the illness is crucial, as potential interventions may have greatly impact on long-term outcome (**Chapter 3**).

The endocannabinoid system

The mentioned effects and consequences of cannabis use are probably mediated by disturbances of the endocannabinoid system, which regulates several biological processes. The endocannabinoid system comprises lipid-derived ligands or endocannabinoids (eCBs), receptors and enzymes participating in the synthesis and degradation of the eCBs (27). The most characterized eCBs are 2-arachidonylglycerol (2-AG) and anandamide (28, 29). Endocannabinoids are synthesized and released postsynaptically and immediately diffuse to nearby cannabinoid G protein-coupled receptors, which are expressed on pre-synaptic terminals (Figure 1.2). This retrograde signalling works 'on-demand', that is, eCBs are released when and where they are needed (30).

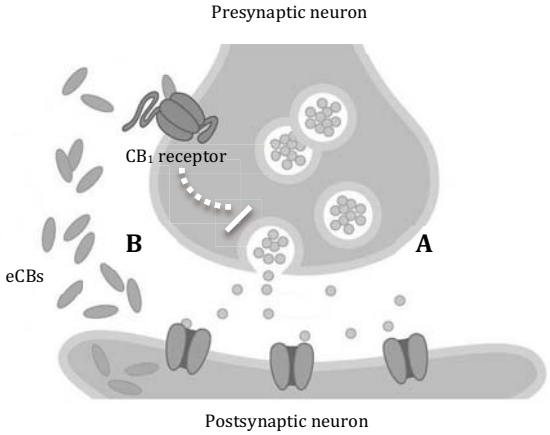


Figure 1.2. **(A)** In the synapse between two neurons, information is transferred from the presynaptic to the postsynaptic neuron through neurotransmitters that cross the synaptic cleft. **(B)** The endoconnabinoid system controls neurotransmitter release mainly in a retrograde manner: endocannabinoids (eCBs) are released postsynaptically and bind to presynaptically located cannabinoid receptors (CB_1).

The best characterized cannabinoid receptors are the cannabinoid receptor-1 (CB_1) and -2 (CB_2) (31, 32). While CB_2 receptors are mainly located peripherally in the cells of the immune system, CB_1 receptors are widely distributed throughout the

brain, mainly in glutamatergic and GABAergic neurons. The highest densities of CB_1 receptors are found in the prefrontal cortex, basal ganglia, medial temporal areas and cerebellum (33). The control of CB_1 receptors over neurotransmitter release in these regions is critical for cognitive processes, reward, motor function and psychiatric symptoms (34).

Moreover, the endocannabinoid system plays an important role in neuromaturation and synaptic pruning (35-37). Ninety per cent of the brain's total volume has developed by around the age of 6 (38) but global cortical development follows an inverted U-shaped trajectory, peaking around 12 to 14 years of age then decreasing in volume and thickness over adolescence (39). This synaptic pruning occurs firstly in primary sensorimotor areas and last in high-order association areas, such as the prefrontal cortex and temporal lobe (39). Given the complex processes going on at this time, it is feasible that the brain may be more vulnerable to disturbances from exogenous cannabinoids that may alter normal brain functioning. Therefore, it is possible that use of cannabis during this neurodevelopmental period may trigger psychiatric disorders, such as psychosis, or cause cognitive deficits or brain alterations in vulnerable subjects (40, 41).

As the neural networks showing functional and structural alterations under cannabis exposure are similar to those during psychotic and pre-psychotic states (42-46), it is relevant to investigate the acute effects of cannabinoids on brain, as well as the possible pathways that may lead to psychosis (**Chapter 4**).

Neurodevelopmental influences

The fact that not all users experience brain abnormalities or psychiatric disorders after chronic cannabis exposure suggests that some factors may play a role amplifying the risk. These factors may be related to disturbances of the neurodevelopmental processes carried by the endocannabinoid system in vulnerable subjects. Age of first exposure to cannabis, duration and amount of cannabis used, along with individuals'

genetic vulnerability may help to understand the mechanisms underlying cannabis-related brain impairment.

Age of onset and dose-related effects

In order to understand how cannabis may interfere with neurodevelopmental processes, animal studies have compared how administration of cannabinoids during developmental periods affects neurocognitive processes in adolescent and adult rats. For instance, rats exposed to synthetic cannabinoids or THC during adolescence experience impaired working memory during adulthood (47-49). These impairments have been correlated with less active synapses in the prefrontal cortex, as well as shorter dendrites and reduced spine densities in the hippocampus (49), suggesting enduring neurobiological consequences of early cannabis exposure. This vulnerability of younger brains is highlighted by the fact that the same amount of THC exposure that led to decreased working memory performance in adolescent rats may have no effect in adult rats (50). Consistently, rats exposed to chronic doses of THC during adolescence but not during late adolescence evidenced deficits in learning during adulthood (51). Taken together, these findings suggest that there may be a critical time during adolescence when cannabis use may have the most negative effects.

This data is consistent with growing evidence in human studies showing that initiation of cannabis during early adolescence may be more detrimental compared to later initiated use. Earlier age of cannabis use onset have been negatively associated with neurocognitive functioning in several studies (15, 16, 52-57). Those who initiate cannabis use before 15 to 17 years of age show more prominent deficits in memory (15, 56), visual attention (53), verbal fluency (15, 55), inhibition (15, 54, 55) and other domains of cognitive functioning (54). Paradoxically, despite the aforementioned evidence and that the onset of cannabis use is typically during early adolescence, there is a lack of neuroimaging studies conducted in adolescent users (58). While the long-term effects of cannabis use may potentially have major implications for social and family life, education and occupational functioning, its effects on brain function and structure have not been well determined.

Actually, current knowledge about chronic cannabis effects on brain structure is mostly inferred from animal studies. It has been demonstrated that THC induces dose-dependent toxicity and structural changes in brain regions rich in cannabinoid CB_1 receptors (50, 59-63). This data is consistent with human studies showing a dose-related neurocognitive (16) and psychiatric (21) effects of cannabis. However, the investigation of the structural effects of long-term cannabis use on the human brain has brought less consistent findings. The discrepancy in the results might be due to heterogeneity in sample characteristics, methodological differences in data processing and inter-individual differences related to amount of consumption and genetic vulnerability. Nevertheless, changes in medial temporal regions, such as the hippocampus/parahippocampal complex and the amygdala, have often been reported (64-67). Importantly, these findings suggest that long-term cannabis use is associated with brain morphology alterations in regions linked to schizophrenia, as well as to memory, executive and affective processing.

One question of interest is whether chronic cannabis use is associated with early effects on brain function and structure. More specifically, whether chronic use of cannabis in the adolescence may lead to early and similar changes in brain function and structure as studies performed in adult population (**Chapter 5**).

Genetic vulnerability

Another explanation as to why only some individuals develop cannabis-related brain impairment is that certain individuals may be especially genetically vulnerable. Variation in the expression of genes implicated in the regulation of neurotransmitters may play an important role in determining individual variability in several outcomes, such as clinical features [e.g. psychosis (68-70), cognition (71)], neuronal activity (72, 73) and brain volume (74-76).

Dopaminergic function has been shown to be involved in psychosis, cognition, brain activity and structure, hence variation in dopaminergic candidate genes might be related to variations in such features (Figure 1.3). Dopamine inactivation from the

extracellular space involves both catechol-O-methyltransferase (COMT) and the dopamine transporter (DAT1) (77). The COMT (*Val*^{158}*Met*, rs4680) gene displays a single-nucleotid polymorphism (SNP), which results in three genotypes (val/val, val/met, and met/met) (78). Whereas the met/met variant shows a 40% lower enzymatic activity, which is associated with high levels of extrasynaptic dopamine, the val/val variant implies higher enzymatic activity, which results in low levels of extrasynaptic dopamine (78). The DAT1 gene displays a polymorphic 40-base pair (bp) variable number of tandem repeats (VNTR) in an untranslated region (UTR). This polymorphism consists of a repetition of 40 bp that leads to several alleles, 9- and 10-repeat alleles being the most common (79). The 10-repeat allele has been associated with increased gene expression (80).

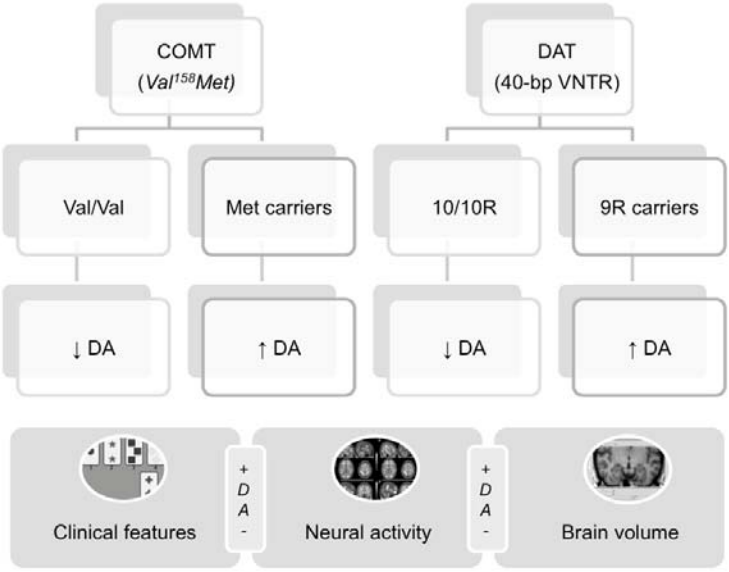


Figure 1.3. Variation in the expression of the dopamine-regulating genes has been related to differences in the dopamine (DA) available in the synapse. Such differences may explain certain variations when exploring several outcomes (e.g. clinical features, neural activity or brain volume) in different populations, such as healthy subjects, psychiatric patients or subjects chronically exposed to certain environmental factors (e.g. cannabis).

Variations in these dopamine-regulating genes may determine variations in clinical outcomes, such as cognitive tasks or psychosis risk. Thus, genetic variants related with low levels of dopamine available in the synapse may result in a combination of two processes. First, reduced dopamine neurotransmission in the prefrontal cortex, which is associated with impairments in working memory, attention, and executive functioning (81, 82). And subsequently, increased levels of mesolimbic dopamine signaling (83) which is hypothesized to result in an increased risk of experiencing psychosis (84).

In the same line, studies assessing the influence of the COMT polymorphism on neural activation during cognitive tasks have also reported variations in brain activity. For instance, studies exploring response inhibition and working memory suggest that *val* carriers, associated with lower dopamine levels, would need larger brain recruitment of certain brain areas in order to perform the task efficiently (72, 85). Moreover, the COMT and DAT1 genotypes have also been shown to interact with each other in the modulation of cortical activity in several brain regions, including the hippocampus (72, 86, 87).

Finally, it has also been reported that these functional polymorphisms may affect brain volume in healthy individuals (74, 75) as well as in subjects at risk for psychosis and with schizophrenia (88-90). Healthy subjects and schizophrenia patients carrying the *val* allele have shown significantly smaller medial temporal lobe volumes relative to met homozygotes (75, 90). However, no previous studies have examined the influence of these dopamine-regulating genes on brain volume in subjects chronically exposed to cannabis.

Considering the potential harmful effect of cannabis in brain structure, especially in early-onset users, it is relevant to investigate whether chronic cannabis use is related to volumetric alterations and whether variation in the dopamine-regulating genes may result in diverse liability to experience brain impairment (**Chapter 6**). In addition, it is interesting to know if these genes interact to moderate individual differences in areas particularly vulnerable to heavy cannabis exposure, such as the

hippocampus, and whether the nature of this association depends on previous cannabis exposure (**Chapter 7**).

2

Aims and hypothesis

Aims

Primary aims

- Assess the influence of drug use on readmission risk in a cohort of patients consecutively admitted to the inpatient unit of a general hospital for experiencing psychotic symptoms for the first time.
- Assess the impact of the acute experimental administration of cannabinoids on brain function in naïve or occasional cannabis users and in animals, focusing on neuroimaging studies that examine patterns of change in dopamine release, brain activation or cerebral blood flow.
- Assess the evidence of the impact of chronic cannabis use on brain structure and function through neuroimaging studies performed in chronic cannabis users with a matched control group.
- Explore the brain morphology of early-onset chronic cannabis users compared to non-using controls while assessing the influence of the COMT genotype in predetermined brain areas.
- Explore whether variation in the COMT and DAT1 genes interact with each other to moderate individual differences in hippocampal volume in early-onset chronic cannabis users compared to non-using controls.

Secondary aims

- Test whether a screening drug scale (the Dartmouth Assessment of Lifestyle Inventory scale (DALI) cannabis/cocaine subscale) is a better instrument than a positive urine sample for predicting readmission.
- Explore whether dopamine release is involved in psychosis induced by cannabis and whether chronic use of cannabis in the adolescence may lead to early and similar changes in brain function and structure as studies performed in adult population.
- Assess morphological brain changes in early-onset chronic cannabis users compared to non-using controls irrespective of genotype, as well as potential correlations between brain volume and cannabis use patterns.

Hypothesis

The hypotheses of the present thesis are:

- Considering that cannabis use may lead to mental health problems and worsen the outcome of established mental disorders, we hypothesize that cannabis use in first-episode psychosis would be associated with worse outcome regarding readmission rates, either measured by a screening drug scale or urinalysis.
- Given that the neural networks showing functional and structural alterations under cannabis exposure may be similar to those during psychotic and pre-psychotic states, we hypothesise that acute and chronic cannabis use would be associated with alterations on brain function and structure in key areas relevant for psychiatric disorders, and that these alterations would also be present in adolescent users.
- Cannabis-psychosis link suggests that variation in the expression of genes implicated in the regulation of dopamine may play a role in determining individual vulnerability to cannabis. We postulate that early-onset chronic cannabis users would show morphologic brain alterations compared to healthy controls, and that variation in the dopamine-regulating genes would result in diverse liability to experience cannabis-related brain impairment.

3

Study 1

Screening for substance use disorders in first-episode psychosis: Implications for readmission

Schizophrenia Research 2013; 146(1-3):125-31

Study 1

Summary

Reference

Title: Screening for substance use disorders in first-episode psychosis: Implications for readmission. Authors: Batalla A, Garcia-Rizo C, Castellvi P, Fernandez-Egea E, Yücel M, Parellada E, Kirkpatrick B, Martín-Santos R, Bernardo M. *Schizophrenia Research 2013; 146(1-3):125-131*. Impact factor 2012: 4.590 (1st quartile psychiatry).

Aims

Based on *hypothesis #1*, the aim of the present study was to assess the influence of drug use on readmission risk in a cohort of patients consecutively admitted to the inpatient unit of a general hospital for experiencing psychotic symptoms for the first time. We further tested whether a screening drug scale (the Dartmouth Assessment of Lifestyle Inventory scale (DALI) cannabis/cocaine subscale) was a better instrument than a positive urine sample for predicting readmission.

Method

First-episode psychotic patients were consecutively recruited at the time of their first clinical contact for non-affective psychotic symptoms at a general academic hospital (Hospital Clínic, Barcelona). This cohort is part of a larger study of metabolic abnormalities and glucose dysregulation in neuropsychiatric disorders (91, 92), conducted with approval of the ethics committee of Hospital Clínic. The final sample consisted of 58 patients, all of whom gave informed consent prior to participating.

Patients included in the present work were recruited from 1st January 2004 to 31st October 2010. After discharge, patients were follow-up by outpatient services of the hospital. The main outcome was the time until the patient was readmitted for the first

time to the hospital's inpatient unit. Therefore, the follow-up time period was defined as days since discharge from the first hospitalisation until readmission or censoring from the study. The end of the study period was the 30th April 2011.

All subjects were interviewed using the Spanish versions of the Structured Clinical Interview for DSM-IV Axis I Disorders, clinician version (SCID-I) (93), the Spanish version of the Positive and Negative Syndrome Scale (PANSS) (94) and the DALI scale (95). The DALI scale, which is based on 18 items, focused on detecting substance use disorders in people with severe mental illness, and includes alcohol and drug screen subscales. The included subjects also underwent blood and urine sampling within 48 hours after admission.

The Kaplan-Meier estimator was applied to estimate the survival curves for bivariate analysis, using time to readmission as a dependent outcome. The Cox proportional hazards model for multivariate analysis was assessed to control for potential confounders. Sensitivity, specificity, positive and negative predictive values of the DALI cannabis/cocaine subscale and urine test were calculated and related to future readmissions, as well as ROC curves. Analyses were performed using Statistical Package for the Social Sciences (SPSS) version 19.

Results

Of the 58 admissions, psychoactive substances on urine/blood samples (excluding benzodiazepines) were detected in 25 subjects (43%). Cannabis was the most common drug found in urinalysis (38%) and self-reported (50%). The DALI cannabis/cocaine subscale classified 29 patients (50%) as being at high risk of cannabis and/or cocaine use disorders and 11 (19%) as at high risk of alcohol use disorders.

Bivariate survival analysis of time to first readmission following the first psychotic episode was significant for the DALI cannabis/cocaine subscale ($p=0.002$) and for urine analysis for cannabis ($p=0.02$) (Figure 3.1 A & B). Younger age ($p=0.03$), male gender ($p=0.04$) and high scores in the PANSS positive subscale ($p<0.001$) were also

associated with readmission during the follow-up period. Alcohol use [positivity in urine/blood samples (p=0.77) and DALI alcohol subscale (p=0.33)] was not associated with readmission.

In the multivariate analysis, the DALI cannabis/cocaine subscale at baseline was a significant predictor of readmission over the study period [HR (Hazard Ratio) = 4.5; 95% CI (Confidence Interval) = 1.1 to 18.7; p=0.036] after controlling for potential confounders (gender, age, duration of untreated psychosis and PANSS positive subscale), while urine analysis for cannabis was not (HR=2.9; 95% CI=0.7 to 5.7; p=0.20).

ROC curve showed greater area under the curve (AUC) for the DALI cannabis/cocaine subscale (0.716; 95% CI=0.572 to 0.860) than the positive urine analysis for cannabis (0.626; 95% CI=0.462 to 0.791) (Figure 3.1 C).

Conclusion

The DALI cannabis/cocaine subscale and urinalysis for cannabis were associated with increased readmission risk in survival curves, mainly during the first five years of follow-up.

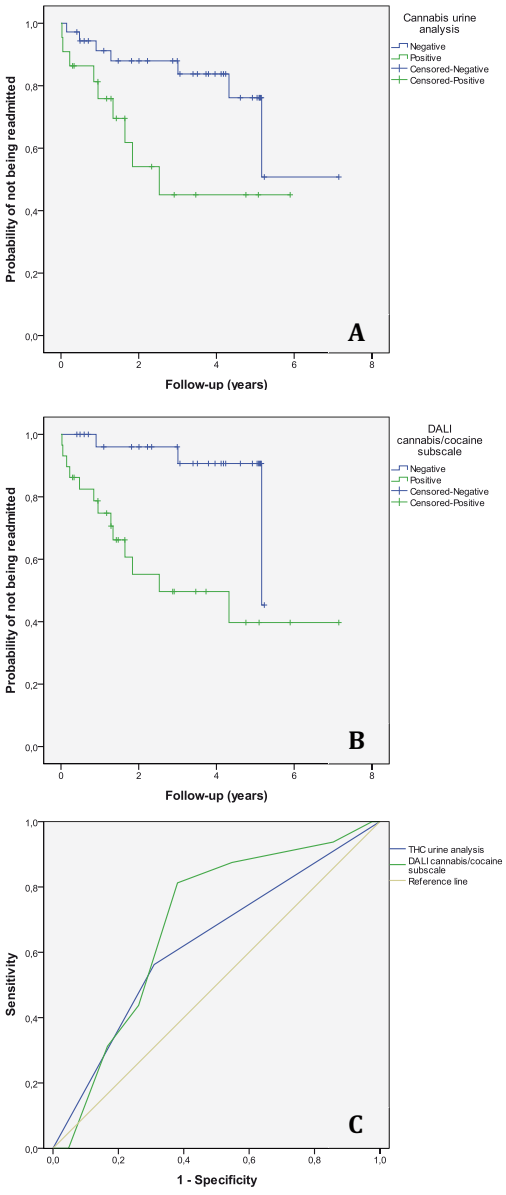


Figure 3.1. (A) & (B) Survival plot of cannabis urine analysis and DALI cannabis/cocaine subscale, respectively. **(C)** ROC curves of DALI cannabis/cocaine subscale compared with positive urine analysis for cannabis for readmission during the whole study period.

Schizophrenia Research 146 (2013) 125–131

Contents lists available at SciVerse ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Screening for substance use disorders in first-episode psychosis: Implications for readmission

Albert Batalla [a,b,*], Clemente Garcia-Rizo [c], Pere Castellví [a,d,e], Emili Fernandez-Egea [f,g], Murat Yücel [h], Eduard Parellada [c], Brian Kirkpatrick [i], Rocío Martin-Santos [a,b], Miguel Bernardo [c]

[a] Department of Psychiatry and Psychology, Clinical Institute of Neuroscience, Hospital Clínic, Institut d'Investigació Biomèdica August Pi i Sunyer (IDIBAPS), Centro de Investigación Biomédica en Red en Salud Mental (CIBERSAM), Spain
[b] Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Villarroel 170, 08036, Barcelona, Spain
[c] Schizophrenia Program, Department of Psychiatry and Psychology, Clinical Institute of Neuroscience, IDIBAPS, CIBERSAM, Hospital Clínic, University of Barcelona, Villarroel 170, 08036, Barcelona, Spain
[d] Health Services Research Unit, IMIM (Hospital del Mar Medical Research Institute), and CIBER Epidemiología y Salud Pública (CIBERESP), Doctor Aiguader 88, 08033, Barcelona, Spain
[e] CIBER Epidemiología y Salud Pública (CIBERESP), Doctor Aiguader 88, 08033, Barcelona, Spain
[f] Good Outcome Schizophrenia Clinic, Cambridgeshire and Peterborough NHS Foundation Trust, UK
[g] Department of Psychiatry and Behavioural and Clinical Neuroscience Institute (BCNI), University of Cambridge, Forvie Site, Cambridge CB2 0SZ, UK
[h] School of Psychology and Psychiatry, Monash University, Clayton Campus, Melbourne, Victoria 3800, Australia
[i] Department of Psychiatry, Texas A&M University College of Medicine, Scott & White Healthcare, 1901 South Veterans Memorial Drive, 76504, Temple, TX, United States

ARTICLE INFO

Article history:
Received 23 October 2012
Received in revised form 5 February 2013
Accepted 24 February 2013
Available online 19 March 2013

Keywords:
Screening
Substance use
Cannabis
First-episode psychosis
Readmission

ABSTRACT

Introduction: Screening of substance use may prove useful to prevent readmission after the first episode of psychosis. The aim of the present study was to evaluate the influence of drug use on readmission risk in a first-episode psychosis sample, and to determine whether the cannabis/cocaine subscale of the Dartmouth Assessment of Lifestyle Inventory (DALI) is a better predictive instrument than urinary analysis.
Methods: After admission, first-episode psychotic patients were interviewed for substance use and assessed with the DALI scale. They also underwent blood and urine sampling. Time to readmission was studied as a dependent outcome. The Kaplan–Meier estimator was applied to estimate the survival curves for bivariate analysis. The Cox proportional hazards model for multivariate analysis was assessed in order to control for potential confounders. ROC curve and validity parameters were used to assess validity to detect readmission.
Results: Fifty-eight patients were included. The DALI cannabis/cocaine subscale and urinalysis were associated with increased readmission risk in survival curves, mainly the first five years of follow-up. After controlling for potential confounding variables for readmission, only the DALI cannabis/cocaine subscale remained as a significant risk factor. In terms of validity, the DALI cannabis/cocaine subscale was more sensitive than urinalysis. Alcohol assessments were not related to readmission.
Conclusions: The findings demonstrated that a quick screening self-report scale for cannabis/cocaine use disorders is superior to urinary analysis for predicting readmission. Future research should consider longitudinal assessments of brief validated screening tests in order to evaluate their benefits in preventing early readmission in first-episode psychosis.

1. Introduction

Identifying modifiable prognostic factors for preventing recurrent psychotic episodes is an extremely important issue (Lambert et al., 2005). Misuse of tobacco, alcohol, cannabis and other illicit substances is common among people with psychotic illnesses (Regier et al., 1990; Kavanagh et al., 2002; Margolese et al., 2004). A high prevalence of substance misuse is also characteristic of patients with first-episode psychosis, with rates varying from 22% to over 50% (Cantwell et al., 1999; Van Mastrigt et al., 2004; Lambert et al., 2005; Larsen et al., 2006; Addington and Addington, 2007; Wade et al., 2007; Baeza et al., 2009; Kamali et al., 2009). Drug misuse, especially cannabis in the early stages of psychosis, has been associated with younger age of onset (Cantwell et al., 1999; Van Mastrigt et al., 2004; Addington and Addington, 2007; Sugranyes et al., 2009), increased symptoms (Lambert et al., 2005; Addington and Addington, 2007; Baeza et al., 2009), poorer treatment compliance (Buhler et al., 2002; Green et al., 2004; Zammit et al.,

* Corresponding author at: Department of Psychiatry and Psychology, Clinical Institute of Neuroscience, Hospital Clínic, IDIBAPS, CIBERSAM, and Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Calle Villarroel, 170, 08036-Barcelona, Spain. Tel.: +34 93 227 55 47; fax: +34 93 227 55 48.
E-mail addresses: batalla@clinic.ub.es (A. Batalla), cgarcia3@clinic.ub.es (C. Garcia-Rizo), ef280@cam.ac.uk (E. Fernandez-Egea), murat@unimelb.edu.au (M. Yücel), eparella@clinic.ub.es (E. Parellada), bkirkpatrick2@aol.com (B. Kirkpatrick), rmsantos@clinic.ub.es (R. Martin-Santos), bernardo@clinic.ub.es (M. Bernardo).

0920-9964/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.schres.2013.02.031

2008), higher rates of relapses and more hospitalizations (Linszen et al., 1994; Cantor-Graae et al., 2001; Salyers and Mueser, 2001; Sorbara et al., 2003; Zammit et al., 2008). Therefore, good screening for substance use during this phase of the illness may prove useful as a predictor of relapse. In spite of this, few longitudinal studies have investigated the impact of substance use on readmission to hospital. Detection and screening of substance use are typically undertaken through clinical interviews, patients' self-reports or toxicological tests. Urinalysis, though reliable and valid, has a narrow window of detection; for their part, structured diagnostic procedures are able to identify a high prevalence of drug use disorders but they are not practical on a day-to-day basis (Bennett, 2009). Research on screeners suggests that brevity is essential for an instrument to be adopted for regular use (Tiet et al., 2008). Although several screening scales are available (Tiet et al., 2008), they are not routinely studied in longitudinal cohorts involving psychotic patients, since these cohorts usually use self-report measures (Grech et al., 2005; Stirling et al., 2005; Hides et al., 2006; Degenhardt et al., 2007), structured interviews (Coldham et al., 2002; Green et al., 2004; Pencer et al., 2005; Wade et al., 2006) or urine drug screening (Grace et al., 2000; Hides et al., 2006). Therefore, their potential influence on outcome measures such as readmission is not frequently considered. Furthermore, screening measures may miss many diagnoses due to their having been developed in the general population or in primary substance abusing samples, with the result that their relevance to people with severe mental illness is doubtful (Bennett, 2009). One potential solution may be the use of screening measures specifically developed for people with psychiatric disorder (Bennett, 2009), such as the Dartmouth Assessment of Lifestyle Inventory (DALI), an 18-item screening questionnaire designed to identify substance use and abuse in people with severe mental illness. The scale contains two subscales: one for assessing the risk of alcohol use disorders and the second for assessing the risk of cannabis and/or cocaine use disorders. The main strengths of the scale are its brevity, as the mean time of administration is approximately 6 min, and its high classificatory accuracy for alcohol, cannabis and cocaine use disorders (Rosenberg et al., 1998; Ford, 2003). However, it has not yet been used to evaluate outcome measures in first-episode psychosis cohorts such as risk for readmission, and its predictive validity has not been explored.

The aim of the present study was to evaluate the influence of drug use on readmission risk in a first-episode psychosis sample, and to establish whether the DALI cannabis/cocaine subscale is a better predictive instrument than a positive urine sample.

2. Methods

2.1. Subjects

Non-affective first-episode psychotic patients were consecutively recruited at the time of their first clinical contact for psychotic symptoms at a general academic hospital (Hospital Clinic, Barcelona). As part of the Spanish National Health System, the hospital offers inpatient and outpatient services to the 560,000 inhabitants who live in the surrounding catchment area. The area is a relatively homogeneous middle/upper-middle class neighborhood in the center of the city, in which Hospital Clinic is the regional referral center for psychosis. The patients met criteria for schizophrenia, schizophreniform disorder, brief psychotic disorder, delusional disorder or psychosis not otherwise specified and had a maximum cumulative (lifetime) antipsychotic exposure of one week and no antipsychotic use in the 30 days prior to the study (although in this particular study, all subjects were drug naïve). Subjects were allowed to receive antianxiety medication (lorazepam) the night before blood was drawn, up to a maximum of 3 mg, but not on the day of the assessment. Additional inclusion and exclusion criteria for all subjects were: 1) age from 18 to 64 years, 2) no history of diabetes or other serious medical or neurological condition associated with glucose intolerance or insulin resistance (e.g. Cushing's disease), and 3) not taking medication associated with insulin resistance (hydrochlorothiazide, furosemide, ethacrynic acid, metolazone, chlorthalidone, beta blockers, glucocorticoids, phenytoin, nicotinic acid, cyclosporine, pentamidine, or narcotics).

One hundred and seven eligible patients were admitted during the study period. After excluding patients who did not have an address in the hospital catchment area (n = 39; 36.4%), patients not discharged during the recruitment period (n = 3; 2.8%) and patients whose blood/urine sample was not collected within 48 h (n = 7; 6.5%), the final sample consisted of 58 patients. There were no differences in baseline socio-demographic or clinical data between the excluded group and the study group: the variables assessed were age, gender, race, marital status, level of education and psychiatric history in first-degree relatives, scores on the Spanish version of the Positive and Negative Syndrome Scale for Schizophrenia (PANSS) (Peralta and Cuesta, 1994) and duration of untreated psychosis (DUP). DSM-IV diagnoses for the subjects included were schizophrenia (n = 40; 69.0%), brief psychotic disorder (n = 5; 8.6%), schizophreniform disorder (n = 4; 6.9%), and psychosis not otherwise specified (n = 9; 15.5%).

2.2. Procedures

Patients experiencing non-affective psychotic symptoms were consecutively admitted to the inpatient unit after their first contact with one of the hospital's psychiatric services. The recruitment period was from 1st January 2004 to 31st October 2010. All patients and their close relatives were carefully interviewed to ensure that inclusion and exclusion criteria were met. After discharge, the patients were followed up by outpatient services. All the interviews, assessments and follow-ups were performed by two fully trained psychiatrists in adult psychiatry (CGR and EFE). The main outcome was the time until first readmission to the hospital's inpatient unit. The follow-up time period was defined as days since discharge from the index admission until readmission or censoring from the study. The end of the study was set at 30th April 2011.

All subjects were interviewed using the Spanish version of the Structured Clinical Interview for DSM-IV Axis I Disorders, clinician version (SCID-I) (First and Spitzer, 1999). They were also administered the Spanish version of the PANSS (Peralta and Cuesta, 1994) and the DALI (Rosenberg et al., 1998). The DALI, which is based on 18 items—three non-scored used to establish the frame for the interview, and 15 scored—focuses on detecting substance use disorders in people with severe mental illness, and includes alcohol and drug screen subscales. The items of the scale were selected from ten instruments, and the scale was validated against the Structured Clinical Interview for DSM-III-R (SCID) (Spitzer et al., 1988) and the Clinician Rating Scale (Drake et al., 1990). The DALI drug screen had a sensitivity = 1.0, specificity = 0.80, positive predictive value (PPV) = 0.56 and negative predictive value (NPV) = 1.0, accuracy rate = 88%, kappa = 0.98, and area under the receiver operating characteristic (ROC) curve (AUC) = 0.93 for cannabis and cocaine disorders (Rosenberg et al., 1998). Among the nine questions related to alcohol, item 7, for example, assesses whether close friends or relatives have shown concern about the subject's alcohol use; and item 9 whether the subject sometimes drinks alcohol soon after getting up. Among the eight questions in the drug scale, item 13 assesses whether marijuana has caused the subject to lose a job; and item 16 whether cocaine use has caused the subject problems with close relatives. The socio-demographic variables recorded included: age, gender, race, marital status, level of education and psychiatric history in first-degree relatives. Self-reported drug use was recorded with a systematic ad hoc protocol which assessed whether tobacco, alcohol, cannabis, cocaine, amphetamines, LSD or ecstasy had been taken in

the last three months. DUP was defined as the interval from first psychotic symptom to first psychiatric hospitalization.

All subjects underwent blood and urine sampling as soon as possible after admission. Admissions during which at least one sample was obtained within 48 h were included in this study. All urine samples were screened for the following substances: benzodiazepines, cannabis, cocaine, amphetamines (amphetamines, methamphetamines and ecstasy), opiates, methadone and lysergic acid diethylamide (LSD), using an enzyme immunoassay method on the *Siemens* ADVIA automated chemistry analyzer. Broadly, urine samples show evidence of drug use between one and four days, although this timeframe may vary according to the chronicity of use and type of drug: for instance, chronic cannabis use may be detected up to three weeks after the last use (Verstraete, 2004). Blood samples were screened for alcohol using an enzymatic assay of alcohol dehydrogenase. Positive screening results were confirmed by gas chromatography (GC-FID). All subjects gave informed consent prior to participating. The study was conducted under the supervision of the ethics committee, and is part of a larger study of metabolic abnormalities and glucose dysregulation in neuropsychiatric disorders (Fernandez-Egea et al., 2009; Garcia-Rizo et al., 2012) and a gene–environment study in first-episode psychosis (Bernardo et al., 2012).

2.3. Statistical analysis

Time to readmission was studied as a dependent outcome. The Kaplan–Meier estimator (using log-rank test) was applied to estimate the survival curves for bivariate analysis. Patients were censored if they moved out of the hospital's recruitment area, died, were lost to follow-up or had not been readmitted by the end of the study. The Cox proportional hazards model for multivariate analysis was assessed to control for potential confounders.

Sensitivity, specificity, positive and negative predictive values of the DALI cannabis/cocaine subscale and urine test were calculated and related to future readmissions. ROC curves were also constructed between the DALI cannabis/cocaine subscale score and future readmission. The area under the curve (AUC) was calculated by means of the trapezoidal rule with 95% CI to find the best cutoff. ROC curves allow the examination of the entire range of sensitivities and specificities at each possible cutoff score. Statistical significance was set at $p = 0.05$. All analyses were performed using SPSS version 19.0 (SPSS version 19.0, for Windows, SPSS, Inc., Chicago, Ill).

3. Results

3.1. Descriptive analysis

Socio-demographic and clinical descriptive data are summarized in Table 1. Of the 58 admissions, psychoactive substances (excluding benzodiazepines) were detected in 25 patients (43.1%; 95% CI = 31.2% to 55.9%) on urine/blood tests. Cannabis was found in 22 patients (37.9%) and alcohol in four (6.9%). No other psychoactive substances were detected in urine/blood samples, although 65.5% (n = 38) of the patients reported having taken at least one substance of abuse (excluding tobacco) in the last three months: 32.8% (n = 19) alcohol, 50% (n = 29) cannabis, 24.1% (n = 14) cocaine, 5.2% (n = 3) amphetamines and 10.3% (n = 6) other substances (LSD or ecstasy). 53.4% (n = 31) reported having taken cannabis and/or cocaine. The DALI cannabis/cocaine subscale classified 29 patients (50%) as being at high risk of cannabis and/or cocaine use disorders and 11 (19.0%) as at high risk of alcohol use disorders. Eight of the eleven patients classified as being at high risk for alcohol use disorder were also classified as at high risk for cannabis/cocaine disorder.

The median (P_{25}–P_{75}) length of follow-up was 888 (348–1556) days in the total sample, 409 (105–861) days in patients readmitted and 1180 (508–1753) days in patients not readmitted. Reasons for censoring from the study were moving/lost to follow-up (n = 7; 12.1%) and end of the study period (n = 35; 60.3%). No patients died. Sixteen patients (27.6%) were readmitted during the whole follow-up period.

3.2. Bivariate analysis

Regarding drug use, bivariate survival analysis of time to first readmission following the first psychotic episode was significant both for urine analyses for cannabis and for the DALI cannabis/cocaine subscale (Table 1, Fig. 1). Younger age, male gender and high scores in the PANSS positive subscale were also significantly associated with readmission during the follow-up period (Table 1). In terms of alcohol use, neither positivity for alcohol urine/blood analysis nor DALI alcohol subscale was associated with readmission ($p = 0.773$ and $p = 0.330$, respectively).

3.3. Multivariate analysis

In the multivariate analysis (using Cox regression), the DALI cannabis/cocaine subscale at baseline was a significant predictor of readmission over the total study period, after controlling for gender, age, DUP and PANSS positive subscale (Hazard Ratio; HR = 4.5; 95% CI = 1.1 to 18.7; $p = 0.036$) while urine analysis for cannabis was not (HR = 2.0; 95% CI = 0.7 to 5.7; $p = 0.20$) (Table 2).

3.4. Validity of screening tests

Regarding the 58 initial admissions, only three (18.8%) readmissions were not recognized by the algorithm-based DALI cannabis/cocaine subscale (false negatives). ROC curve showed a greater AUC for the DALI cannabis/cocaine subscale (0.716; 95% CI = 0.572 to 0.860) than the positive urine analysis for cannabis (0.626; 95% CI = 0.462 to 0.791) (Fig. 1). The optimum cutoff point for DALI cannabis/cocaine subscale to predict readmission was above minus one. Using this cutoff in our sample, sensitivity and specificity for the DALI cannabis/cocaine subscale [0.81 (CI = 0.57–0.93) and 0.62 (0.47–0.75), respectively] showed better validity than those for the urine test [0.56 (CI = 0.33–0.77) and 0.69 (CI = 0.54–0.81), respectively], suggesting that this subscale is appropriate to predict readmission in this population (Table 3). Other measures to describe the validity of both screening tests are presented in Table 3.

4. Discussion

This study compared the efficacy of the DALI cannabis/cocaine subscale and urinalysis as predictors of readmission among adults with first-episode psychosis. Overall, both assessments were associated with increased risk of readmission, especially during the first five years of follow-up. However, after controlling for potential confounding variables for readmission, only the DALI cannabis/cocaine subscale remained a significant predictor. In terms of validity, the DALI cannabis/cocaine subscale was more sensitive than urinalysis. Alcohol assessments (DALI subscale and blood samples) were not related to readmission.

We found that nearly two thirds of our sample reported having taken at least one substance of abuse (apart from tobacco) in the last three months, while just under half recorded a positive result in the urine/blood analysis (excluding benzodiazepines). In agreement with other recent European studies in first-episode psychosis samples (Cantwell et al., 1999; Barnes et al., 2006; Larsen et al., 2006; Kamali et al., 2009; Van Dorn et al., 2012), cannabis was the most frequently reported substance of abuse, followed by alcohol and cocaine. The DALI cannabis/cocaine subscale showed that 50% of individuals with first-episode psychosis were at risk of a cannabis and/or cocaine use disorder and 19.0% at risk of alcohol use disorders, a rate that is in the

Table 1
Sample characteristics and bivariate survival analysis (Kaplan–Meier).

Variable	Descriptive	Probability to be readmitted	95% CI	p
Age: Mean (SD; range)	27.6 (6.6; 18–45)			0.03
18–23 years old: N (%)	19 (32.8)	0.54	0.48 to 0.60	
24–29 years old: N (%)	20 (34.5)	0.32	0.25 to 0.39	
>29 years old: N (%)	19 (32.8)	0.13	0.09 to 0.16	
Gender				0.04
Male: N (%)	39 (67.2)	0.60	0.55 to 0.65	
Female: N (%)	19 (32.8)	0.19	0.13 to 0.25	
Caucasian: N (%)	51 (87.9)	0.51	0.47 to 0.55	0.97
Single: N (%)	46 (79.3)	0.53	0.49 to 0.57	0.48
Level of education: N (%)				0.83
Primary education	13 (23.2)	0.67	0.54 to 0.80	
High school certificate	21 (37.5)	0.29	0.24 to 0.34	
Vocational training	9 (16.1)	0.33	0.19 to 0.47	
University graduate	13 (23.2)	0.35	0.25 to 0.45	
First-degree relatives with psychiatric history: N (%)	7 (12.1)	0.33	0.30 to 0.36	0.93
DUP: Mean (SD; range)	14.7 (19.8; 01–83)			0.77
≤12 months: N (%)	36 (62.1)	0.43	0.38 to 0.48	
>12 months: N (%)	22 (37.9)	0.53	0.45 to 0.61	
PANSS Positive subscale: Mean (SD)	26.0 (5.7)			<0.001
≤25	11 (19.0)	0.39	0.30 to 0.48	
Percentile N (%): 25–75	38 (65.5)	0.47	0.40 to 0.54	
≥75	9 (15.5)	0.80	0.69 to 0.91	
Cannabis urine analysis				0.021
Positive: N (%)	22 (37.9)	0.55	0.49 to 0.61	
Negative: N (%)	38 (62.1)	0.49	0.42 to 0.56	
Alcohol blood/urine analysis				0.773
Positive: N (%)	4 (6.9%)	0.46	0.41 to 0.51	
Negative: N (%)	54 (93.1)	0.51	0.44 to 0.58	
DALI cannabis/cocaine subscale				0.002
Positive: N (%)	29 (50.0)	0.60	0.55 to 0.65	
Negative: N (%)	29 (50.0)	0.55	0.42 to 0.68	
DALI alcohol subscale				0.330
Positive: N (%)	11 (19.0)	0.41	0.32 to 0.50	
Negative: N (%)	47 (81.0)	0.49	0.44 to 0.54	

CI: confidence interval; DUP: duration of untreated psychosis.

upper range for these studies (Cantwell et al., 1999; Barnes et al., 2006; Larsen et al., 2006; Kamali et al., 2009; Van Dorn et al., 2012). This may be explained by local and national differences in the pattern of substance misuse, as Spain is among the countries with the highest prevalence of alcohol, cannabis and cocaine use (European Monitoring Centre for Drugs and Drug Addiction, 2011). The finding that urinary analysis and blood samples under-detected cannabis, cocaine and alcohol use compared with the self-report supports the validity of self-report data among first-episode psychosis patients (Van Dorn et al., 2012). On the other hand, the self-report over-detected the risk of substance use compared with the DALI subscales, although it was only slightly higher for cannabis/cocaine use. In fact, most patients who reported recent cannabis and/or cocaine use obtained a positive result on the DALI subscale (80%). Taking this into consideration, these findings indicate that the presence of alcohol use in first-episode psychosis may be a poor proxy for the risk of alcohol use disorder, and that the use of other illicit drugs may represent a better approach in this population. However, another study concluded that self-reported illicit drug use was a poor proxy for disordered drug use in a sample of adults with schizophrenia from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) trial (Van Dorn et al., 2012). These discrepancies may in fact reflect contextual and sample differences, as Van Dorn et al.'s sample was recruited from over fifty sites across the United States and was much older on average (~15 years) than our sample.

In agreement with other literature reports (Addington et al., 2010), we found that younger age, male gender and higher scores on the PANSS positive subscale were associated with readmission throughout the study period. We did not find associations between other socio-demographic or clinical variables and readmission. Nevertheless, considering the significant heterogeneity across studies regarding the influence of DUP on relapses and readmission (Cougnard et al., 2006; Alvarez-Jimenez et al., 2011; Alvarez-Jimenez et al., 2012), we included DUP as a potential confounding factor in our multivariate analysis. Significantly, both positive urine analyses for cannabis and the DALI cannabis/cocaine subscale were associated with readmission, highlighting the importance of drug use in relapses and readmissions (Alvarez-Jimenez et al., 2012). However, after controlling for potential confounding variables, such as gender, age, PANSS positive subscale and DUP, only the DALI cannabis/cocaine subscale remained as a predictor of readmission, a finding that supports the utility of this screening test over laboratory parameters. Our results suggest an overall 4.5-fold increase in risk of readmission for patients at a high risk for cannabis/cocaine disorders, in agreement with other studies which have reported three to five-fold increases in the risk of relapse also when controlling for potential confounders (Wade et al., 2006; Malla et al., 2008; Turkington et al., 2009).

It is interesting that survival plots (Fig. 1) showed the greatest difference in readmission rates during the first five years of the follow-up. Considering that relapse prevention during the first years of illness has a critical impact on life-long outcomes in schizophrenia, avoidance of this modifiable risk factor should be a priority for clinicians and intervention programs. Several studies have reported that comorbid diagnosis of a drug use disorder may enhance the risk of relapse, particularly during the early stages of the illness (Hides et al., 2006; Wade et al., 2006; Malla et al., 2008), and that abstaining from use after the first psychotic episode may contribute to a clear

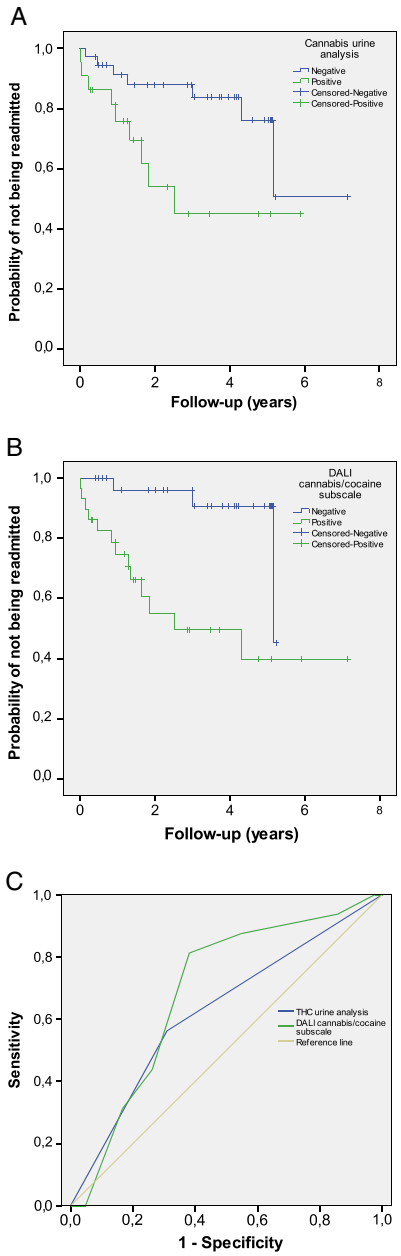


Fig. 1. (A) & (B) Survival plot of cannabis urine analysis and DALI cannabis/cocaine subscale, respectively. (C) ROC curves of DALI cannabis/cocaine subscale compared with positive urine analysis for cannabis for readmission during the whole study period.

improvement in outcome (Sorbara et al., 2003; Grech et al., 2005; Baeza et al., 2009; Turkington et al., 2009; Gonzalez-Pinto et al., 2011). In fact, cohort studies involving subjects with first-episode psychosis reported that approximately half the subjects become abstinent or significantly reduce their alcohol and drug use, in most cases in a stable manner (Wisdom et al., 2011). Furthermore, while those who become abstinent reduce their rates of relapse and hospitalization, those with persistent substance use disorders present increased rates (Wisdom et al., 2011).

Cannabis use is frequently associated with alcohol consumption (Cantwell et al., 1999), which itself has been associated with deleterious effect and worse outcome in first-episode psychosis and schizophrenia (Wade et al., 2007; Turkington et al., 2009). However, alcohol assessments (DALI subscale and blood samples) were not related to readmission when studied separately. One explanation may be the differences in the severity of substance use, since it has been reported that heavy, but not mild, substance use disorders may be associated with poorer functional outcome (Wade et al., 2007). As the DALI scale does not assess the severity of substance use, such differences cannot be excluded. In any case, the contribution of alcohol to the overall findings cannot be ruled out as most of the patients who were at risk for alcohol use disorder were also at risk for cannabis/cocaine use disorder. However, despite the mentioned overlap, the limited number of positive results obtained in both the alcohol subscale and the blood tests does not allow us to reach any firm conclusion.

As the predictive validity of the DALI scale for readmission risk was not assessed in the original validation (Rosenberg et al., 1998), we deemed it essential to establish the optimum cutoff point in our sample since the use of an incorrect cutoff would lead to misclassification and an inaccurate prediction of the readmission risk. Our results showed that DALI has good psychometric properties for predicting readmission. Compared to urinalysis, the DALI cannabis/cocaine subscale showed a greater AUC due to its higher sensitivity. Sensitivity assesses the proportion of readmitted subjects who are correctly identified as having a condition. False negatives assess the proportion of readmitted subjects whom the subscale is not able to identify. Therefore, the scale's higher predictive validity may indicate that it is a better detector of patients at risk of readmission than urine samples. In addition to its significant reduction in costs and its efficiency of administration, a positive result on this screening scale may be more reliable for detecting current use and misuse, and even for predicting readmission, than a urine sample. The availability of a brief and practical screening test means that more patients with drug-related problems can be identified and

Table 2
Multivariate analysis (Cox regression).

Adjusted readmission model	Crude HR	95% CI	Adjusted HR	95% CI	p
Cannabis urine analysis	3.08	1.13 to 8.39	1.99	0.69 to 5.72	0.20
Male gender	4.16	0.94 to 18.39	2.90	0.61 to 13.84	0.18
Age	0.90	0.81 to 1.00	0.95	0.85 to 1.06	0.35
DUP	0.84	0.61 to 1.17	0.90	0.62 to 1.30	0.55
PANSS positive subscale	1.03	0.93 to 1.14	1.02	0.93 to 1.11	0.74
DALI cannabis/cocaine subscale	6.09	1.72 to 21.54	4.55	1.11 to 18.72	0.036
Male gender	4.16	0.94 to 18.39	2.63	0.55 to 12.47	0.22
Age	0.90	0.81 to 1.00	0.99	0.88 to 1.11	0.89
DUP	0.84	0.61 to 1.17	0.82	0.58 to 1.17	0.27
PANSS positive subscale	1.03	0.93 to 1.14	1.02	0.93 to 1.12	0.67

CI: confidence interval.

Table 3
Predictive validity of the screening tests.

Screening test	Se (95% CI)	Sp (95% CI)	PPV (95% CI)	NPV (95% CI)	AUC (95% CI)
DALI cannabis/cocaine subscale	0.81 (0.57–0.93)	0.62 (0.47–0.75)	0.45 (0.28–0.62)	0.90 (0.74–0.96)	0.72 (0.57–0.86)
Positive urine sample for cannabis	0.56 (0.33–0.77)	0.69 (0.54–0.81)	0.41 (0.23–0.61)	0.81 (0.65–0.90)	0.63 (0.46–0.79)

Se: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; AUC: area under curve; CI: confidence interval.

appropriately managed and treated, either within the psychiatric care system, in dual diagnosis programs, or in substance use disorder specialty care (Tiet et al., 2008).

Our study has several limitations, including a relatively small sample size, limited generalizability to non-affective psychosis, and the inability to quantify drug use precisely as we had only self-reported information on drug use in the last three months. With regard to the perceived problems related to non-disclosure, especially among patients with severe mental illness, it is interesting that studies rely, in the main, on self-reports (Van Dorn et al., 2012). In this regard, our results favor the use of self-reports of drug use over laboratory tests. However, given the implications for research and clinical practice, further work is needed to evaluate the accuracy of reported substance use in subjects with severe mental illness, and to assess whether biological measures provide more accurate data. Another limitation is the fact that drug assessment was only conducted at baseline; as a result, we were unable to obtain a clear picture of the temporal relationship between substance misuse and readmission during the follow-up. Longitudinal studies with periodical drug assessments may prove useful in the search for a convergent and standardized methodology for recruitment, assessment and treatment strategies (Wisdom et al., 2011). Another limitation is that the DALI scales have been validated for the most prevalent drugs only (alcohol, cannabis and cocaine), and their performance in patients with other drug disorders is unknown at present. In addition, we compared a subscale that measures cannabis and cocaine consumption with positive urinary analysis for cannabis alone, as no positive results were detected for cocaine. In this regard, it might have been more illuminating to assess each drug separately in order to establish its individual effect. Finally, other well known factors related to relapse, such as medication adherence (Alvarez-Jimenez et al., 2012; Caseiro et al., 2012), were not assessed in the current study. As such, the influence of these variables on the current results cannot be ruled out.

The findings of this study demonstrate that a quick screening self-report scale for cannabis and cocaine use disorders is more useful than urinary analysis for predicting readmission. Indeed, scoring in the "at risk" range for these drug disorders at admission was found to increase the readmission risk in first-episode psychosis by 4.5 times. This finding has direct clinical implications for preventing readmission during the early course of psychosis, when intervention may have the greatest impact on long-term outcomes. After patients are screened, they can be referred to specialty substance use disorder or dual diagnosis integrative care, which may decrease readmission and improve outcome. Future research should consider longitudinal assessment of brief validated screening tests in order to evaluate their benefits in prevention of early readmission in first-episode psychosis.

Role of funding source

This work was supported by the Government of Catalonia, Comissionat per Universitats I Recerca del Departament d'Innovació, Universitats I Empresa (DIUE) 2009SGR1295, and 2009SGR1435, by the Esther Koplowitz Center (Barcelona), by the Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM, and by the Ministerio de Sanidad y Consumo, PNSD: PI101/2006 and PNSD: PI041731/2011. Prof Yücel is supported by a NHMRC fellowship (#1021973). The founding agencies played no role in the direction of this project.

Contributors

AB, CGR, EFE and MB contributed substantially to conception and design; AB, PC and MY contributed to analysis and interpretation of data; AB, CGR and MY drafted the article; EP, BK, RMS and MB revised it critically for important intellectual content. All authors gave final approval of the version to be published.

Conflict of interest

All authors declare that they have no conflicts of interest.

Acknowledgments

Government of Catalonia, Comissionat per Universitats I Recerca del Departament d'Innovació, Universitats I Empresa (DIUE) 2009SGR1295, 2009SGR1435, Esther Koplowitz Center (Barcelona), Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM, and Ministerio de Sanidad y Consumo, PNSD: PI101/2006 and PNSD: PI041731/2011.

References

Addington, J., Addington, D., 2007. Patterns, predictors and impact of substance use in early psychosis: a longitudinal study. Acta Psychiatr. Scand. 115 (4), 304–309.
Addington, D.E., Beck, C., Wang, J., Adams, B., Pryce, C., Zhu, H., Kang, J., McKenzie, E., 2010. Predictors of admission in first-episode psychosis: developing a risk adjustment model for service comparisons. Psychiatr. Serv. 61 (5), 483–488.
Alvarez-Jimenez, M., Parker, A.G., Hetrick, S.E., McGorry, P.D., Gleeson, J.F., 2011. Preventing the second episode: a systematic review and meta-analysis of psychosocial and pharmacological trials in first-episode psychosis. Schizophr. Bull. 37 (3), 619–630.
Alvarez-Jimenez, M., Priede, A., Hetrick, S.E., Bendall, S., Killackey, E., Parker, A.G., McGorry, P.D., Gleeson, J.F., 2012. Risk factors for relapse following treatment for first episode psychosis: a systematic review and meta-analysis of longitudinal studies. Schizophr. Res. 139 (1–3), 116–128.
Baeza, I., Graell, M., Moreno, D., Castro-Fornieles, J., Parellada, M., Gonzalez-Pinto, A., Paya, B., Soutullo, C., de la Serna, E., Arango, C., 2009. Cannabis use in children and adolescents with first episode psychosis: influence on psychopathology and short-term outcome (CAFEPS study). Schizophr. Res. 113 (2–3), 129–137.
Barnes, T.R., Mutsatsa, S.H., Hutton, S.B., Watt, H.C., Joyce, E.M., 2006. Comorbid substance use and age at onset of schizophrenia. Br. J. Psychiatry 188, 237–242.
Bennett, M.E., 2009. Assessment of substance use and substance-use disorders in schizophrenia. Clin. Schizophr. Relat. Psychoses 3 (1), 50–63.
Bernardo, M., Bioque, M., Parellada, M., Saiz, R.J., Cuesta, M.J., Llerena, A., Sanjuan, J., Castro-Fornieles, J., Arango, C., Cabrera, B., 2012. Assessing clinical and functional outcomes in a gene–environment interaction study in first episode of psychosis (PEPs). Rev. Psiquiatr. Salud Ment. 6 (1), 4–16 (Nov 30.).
Buhler, B., Hambrecht, M., Loffler, W., an der, H.W., Hafner, H., 2002. Precipitation and determination of the onset and course of schizophrenia by substance abuse—a retrospective and prospective study of 232 population-based first illness episodes. Schizophr. Res. 54 (3), 243–251.
Cantor-Graae, E., Nordstrom, L.G., McNeil, T.F., 2001. Substance abuse in schizophrenia: a review of the literature and a study of correlates in Sweden. Schizophr. Res. 48 (1), 69–82.
Cantwell, R., Brewin, J., Glazebrook, C., Dalkin, T., Fox, R., Medley, I., Harrison, G., 1999. Prevalence of substance misuse in first-episode psychosis. Br. J. Psychiatry 174, 150–153.
Caseiro, O., Perez-Iglesias, R., Mata, I., Martinez-Garcia, O., Pelayo-Teran, J.M., Tabares-Seisdedos, R., de la Foz, Ortiz-Garcia, Vazquez-Barquero, J.L., Crespo-Facorro, B., 2012. Predicting relapse after a first episode of non-affective psychosis: a three-year follow-up study. J. Psychiatr. Res. 46 (8), 1099–1105.
Coldham, E.L., Addington, J., Addington, D., 2002. Medication adherence of individuals with a first episode of psychosis. Acta Psychiatr. Scand. 106 (4), 286–290.
Cougnard, A., Parrot, M., Grolleau, S., Kalmi, E., Desage, A., Misdrahi, D., Brun-Rousseau, H., Verdoux, H., 2006. Pattern of health service utilization and predictors of readmission after a first admission for psychosis: a 2-year follow-up study. Acta Psychiatr. Scand. 113 (4), 340–349.
Degenhardt, L., Tennant, C., Gilmour, S., Schofield, D., Nash, L., Hall, W., McKay, D., 2007. The temporal dynamics of relationships between cannabis, psychosis and depression among young adults with psychotic disorders: findings from a 10-month prospective study. Psychol. Med. 37 (7), 927–934.
Drake, R.E., Osher, F.C., Noordsy, D.L., Hurlbut, S.C., Teague, G.B., Beaudett, M.S., 1990. Diagnosis of alcohol use disorders in schizophrenia. Schizophr. Bull. 16 (1), 57–67.
European Monitoring Centre for Drugs and Drug Addiction, 2011. The state of the drugs problem in Europe. EMCDDA, Lisbon. Access date: 2-2-2012. Available from http://www.emcdda.europa.eu/publications/annual-report/2011.
Fernandez-Egea, E., Bernardo, M., Donner, T., Conget, I., Parellada, E., Justicia, A., Esmatjes, E., Garcia-Rizo, C., Kirkpatrick, B., 2009. Metabolic profile of antipsychotic-naive individuals with non-affective psychosis. Br. J. Psychiatry 194 (5), 434–438.

First, M., Spitzer, R., 1999. SCID-I Structured Clinical Interview for the DSM-IV Axis I Disorders [Spanish] (trans. J Blanch, I Andreu), Masson edn.
Ford, P., 2003. An evaluation of the Dartmouth Assessment of Lifestyle Inventory and the Leeds Dependence Questionnaire for use among detained psychiatric inpatients. Addiction 98 (1), 111–118.
Garcia-Rizo, C., Fernandez-Egea, E., Oliveira, C., Justicia, A., Parellada, E., Bernardo, M., Kirkpatrick, B., 2012. Prolactin concentrations in newly diagnosed, antipsychotic-naive patients with nonaffective psychosis. Schizophr. Res. 134 (1), 16–19.
Gonzalez-Pinto, A., Alberich, S., Barbeito, S., Gutierrez, M., Vega, P., Ibanez, B., Haidar, M.K., Vieta, E., Arango, C., 2011. Cannabis and first-episode psychosis: different long-term outcomes depending on continued or discontinued use. Schizophr. Bull. 37 (3), 631–639.
Grace, R.F., Shenfield, G., Tennant, C., 2000. Cannabis and psychosis in acute psychiatric admissions. Drug Alcohol Rev. 19 (3), 287–290.
Grech, A., van, O.J., Jones, P.B., Lewis, S.W., Murray, R.M., 2005. Cannabis use and outcome of recent onset psychosis. Eur. Psychiatry. 20 (4), 349–353.
Green, A.I., Tohen, M.F., Hamer, R.M., Strakowski, S.M., Lieberman, J.A., Glick, I., Clark, W.S., 2004. First episode schizophrenia-related psychosis and substance use disorders: acute response to olanzapine and haloperidol. Schizophr. Res. 66 (2–3), 125–135.
Hides, L., Dawe, S., Kavanagh, D.J., Young, R.M., 2006. Psychotic symptom and cannabis relapse in recent-onset psychosis. Prospect. Study Br. J. Psychiatry 189, 137–143.
Kamali, M., Mctigue, O., Whitty, P., Gervin, M., Clarke, M., Browne, S., Larkin, C., O'callaghan, E., 2009. Lifetime history of substance misuse in first-episode psychosis: prevalence and its influence on psychopathology and onset of psychotic symptoms. Early Interv. Psychiatry 3 (3), 198–203.
Kavanagh, D.J., McGrath, J., Saunders, J.B., Dore, G., Clark, D., 2002. Substance misuse in patients with schizophrenia: epidemiology and management. Drugs 62 (5), 743–755.
Lambert, M., Conus, P., Lubman, D.I., Wade, D., Yuen, H., Moritz, S., Naber, D., McGorry, P.D., Schimmelmann, B.G., 2005. The impact of substance use disorders on clinical outcome in 643 patients with first-episode psychosis. Acta Psychiatr. Scand. 112 (2), 141–148.
Larsen, T.K., Melle, I., Auestad, B., Friis, S., Haahr, U., Johannessen, J.O., Opjordsmoen, S., Rund, B.R., Simonsen, E., Vaglum, P., McGlashan, T.H., 2006. Substance abuse in first-episode non-affective psychosis. Schizophr. Res. 88 (1–3), 55–62.
Linszen, D.H., Dingemans, P.M., Lenior, M.E., 1994. Cannabis abuse and the course of recent-onset schizophrenic disorders. Arch. Gen. Psychiatry 51 (4), 273–279.
Malla, A., Norman, R., Bechard-Evans, L., Schmitz, N., Manchanda, R., Cassidy, C., 2008. Factors influencing relapse during a 2-year follow-up of first-episode psychosis in a specialized early intervention service. Psychol. Med. 38 (11), 1585–1593.
Margolese, H.C., Malchy, L., Negrete, J.C., Tempier, R., Gill, K., 2004. Drug and alcohol use among patients with schizophrenia and related psychoses: levels and consequences. Schizophr. Res. 67 (2–3), 157–166.
Pencer, A., Addington, J., Addington, D., 2005. Outcome of a first episode of psychosis in adolescence: a 2-year follow-up. Psychiatry Res. 133 (1), 35–43.
Peralta, V., Cuesta, M.J., 1994. Psychometric properties of the positive and negative syndrome scale (PANSS) in schizophrenia. Psychiatry Res. 53 (1), 31–40.
Regier, D.A., Farmer, M.E., Rae, D.S., Locke, B.Z., Keith, S.J., Judd, L.L., Goodwin, F.K., 1990. Comorbidity of mental disorders with alcohol and other drug abuse. Results from the Epidemiologic Catchment Area (ECA) Study. JAMA 264 (19), 2511–2518.
Rosenberg, S.D., Drake, R.E., Wolford, G.L., Mueser, K.T., Oxman, T.E., Vidaver, R.M., Carrieri, K.L., Luckoor, R., 1998. Dartmouth Assessment of Lifestyle Instrument (DALI): a substance use disorder screen for people with severe mental illness. Am. J. Psychiatry 155 (2), 232–238.
Salyers, M.P., Mueser, K.T., 2001. Social functioning, psychopathology, and medication side effects in relation to substance use and abuse in schizophrenia. Schizophr. Res. 48 (1), 109–123.
Sorbara, F., Liraud, F., Assens, F., Abalan, F., Verdoux, H., 2003. Substance use and the course of early psychosis: a 2-year follow-up of first-admitted subjects. Eur. Psychiatry 18 (3), 133–136.
Spitzer, R., Williams, J.B.W., Gibbon, M., First, M., 1988. Instruction Manual for the Structured Clinical Interview for DSM-III-R. State Psychiatric Institute, Biometrics Research, New York.
Stirling, J., Lewis, S., Hopkins, R., White, C., 2005. Cannabis use prior to first onset psychosis predicts spared neurocognition at 10-year follow-up. Schizophr. Res. 75 (1), 135–137.
Sugranyes, G., Flamarique, I., Parellada, E., Baeza, I., Goti, J., Fernandez-Egea, E., Bernardo, M., 2009. Cannabis use and age of diagnosis of schizophrenia. Eur. Psychiatry 24 (5), 282–286.
Tiet, Q.Q., Finney, J.W., Moos, R.H., 2008. Screening psychiatric patients for illicit drug use disorders and problems. Clin. Psychol. Rev. 28 (4), 578–591.
Turkington, A., Mulholland, C.C., Rushe, T.M., Anderson, R., McCaul, R., Barrett, S.L., Barr, R.S., Cooper, S.J., 2009. Impact of persistent substance misuse on 1-year outcome in first-episode psychosis. Br. J. Psychiatry 195 (3), 242–248.
Van Dorn, R.A., Desmarais, S.L., Scott, Y.M., Sellers, B.G., Swartz, M.S., 2012. Assessing illicit drug use among adults with schizophrenia. Psychiatry Res. (Dec 30. http://dx.doi.org/10.1016/j.psychres.2012.05.028).
Van Mastrigt, S., Addington, J., Addington, D., 2004. Substance misuse at presentation to an early psychosis program. Soc. Psychiatry Psychiatr. Epidemiol. 39 (1), 69–72.
Verstraete, A.G., 2004. Detection times of drugs of abuse in blood, urine, and oral fluid. Ther. Drug Monit. 26 (2), 200–205.
Wade, D., Harrigan, S., Edwards, J., Burgess, P.M., Whelan, G., McGorry, P.D., 2006. Substance misuse in first-episode psychosis: 15-month prospective follow-up study. Br. J. Psychiatry 189, 229–234.
Wade, D., Harrigan, S., McGorry, P.D., Burgess, P.M., Whelan, G., 2007. Impact of severity of substance use disorder on symptomatic and functional outcome in young individuals with first-episode psychosis. J. Clin. Psychiatry 68 (5), 767–774.
Wisdom, J.P., Manuel, J.I., Drake, R.E., 2011. Substance use disorder among people with first-episode psychosis: a systematic review of course and treatment. Psychiatr. Serv. 62 (9), 1007–1012.
Zammit, S., Moore, T.H., Lingford-Hughes, A., Barnes, T.R., Jones, P.B., Burke, M., Lewis, G., 2008. Effects of cannabis use on outcomes of psychotic disorders: systematic review. Br. J. Psychiatry 193 (5), 357–363.

4

Study 2

Neuroimaging studies of acute effects of THC and CBD in humans and animals: a systematic review

Current Pharmaceutical Design 2014; 20(13):2168-85

Study 2

Summary

Reference

Title: Neuroimaging Studies of Acute Effects of THC and CBD in humans and animals: a Systematic Review. Authors: Batalla A, Crippa JA, Busatto GF, Guimarães FS, Zuardi AW, Valverde O, Atakan Z, McGuire PK, Bhattacharyya S, Martín-Santos R. *Current Pharmaceutical Design 2014; 20(13):2168-85*. Impact factor 2012: 3.311 (1st quartile pharmacology & pharmacy).

Aims

Centred in *hypothesis* #2, a systematic review to assess the impact of acute experimental administration of cannabinoids on brain function in naïve or occasional cannabis users and in animals was performed. This review focused on neuroimaging studies that examined patterns of change in dopamine release, brain activation or cerebral blood flow during performance of different cognitive tasks and resting state after administration of cannabinoids.

Method

Papers published until June 2012 were included from four databases (EMBASE, Medline, PubMed, LILACS) following a comprehensive search strategy and pre-determined protocol in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (96). Only neuroimaging studies of experimental administration of cannabinoids involving animals naïve to cannabinoids or naïve/occasional cannabis users were included. Studies involving participants with psychiatric use disorders, including drug use disorders, were excluded. Recreational or occasional cannabis users were defined as persons who used cannabis sporadically

(less than four times a month) and naïve users as persons who used cannabis less than 25 times lifetime.

The primary neuroimaging measures of interest were: global and regional activity (cerebral blood flow; regional cerebral blood flow; blood oxygen level dependant signal); local cerebral glucose utilisation in animal studies; and measures of dopamine release (dialysate dopamine levels or cell firing rate in animal studies; and non-displaceable binding potential in human studies).

Results

Forty-five studies met the inclusion criteria, of which 24 were in humans and 21 in animals. Despite the considerable degree of methodological heterogeneity, the studies included reported modulation of resting state activity, in particular increases mainly in CB_1-rich areas implicated in cognitive processes and reward, and altered neural activity during performance of several cognitive tasks, reflecting a different recruitment of brain areas after THC challenge. In addition, THC and CBD showed opposite neurophysiological properties, which is consistent with their opposite symptomatic effects. Finally, in contrast with findings in animals, the few neurochemical studies carried out in humans did not clearly support an increased dopaminergic activity in THC-induced psychosis.

Conclusion

The functional neuroimaging studies reviewed provided extensive evidence for the acute modulation of brain function by cannabinoids. However, further studies are needed in order to detail the mechanisms underlying these effects. The present review also raised the great need for replication of current findings, considering the use of convergent methodology.

Send Orders for Reprints to reprints@benthamscience.net

2168 *Current Pharmaceutical Design,* 2014, *20,* 2168-2185

Neuroimaging Studies of Acute Effects of THC and CBD in Humans and Animals: a Systematic Review

A. Batalla[1], J.A. Crippa[2,3,4], G.F. Busatto[4,5], F.S. Guimarães[3,4,6], A.W. Zuardi[2,3,4], O. Valverde[7], Z. Atakan[8], P.K. McGuire[8], S. Bhattacharyya[8] and R. Martín-Santos[1,3*]

[1]Psychiatry and Psychology, Institute of Neurosciences, Hospital Clínic, IDIBAPS, CIBERSAM, Barcelona, and Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Spain; [2]Department of Neuroscience and Behavior, Ribeirão Preto Medical School, University of São Paulo, Brazil; [3]INCT Translational Medicine (CNPq), Ribeirão Preto, Brazil; [4]Center for Interdisciplinary Research on Applied Neurosciences (NAPNA), University of São Paulo, Brazil; [5]Department and Institute of Psychiatry, Faculty of Medicine, University of São Paulo, São Paulo, SP, Brazil; [6]Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo. 3900 Av. Bandeirantes, Monte Alegre, Ribeirão Preto- São Paulo, Brazil; [7]Department of Experimental and Health Sciences, Pompeu Fabra University, Barcelona, IMIM Hospital del Mar Clinical Research Institute, and Red de Trastornos Adictivos (RETIC), Spain; [8]Department of Psychosis Studies, King's College London, Institute of Psychiatry, London, UK

Abstract: Background: In recent years, growing concerns about the effects of cannabis use on mental health have renewed interest in cannabis research. In particular, there has been a marked increase in the number of neuroimaging studies of the effects of cannabinoids. We conducted a systematic review to assess the impact of acute cannabis exposure on brain function in humans and in experimental animals.

Methods: Papers published until June 2012 were included from EMBASE, Medline, PubMed and LILACS databases following a comprehensive search strategy and pre-determined set of criteria for article selection. Only pharmacological challenge studies involving the *acute* experimental administration of cannabinoids in occasional or naïve cannabis users, and naïve animals were considered.

Results: Two hundred and twenty-four studies were identified, of which 45 met our inclusion criteria. Twenty-four studies were in humans and 21 in animals. Most comprised studies of the acute effects of cannabinoids on brain functioning in the context of either resting state activity or activation during cognitive paradigms. In general, THC and CBD had opposite neurophysiological effects. There were also a smaller number of neurochemical imaging studies: overall, these did not support a central role for increased dopaminergic activity in THC-induced psychosis. There was a considerable degree of methodological heterogeneity in the imaging literature reviewed.

Conclusion: Functional neuroimaging studies have provided extensive evidence for the acute modulation of brain function by cannabinoids, but further studies are needed in order to understand the neural mechanisms underlying these effects. Future studies should also consider the need for more standardised methodology and the replication of findings.

Keywords: Animals, cannabis, cannabis users, THC, CBD, brain function, neuroimaging, systematic review, CB_1 cannabinoid receptors.

INTRODUCTION

Cannabis remains the most commonly used illegal drug with estimated annual prevalence of 125 to 203 million people worldwide [1]. Following steady increases throughout the 1990s and early 2000s, the prevalence of cannabis use has stabilized more recently but still remains disturbingly high [1, 2]. The extract of *Cannabis sativa* contains multiple compounds, with over 60 different cannabinoids reported, of which delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are the most studied ones [3, 4]. THC, the main psychoactive constituent of cannabis, is thought to be responsible for most of its psychotropic effects [5]. Its administration in healthy subjects can induce intoxication, anxiety, psychotic symptoms [6], as well as modulatory effects on different cognitive domains [4], such as learning and memory [7], psychomotor control [8] and attention [9]. In contrast, CBD is the major non-psychotomimetic constituent of cannabis, and it has been found to induce anxiolytic effects both in animals and humans [10, 11], and even antipsychotic properties [12, 13] without impairing memory or other cognitive functions [4, 14]. Thus, CBD may be potentially able to reduce some symptomatic effects of THC such as anxiety and psychosis [12]. However, it is relevant to note that concentrations of THC and CBD in the different preparations of cannabis (marihuana, hashish, skunk) have changed in the last few years, with claims of a sharp increase in the THC/CBD rate [1, 15]. This may result in a heightened risk of psychiatric symptoms, such as psychosis [16, 17].

Although it is thought that the endocannabinoid system may play a critical role in the mechanism of action of cannabis, the neurophysiological basis of the different and even opposite psychiatric and cognitive effects of cannabis outlined above still remains uncertain. The vast majority of CB_1 receptors are located in the central nervous system, particularly in brain regions that are critical for executive functioning, attention, memory and reward processing, such as the prefrontal cortex, anterior cingulate cortex, basal ganglia, medial temporal areas (e.g., hippocampus and amygdala) and cerebellum [18]. CB_1 receptors are mainly localized in gamma-aminobutyric acid (GABA) and glutamatergic terminals, where they inhibit neurotransmitter release [19, 20]. However, CB_1 receptor activation also affects the release of other neurotransmitters, such as dopamine, which may be related to the reinforcing effects of cannabinoids [21], as well as to an increased risk of psychosis [22]. CB_2 receptors are primarily expressed in peripheral cells of the immune system, but recent evidence indicates that they are also present within the central nervous system [23]. Although the effects of THC are thought to be mediated by a partial agonism at the central CB_1 receptors [24], CBD has low affinity for CB_1 receptors

*Address correspondence to this author at the Department of Psychiatry, Hospital Clínic, IDIBAPS, CIBERSAM; and Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Catalonia, Spain; Tel:+34 932275400; Fax: +34 93 2275548; E-mail: rmsantos@clinic.ub.es

1873-4286/14 $58.00+.00

[25] and its precise molecular mechanism of action, which may involve a wide variety of mechanisms [4, 26], remains unclear.

Neuroimaging techniques provide a highly useful approach to investigate the neural basis of the effects of cannabinoids. In recent years, renewed interest in gene-environment interplay, such as the cannabis-psychosis link [27-29], and the potential therapeutic effect of certain cannabinoids (such as CBD [12]), have led to a significant increase in the number of human studies using neuroimaging techniques to determine the functional and structural brain effects of cannabinoids. Several recent reviews have examined this topic, especially regarding chronic cannabis use [30-38].

Additionally, pharmacological challenge studies involving the *acute* experimental administration of cannabinoids or their synthetic equivalents, in combination with neuroimaging methods, offer novel opportunities to study *in vivo* the effects of these substances on brain functioning [30]. In the present review, we have conducted a systematic literature search of neuroimaging studies investigating the acute effects of cannabinoids on brain functioning both in animals and in humans (naïve or occasional users). These papers have examined patterns of change in dopamine release, brain activation or cerebral blood flow either at rest or during different cognitive paradigms, after acute experimental administration of cannabinoids. Papers published until June 2012 were included, following a comprehensive search strategy and pre-determined protocol in accordance with PRISMA guidelines [39].

1. METHODS

1.1. Search Strategy

Electronic searches for published reports were performed using EMBASE (1980-June 2012), Medline (1966-June 2012), PubMed (1966-June 2012) and LILACS (1982-June 2012) databases, without language restriction. Abstracts, review articles, clinical observations, and unpublished data were not included. For human studies, a combination of two of the following key words was used: cannabis; marijuana; delta-9-tetrahydrocannabinol; THC; cannabidiol, CBD; or cannabinoid. These terms were combined with: neuroimaging; brain imaging; magnetic resonance, MRI; single photon emission tomography, SPECT; functional magnetic resonance, fMRI; positron emission tomography, PET; spectroscopy, MRS. For animal studies, a combination of three of the following key words were used: animal; or rat. These were combined with: cannabis; marijuana; delta-9-tetrahydrocannabinol; THC; cannabidiol, CBD; or cannabinoid; and cerebral blood flow; cerebral glucose utilization; microdialysis; electrophysiological; dopamine release; single photon emission tomography, SPECT; or positron emission tomography, PET. All studies published up to June 2012 were included. The references of selected papers were also screened for relevant articles, yielding three further papers.

1.2. Selection Criteria

A general review of all functional neuroimaging studies involving cannabinoids in animals and humans was initially performed. We obtained a total of 224 published papers (Fig. **1**). In order to homogenize the selection and facilitate comparisons, studies were only included if they met the following inclusion criteria: (i) use of functional neuroimaging techniques involving animals naïve to cannabinoids or naïve/occasional cannabis users; (ii) acute experimental administration of cannabinoids; for human studies: (iii) same gender, age, handedness in all subjects; for animal studies: (iv) *in vivo* studies involving cannabinoid effects on blood flow, cerebral metabolism or dopamine release. Exclusion criteria were: (i) non-neuroimaging studies of experimental administration of cannabinoids; for human studies: (ii) neuroimaging studies that involved participants who had other neurological or psychiatric disorders, or individuals with substance abuse disorders; (iii) neuroimaging studies with chronic cannabis users; for animals: (iv) *in vitro* experiments; (v) chronic or combined drug administration; (vi) anesthetized animals during the experimental procedure.

We defined recreational (or occasional) cannabis users as persons who used cannabis sporadically (less than four times a month), and naïve users as persons who used cannabis less than 25 times in their lifetime, according to strict standardized criteria. Chronic cannabis users were defined as persons who used cannabis several times a week and who had done so for at least two years.

A publication that reported administration of different cannabinoids to the same subjects or animals (e.g., THC and CBD), or examined the same subjects with two different tasks (e.g., verbal working memory and visual attention task), was considered as two separate studies. A publication that reported two different analysis methods to the same sample (e.g., arterial spin labeling and fMRI) was considered as a single study.

1.3. Data Extraction

Data was extracted independently by two researchers. From the articles included, we recorded: socio-demographic information (e.g., sample size, gender; handedness; species); patterns of cannabis use (e.g., duration, age of onset, frequency of cannabis use); cannabinoid administration characteristics (e.g., dose, route); imaging type and design of the study (e.g., randomized, single/double blind, placebo controlled); exclusion criteria (for neurological, psychiatric or drug history); information on abstinence to other drugs (checked by urine test); use of rest/active condition for human functional imaging studies; type of task performed during functional imaging; and psychopathological variables (e.g., depersonalization, level of subjective intoxication, or psychotic symptoms).

For functional imaging data, the primary measures of interest were: global and regional activity [cerebral blood flow (CBF); regional CBF (rCBF); blood oxygen level dependent signal (BOLD)]; local cerebral glucose utilization (LCGU) in animal studies; and measures of dopamine release [dialysate dopamine levels or cell firing rate in animal studies; and non-displaceable binding potential (BP_{ND}) in human studies].

2. RESULTS

From the 224 studies identified, seventy-five did not meet the *a priori* selection criteria [40-114], whereas one hundred and two met the exclusion criteria [10, 21, 115-214], or were case/series reports [215, 216] (for more detailed information, see Fig. **1**). The 45 studies included in the review were classified according to: group (naïve/occasional cannabis users and animal studies); drug administered (THC, endogenous cannabinoids or other CB_1 agonists, CBD, opiates); and type of functional imaging measure [CBF (during resting state or cognitive task) or dopamine release]. The human studies comprised 26 articles evaluating acute effects of cannabinoids on CBF (8 at rest and 18 during cognitive tasks; 20 after THC and 6 after CBD administration); and 3 studies evaluating acute effects on dopamine release after THC administration. The animal studies included 9 articles evaluating acute effects of cannabinoids on CBF or brain glucose metabolism (5 after THC, 3 after other CB_1 agonists and 1 after endogenous cannabinoid administration); and 21 articles addressing acute effects on dopamine release (9 after THC, 6 after other CB_1 agonists, 3 after endogenous cannabinoid, 2 after opioids and 1 after CBD administration).

2.1. Human Studies

2.1.1. Acute Effects of Cannabinoids on Cerebral Blood Flow in the Resting State

We identified eight functional resting-state imaging studies of the acute effects of cannabinoids, seven of which involved THC challenge in occasional cannabis users (Table **1**) and one after CBD challenge in naïve cannabis users (Table **2**).

2.1.1.1. Occasional Cannabis Users

We included seven studies comparing resting rCBF in occasional cannabis users before and following THC or placebo administration (Table **1**). Only Volkow *et al*. (1991) [217] was not placebo-controlled. Four different imaging methods were used: ^{18}F-FDG-PET [217], ^{133}Xe-SPECT [218, 219], $H_2{}^{15}O$-PET [220-222] and fMRI [223]. Regional differences in resting brain activity were reported in all of them when compared to placebo or to the baseline state before THC administration, with five studies reporting global CBF increase [217-220, 222].

Marijuana-cigarette administration. Two ^{133}Xe-SPECT studies [218, 219] examined resting state CBF before and after subjects smoked a marijuana cigarette with controlled THC dose (Table **1**). These studies described a dose-dependent increase in regional resting state brain activity (maximal after 30 minutes) either relative to the baseline state before THC use [219] or in comparison to smoking a marijuana cigarette without THC [218]. Overall, marijuana smoking was associated with bilateral CBF increases, with stronger activations in the anterior part of each brain hemisphere [218, 219]. Subjective levels of intoxication [218, 219], depersonalization [as assessed with the Depersonalization Inventory (DPI)] [219], dissociative experiences [measured with the Temporal Disintegration Inventory (TDI)] [219] and measures of confusion [219] were correlated with increased global CBF. Plasma levels and pulse rate were also positively correlated with global CBF [218].

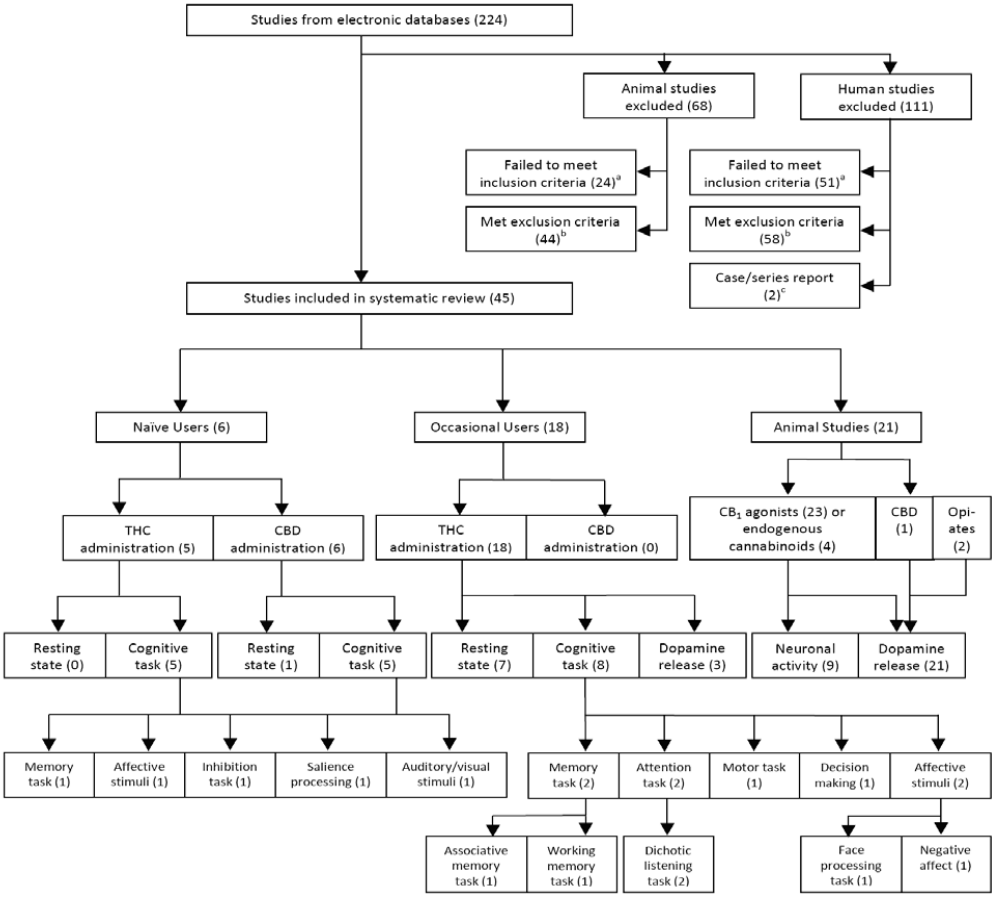


Fig. (1). Flow diagram of included functional neuroimaging studies.
[a]Animal studies not involving cannabinoid effects on blood flow, cerebral metabolism or dopamine release: [40-63]. Human studies not involving experimental administration of cannabinoids: [64-113] or no handedness matched [114]. [b]Animal *in vitro* studies: [115-133]; non-acute or combined drug administration of cannabinoids: [21, 134-146]; anesthetized animals during the experimental procedure [147-157]. Human studies involving chronic cannabis users: [158-214]; psychiatric, other abuse or medical disorder: [10]. [C]Case/series report: [215, 216].

Table 1. Acute effects of THC and CBD in humans: Functional neuroimaging studies in occasional cannabis users.

Author (yr.)	Method	Design	M/F	Mean (SD) age	Image analysis	Condition	Drug	Dose`; Route	Time to imaging method	Comparison (placebo/ baseline)	Results		
											Brain area	Detailed results	Correlations with clinical variables
OCCASIONAL Users													
Acute effects on cerebral blood flow in resting state											FL-PL-TL-OL-I-BD-Cb		
Volkow *et al.* (1991) [217]	^{18}F-FDG-PET	NB, NC, NR, WS	8/0	34.0 (8.0)	ROI	Resting state	THC	2 mg; IV	30-40'	Baseline	○○○○●○●	↑ global CBF ↑ rCBF L/R cerebellum	↑ rCBF L/R cerebellum with intoxication (AIS)
Mathew *et al.* (1992) [218]	^{133}Xe-SPECT	DB, PC, R, WS	20/0	25.3 (6.4)	Scinti-llation detector	Resting state	MC THC S	1.75% 3.55%; S	30', 60' and 120'	MC without THC (Placebo)	●●●○●●●	↑ global CBF 30': 1.75%: ↑ rCBF R frontal lobe; 3.55%: ↑ rCBF R frontal, R temporal regions and L parietal lobe	↑ global CBF with intoxication (AIS), plasma levels and pulse rate
											●○○○●●●	60': 1.75%: NS effect; 3.55%: ↑ rCBF R frontal lobe	
											○○●○●●●	120': 1.75%: ↑ rCBF temporal lobe; 3.55%: NS effect	
Mathew *et al.* (1993) [219]	^{133}Xe-SPECT	DB, PC, R, WS	35/0	21.7 (8.0)	Scinti-llation detector	Resting state	MC THC S	1.75% 3.55%; S	30', 60' and 120'	Baseline	●●●●●●●	↑ global CBF 1.75%: global CBF was significantly higher at 30' from baseline 3.55%: global CBF was significantly higher at 30' and 60' from baseline ↑ rCBF were stronger in the anterior part of each hemisphere	↑ global CBF with intoxication (AIS), depersonalization (DPI), dissociative experiences (TDI) and confusion
Mathew *et al.* (1997) [220]	$H_2{}^{15}O$-PET	DB, PC, R, BS	20/12	32.5 (7.6)	ROI	Resting state	THC	3 and 5 mg; IV	30' and 60'	Placebo	●●●●●●○	↑ global CBF 30': 3 mg: ↑ rCBF R frontal, cingulate and L insula; 5 mg: ↑ rCBF in most studied regions (NE)	↑ global CBF and rCBF (anterior frontal lobe and ACC) with intoxication (AIS)
											●●●○●●○	60': 3 mg: ↑ rCBF L/R frontal, parietal, temporal, cingulate, insula, basal ganglia and L amygdala; 5 mg: ↑ rCBF in same regions and thalamus	
Mathew *et al.* (1998) [221]	$H_2{}^{15}O$-PET	DB, PC, R, WS	22/24	29.9 (6.5)	ROI	Resting state	THC	3 and 5 mg; IV	30' and 60'	Baseline	●●●●●●●	↑ rCBF greater at 30' for all regions studied, with greatest increases in ACC, insula and cerebellum but also in frontal, temporal, parietal and occipital cortices	↓ rCBF cerebellum with greater increase of disturbance of time sense (TDI)
Mathew *et al.* (1999) [222]	$H_2{}^{15}O$-PET	DB, PC, R, WS	33/26	31.8 (7.5)	ROI*	Resting state	THC	3 and 5 mg; IV	30', 60' and 120'	Baseline	●●●●●●●	↑ global CBF 30': 3 mg: ↑ rCBF L/R frontal, parietal, occipital, cingulate and L insula; 5 mg: ↑ rCBF L/R frontal, parietal, occipital, R cingulate and L insula	↑ rCBF R frontal and R ACC with depersonalization (DPI) ↑ rCBF R frontal with intoxication (AIS)
											●●●●●●●	60': 3 mg: ↑ rCBF L frontal, R parietal, R occipital, L/R cingulate and L insula; 5 mg: ↑ rCBF R frontal, L/R parietal, occipital, insula and R cingulate	
												120': 3 mg: NS effect; 5 mg: ↑ rCBF R insula.	
											○○○○●○○	↓ rCBF basal ganglia, thalamus, amygdala and hippocampus	
van Hell *et al.* (2011) [223]	fMRI 3T	DB, PC, R, WS	20/0	21.1 (2.2)	ASL - Whole Brain ROI	Resting state	THC	6 mg; I	5'	Placebo	●●○●●○○	↑ rCBF ACC, L superior frontal cortex and L/R insula ↓ rCBF R post-central gyrus and L/R occipital gyrus	↑ rCBF L superior frontal cortex with decreased feeling high effect (AIS)
					Whole Brain ROI	Resting state	THC	6 mg; I	5'	Placebo	○○○○●●●	↓ tSNR (↑ BOLD) R insula, L substantia nigra and L cerebellum	
Acute effects on cerebral blood flow during cognitive tasks													
O'Leary *et al.* (2002) [225]	$H_2{}^{15}O$-PET	DB, PC, NR, WS	6/6	30.5 (8.6)	Whole Brain	Dichotic listening task	MC THC S	20 mg; S	10-15'	MC without THC (Placebo)	●○●●●○●	↑ rCBF L/R ventral frontal and temporal lobes, ACC, insula, cerebellum ↓ rCBF L/R frontal, L superior temporal gyrus and R occipital lobe	
O'Leary *et al.* (2003) [226]	$H_2{}^{15}O$-PET	DB, PC, NR, WS	6/6	21.7 (1.4)	Whole Brain	Self-paced counting task	MC THC S	20 mg; S	10-15'	Baseline	●●●●○○○	↑ rCBF ACC, R cerebellum and L OFC, L/R ventral and mesial frontal lobe, R DLPFC, R middle temporal and R parietal lobe ↓ rCBF R occipital, temporal and frontal lobe	↑ rCBF cerebellum with increased self-pace tapping and counting rate
O'Leary *et al.* (2007) [227]	$H_2{}^{15}O$-PET	DB, PC, NR, WS	6/6	23.5 (4.3)	Whole Brain	Dichotic listening task	MC THC S	20 mg; S	10-15'	MC without THC (Placebo)	●○●●●○●	↑ rCBF R OFC, ACC, L/R temporal poles, insula, cerebellum ↓ rCBF R superior temporal gyrus and mesial occipital lobe	
Phan *et al.* (2008) [230]	fMRI 3T	DB, PC, R, WS	8/8	20.8 (2.6)	ROI*	Emotional face processing task	THC	7.5 mg; O	120'	Placebo	●●●●●●●	↓ BOLD R amygdala (angry/fearful faces)	↓ BOLD R amygdala with increase in DEQ "feel drug" (trend)
Rabinak *et al.* (2012) [231]	fMRI 3T	DB, PC, R, WS	8/8	20.8 (2.6)	Whole Brain ROI*	Affective stimuli	THC	7.5 mg; O	120'	Placebo	●●○●○○●	↓ BOLD L middle occipital gyrus, L/R postcentral gyrus, L/R superior frontal and R parietal gyrus, R cerebellum, R subgenual ACC (negative affect) ACC: ↓ BOLD subgenual ACC (negative affect)	
van Hell *et al.* (2012) [232]	fMRI 3T	DB, PC, R, WS	11/0	21.7 (2.3)	ROI*	Monetary incentive delay task	THC	6 mg; I	5'	Placebo	●●●○○○○	↓ BOLD L/R inferior parietal and temporal cortices, middle OFC, R medial superior frontal cortex, posterior and anterior cingulate (reward feedback)	
Bossong *et al.* (2012) [228]	fMRI 3T	DB, PC, R, WS	13/0	21.6 (2.1)	ROI	Associative memory task	THC	6 mg; I	5'	Placebo	●○○●○○○	Encoding: ↓ BOLD R insula, R inferior frontal gyrus, L middle occipital gyrus	
											○●○●○○○	Recall: ↑ BOLD L/R cuneus and precuneus	
Bossong *et al.* (2015) [229]	fMRI 3T	DB, PC, R, WS	17/0	21.4 (2.1)	ROI*	Working memory (WM)	THC	6 mg; I	5'	Placebo	●●●○○○●	↑ BOLD for low WM loads and reduced the linear relationship between WM load and activity observed in placebo condition in the WM system, particularly in L	

Acute effects on dopamine release											DLPFC, inferior temporal and parietal gyrus and cerebellum
Bossong *et al.* (2009) [239]	[^{11}C]raclopride PET	DB, PC, R, WS	7/0	21.9 (2.7)	ROI[a]	Resting state	THC	8 mg; I	40'	Placebo	↓ BP_{ND} (↑ DA release) ventral striatum and precommissural dorsal putamen
Stokes *et al.* (2009) [240]	[^{11}C]raclopride PET	SB, PC, R, WS	7/6	33.0 (7.0)	ROI[a]	Resting state	THC	10 mg; O	90'	Placebo	↓ percentage change in BP_{ND} in whole striatum with ↑ THC plasma levels
Barkus *et al.* (2011) [241]	[123]-IBZM SPECT	DB, PC, R, WS	9/0	26.3 (4.2)	ROI	Resting state	THC	2.5 mg; IV	30'	Placebo	

Note: Yr. = years; M = male; F = female; SD = standard deviation; FL = frontal lobe; PL = parietal lobe; TL = temporal lobe; OL = occipital lobe; I = insula; BD = basal ganglia and diencephalon; Cb = cerebellum; fMRI = functional magnetic resonance imaging; SPECT = single photon emission tomography; PET = positron emission tomography; FDG = fludeoxyglucose; IBZM = iodobenzamide; SB = single-blinded; DB = double-blinded; NB = non-blinded; PC = placebo-controlled; NC = non-controlled; R = randomized; NR = non-randomized; BS = between-subjects; WS = within-subjects; ASL = arterial spin labelling; MC = marijuana cigarette (contain other cannabinoids); O = oral; I = inhaled; S = smoking; IV = intravenous; L = left hemisphere; R = right hemisphere; ROI = region of interest; CBF = global cerebral blood flow; rCBF = regional cerebral blood flow; BOLD = blood oxygenation-level dependent; tSNR = temporal signal-to-noise ratio; BP_{ND} = non-displaceable binding potential; NS = Non-significant; NE = not specified; PFC = prefrontal cortex; DLPFC = dorsolateral prefrontal cortex; OFC = orbitofrontal cortex; ACC = anterior cingulated cortex; AIS = analog intoxication scale; DPI = depersonalization inventory; TDI = temporal disintegration inventory; DEQ = drug effects questionnaire; COMT = Cathecol O-Methyltransferase.

[a] mg of THC/CBD *per os*, mg/ml of THC/CBD intravenously administered or % THC/CBD in cannabis cigarettes.

● = significant increase; ● = significant decrease; ○ = non-significant difference; ● = not examined.

[a] Multiple comparison correction.

Intravenous administration. Four studies examined resting state CBF before and after intravenous infusion of 2 to 5 mg of THC, three of which used $H_2{}^{15}O$-PET [220-222] and one used ^{18}F-FDG-PET [217] (Table **1**). Volkow *et al*. (1991) [217] was not placebo-controlled. Similar to the results described above, these studies described dose-dependent [220-222] increases in regional brain activity at rest following the administration of THC, relative to baseline or placebo use. The greatest increases were described in the anterior cingulate cortex [220-222], insula [220-222] and cerebellum [217, 221], even though increased activation was also reported in the basal ganglia, thalamus and along the frontal, parietal, temporal and occipital cortices [220-222]. Mathew *et al*. (1999) [222] reported decreased activation in the basal ganglia, thalamus, amygdala and hippocampus (Table **1**). The subjective levels of intoxication were positively correlated with global CBF [220, 221] and rCBF in the cerebellum [217], anterior frontal lobe and cingulate cortex [220, 221]. Furthermore, disturbance of time sense as assessed with the TDI was negatively correlated with rCBF in the cerebellum [221], and DPI-measured levels of depersonalization were positively correlated with rCBF in the frontal and right anterior cingulate cortices [222].

Inhaled administration. Van Hell *et al*. (2011) [223] assessed the effects of inhaled THC on baseline brain perfusion using arterial spin labeling (ASL), as well as brain activity using resting-state fMRI. Consistent with previous PET and SPECT studies, ASL showed increased perfusion in the anterior cingulate cortex, superior frontal cortex and insula but also reduced perfusion in the postcentral and occipital gyri after THC [223]. Interestingly, resting state fMRI showed increased baseline brain activity in the insula but also in the substantia nigra and cerebellum, suggesting that baseline perfusion measures may not simply amplify resting-state fluctuations [223]. However, contrary to the findings described above in relation to intoxication and depersonalization [220, 222], perfusion changes in the frontal cortex were negatively correlated with ratings of "feeling high" (Visual Analogue Scale) [223].

2.1.1.2. Naïve Cannabis Users

Crippa *et al*. (2004) [224] explored the acute effect of oral CBD relative to placebo in a sample of naïve cannabis users using ^{9m}Tc-ECD SPECT. CBD decreased rCBF in two of the *a priori* selected brain regions where CBD effects had been expected: the amygdala-hippocampal complex extending to the hypothalamus, and the posterior cingulate gyrus. There was also a cluster of greater activity in the left parahippocampal gyrus. Other unpredicted foci of decreased CBF were described in the whole brain analysis but none of these remained significant after correction for multiple comparisons. No significant correlations were observed between blood flow and subjective anxiety ratings (as measured by the Visual Analogue Mood Scale) in the study [224].

2.1.2. Acute Effects of Cannabinoids on Cerebral Blood Flow During Cognitive Tasks

2.1.2.1. Occasional Cannabis Users

Eight double-blinded placebo-controlled studies comparing CBF during cognitive paradigms before and following THC administration in occasional users were included in the present review (Table **1**). Methods used were either $H_2{}^{15}O$-PET [225-227] or fMRI [228-232].

*Memory task*s. Two pharmacological fMRI studies investigated the effects of inhaled THC on memory-related brain functioning compared to placebo [228, 229], particularly on associative and working memory tasks. Only working memory performance was significantly reduced after THC administration [229]. During the associative memory task [228], THC caused reductions in activity during encoding in the right insula, right inferior frontal gyrus and left middle occipital gyrus, as well as increases in brain activity during recall which were most prominent in the cuneus and precuneus bilaterally. The working memory paradigm included five difficulty levels to induce a gradual working memory load [229]. While brain activity increased linearly with rising memory loads in the placebo condition, the use of THC led to enhanced brain activity for low memory loads and reduced this linear relationship in brain areas related to working memory, such as the left dorsolateral prefrontal cortex, inferior temporal and parietal gyri and cerebellum. Performance started to declined at a lower memory load after THC administration, possibly indicating that a perturbation of the endocannabinoid system may affect working memory function [229].

Attention tasks. Two $H_2{}^{15}O$-PET studies conducted by the same group assessed rCBF changes using a dichotic listening task after subjects smoked marijuana or placebo cigarettes [225, 227]. In both studies, marijuana-cigarettes did not impair behavioural performance on the attention task but caused significant increases in rCBF in the anterior cingulate cortex, mesial and orbital frontal lobes, insula, temporal poles and cerebellum [225, 227]. Decreased activity was described in auditory regions of the temporal lobe but also in the mesial portion of the occipital and parietal lobes, including the precuneus and visual cortex, despite the fact that subjects had their eyes closed and covered by a cloth [225, 227]. These data showed that despite marked effects on rCBF, marijuana smoking had a relatively modest effect on behavioural performance during this auditory focused attention task.

Motor tasks. The above group also studied the acute effects of marijuana cigarettes during a self-paced counting task using $H_2{}^{15}O$-PET in groups of occasional or chronic cannabis users [226]. Smoked THC was associated with faster response times as well as with increased activation mainly in the cerebellum and ventral frontal lobe. Overall, the magnitude of the effects on activation was

Table 2. Acute effects of THC and CBD in humans: Functional neuroimaging studies in naïve cannabis users.

Author (yr.)	Method	Design	M/F	Mean (SD) age	Image analysis	Condition	Drug	Dose*; Route	Time to imaging method	Comparison (placebo/drug /baseline)	Results		
											Brain area	Detailed results	Correlations with clinical variables
NAÏVE Users													
Acute effects on cerebral blood flow in resting state											FL-PL-TL-OL-I-BD-Cb		
Crippa *et al.* (2004) [224]	^{99m}Tc-ECD SPECT	DB, PC, R, WS	10/0	29.8 (5.1)	ROI Whole brain	Resting state	CBD	400 mg; O	110'	Placebo	○●●○○●○	↓ rCBF L amygdala, hippocampus, hypothalamus ↓ rCBF L posterior cingulate gyrus ↑ rCBF L parahippocampus, fusiform gyrus	
Acute effects on cerebral blood flow during cognitive tasks													
Borgwardt *et al.* (2008) [235]	fMRI 1.5T	DB, PC, R, WS	15/0	26.7 (5.7)	Whole brain	Response inhibition task	THC	10 mg; O	1-2h	Placebo	●●○○○●○	↓ BOLD R inferior frontal gyrus; L/R ACC and precuneus ↑ BOLD R hippocampus, transverse temporal and fusiform gyrus, parahippocampus and caudate; L precuneus and posterior cingulate	
							CBD	600 mg; O	1-2h	Placebo	○○●○●○○	↓ BOLD L insula and superior and transverse temporal gyrus	
Fusar-Poli *et al.* (2009) [236]	fMRI 1.5T	DB, PC, R, WS	15/0	26.7 (5.7)	Whole brain	Affective stimuli	THC	10 mg; O	1-2h	Placebo	○●○○○○○ ●○○○○○○ ●○○○○○○	Neutral face: ↑ BOLD posterior temporal and L inferior parietal lobule Mildly fearful face: ↑ BOLD R inferior parietal lobule; ↓ BOLD L frontal gyrus Intensely fearful face: ↓ BOLD L/R frontal gyrus, posterior cingulate gyrus; ↑ BOLD L precuneus, L/R primary sensorimotor cortex	
							CBD	600 mg; O	1-2h	Placebo	○○○○○○○ ○○○○○○● ●●●○○●●	Neutral face: NS differences to placebo Mildly fearful face: ↓ BOLD L/R posterior lobe cerebellum Intensely fearful face: ↓ BOLD amygdala, anterior parahippocampus, anterior and posterior cingulate gyrus, L occipital gyurs, R posterior lobe cerebellum	↓ BOLD amygdala and anterior cingulate gyrus with reduction in SCR fluctuations
Bhattacharyya *et al.* (2009) [233]	fMRI 1.5T	DB, PC, R, WS	15/0	26.7 (5.7)	Whole brain	Verbal paired associate task	THC	10 mg; O	1-2h	Placebo	○○●○○○○ ●○○○○●○	Encoding block 1: BOLD ↓ parahippocampus; blocks 2 and 3: ↑ BOLD parahippocampus Recall block 1 and 2: NS differences; block 3: ↑ BOLD L dorsoanterior cingulate and medioprefrontal cortex; ↓ BOLD L/R striatum and L rostroanterior cingulate	↓ BOLD striatum and rostroanterior cingulated cortex with severity of psychotic symptoms (PANSS)
						Verbal paired associate task	CBD	600 mg; O	1-2h	Placebo	○○○○○○○	NS differences to placebo	
Winton-Brown *et al.* (2011) [237]	fMRI 1.5T	DB, PC, R, WS	14/0	26.7 (5.7)	Whole Brain	Auditory and visual stimuli	THC	10 mg; O	1-2h	Placebo	●●●○●○●	Auditory: ↓ BOLD L/R anterior and posterior superior and middle temporal gyrus, insula, supramarginal gyrus, R inferior frontal gyrus and L cerebellum	↓ BOLD temporal (auditory) with increase in PANSS total and PANSS positive
											○○●●○○○	Visual: ↓ BOLD extrastriate visual cortex; ↑ BOLD R lingual and occipital gyrus, L lingual and fusiform gyrus	↑ BOLD visual cortex with increase in PANSS positive
						Auditory and visual stimuli	CBD	600 mg; O	1-2h	Placebo	○○●○●○○	Auditory: ↑ BOLD L/R temporal cortex, insula, parahippocampus and hippocampus; ↓ BOLD L superior temporal gyrus, insula, posterior temporal and supramarginal gyrus	
											○○○●○○○	Visual: ↑ BOLD R middle and inferior occipital gyrus, lingual gyrus and cuneus	
						Auditory and visual stimuli	THC	10 mg; O	1-2h	600 mg CBD; O	○○●○○○○ ○○○●○○○	Auditory: relative to CBD, THC ↓ BOLD in R superior and middle temporal gyrus Visual: relative to CBD, THC ↑ BOLD L lingual and middle occipital gyrus and ↓ BOLD L/R occipital regions	
Bhattacharyya *et al.* (2012) [234]	fMRI 1.5T	DB, PC, R, WS	15/0	26.7 (5.7)	Whole Brain	Salience processing	THC	10 mg; O	1-2h	Placebo	●○○○●●○	↑ BOLD R PFC, OFC and frontal pole ↓ BOLD R head of caudate, putamen, insula and thalamus	↓ BOLD R caudate with severity of psychotic symptoms (PANSS) and with increased response latency ↑ BOLD PFC with greater effects on response latency
						Salience processing	CBD	600 mg; O	1-2h	Placebo	●○○○●●○	↓ BOLD R PFC ↑ BOLD L caudate, parahippocampus, insula, precentral gyrus and thalamus	
						Salience processing	THC	600 mg; O	1-2h	600 mg CBD; O	●○○●○○●	Relative to CBD, THC ↑ BOLD R OFC and ↓ BOLD L parahippocampus, caudate, putamen, thalamus and lingual gyrus	

Note: Yr. = years; M = male; F = female; SD = standard deviation; FL = frontal lobe; PL = parietal lobe; TL = temporal lobe; OL = occipital lobe; I = insula; BD = basal ganglia and diencephalon; Cb = cerebellum; fMRI = functional magnetic resonance imaging; SPECT = single photon emission tomography; PET = positron emission tomography; ; ^{99m}Tc-ECD = ethyl-cisteinate-dimer labelled with technetium-99 m; DB = double-blinded; PC = placebo-controlled; R = randomized; WS = within-subject; O = oral; I = inhaled; S = smoking; IV = intravenous; L = left hemisphere; R = right hemisphere; ROI = region of interest; CBF = global cerebral blood flow; rCBF = regional cerebral blood flow; BOLD = blood oxygenation-level dependent; NS = Non-significant; SCR = skin conductance responses; PFC = prefrontal cortex; OFC = orbitofrontal cortex; ACC = anterior cingulated cortex; PANSS = positive and negative syndrome scale.

* mg of THC/CBD *per os*, mg/ml of THC/CBD intravenously administered or % THC/CBD in cannabis cigarettes.

● = significant increase; ● = significant decrease; ● = significant connectivity disruption; ○ = non-significant difference; ◎ = not examined.

a Multiple comparison correction.

greater in chronic cannabis users, except in the ventral frontal lobe, where brain activity was larger in the occasional users group [226]. The speeding-up in behavioural performance were correlated across subjects with rCBF changes in the cerebellum, suggesting that marijuana may increase the activity of an internal cerebellar clock [226].

Decision-making tasks. Van Hell *et al*. (2012) [232] used fMRI to compare anticipatory and feedback-related brain activity changes after placebo or inhaled THC, using a monetary incentive delay task. Subjects showed faster reaction times during reward trials compared to neutral trials but this effect was not altered by THC. However, THC induced attenuation of the brain response to feed-

back in reward trials in the parietal and temporal cortices, middle orbitofrontal cortex, medial superior frontal cortex and anterior and posterior cingulate gyrus [232]. These findings suggest that THC may affect the appreciation of obtaining a monetary reward, which may be relevant for addictive disorders (in which appreciation of natural rewards may be affected) [232].

Affective processing tasks. Two fMRI studies conducted in the same sample assessed emotional processing after oral THC challenge [230, 231]. First, the effects of THC were evaluated on amygdala reactivity to social signs of threat (fearful and angry faces), and THC was found to significantly attenuate amygdala activation to threatening faces [230]. In the second study, the effects of THC were assessed on subjective and brain activity indices during stimulus-induced negative affect [231]. Within the *a priori* brain regions selected, THC reduced subgenual anterior cingulate cortex activity [231]. No significant correlations between brain activity and subjective drug effects were reported [230, 231], apart from a trend towards reduced amygdala activation related to an increase in "feel drug" [as assessed using the drug effect questionnaire (DEQ)] [230].

2.1.2.2. Naïve Cannabis Users

We included in this review five double-blind, placebo-controlled fMRI studies in which brain activity was measured during performance of cognitive tasks before and following oral THC or CBD administration in naïve cannabis users (Table **2**) [233-237].

Memory and verbal learning tasks. Bhattacharyya *et al*. (2009) [233] investigated the effects of THC and CBD on regional brain functioning during verbal paired associate learning. As the same stimuli was presented during four blocks of encoding, only the imaging results for the first presentation stimuli are comparable to the above described study carried out with occasional cannabis users [229]. However, in both studies there was no significant effect of cannabinoids on task performance [229, 233]. The expected linear activity decrease in the parahippocampal gyrus seen over repeated encoding blocks was no longer evident after oral THC administration (Table **1**), and this may reflect increased demands on encoding under the influence of THC [30, 233]. During recall, THC augmented activation in left medial prefrontal and dorsal anterior cingulate cortices and also attenuated activity in the left rostral anterior cingulate cortex and bilateral striatum. Striatal effects were directly correlated with the severity of psychotic symptoms [233]. In contrast, CBD administration modulated activation in a different set of areas in the brain during repeated encoding and recall blocks, but these areas did not reach the statistical threshold established.

Affective processing tasks. The acute effects of oral THC and CBD in naïve cannabis users have also been investigated during the processing of fearful faces using fMRI [236]. THC administration was associated with increased activation in the right inferior parietal lobule and attenuation of the engagement of the left medial frontal gyrus while viewing mildly fearful faces. When subjects were presented with intensely fearful faces, THC increased brain activity in the left precuneus and primary sensorimotor cortex bilaterally, and decreased activity in the middle frontal gyrus and posterior cingulate gyrus bilaterally. Inconsistently with results reported previously in occasional cannabis users [230], there were no effects on amygdala activity, and this negative finding may be attributed to a modestly powered sample [30, 236]. On the other hand, a direct comparison of the effects of THC and CBD found that THC had an effect on amygdala activation, again possibly suggesting that lack of effect on direct comparison of THC versus placebo was a power issue [13].

In contrast, CBD showed an attenuation of the activation in the amygdala as well as in the anterior and posterior cingulate cortices [236]. Moreover, these effects were associated with the effect of CBD on autonomic arousal [indicated by the number of fluctuations in skin conductance response (SCR)]. This result, consistent with the findings shown above of CBD administration in occasional cannabis users [224], provides further evidence of the potential role of CBD as an anxiolytic agent [236]. In this sense, it has been suggested that the disruption of prefrontal-subcortical connectivity by CBD, but not THC, during the neural response to fearful faces may represent neurophysiological correlates of its anxiolytic properties [238].

Response inhibition tasks. During a motor inhibition task (Go/No-Go), THC was associated with a decrease in the normal activation associated with response inhibition in the right inferior frontal gyrus and anterior cingulate cortex [235], key regions implicated in inhibitory control. However, THC also enhanced activation in brain areas not implicated in response inhibition such as the right hippocampus, transverse temporal gyrus and fusiform gyrus. CBD deactivated the left temporal cortex and insula [235], brain areas also not usually implicated in this cognitive process.

Salience processing tasks. Bhattacharyya *et al*. (2012) [234] reported effects of THC and CBD on the processing of salience, as well as its relation with psychotic symptoms. Employing a visual oddball detection task, THC attenuated activation in the right caudate but augmented activity in the right prefrontal cortex, including the inferior frontal gyrus [234]. THC also reduced the response latency to standard stimuli relative to oddball stimuli, suggesting that THC may have made the non-salient stimuli to appear relatively more salient. These findings help in the understanding of the brain effects whereby cannabis may contribute to the induction of psychotic symptoms [30, 234]. Moreover, the effect of THC in the right caudate nucleus was negatively correlated with the severity of psychotic symptoms and the changes in response latency [234]. Interestingly, as shown in Table **2**, the effects of CBD on task-related brain activation during the same task in this study were in the opposite direction to those of THC: relative to placebo, CBD augmented activity in the left caudate and hippocampus, but attenuated right prefrontal activation [234].

Sensory processing tasks. Finally, Winton-Brown *et al*. (2011) [237] used fMRI to assess the modulation of brain activation during auditory and visual processing. THC attenuated activation bilaterally in the anterior and posterior superior temporal gyrus and middle temporal gyrus, insula, supramarginal gyrus, right inferior frontal gyrus and left cerebellum during auditory processing. The attenuating effect of THC on temporal cortical activity was correlated with the severity of psychotic symptoms [237]. Although this investigation involved administration of pure cannabinoids and used different task and imaging methods, their findings are consistent with the studies discussed above that used $H_2{}^{15}O$-PET to measure the effect of marijuana cigarettes on rCBF during a dichotic auditory listening task in occasional cannabis users [225, 227], where reduced blood flow in the temporal cortices bilaterally was observed. In the fMRI study presented herein, CBD showed opposing effects when compared to THC on temporal cortical activation, particularly in the right superior and middle temporal gyri, as well as in the supramarginal gyrus and insula (Table **2**).

During visual stimulation, THC attenuated activation in the extrastriate visual cortex and increased activation in the lingual and the primary visual cortex on the right side, as well as in portions of the lingual and fusiform gyrus on the left side [237]. Increased activation in primary visual cortex was correlated with the severity of psychotic symptoms. Relative to CBD, THC increased activation in the left lingual and primary visual cortices and attenuated activation in other occipital regions bilaterally [237] (Table **2**).

2.1.3. Acute Effects of Cannabinoids on Dopamine Release

Three recently published studies have used neurochemical imaging to measure dopamine release in occasional cannabis users following THC administration (Table **1**), two of which used [^{11}C]raclopride PET [239, 240] and one [123]-IBZM SPECT [241]. These studies investigated whether THC can induce dopamine re-

lease in the striatum using PET and dopamine D_2/D_3 receptor radioligands. With this method, an increase in synaptic dopamine concentrations can be determined by a reduction in ligand binding.

Bossong *et al*. (2009) [239] showed an approximately 3.5% decrease of [^{11}C]raclopride binding in the ventral striatum and precommisural dorsal putamen after THC inhalation, consistent with an increase in dopamine levels in these regions. However, in a larger PET study, Stokes *et al*. (2009) [240] found no significant differences in striatal [^{11}C]raclopride binding between oral THC and placebo administration. Finally, Barkus *et al*. (2011) [241], using [123]-IBZM SPECT, found no significant differences in radioligand binding indices in the caudate or putamen under the THC condition when compared to the placebo condition. Overall, these three studies suggest that a single-dose THC challenge may have only a modest effect on dopamine release in humans, as measured by neurochemical imaging. Thereby these findings do not support a central role for increased striatal dopaminergic activity in THC-induced psychosis.

2.2. Animal Studies

2.2.1. Acute Effects of Cannabinoids on Cerebral Blood Flow and Brain Glucose Metabolism

We identified eight functional imaging studies in animals assessing neuronal activity changes after administration of THC, CB_1 agonists or endogenous cannabinoids. Seven of these investigations were *ex vivo* animal studies using 2-DG [241-245] or [^{14}C]iodoantipyrine (IAP) autoradiography [246-248], and one was an *in vivo* ^{18}F-FDG PET study [249] (Table **3**).

Autoradiographic studies. Studies using IAP autoradiography have reported an acute and dose-dependent reduction in rCBF in the rat brain after intravenous doses of THC (from 0.5 to 16 mg/kg) [246, 247], or anandamide (from 10 mg/kg to 30 mg/kg), an endocannabinoid ligand [248]. Brain areas affected included regions with high density of cannabinoid receptors, which are thought to be involved in the characteristic behavioural actions of THC. Administration of the active metabolite 11-OH-THC (4 mg/kg) also induced CBF reductions in a regionally specific manner [246] (Table **3**). Bloom *et al*. (1997) [246] reported increased blood flow in the arcuate nucleus after 4 mg/kg of THC.

The studies that employed 2-DG autoradiography have also reported acute and dose-dependent reductions in brain glucose metabolism after the administration of THC [242, 243, 245] or the CB_1 agonist WIN 55212-2 [244]. Margulies *et al*. (1991) [243] reported findings of altered 2-DG uptake in limbic structures in the rat brain in a biphasic manner: increases at low doses (0.2-0.5 mg/kg) and decrements at high doses (2-10 mg/kg) of THC. A similar study by Freedland and colleagues (2002) [242] showed altered cerebral glucose metabolism but no brain activity changes at similar low doses (0.25 mg/kg). However, a dose-dependent decrease was observed at higher doses of THC [242, 245]. Using a different drug (the CB_1 agonist WIN 55212-2) and a lower dose (0.15-0.30 mg/kg), Poniteri *et al*. (1999) [244] also described a biphasic pattern in brain glucose utilization: an increase at low doses in the nucleus accumbens and a decrease at high doses in the hippocampus and thalamus.

Only two studies have measured the temporal course of the effects of acute administration of cannabinoids on brain functional indices [245, 248]. Stein *et al*. (1998) [248] measured time-course of changes after anandamide administration at 15, 20 and 60 minutes: at later time points during the study (60 minutes), the widespread changes in blood flow that had been detected initially became largely restricted to parts of the extended amygdala. In the same line, Whitlow *et al*. (2002) [245] studied the temporal course of the effects of acute administration of THC (2.5 and 10 mg/kg). THC also produced widespread dose-dependent reductions in rates of cerebral metabolism when 2-DG was applied fifteen minutes after treatment. However, when the 2-DG method was applied at 6 hours, a more limited set of brain structures were affected (Table **3**). Finally, at 24 hours, glucose utilization remained depressed within mesolimbic and amygdalar areas. Despite differences in the half-lives of the cannabinoid agonists investigated, these findings are robust and may highlight region-specific effects of cannabinoids within amygdala and extended regions [245].

PET studies. Nguyen *et al*. (2012) [249] examined the short-term effects of a single-dose injection of the synthetic cannabinoid agonist HU210 on glucose metabolism in the rat brain using ^{18}F-FDG PET. In contrast with the above autoradiography studies but consistent with the human studies [217-220, 222], globally increased brain metabolism was found shortly after drug administration. This effect was not apparent 24 hours later, and no changes were detected in individual brain regions (neither after fifteen minutes nor after 24 hours following administration of the CB_1 agonist) [249].

2.2.2. Acute Effects of Cannabinoids on Dopamine Release

Thirteen studies have employed neurochemical [250-258] or electrophysiological [259-262] imaging methods to measure dopamine release in animals following the administration of cannabinoids (Table **3**).

Electrochemical studies. *In vivo* microdialysis experiments seem to indicate that cannabinoid receptor activation markedly increases dopamine release in the nucleus accumbens [253, 255-258], as well as in the striatum [254], ventral tegmental area [252] and prefrontal cortex [251]. Cannabinoid-induced increase in dopamine release was seen after systemic administration of THC [251-254, 258], cannabinoid agonist WIN 55212-2 [258], the endogenous cannabinoids anandamide and methanandamide [256, 257], CBD [255] and heroin [258]. These effects were attributed to an action on CB_1 cannabinoid receptors because they were prevented by the administration of CB_1 antagonists [254, 256, 258] and even potentiated by agonists [256, 257]. Moreover, Tanda *et al*. (1997) [258] reported that although CB_1 antagonists prevented the action of cannabinoids but not of opiates, the opioid antagonists prevented the effects of both, suggesting the existence of an interaction between opioid and cannabinoid systems [263-265]. On the other hand, Malone *et al*. (1999) [254] observed that pretreatment with fluoxetine also abolished the THC-induced dopamine release. However, when fluoxetine was administered locally into the striatum after THC administration, the effect was potentiated. Thereby, these studies suggest that dopamine release induced by THC may be modulated by opioid and serotoninergic transmitter systems.

Negative results were reported after gavage administration of THC (1 and 10 mg/kg) [250] and intraperitoneal administration of anandamide (10 mg/kg) [257]. Finally, Tanda *et al*. (1997) [258] also administered a non-psychoactive cannabinoid (cannabinol), which, as expected, failed to modify dialysate dopamine levels in the nucleus accumbens.

Electrophysiological studies. Consistent with the above microdialysis experiments, studies recording neural spike activity in awake rats have reported dose-dependent increases in the firing rates of dopaminergic neurons in the nucleus accumbens [259, 261, 262], substantia nigra pars reticulata [262], and prefrontal cortex [260]. Such effects have been reported after systemic administration of THC [260-262], the cannabinoid agonists WIN 55212-2 [259-261] and CP55940 [261], and morphine [262]. This effect was attributed to an action on specific cannabinoid receptors because it was prevented by the administration of CB_1 antagonists [259, 261, 262]. However, in contrast with the findings of microdialysis studies [258], the role of opioid neurotransmission on the action of cannabinoids was not supported by this investigation, as the opioid antagonist naloxone did not prevent the effect of THC on cell firing rates [262].

Table 3. Acute effects of THC, CB_1 agonists and CBD in animals: Functional neuroimaging studies.

Author (yr.)	Method	Species	EG M/F	CG M/F	Image analysis	Drug	Dose; Route	Time to imaging method	Comparison (vehicle/ baseline)	Results	
										Brain area	Detailed results
ANIMAL studies											
Acute effects on cerebral blood flow and brain glucose metabolism										FL-PL-TL-OL-I-BD-Cb	
Goldman *et al.* (1975) [247]	[^{14}C]IAP	UnR rats	13/0	22/0	Autoradio-graphy ROI	THC	1 mg/kg; IV	20'	Vehicle	○○●○○●●●	↓ rCBF cerebellum, hypothalamus, basal ganglia, dorsal hippocampus
Margulies *et al.* (1991) [243]	[^{3}H]2-DG	SD rats	20/0	4/0	Autoradio-graphy ROI	THC	0.2-10 mg/kg; IV	10'	Vehicle	●●●●●○○●	0.2 mg/kg: ↑ LCGU in limbic and cortical areas
										●●●●●○○●	>2 mg/kg: ↓ LCGU in limbic and cortical areas except for auditory cortex. NS effect observed in diencephalon and brainstem structures
Bloom *et al.* (1997) [246]	[^{14}C]IAP	SD rats	25/0	5/0	Autoradio-graphy ROI	THC	0.5-16 mg/kg; IV	30'	Vehicle	●○○○○○●○	< 4 mg/kg: ↓ rCBF in claustrum, NAcc, medial PFC
										●○●○○●○	4 mg/kg: ↓ rCBF hippocampus, NAcc, claustrum, medial PFC; ↑ rCBF arcuate nucleus
										●○●○○●●○	16 mg/kg: ↓ rCBF dentate gyrus, hippocampus, NAcc, claustrum, entorhinal cortex, globus pallidus and medial PFC
						11-OH-THC	4 mg/kg; IV	30'	Vehicle	●○●○●●●○	↓ rCBF amygdala, hippocampus, NAcc, insula, claustrum, enthorrinal cortex and medial PFC
Stein *et al.* (1998) [248]	[^{14}C]IAP	SD rats	33/0	11/0	Autoradio-graphy ROI	AEA	3-30 mg/kg; IV	15', 20', 60'	Vehicle	●○●●●●●○	After 15': 3 mg/kg: NS rCBF effect; 10 mg/kg: ↓ rCBF in 7/59 areas: basomedial and lateral amygdala, cingulate, frontal, agranular preinsular, prepyriform and primary auditory cortex; 30 mg/kg: 16 additional regions: hippocampus, NAcc, caudate, diagonal brand of Broca and amygdala
										●○●●●●●○	After 20' and 30 mg/kg: NS differences with 15' group
										○○●●●●○○	After 60' and 30 mg/kg: ↓ rCBF in 4/59 areas: basomedial and lateral amygdala, hippocampus and agranular preinsular.
Pontieri *et al.* (1999) [244]	[^{14}C]2-DG	SD rats	8/0	4/0	Autoradio-graphy ROI	WIN 55212-2	0.15 and 0.30 mg/kg; IV	5'	Vehicle	○●○○●●●○	0.15 mg/kg: ↑ LCGU shell of NAcc
										○●●○●●●○	0.30 mg/kg: ↓ LCGU dentate gyrus, hippocampus, ventromedial thalamus
Whitlow *et al.* (2002) [245]	[^{14}C]2-DG	SD rats	10/0	5/0	Autoradio-graphy ROI	THC	2.5 and 10 mg/kg; IP	15', 6h, 24h	Vehicle	●●●●●●●●	After 15': 2.5 mg/kg: ↓ LCGU in 17/35 areas: motor (caudate, SN, cerebellum) and sensory (medial geniculate, auditory cortex, superior culliculus) systems and corticolimbic structures (ACC, hippocampus, basolateral amygdala, septum, olfactory tubercle); 10 mg/kg: ↓ LCGU in 31/35 areas: somatomotor cortex, globus pallidus, agranular insular cortex, NAcc, thalamus, stria terminalis, dorsal and median raphe and locus coeruleus
										●○●○○●●○	After 6h: 2.5 mg/kg: ↓ LCGU in 11/35 areas: cerebellum, auditory cortex, superior culliculus, ACC, olfactory tubercle, caudate, basolateral and central amygdala, septum, median raphe; 10 mg/kg: ↓ LCGU in 4/35 areas: SN, caudate, basolateral amygdala, median raphe
										○○●○○●○○	After 24h: 2.5 mg/kg: ↓ LCGU in 7/35 areas: basolateral and central amygdala, auditory cortex, infralimbic cortex, NAcc, caudate, superior colliculus; 10 mg/kg: ↓ LCGU in 2/35 areas: infralimbic cortex, central amygdala
Freedland *et al.* (2002) [242]	[^{14}C]2-DG	SD rats	16/0	3/0	Autoradio-graphy ROI	THC	0.25-2.5 mg/kg; IV	15'	Vehicle	○○○○○○●○	0.25 mg/kg: NS LCGU effect
										●●●○○●○	1 mg/kg: ↓ LCGU in 10/38 areas: rostral NAcc, ACC, ventral caudate, lateral septum, stria terminalis, thalamus, central and basolateral amygdala, auditory cortex and medial geniculate
										●●●○○●●	2.5 mg/kg: ↓ LCGU in 28/38 areas: infralimbic cortex, ACC, dorsolateral and ventral caudate, thalamus, basolateral amygdala, peraqueductal gray, cerebellum, rostral accumbens, olfactory tubercle, motor cortex, septum, stria terminalis, hippocampus, subthalamic nucleus, auditory cortex, medial geniculate, SN pars reticulata, VTA, superior colliculus and median raphe nucleus
Nguyen *et al.* (2012) [249]	^{18}F-FDG PET	Wistar rats	7/0	5/0	ROI	HU210	100 µg/kg; IV	15', 24h	Vehicle	○○○○○○○○	After 15':↑ global brain glucose metabolism. NS effect observed in individual brain regions
											After 24h: NS effect observed
Acute effects on dopamine release											
Chen *et al.* (1990) [251]	Micro-dialysis	Lewis rats	8/0	4/0	EC detection	THC	1 and 2 mg/kg; IV	-	Vehicle	●○○○○○○○	↑ presynaptic DA efflux in medial PFC
Castañeda *et al.* (1991) [250]	Micro-dialysis	LE rats	12/0	11/0	Coulome-tric detector	THC	1 and 10 mg/kg; G	-	Vehicle	○○○○○○○○	NS of dialysate DA (and metabolites) in the striatum and NAcc
Chen *et al.* (1991) [253]	Micro-dialysis	Lewis and SD rats	8/0 and 10/0	3/0 and 5/0	Coulome-tric detector	THC	0.5 and 1 mg/kg; IP	-	Vehicle	○○○○○●○○	↑ dialysate DA in the NAcc in Lewis rats (compared to S-D rats)
Chen *et al.* (1993) [252]	Micro-dialysis	Lewis rats	18/0	39/0	EC detection	THC	12 or 24 µg; MI	-	Vehicle	○○○○○●○○	↑ dialysate DA in VTA(dose-dependent)
											↑ dialysate DA in NAcc (dose-dependent)
Tanda *et al.* (1997) [258]	Micro-dialysis	SD rats	NE	NE	Coulome-tric detector	THC	0.15 and 0.30 mg/kg; IV	-	Vehicle	○○○○○●○○	0.15 and 0.30 mg/kg: ↑ dialysate DA in the NAcc shell (dose- and time-dependent). NS effect in the NAcc core.
											The increase was prevented by pretreatment with SR141716-A and naloxone
						WIN 55212-2	0.15 and 0.30 mg/kg; IV	-	Vehicle	○○○○○●○○	0.15 and 0.30 mg/kg: ↑ dialysate DA in the NAcc shell (dose- and time-dependent). NS effect in the NAcc core.
											The increase was prevented by pretreatment with SR141716-A and naloxone
						Cannabi-nol	0.30 and 1.0 mg/kg; IV	-	Vehicle	○○○○○○○○	0.15 and 0.30 mg/kg: NS effect on dialysate DA in NAcc
						Heroin	0.018 and 0.030 mg/kg; IV	-	Vehicle	○○○○○●○○	0.018 and 0.030 mg/kg: ↑ dialysate DA in the NAcc shell (dose- and time-dependent). NS effect in the NAcc core.
											The increase was prevented by pretreatment with naloxone but not with SR141716-A
Diana *et al.* (1998) [260]	Electro-physiolo-gical recording	SD rats	7/0	-	Neural spike activity	THC	0.0625-1 mg/kg; IV	-	Baseline	●○○○○○○○	↑ DA cell firing (dose-dependent) projecting to PFC
			14/0			WIN 55212-2	62.5-500 µg/kg; IV	-	Baseline	●○○○○○○○	↑ DA cell firing (dose-dependent) projecting to PFC
Gessa *et al.* (1998) [261]	Electro-physiolo-	SD rats	13/0	-	Neural spike	THC	0.0625-1 mg/kg;	-	Baseline	○○○○○○●○	↑ DA cell firing (dose-dependent) projecting to NAcc. Administration of SR141716 suppressed the effect

	gical recording				activity		IV			
			13/0			WIN 55212-2	0.0625-1 mg/kg; IV	-	Baseline	↑ DA cell firing (dose-dependent) projecting to NAcc. Administration of SR141716 suppressed the effect
			6/0			CP55940	0.0625-1 mg/kg; IV	-	Baseline	↑ DA cell firing (dose-dependent) projecting to NAcc. Administration of SR141716 suppressed the effect
Malone and Taylor (1999) [254]	Micro-dialysis	Wistar rats	25/0	10/0	EC detection	THC	0.5-5 mg/kg; IV	-	Vehicle	↑ DA release in striatum (dose-dependent) Pretreatment with SR141716 and fluoxetine decreased DA levels Local perfusion of fluoxetine increased the effect of THC
Melis *et al.* (2000) [262]	Electro-physiolo-gical recording	SD rats	16/0	-	Neural spike activity	Morphine	1-4 mg/kg; IV	-	Baseline	↑ DA cell firing (dose-dependent) projecting to NAcc and in SN pars reticulate. Naloxone antagonized effect in both systems. SR141716-A did not affect firing rate
			16/0		Neural spike activity	THC	0.125-0.5 mg/kg; IV	-	Baseline	↑ DA cell firing (dose-dependent) projecting to NAcc and in SN pars reticulate. SR141716-A antagonized effect in both systems. Naloxone did not affect firing rate
Cheer *et al.* (2004) [259]	Cyclic voltam-metry	SD rats	14/0	7/0	Voltam-metry	WIN 55212-2	125-250 µg/kg; IV	-	Vehicle	↑ frequency/amplitude (dose-dependent) of rapid DA transients in the NAcc (↑ extracellular DA). Pretreatment with SR141716-A reversed the effect
Solinas *et al.* (2006) [256]	Micro-dialysis	SD rats	5/0	4/0	Coulome-tric detector	AEA	0.3-10 mg/kg; IV	-	Vehicle	0.3-10 mg/kg: ↑ dialysate DA in the NAcc shell (dose- and time-dependent) Pretreatment with SR141716 significantly reduced the effects of 3 mg/kg of AEA in the shell of NAcc; with URB597 increased the effects; and with capsazine had no effects. TTX and Ca^{2+} depletion blocked the effects of AEA on DA levels
			5/0			MAEA	0.3-10 mg/kg; IV	-	Vehicle	<3.0 mg/kg: NS effect in the NAcc shell ≥3.0-10 mg/kg: ↑ dialysate DA in the NAcc shell (dose- and time-dependent) Pretreatment with SR141716 completely blocked the effects of 3 mg/kg of MAEA in the shell of NAcc
Murillo-Rodriguez *et al.* (2006) [255]	Micro-dialysis	Wistar rats	7/0	7/0	Coulome-tric detector	CBD	10 µg/5µl; ICV	-	Vehicle	↑ dialysate DA in the NAcc shell. Also noradrenaline, epinephrine, serotonin and 5-hydroxy-indoleacetic acid (5-HIAA) increased, whereas 3,4-dihydroxy-L-phenylalanine (L-DOPA) extracellular levels decreased
Solinas *et al.* (2007) [257]	Micro-dialysis	SD rats	NE	NE	Coulome-tric detector	AEA	3 mg/kg; IV or 10 mg/kg; IP	-	Vehicle	3 mg/kg IV: ↑ dialysate DA in the NAcc shell. The effect was potentiated by URB-597 but no by AM-404 10 mg/kg IP: NS effect. Pretreatment with URB-597 produced a small increase in dopamine levels

Note: Yr. = years; EG = Experimental group; CG = Control gorups; ; M = male; F = female; FL = frontal lobe; PL = parietal lobe; TL = temporal lobe; OL = occipital lobe; I = insula; BD = basal ganglia and diencephalon; Cb = cerebellum; NAcc = nucleus accumbens; SN = substantia nigra; PET = positron emission tomography; UnR = unrestrained; LE = long evans; SD = Sprague-Dawley; THC = tetrahydrocannabinol; CBD = cannabidiol; 11-OH-THC = 11-hydroxy-tetrahydrocannabinol; WIN 55212-2, HU210 and CP55940 = cannabinoid CB_1 receptor agonist; SR141716-A = cannabinoid CB_1 receptor antagonist; AEA = anandamide; MAEA = methanoanandamide; URB597 = inhibitor of the fatty acid amide hydrolase (FAAH) enzyme; AM-404 = anandamide transport inhibitors; TTX = tetrodotoxin; DA = dopamine; IV = intravenous; G =gavage; MI = microinjection; IP = intraperitoneally; SC = subcutaneously; ICV = intracerebroventricular; 2-DG = deoxyglucose; FDG = fludeoxyglucose; IAP = iodoantipyrine; EC detection = electrochemical detection; L = left hemisphere; R = right hemisphere; LCGU = local cerebral glucose utilization; ROI = region of interest; CBF = global cerebral blood flow; rCBF = regional cerebral blood flow; NS = Non-significant; PFC = prefrontal cortex; ACC = anterior cingulated cortex; NAcc = nucleus accumbens; VTA = ventral tegmental area.

● = significant increase; ● = significant decrease; ○ = non-significant difference; ● = not examined.

3. DISCUSSION

In this systematic review, 45 studies were found suitable for inclusion that examined the acute effect of cannabinoids on several aspects of brain function in rodents and in humans, encompassing changes in dopamine release, brain activation or cerebral blood flow, either at rest or during several different types of cognitive paradigms. However, there were important methodological differences across these studies and this limits what can be learned from direct comparisons between them. Although we used strict inclusion and exclusion criteria for study selection in an attempt to avoid excessive heterogeneity between samples, the investigations that were included often differed in study design (e.g. between-subject as opposed to within-subject comparisons), the imaging methods used (e.g. PET, SPECT, ASL), and the dose, route and type of drug administered (e.g. use of marijuana cigarettes, which may contain other cannabinoids as opposed to pure THC or CBD administration). Another methodological limitation was that some human studies involved small samples, often below the threshold that would be regarded as acceptable in a neuroimaging study [35]. Moreover, a diversity of cognitive paradigms has been used in functional imaging investigations; in these investigations, the definition of regions of interest has been often variable, again hampering comparisons between separate studies. However, despite the fact that the accurate comparison between studies was often prevented, the studies reviewed herein offer a global picture indicating that cannabinoids have modulatory effects over widely distributed neural networks in animal and man, and provide evidence of the neural substrates for the symptomatic effects of cannabinoids. Finally, by including only published data, we cannot exclude publication bias. However, we attempted to minimize this by making our literature search as complete as possible, including studies without language restriction from several databases.

Despite the above limitations, a number of important findings stand out, and these are discussed in detail below.

3.1. Acute Effects of Cannabinoids on Resting Cerebral Blood Flow: Human and Animals Studies

Imaging studies that measured the acute effects of THC on baseline brain perfusion in humans have consistently shown an increase in CBF, mainly in the prefrontal, insular, cerebellar and anterior cingulate regions [217-223]. These areas are known to be enriched with cannabinoid receptors [18], and they have been implicated in several cognitive functions, as well as playing an important role in the neurobiology of addiction [266]. Furthermore, changes in CBF have been associated with many aspects of acute THC-induced behavioural effects, such as a changes in time perception [221], depersonalization [219, 222], increased anxiety [219], intoxication levels [217-220, 222], and 'feeling high' effects [223].

Measures of perfusion and brain activity were obtained in the same sample in one multimodal study [223]. Interestingly, direct comparisons between baseline perfusion and resting-state BOLD signal (as assessed with fMRI) showed that CBF does not simply amplify resting-state fluctuations as one would expect, as values of activity were similar for THC and placebo in regions where perfusion measures showed differences between the two drugs. However, both methods converged in showing increased perfusion and signal fluctuation in the anterior insula (Table **1**).

In contrast to human studies, *ex vivo* autoradiography experiments in animals have shown a dose-dependent decrease in brain CBF and metabolism after cannabinoid challenge [242-248]. Only two studies reported an increase at low doses after THC [242] and CB_1 agonist WIN 55212-2 [244] on limbic structures. However, the only PET study in animals to date [249], although using an experimental design similar to the microdialysis experiments, showed results that are in line with the findings of human studies, reporting

a global increase in brain metabolism after administration of the CB_1 agonist HU210. Discrepancies between these studies may be attributed to several reasons, including methodological differences in the techniques employed or methods of imaging quantification/analysis [249]. In addition, the use of different CB_1 agonists, with different potencies and pharmacokinetic proprieties, may have also contributed to these controversial findings.

The acute effects of CBD on resting CBF have been explored only in humans to date, in naïve cannabis users [224]. Consistent with its anxiolytic effect [12], CBD significantly modulated resting brain activity predominantly in limbic and paralimbic cortical areas, which are known to be implicated in the pathophysiology of anxiety [224].

3.2. Acute Effects of THC on Cerebral Blood Flow During Cognitive Tasks in Occasional and Naïve Cannabis Users

Functional neuroimaging studies comparing CBF during cognitive paradigms before and following THC administration indicate that the perturbation of the endocannabinoid system may affect neural activity during several different types of cognitive tasks (Table 1 and 2).

Three studies examined the acute effects of THC on memory-related brain function, two of which were in occasional cannabis users [228, 229] and one in naïve cannabis users [233], employing different doses and routes of administration (Tables 1 and 2). When assessing associative memory [228, 233], two studies reported a THC-induced reduction in encoding activity in the first block, while differences in recall were reported only by one study [228]. However, THC augmented activation in the parahippocampal gyrus in the subsequent encoding blocks, such that the normal linear decrement in activation across repeated encoding blocks was no longer apparent [233]. These results may reflect recruitment of additional brain areas during memory encoding as a compensatory mechanism under the influence of THC. These investigations also provided evidence that impairments in learning and memory induced by THC are mediated through its effects on medial temporal and prefrontal functioning. The third study was the only one reporting abnormal cognitive performance after THC challenge [229]. Using a working memory task, Bossong *et al.* 2012 [229] demonstrated a decline in performance at lower memory loads after THC challenge together with an increased activity in brain areas related to working memory, such as the dorsolateral prefrontal, inferior temporal and parietal cortices. Overall, these imaging studies seem to indicate a clear involvement of the endocannabinoid system in learning and memory processes.

With regard to affective processing, two studies have examined the effect of THC while subjects viewed fearful faces but using different image analysis approaches [230, 236]. THC was found to increase brain activity in the left precuneus and primary sensorimotor cortex, as well as decreasing activity in the middle frontal gyrus and posterior cingulate gyrus [236]. Inconsistent results were reported in the amygdala: while an attenuation of activation under the influence of THC was described in occasional cannabis users [230], no effect was found in naïve subjects [236]. However, methodological differences may have influenced in such disparity of results, especially regarding to the limited sample size [30]. Finally, the effect of THC was assessed during a task evoking negative emotions in occasional cannabis users [231], and reductions in subgenual anterior cingulate cortex activation were observed. Overall, these results suggest that THC may have centrally mediated effects on mood processing. The findings support the idea that endogenous cannabinoids may play a role in modulating affect.

Besides memory and affective processing, abnormal brain activity has also been reported during the performance tasks related to attention [225, 227], motor function [226] and reward [232] in occasional cannabis users, as well as response inhibition [235], salience [234] and sensory processing [237] in naïve cannabis users. Among these studies, only one reported impaired task performance after THC administration. O'Leary 2003 *et al.* [226] reported a pattern of faster response times in a self-paced counting task that was directly related to an increase in the cerebellum activity, suggesting that cannabis may increase the activity of an internal cerebellar clock. As the remaining studies did not report a significant effect of THC on task performance, the interpretation of the neural effect may be attributed to the pharmacological effects of the drug rather than being confounded by differential task performance.

3.3. Acute Effects of CBD on Cerebral Blood Flow During Cognitive Tasks: Opposite Effects to THC

Opposite symptomatic effects of THC and CBD have been previously described, particularly regarding psychotic [11] and anxiety [224] features. The series of studies included herein extend these findings by showing, for the first time, the modulating effects of these drugs on brain activation during cognitive tasks. Five studies examined the effects of CBD and THC on different cognitive processes in naïve cannabis users [224, 233-237]. As these studies also reported the effects of a THC challenge, direct comparison between drugs was feasible. Remarkably, opposite effects on activation in the same brain regions were observed in all these studies. The only exception was one investigation involving memory and verbal learning, where CBD had no significant effect [233], consistent with evidence that CBD does not affect learning and memory [14]. In line with the opposite clinical effects observed, these studies provide evidence of the opposite neurophysiological properties of THC and CBD during tasks involving response inhibition, affective, sensory and salience processing [234-237], as recently reviewed by Bhattacharyya and colleagues [30].

3.4. Acute Effects of Cannabinoids on Dopamine Release: Link between Cannabis and Psychosis

The imaging studies discussed above showed that the acute effects of THC often involved activity changes in striatal structures. Furthermore, these effects on striatal activation have even demonstrated being related to the severity of psychotic symptoms in some paradigms [233, 234]. Although the precise neurochemical mechanism underlying this effect remains unclear, perturbed dopamine function may be a key factor in the inappropriate attribution of salience to environmental stimuli [267]. It has been suggested that psychosis stems from a psychological state of aberrant salience, which itself arises from excessive stimulation of dopamine in the corpus striatum [22, 267]. Therefore, it is possible that THC leads to perturbed salience processing and in the induction of psychotic symptoms through its effects on central dopamine function.

While the animal studies reviewed in this article seem to indicate that cannabinoids stimulate dopamine release in striatal areas when measured by electrochemical or electrophysiological methods, human neurochemical imaging studies have reported inconsistent results. With two negatives studies [240, 241] and one study reporting a modest increase in dopamine striatal levels after THC administration [239], it seems feasible that the psychomimetic properties of THC arise from direct actions at CB_1 receptors on glutamate and GABA-ergic terminals rather than via dopamine signalling [22, 239, 268].

CONCLUSIONS AND FUTURE DIRECTIONS

Despite the considerable degree of methodological heterogeneity in the imaging literature reviewed herein, the studies carried out so far have shown a number of consistent findings regarding the acute effects of cannabinoids on brain functioning, including: (1) Modulation of resting state activity, with increases mainly detected in CB_1-rich areas implicated in several cognitive functions and in the addictive process; (2) Altered neural activity during performance of several different types of cognitive paradigms, possibly reflecting a different recruitment of brain areas during the task; (3)

THC and CBD showed opposite neurophysiological properties, consistent with their opposite symptomatic effects; and (4) While the psychotomimetic effects of THC in humans are likely to arise from direct actions at CB_1 receptors, it is unclear whether this occurs through a modulatory effect on dopamine signalling.

A further important issue pointed out in this review is that there is a great need for replication of findings in future studies, which should consider the use of convergent methodologies. Functional neuroimaging studies have provided extensive evidence for the modulation of cognitive processes by cannabinoids, but further studies are needed in order to delineate the precise neural mechanisms underlying these distinct (or even opposite) effects. These studies may help to inspire new research regarding the potential therapeutic applications of cannabinoids, such as the use of CBD for anxiety and psychotic disorders, and may also offer a better understanding of the neurophysiological mechanisms underlying mental health disorders.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This study has been done in part with Spanish grants: Plan Nacional sobre Drogas, Ministerio de Sanidad y Consumo PNSD/2011/050 (R Martín-Santos) and PNSD/2010/018 (O Valverde); and the support Agency of University and Research Funding Management of the Catalonia Government SGR2009/1435 (R Martín-Santos), and SGR2009/684 (O Valverde); and INCT Translational Medicine (INCT-TM, CNPq), Brazil (JA Crippa); S Bhattacharyya is supported by a Clinician Scientist award from the National Institute of Health Research, UK; and JA Crippa and AW Zuardi receives a CNPq (Brazil) productivity award (IC).

FINANCIAL DISCLOSURES

The authors disclose no competing financial interests.

REFERENCES

[1] United Nations Office on Drugs and Crime. World drug report 2011. UNODC, Vienna 2011. Access date: 2-2-2012. Available from: http://www.unodc.org/unodc/en/data-and-analysis/WDR-2011.html.

[2] European Monitoring Centre for Drugs and Drug Addiction. The state of the drugs problem in Europe. EMCDDA, Lisbon 2011. Access date: 2-2-2012. Available from: http://www.emcdda.europa.eu/publications/annual-report/2011.

[3] Mechoulam R, Gaoni Y. Recent advances in the chemistry of hashish. Fortschr Chem Org Naturst 1967; 25: 175-213.

[4] Mechoulam R, Parker LA. The Endocannabinoid System and the Brain. Annu Rev Psychol 2012; Jul 12. [Epub ahead of print].

[5] Gaoni Y, Mechoulam R. The isolation and structure of delta-1-tetrahydrocannabinol and other neutral cannabinoids from hashish. J Am Chem Soc 1971; 93(1): 217-24.

[6] Martin-Santos R, Crippa JA, Batalla A, *et al*. Acute effects of a single, oral dose of d9-tetrahydrocannabinol (THC) and cannabidiol (CBD) administration in healthy volunteers. Curr Pharm Des 2012; 18(32): 4966-79.

[7] Ranganathan M, D'Souza DC. The acute effects of cannabinoids on memory in humans: a review. Psychopharmacology (Berl) 2006; 188(4): 425-44.

[8] Ramaekers JG, Kauert G, Theunissen EL, Toennes SW, Moeller MR. Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. J Psychopharmacol 2009; 23(3): 266-77.

[9] Hall W, Solowij N. Adverse effects of cannabis. Lancet 1998; 352(9140): 1611-6.

[10] Crippa JA, Derenusson GN, Ferrari TB, *et al*. Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. J Psychopharmacol 2011; 25(1): 121-30.

[11] Zuardi AW, Crippa JA, Hallak JE, Moreira FA, Guimaraes FS. Cannabidiol, a Cannabis sativa constituent, as an antipsychotic drug. Braz J Med Biol Res 2006; 39(4): 421-9.

[12] Zuardi AW, Crippa JA, Hallak JE, *et al*. A critical review of the antipsychotic effects of Cannabidiol: 30 years of a translational investigation. Curr Pharm Des 2012; 18(32): 5131-40.

[13] Bhattacharyya S, Morrison PD, Fusar-Poli P, *et al*. Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. Neuropsychopharmacology 2010; 35(3): 764-74.

[14] Ilan AB, Gevins A, Coleman M, ElSohly MA, de WH. Neurophysiological and subjective profile of marijuana with varying concentrations of cannabinoids. Behav Pharmacol 2005; 16(5-6): 487-96.

[15] Potter DJ, Clark P, Brown MB. Potency of delta 9-THC and other cannabinoids in cannabis in England in 2005: implications for psychoactivity and pharmacology. J Forensic Sci 2008; 53(1): 90-4.

[16] Di Forti M, Morgan C, Dazzan P, *et al*. High-potency cannabis and the risk of psychosis. Br J Psychiatry 2009; 195(6): 488-91.

[17] Batalla A, Garcia-Rizo C, Castellvi P, *et al*. Screening for substance use disorders in first-episode psychosis: Implications for readmission. Schizophr Res 2013; 146(1-3): 125-31.

[18] Burns HD, Van LK, Sanabria-Bohorquez S, *et al*. [18F]MK-9470, a positron emission tomography (PET) tracer for *in vivo* human PET brain imaging of the cannabinoid-1 receptor. Proc Natl Acad Sci USA 2007; 104(23): 9800-5.

[19] El Khoury MA, Gorgievski V, Moutsimilli L, Giros B, Tzavara ET. Interactions between the cannabinoid and dopaminergic systems: Evidence from animal studies. Prog Neuropsychopharmacol Biol Psychiatry 2012; 38(1): 36-50.

[20] Reguero L, Puente N, Elezgarai I, *et al*. GABAergic and cortical and subcortical glutamatergic axon terminals contain CB1 cannabinoid receptors in the ventromedial nucleus of the hypothalamus. PLoS One 2011; 6(10): e26167.

[21] Fadda P, Scherma M, Spano MS, *et al*. Cannabinoid self-administration increases dopamine release in the nucleus accumbens. Neuroreport 2006; 17(15): 1629-32.

[22] Morrison PD, Murray RM. From real-world events to psychosis: the emerging neuropharmacology of delusions. Schizophr Bull 2009; 35(4): 668-74.

[23] Van Sickle MD, Duncan M, Kingsley PJ, *et al*. Identification and functional characterization of brainstem cannabinoid CB2 receptors. Science 2005; 310(5746): 329-32.

[24] Pertwee RG. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. Br J Pharmacol 2008; 153(2): 199-215.

[25] Thomas BF, Gilliam AF, Burch DF, Roche MJ, Seltzman HH. Comparative receptor binding analyses of cannabinoid agonists and antagonists. J Pharmacol Exp Ther 1998; 285(1): 285-92.

[26] Mechoulam R, Peters M, Murillo-Rodriguez E, Hanus LO. Cannabidiol--recent advances. Chem Biodivers 2007; 4(8): 1678-92.

[27] van OJ, Kenis G, Rutten BP. The environment and schizophrenia. Nature 2010; 468(7321): 203-12.

[28] Bhattacharyya S, Atakan Z, Martin-Santos R, *et al*. 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 2012; 17(12): 1152-5.

[29] Batalla A, Soriano-Mas C, Lopez-Sola M, *et al*. Modulation of brain structure by catechol-O-methyltransferase Val(158) Met polymorphism in chronic cannabis users. Addict Biol 2013; Jan 14. [Epub ahead of print].

[30] Bhattacharyya S, Atakan Z, Martin-Santos R, Crippa JA, McGuire PK. Neural Mechanisms for the Cannabinoid Modulation of Cognition and Affect in Man: a Critical Review of Neuroimaging Studies. Curr Pharm Des 2012; 18(32): 5045-54.

[31] Chang L, Chronicle EP. Functional imaging studies in cannabis users. Neuroscientist 2007; 13(5): 422-32.

[32] Gonzalez R. Acute and non-acute effects of cannabis on brain functioning and neuropsychological performance. Neuropsychol Rev 2007; 17(3): 347-61.

[33] Jager G, Ramsey NF. Long-term consequences of adolescent cannabis exposure on the development of cognition, brain structure

and function: an overview of animal and human research. Curr Drug Abuse Rev 2008; 1(2): 114-23.

[34] Lorenzetti V, Lubman DI, Whittle S, Solowij N, Yucel M. Structural MRI findings in long-term cannabis users: what do we know? Subst Use Misuse 2010; 45(11): 1787-808.

[35] Martin-Santos R, Fagundo AB, Crippa JA, *et al*. Neuroimaging in cannabis use: a systematic review of the literature. Psychol Med 2010; 40(3): 383-98.

[36] Quickfall J, Crockford D. Brain neuroimaging in cannabis use: a review. J Neuropsychiatry Clin Neurosci 2006; 18(3): 318-32.

[37] Bhattacharyya S, Crippa JA, Martin-Santos R, Winton-Brown T, Fusar-Poli P. Imaging the neural effects of cannabinoids: current status and future opportunities for psychopharmacology. Curr Pharm Des 2009; 15(22): 2603-14.

[38] Batalla A, Bhattacharyya S, Yucel M, *et al*. Structural and functional imaging studies in chronic cannabis users: a systematic review of adolescent and adult findings. PLoS One 2013; 8(2): e55821.

[39] Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009; 6(7): e1000097.

[40] Acquas E, Pisanu A, Marrocu P, Di CG. Cannabinoid CB(1) receptor agonists increase rat cortical and hippocampal acetylcholine release *in vivo*. Eur J Pharmacol 2000; 401(2): 179-85.

[41] Acquas E, Pisanu A, Marrocu P, Goldberg SR, Di CG. Delta9-tetrahydrocannabinol enhances cortical and hippocampal acetylcholine release *in vivo*: a microdialysis study. Eur J Pharmacol 2001; 419(2-3): 155-61.

[42] Carta G, Nava F, Gessa GL. Inhibition of hippocampal acetylcholine release after acute and repeated Delta9-tetrahydrocannabinol in rats. Brain Res 1998; 809(1): 1-4.

[43] Cenni G, Blandina P, Mackie K, *et al*. Differential effect of cannabinoid agonists and endocannabinoids on histamine release from distinct regions of the rat brain. Eur J Neurosci 2006; 24(6): 1633-44.

[44] Chikai T, Oishi R, Saeki K. Microdialysis study of the effects of sedative drugs on extracellular histamine in the striatum of freely moving rats. J Pharmacol Exp Ther 1993; 266(3): 1277-81.

[45] Degroot A, Kofalvi A, Wade MR, *et al*. CB1 receptor antagonism increases hippocampal acetylcholine release: site and mechanism of action. Mol Pharmacol 2006; 70(4): 1236-45.

[46] Egashira N, Mishima K, Katsurabayashi S, *et al*. Involvement of 5-hydroxytryptamine neuronal system in Delta(9)-tetrahydrocannabinol-induced impairment of spatial memory. Eur J Pharmacol 2002; 445(3): 221-9.

[47] Ferraro L, Tomasini MC, Cassano T, *et al*. Cannabinoid receptor agonist WIN 55,212-2 inhibits rat cortical dialysate gamma-aminobutyric acid levels. J Neurosci Res 2001; 66(2): 298-302.

[48] Gessa GL, Casu MA, Carta G, Mascia MS. Cannabinoids decrease acetylcholine release in the medial-prefrontal cortex and hippocampus, reversal by SR 141716A. Eur J Pharmacol 1998; 355(2-3): 119-24.

[49] Inui K, Egashira N, Mishima K, *et al*. The serotonin1A receptor agonist 8-OHDPAT reverses delta 9-tetrahydrocannabinol-induced impairment of spatial memory and reduction of acetylcholine release in the dorsal hippocampus in rats. Neurotox Res 2004; 6(2): 153-8.

[50] Merroun I, Errami M, Hoddah H, *et al*. Influence of intracerebroventricular or intraperitoneal administration of cannabinoid receptor agonist (WIN 55,212-2) and inverse agonist (AM 251) on the regulation of food intake and hypothalamic serotonin levels. Br J Nutr 2009; 101(10): 1569-78.

[51] Mishima K, Egashira N, Matsumoto Y, Iwasaki K, Fujiwara M. Involvement of reduced acetylcholine release in Delta9-tetrahydrocannabinol-induced impairment of spatial memory in the 8-arm radial maze. Life Sci 2002; 72(4-5): 397-407.

[52] Murillo-Rodriguez E, Blanco-Centurion C, Sanchez C, Piomelli D, Shiromani PJ. Anandamide enhances extracellular levels of adenosine and induces sleep: an *in vivo* microdialysis study. Sleep 2003; 26(8): 943-7.

[53] Oropeza VC, Page ME, Van Bockstaele EJ. Systemic administration of WIN 55,212-2 increases norepinephrine release in the rat frontal cortex. Brain Res 2005; 1046(1-2): 45-54.

[54] Page ME, Oropeza VC, Sparks SE, *et al*. Repeated cannabinoid administration increases indices of noradrenergic activity in rats. Pharmacol Biochem Behav 2007; 86(1): 162-8.

[55] Page ME, Oropeza VC, Van Bockstaele EJ. Local administration of a cannabinoid agonist alters norepinephrine efflux in the rat frontal cortex. Neurosci Lett 2008; 431(1): 1-5.

[56] Pisanu A, Acquas E, Fenu S, Di CG. Modulation of Delta(9)-THC-induced increase of cortical and hippocampal acetylcholine release by micro opioid and D(1) dopamine receptors. Neuropharmacology 2006; 50(6): 661-70.

[57] Sano K, Mishima K, Koushi E, *et al*. Delta 9-tetrahydrocannabinol-induced catalepsy-like immobilization is mediated by decreased 5-HT neurotransmission in the nucleus accumbens due to the action of glutamate-containing neurons. Neuroscience 2008; 151(2): 320-8.

[58] Solinas M, Zangen A, Thiriet N, Goldberg SR. Beta-endorphin elevations in the ventral tegmental area regulate the discriminative effects of Delta-9-tetrahydrocannabinol. Eur J Neurosci 2004; 19(12): 3183-92.

[59] Tzavara ET, Perry KW, Rodriguez DE, Bymaster FP, Nomikos GG. The cannabinoid CB(1) receptor antagonist SR141716A increases norepinephrine outflow in the rat anterior hypothalamus. Eur J Pharmacol 2001; 426(3): R3-R4.

[60] Tzavara ET, Davis RJ, Perry KW, *et al*. The CB1 receptor antagonist SR141716A selectively increases monoaminergic neurotransmission in the medial prefrontal cortex: implications for therapeutic actions. Br J Pharmacol 2003; 138(4): 544-53.

[61] Valverde O, Noble F, Beslot F, *et al*. Delta9-tetrahydrocannabinol releases and facilitates the effects of endogenous enkephalins: reduction in morphine withdrawal syndrome without change in rewarding effect. Eur J Neurosci 2001; 13(9): 1816-24.

[62] Verrico CD, Jentsch JD, Dazzi L, Roth RH. Systemic, but not local, administration of cannabinoid CB1 receptor agonists modulate prefrontal cortical acetylcholine efflux in the rat. Synapse 2003; 48(4): 178-83.

[63] Wade MR, Tzavara ET, Nomikos GG. Cannabinoids reduce cAMP levels in the striatum of freely moving rats: an *in vivo* microdialysis study. Brain Res 2004; 1005(1-2): 117-23.

[64] Aasly J, Storsaeter O, Nilsen G, Smevik O, Rinck P. Minor structural brain changes in young drug abusers. A magnetic resonance study. Acta Neurol Scand 1993; 87(3): 210-4.

[65] Amen DG, Waugh M. High resolution brain SPECT imaging of marijuana smokers with AD/HD. J Psychoactive Drugs 1998; 30(2): 209-14.

[66] Becker B, Wagner D, Gouzoulis-Mayfrank E, Spuentrup E, Daumann J. The impact of early-onset cannabis use on functional brain correlates of working memory. Prog Neuropsychopharmacol Biol Psychiatry 2010; 34(6): 837-45.

[67] Becker B, Wagner D, Gouzoulis-Mayfrank E, Spuentrup E, Daumann J. Altered parahippocampal functioning in cannabis users is related to the frequency of use. Psychopharmacology (Berl) 2010; 209(4): 361-74.

[68] Campbell AM, Evans M, Thomson JL, Williams MJ. Cerebral atrophy in young cannabis smokers. Lancet 1971; 2(7736): 1219-24.

[69] Cheetham A, Allen NB, Whittle S, *et al*. Orbitofrontal Volumes in Early Adolescence Predict Initiation of Cannabis Use: A 4-Year Longitudinal and Prospective Study. Biol Psychiatry 2011.

[70] Chung T, Geier C, Luna B, *et al*. Enhancing response inhibition by incentive: comparison of adolescents with and without substance use disorder. Drug Alcohol Depend 2011; 115(1-2): 43-50.

[71] Churchwell JC, Lopez-Larson M, Yurgelun-Todd DA. Altered frontal cortical volume and decision making in adolescent cannabis users. Front Psychol 2010; 1: 225.

[72] Cousijn J, Goudriaan AE, Ridderinkhof KR, *et al*. Neural responses associated with cue-reactivity in frequent cannabis users. Addict Biol 2012; Jan 20. [Epub ahead of print].

[73] Demirakca T, Sartorius A, Ende G, *et al*. Diminished gray matter in the hippocampus of cannabis users: Possible protective effects of cannabidiol. Drug Alcohol Depend 2011; 114(2-3): 242-5.

[74] Filbey FM, Schacht JP, Myers US, Chavez RS, Hutchison KE. Marijuana craving in the brain. Proc Natl Acad Sci USA 2009; 106(31): 13016-21.

[75] Filbey FM, Schacht JP, Myers US, Chavez RS, Hutchison KE. Individual and additive effects of the CNR1 and FAAH genes on

brain response to marijuana cues. Neuropsychopharmacology 2010; 35(4): 967-75.

[76] Hermann D, Sartorius A, Welzel H, *et al*. Dorsolateral prefrontal cortex N-acetylaspartate/total creatine (NAA/tCr) loss in male recreational cannabis users. Biol Psychiatry 2007; 61(11): 1281-9.

[77] Jacobsen LK, Mencl WE, Westerveld M, Pugh KR. Impact of cannabis use on brain function in adolescents. Ann N Y Acad Sci 2004; 1021: 384-90.

[78] Leroy C, Karila L, Martinot JL, *et al*. Striatal and extrastriatal dopamine transporter in cannabis and tobacco addiction: a high-resolution PET study. Addict Biol 2012; 17(6): 981-90.

[79] Mata I, Perez-Iglesias R, Roiz-Santianez R, *et al*. Gyrification brain abnormalities associated with adolescence and early-adulthood cannabis use. Brain Res 2010; 1317: 297-304.

[80] Medina KL, Nagel BJ, Park A, McQueeny T, Tapert SF. Depressive symptoms in adolescents: associations with white matter volume and marijuana use. J Child Psychol Psychiatry 2007; 48(6): 592-600.

[81] Murphy K, Dixon V, LaGrave K, *et al*. A validation of event-related FMRI comparisons between users of cocaine, nicotine, or cannabis and control subjects. Am J Psychiatry 2006; 163(7): 1245-51.

[82] Nestor L, Roberts G, Garavan H, Hester R. Deficits in learning and memory: parahippocampal hyperactivity and frontocortical hypoactivity in cannabis users. Neuroimage 2008; 40(3): 1328-39.

[83] Parkar SR, Ramanathan S, Nair N, *et al*. Cannabis dependence: Effects of cannabis consumption on inter-regional cerebral metabolic relationships in an Indian population. Indian J Psychiatry 2010; 52(3): 236-42.

[84] Prescot AP, Locatelli AE, Renshaw PF, Yurgelun-Todd DA. Neurochemical alterations in adolescent chronic marijuana smokers: a proton MRS study. Neuroimage 2011; 57(1): 69-75.

[85] Schneider M. Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure. Addict Biol 2008; 13(2): 253-63.

[86] Smith AM, Longo CA, Fried PA, Hogan MJ, Cameron I. Effects of marijuana on visuospatial working memory: an fMRI study in young adults. Psychopharmacology (Berl) 2010; 210(3): 429-38.

[87] Wiesbeck GA, Taeschner KL. A cerebral computed tomography study of patients with drug-induced psychoses. Eur Arch Psychiatry Clin Neurosci 1991; 241(2): 88-90.

[88] Wilson W, Mathew R, Turkington T, *et al*. Brain morphological changes and early marijuana use: a magnetic resonance and positron emission tomography study. J Addict Dis 2000; 19(1): 1-22.

[89] Cohen M, Rasser PE, Peck G, *et al*. Cerebellar grey-matter deficits, cannabis use and first-episode schizophrenia in adolescents and young adults. Int J Neuropsychopharmacol 2011; 1-11.

[90] Cornelius JR, Aizenstein HJ, Hariri AR. Amygdala reactivity is inversely related to level of cannabis use in individuals with comorbid cannabis dependence and major depression. Addict Behav 2010; 35(6): 644-6.

[91] Cowan RL, Joers JM, Dietrich MS. N-acetylaspartate (NAA) correlates inversely with cannabis use in a frontal language processing region of neocortex in MDMA (Ecstasy) polydrug users: a 3 T magnetic resonance spectroscopy study. Pharmacol Biochem Behav 2009; 92(1): 105-10.

[92] Dekker N, Schmitz N, Peters BD, *et al*. Cannabis use and callosal white matter structure and integrity in recent-onset schizophrenia. Psychiatry Res 2010; 181(1): 51-6.

[93] Habets P, Marcelis M, Gronenschild E, Drukker M, van OJ. Reduced cortical thickness as an outcome of differential sensitivity to environmental risks in schizophrenia. Biol Psychiatry 2011; 69(5): 487-94.

[94] Ho BC, Wassink TH, Ziebell S, Andreasen NC. Cannabinoid receptor 1 gene polymorphisms and marijuana misuse interactions on white matter and cognitive deficits in schizophrenia. Schizophr Res 2011; 128(1-3): 66-75.

[95] Jacobus J, McQueeny T, Bava S, *et al*. White matter integrity in adolescents with histories of marijuana use and binge drinking. Neurotoxicol Teratol 2009; 31(6): 349-55.

[96] James A, Hough M, James S, *et al*. Greater white and grey matter changes associated with early cannabis use in adolescent-onset schizophrenia (AOS). Schizophr Res 2011; 128(1-3): 91-7.

[97] Kumra S, Robinson P, Tambyraja R, *et al*. Parietal lobe volume deficits in adolescents with schizophrenia and adolescents with cannabis use disorders. J Am Acad Child Adolesc Psychiatry 2012; 51(2): 171-80.

[98] Li CS, Milivojevic V, Constable RT, Sinha R. Recent cannabis abuse decreased stress-induced BOLD signals in the frontal and cingulate cortices of cocaine dependent individuals. Psychiatry Res 2005; 140(3): 271-80.

[99] Parkar SR, Ramanathan S, Nair N, *et al*. Are the effects of cannabis dependence on glucose metabolism similar to schizophrenia? An FDG PET understanding. Indian J Psychiatry 2011; 53(1): 13-20.

[100] Peters BD, de HL, Vlieger EJ, *et al*. Recent-onset schizophrenia and adolescent cannabis use: MRI evidence for structural hyperconnectivity? Psychopharmacol Bull 2009; 42(2): 75-88.

[101] Rais M, van Haren NE, Cahn W, *et al*. Cannabis use and progressive cortical thickness loss in areas rich in CB1 receptors during the first five years of schizophrenia. Eur Neuropsychopharmacol 2010; 20(12): 855-65.

[102] Roberts GM, Garavan H. Evidence of increased activation underlying cognitive control in ecstasy and cannabis users. Neuroimage 2010; 52(2): 429-35.

[103] Safont G, Corripio I, Escarti MJ, *et al*. Cannabis use and striatal D2 receptor density in untreated first-episode psychosis: an *in vivo* SPECT study. Schizophr Res 2011; 129(2-3): 169-71.

[104] Schweinsburg AD, Schweinsburg BC, Cheung EH, *et al*. fMRI response to spatial working memory in adolescents with comorbid marijuana and alcohol use disorders. Drug Alcohol Depend 2005; 79(2): 201-10.

[105] Solowij N, Yucel M, Respondek C, *et al*. Cerebellar white-matter changes in cannabis users with and without schizophrenia. Psychol Med 2011; 41(11): 2349-59.

[106] Sundram S. Cannabis and neurodevelopment: implications for psychiatric disorders. Hum Psychopharmacol 2006; 21(4): 245-54.

[107] Takagi M, Lubman DI, Walterfang M, *et al*. Corpus callosum size and shape alterations in adolescent inhalant users. Addict Biol 2011; Sep 29. [Epub ahead of print].

[108] Tanabe J, Thompson L, Claus E, *et al*. Prefrontal cortex activity is reduced in gambling and nongambling substance users during decision-making. Hum Brain Mapp 2007; 28(12): 1276-86.

[109] Voytek B, Berman SM, Hassid BD, *et al*. Differences in regional brain metabolism associated with marijuana abuse in methamphetamine abusers. Synapse 2005; 57(2): 113-5.

[110] Welch KA, McIntosh AM, Job DE, *et al*. The impact of substance use on brain structure in people at high risk of developing schizophrenia. Schizophr Bull 2011; 37(5): 1066-76.

[111] Welch KA, Stanfield AC, McIntosh AM, *et al*. Impact of cannabis use on thalamic volume in people at familial high risk of schizophrenia. Br J Psychiatry 2011; 199: 386-90.

[112] Wobrock T, Sittinger H, Behrendt B, D'Amelio R, Falkai P. Comorbid substance abuse and brain morphology in recent-onset psychosis. Eur Arch Psychiatry Clin Neurosci 2009; 259(1): 28-36.

[113] Yucel M, Zalesky A, Takagi MJ, *et al*. White-matter abnormalities in adolescents with long-term inhalant and cannabis use: a diffusion magnetic resonance imaging study. J Psychiatry Neurosci 2010; 35(6): 409-12.

[114] O'Leary DS, Block RI, Flaum M, *et al*. Acute marijuana effects on rCBF and cognition: a PET study. Neuroreport 2000; 11(17): 3835-41.

[115] Cadogan AK, Alexander SP, Boyd EA, Kendall DA. Influence of cannabinoids on electrically evoked dopamine release and cyclic AMP generation in the rat striatum. J Neurochem 1997; 69(3): 1131-7.

[116] Chan PK, Chan SC, Yung WH. Presynaptic inhibition of GABAergic inputs to rat substantia nigra pars reticulata neurones by a cannabinoid agonist. Neuroreport 1998; 9(4): 671-5.

[117] Cheer JF, Marsden CA, Kendall DA, Mason R. Lack of response suppression follows repeated ventral tegmental cannabinoid administration: an *in vitro* electrophysiological study. Neuroscience 2000; 99(4): 661-7.

[118] Foy MR, Teyler TJ, Vardaris RM. delta 9-THC and 17-beta-estradiol in hippocampus. Brain Res Bull 1982; 8(4): 341-5.

[119] Jentsch JD, Andrusiak E, Tran A, Bowers MB, Jr., Roth RH. Delta 9-tetrahydrocannabinol increases prefrontal cortical catecholaminergic utilization and impairs spatial working memory in the rat: blockade of dopaminergic effects with HA966. Neuropsychopharmacology 1997; 16(6): 426-32.

[120] Jentsch JD, Wise A, Katz Z, Roth RH. Alpha-noradrenergic receptor modulation of the phencyclidine- and delta9-

tetrahydrocannabinol-induced increases in dopamine utilization in rat prefrontal cortex. Synapse 1998; 28(1): 21-6.

[121] Ma YL, Weston SE, Whalley BJ, Stephens GJ. The phytocannabinoid Delta(9)-tetrahydrocannabivarin modulates inhibitory neurotransmission in the cerebellum. Br J Pharmacol 2008; 154(1): 204-15.

[122] Melis M, Pistis M, Perra S, *et al*. Endocannabinoids mediate presynaptic inhibition of glutamatergic transmission in rat ventral tegmental area dopamine neurons through activation of CB1 receptors. J Neurosci 2004; 24(1): 53-62.

[123] Miller AS, Walker JM. Electrophysiological effects of a cannabinoid on neural activity in the globus pallidus. Eur J Pharmacol 1996; 304(1-3): 29-35.

[124] O'Neill C, Evers-Donnelly A, Nicholson D, O'Boyle KM, O'Connor JJ. D2 receptor-mediated inhibition of dopamine release in the rat striatum *in vitro* is modulated by CB1 receptors: studies using fast cyclic voltammetry. J Neurochem 2009; 108(3): 545-51.

[125] Pandolfo P, Silveirinha V, dos Santos-Rodrigues A, *et al*. Cannabinoids inhibit the synaptic uptake of adenosine and dopamine in the rat and mouse striatum. Eur J Pharmacol 2011; 655(1-3): 38-45.

[126] Poddar MK, Dewey WL. Effects of cannabinoids on catecholamine uptake and release in hypothalamic and striatal synaptosomes. J Pharmacol Exp Ther 1980; 214(1): 63-7.

[127] Rodriguez de FF, Fernandez-Ruiz JJ, Murphy LL, *et al*. Acute effects of delta-9-tetrahydrocannabinol on dopaminergic activity in several rat brain areas. Pharmacol Biochem Behav 1992; 42(2): 269-75.

[128] Sakurai-Yamashita Y, Kataoka Y, Fujiwara M, Mine K, Ueki S. Delta 9-tetrahydrocannabinol facilitates striatal dopaminergic transmission. Pharmacol Biochem Behav 1989; 33(2): 397-400.

[129] Sperlagh B, Windisch K, Ando RD, Sylvester VE. Neurochemical evidence that stimulation of CB1 cannabinoid receptors on GABAergic nerve terminals activates the dopaminergic reward system by increasing dopamine release in the rat nucleus accumbens. Neurochem Int 2009; 54(7): 452-7.

[130] Szabo B, Dorner L, Pfreundtner C, Norenberg W, Starke K. Inhibition of GABAergic inhibitory postsynaptic currents by cannabinoids in rat corpus striatum. Neuroscience 1998; 85(2): 395-403.

[131] Szabo B, Muller T, Koch H. Effects of cannabinoids on dopamine release in the corpus striatum and the nucleus accumbens *in vitro*. J Neurochem 1999; 73(3): 1084-9.

[132] Szabo B, Wallmichrath I, Mathonia P, Pfreundtner C. Cannabinoids inhibit excitatory neurotransmission in the substantia nigra pars reticulata. Neuroscience 2000; 97(1): 89-97.

[133] Szabo B, Siemes S, Wallmichrath I. Inhibition of GABAergic neurotransmission in the ventral tegmental area by cannabinoids. Eur J Neurosci 2002; 15(12): 2057-61.

[134] Bambico FR, Nguyen NT, Katz N, Gobbi G. Chronic exposure to cannabinoids during adolescence but not during adulthood impairs emotional behaviour and monoaminergic neurotransmission. Neurobiol Dis 2010; 37(3): 641-55.

[135] Cadoni C, Valentini V, Di CG. Behavioral sensitization to delta 9-tetrahydrocannabinol and cross-sensitization with morphine: differential changes in accumbal shell and core dopamine transmission. J Neurochem 2008; 106(4): 1586-93.

[136] Higuera-Matas A, Miguens M, Coria SM, *et al*. Sex-specific disturbances of the glutamate/GABA balance in the hippocampus of adult rats subjected to adolescent cannabinoid exposure. Neuropharmacology 2012; 62(5-6): 1975-84.

[137] Hill MN, Froc DJ, Fox CJ, Gorzalka BB, Christie BR. Prolonged cannabinoid treatment results in spatial working memory deficits and impaired long-term potentiation in the CA1 region of the hippocampus *in vivo*. Eur J Neurosci 2004; 20(3): 859-63.

[138] Hoffman AF, Oz M, Caulder T, Lupica CR. Functional tolerance and blockade of long-term depression at synapses in the nucleus accumbens after chronic cannabinoid exposure. J Neurosci 2003; 23(12): 4815-20.

[139] Hoffman AF, Oz M, Yang R, Lichtman AH, Lupica CR. Opposing actions of chronic Delta9-tetrahydrocannabinol and cannabinoid antagonists on hippocampal long-term potentiation. Learn Mem 2007; 14(1-2): 63-74.

[140] Lecca D, Cacciapaglia F, Valentini V, Di CG. Monitoring extracellular dopamine in the rat nucleus accumbens shell and core during acquisition and maintenance of intravenous WIN 55,212-2 self-administration. Psychopharmacology (Berl) 2006; 188(1): 63-74.

[141] Mato S, Robbe D, Puente N, Grandes P, Manzoni OJ. Presynaptic homeostatic plasticity rescues long-term depression after chronic Delta 9-tetrahydrocannabinol exposure. J Neurosci 2005; 25(50): 11619-27.

[142] Nava F, Carta G, Colombo G, Gessa GL. Effects of chronic Delta(9)-tetrahydrocannabinol treatment on hippocampal extracellular acetylcholine concentration and alternation performance in the T-maze. Neuropharmacology 2001; 41(3): 392-9.

[143] Robledo P, Trigo JM, Panayi F, de la Torre R, Maldonado R. Behavioural and neurochemical effects of combined MDMA and THC administration in mice. Psychopharmacology (Berl) 2007; 195(2): 255-64.

[144] Tanda G, Loddo P, Di CG. Dependence of mesolimbic dopamine transmission on delta9-tetrahydrocannabinol. Eur J Pharmacol 1999; 376(1-2): 23-6.

[145] Whitlow CT, Freedland CS, Porrino LJ. Functional consequences of the repeated administration of Delta9-tetrahydrocannabinol in the rat. Drug Alcohol Depend 2003; 71(2): 169-77.

[146] Wu X, French ED. Effects of chronic delta9-tetrahydrocannabinol on rat midbrain dopamine neurons: an electrophysiological assessment. Neuropharmacology 2000; 39(3): 391-8.

[147] Cheer JF, Kendall DA, Mason R, Marsden CA. Differential cannabinoid-induced electrophysiological effects in rat ventral tegmentum. Neuropharmacology 2003; 44(5): 633-41.

[148] French ED. delta9-Tetrahydrocannabinol excites rat VTA dopamine neurons through activation of cannabinoid CB1 but not opioid receptors. Neurosci Lett 1997; 226(3): 159-62.

[149] French ED, Dillon K, Wu X. Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. Neuroreport 1997; 8(3): 649-52.

[150] Gifford AN, Gardner EL, Ashby CR, Jr. The effect of intravenous administration of delta-9-tetrahydrocannabinol on the activity of A10 dopamine neurons recorded *in vivo* in anesthetized rats. Neuropsychobiology 1997; 36(2): 96-9.

[151] Massi L, Elezgarai I, Puente N, *et al*. Cannabinoid receptors in the bed nucleus of the stria terminalis control cortical excitation of midbrain dopamine cells *in vivo*. J Neurosci 2008; 28(42): 10496-508.

[152] Murillo-Rodriguez E, Palomero-Rivero M, Millan-Aldaco D, Mechoulam R, Drucker-Colin R. Effects on sleep and dopamine levels of microdialysis perfusion of cannabidiol into the lateral hypothalamus of rats. Life Sci 2011; 88(11-12): 504-11.

[153] Ng Cheong Ton JM, Gerhardt GA, Friedemann M, *et al*. The effects of delta 9-tetrahydrocannabinol on potassium-evoked release of dopamine in the rat caudate nucleus: an *in vivo* electrochemical and *in vivo* microdialysis study. Brain Res 1988; 451(1-2): 59-68.

[154] Pistis M, Porcu G, Melis M, Diana M, Gessa GL. Effects of cannabinoids on prefrontal neuronal responses to ventral tegmental area stimulation. Eur J Neurosci 2001; 14(1): 96-102.

[155] Pistis M, Muntoni AL, Pillolla G, Gessa GL. Cannabinoids inhibit excitatory inputs to neurons in the shell of the nucleus accumbens: an *in vivo* electrophysiological study. Eur J Neurosci 2002; 15(11): 1795-802.

[156] Pistis M, Ferraro L, Pira L, *et al*. Delta(9)-tetrahydrocannabinol decreases extracellular GABA and increases extracellular glutamate and dopamine levels in the rat prefrontal cortex: an *in vivo* microdialysis study. Brain Res 2002; 948(1-2): 155-8.

[157] Wu WC, Wang Y, Chai CY. Acute effects of the cannabinoid receptor agonist WIN55212-2 on dopamine release in rat: an *in vivo* electrochemical study. Chin J Physiol 2008; 51(3): 152-9.

[158] Arnone D, Barrick TR, Chengappa S, *et al*. Corpus callosum damage in heavy marijuana use: preliminary evidence from diffusion tensor tractography and tract-based spatial statistics. Neuroimage 2008; 41(3): 1067-74.

[159] Ashtari M, Cervellione K, Cottone J, *et al*. Diffusion abnormalities in adolescents and young adults with a history of heavy cannabis use. J Psychiatr Res 2009; 43(3): 189-204.

[160] Ashtari M, Avants B, Cyckowski L, *et al*. Medial temporal structures and memory functions in adolescents with heavy cannabis use. J Psychiatr Res 2011; 45(8): 1055-66.

[161] Bava S, Frank LR, McQueeny T, *et al*. Altered white matter microstructure in adolescent substance users. Psychiatry Res 2009; 173(3): 228-37.

[162] Bava S, Jacobus J, Mahmood O, Yang TT, Tapert SF. Neurocognitive correlates of white matter quality in adolescent substance users. Brain Cogn 2010; 72(3): 347-54.

[163] Block RI, O'Leary DS, Ehrhardt JC, *et al*. Effects of frequent marijuana use on brain tissue volume and composition. Neuroreport 2000; 11(3): 491-6.

[164] Block RI, O'Leary DS, Hichwa RD, *et al*. Cerebellar hypoactivity in frequent marijuana users. Neuroreport 2000; 11(4): 749-53.

[165] Block RI, O'Leary DS, Hichwa RD, *et al*. Effects of frequent marijuana use on memory-related regional cerebral blood flow. Pharmacol Biochem Behav 2002; 72(1-2): 237-50.

[166] Clark DB, Chung T, Thatcher DL, Pajtek S, Long EC. Psychological dysregulation, white matter disorganization and substance use disorders in adolescence. Addiction 2012; 107(1): 206-14.

[167] Co BT, Goodwin DW, Gado M, Mikhael M, Hill SY. Absence of cerebral atrophy in chronic cannabis users. Evaluation by computerized transaxial tomography. JAMA 1977; 237(12): 1229-30.

[168] Cousijn J, Wiers RW, Ridderinkhof KR, *et al*. Grey matter alterations associated with cannabis use: Results of a VBM study in heavy cannabis users and healthy controls. Neuroimage 2012; 59(4): 3845-51.

[169] DeLisi LE, Bertisch HC, Szulc KU, *et al*. A preliminary DTI study showing no brain structural change associated with adolescent cannabis use. Harm Reduct J 2006; 3: 17.

[170] Gruber SA, Yurgelun-Todd DA. Neuroimaging of marijuana smokers during inhibitory processing: a pilot investigation. Brain Res Cogn Brain Res 2005; 23(1): 107-18.

[171] Gruber SA, Rogowska J, Yurgelun-Todd DA. Altered affective response in marijuana smokers: an FMRI study. Drug Alcohol Depend 2009; 105(1-2): 139-53.

[172] Gruber SA, Silveri MM, Dahlgren MK, Yurgelun-Todd D. Why so impulsive? White matter alterations are associated with impulsivity in chronic marijuana smokers. Exp Clin Psychopharmacol 2011; 19(3): 231-42.

[173] Hannerz J, Hindmarsh T. Neurological and neuroradiological examination of chronic cannabis smokers. Ann Neurol 1983; 13(2): 207-10.

[174] Jager G, Kahn RS, van den Brink W, van Ree JM, Ramsey NF. Long-term effects of frequent cannabis use on working memory and attention: an fMRI study. Psychopharmacology (Berl) 2006; 185(3): 358-68.

[175] Jager G, van Hell HH, De Win MM, *et al*. Effects of frequent cannabis use on hippocampal activity during an associative memory task. Eur Neuropsychopharmacol 2007; 17(4): 289-97.

[176] Kim DJ, Skosnik PD, Cheng H, *et al*. Structural network topology revealed by white matter tractography in cannabis users: a graph theoretical analysis. Brain Connect 2011; 1(6): 473-83.

[177] Kuehnle J, Mendelson JH, Davis KR, New PF. Computed tomographic examination of heavy marijuana smokers. JAMA 1977; 237(12): 1231-2.

[178] Lopez-Larson MP, Bogorodzki P, Rogowska J, *et al*. Altered prefrontal and insular cortical thickness in adolescent marijuana users. Behav Brain Res 2011; 220(1): 164-72.

[179] Mathew RJ, Wilson WH, Tant SR. Acute changes in cerebral blood flow associated with marijuana smoking. Acta Psychiatr Scand 1989; 79(2): 118-28.

[180] Mathew RJ, Wilson WH, Turkington TG, *et al*. Time course of tetrahydrocannabinol-induced changes in regional cerebral blood flow measured with positron emission tomography. Psychiatry Res 2002; 116(3): 173-85.

[181] Matochik JA, Eldreth DA, Cadet JL, Bolla KI. Altered brain tissue composition in heavy marijuana users. Drug Alcohol Depend 2005; 77(1): 23-30.

[182] McQueeny T, Padula CB, Price J, *et al*. Gender effects on amygdala morphometry in adolescent marijuana users. Behav Brain Res 2011; 224(1): 128-34.

[183] Medina KL, McQueeny T, Nagel BJ, *et al*. Prefrontal cortex morphometry in abstinent adolescent marijuana users: subtle gender effects. Addict Biol 2009; 14(4): 457-68.

[184] Medina KL, Nagel BJ, Tapert SF. Abnormal cerebellar morphometry in abstinent adolescent marijuana users. Psychiatry Res 2010; 182(2): 152-9.

[185] Tzilos GK, Cintron CB, Wood JB, *et al*. Lack of hippocampal volume change in long-term heavy cannabis users. Am J Addict 2005; 14(1): 64-72.

[186] Ward PB, Solowij N, Peters R, *et al*. An fMRI study of regional brain volumes in long-term cannabis users. Journal of Psychopharmacology 2002; 16 (Suppl. 3)(A56).

[187] Yucel M, Solowij N, Respondek C, *et al*. Regional brain abnormalities associated with long-term heavy cannabis use. Arch Gen Psychiatry 2008; 65(6): 694-701.

[188] Abdullaev Y, Posner MI, Nunnally R, Dishion TJ. Functional MRI evidence for inefficient attentional control in adolescent chronic cannabis abuse. Behav Brain Res 2010; 215(1): 45-57.

[189] Bolla KI, Eldreth DA, Matochik JA, Cadet JL. Neural substrates of faulty decision-making in abstinent marijuana users. Neuroimage 2005; 26(2): 480-92.

[190] Chang L, Yakupov R, Cloak C, Ernst T. Marijuana use is associated with a reorganized visual-attention network and cerebellar hypoactivation. Brain 2006; 129(Pt 5): 1096-112.

[191] Eldreth DA, Matochik JA, Cadet JL, Bolla KI. Abnormal brain activity in prefrontal brain regions in abstinent marijuana users. Neuroimage 2004; 23(3): 914-20.

[192] Harding IH, Solowij N, Harrison BJ, *et al.* Functional connectivity in brain networks underlying cognitive control in chronic cannabis users. Neuropsychopharmacology 2012; 37(8): 1923-33.

[193] Hester R, Nestor L, Garavan H. Impaired error awareness and anterior cingulate cortex hypoactivity in chronic cannabis users. Neuropsychopharmacology 2009; 34(11): 2450-8.

[194] Hirvonen J, Goodwin RS, Li CT, *et al*. Reversible and regionally selective downregulation of brain cannabinoid CB(1) receptors in chronic daily cannabis smokers. Mol Psychiatry 2012; 17(6): 642-9.

[195] Jager G, Block RI, Luijten M, Ramsey NF. Cannabis use and memory brain function in adolescent boys: a cross-sectional multicenter functional magnetic resonance imaging study. J Am Acad Child Adolesc Psychiatry 2010; 49(6): 561-72, 572.

[196] Kanayama G, Rogowska J, Pope HG, Gruber SA, Yurgelun-Todd DA. Spatial working memory in heavy cannabis users: a functional magnetic resonance imaging study. Psychopharmacology (Berl) 2004; 176(3-4): 239-47.

[197] King GR, Ernst T, Deng W, *et al*. Altered brain activation during visuomotor integration in chronic active cannabis users: relationship to cortisol levels. J Neurosci 2011; 31(49): 17923-31.

[198] Lundqvist T, Jonsson S, Warkentin S. Frontal lobe dysfunction in long-term cannabis users. Neurotoxicol Teratol 2001; 23(5): 437-43.

[199] Nestor L, Hester R, Garavan H. Increased ventral striatal BOLD activity during non-drug reward anticipation in cannabis users. Neuroimage 2010; 49(1): 1133-43.

[200] Padula CB, Schweinsburg AD, Tapert SF. Spatial working memory performance and fMRI activation interaction in abstinent adolescent marijuana users. Psychol Addict Behav 2007; 21(4): 478-87.

[201] Pillay SS, Rogowska J, Kanayama G, *et al*. Neurophysiology of motor function following cannabis discontinuation in chronic cannabis smokers: an fMRI study. Drug Alcohol Depend 2004; 76(3): 261-71.

[202] Schweinsburg AD, Nagel BJ, Schweinsburg BC, *et al*. Abstinent adolescent marijuana users show altered fMRI response during spatial working memory. Psychiatry Res 2008; 163(1): 40-51.

[203] Schweinsburg AD, Schweinsburg BC, Medina KL, *et al*. The influence of recency of use on fMRI response during spatial working memory in adolescent marijuana users. J Psychoactive Drugs 2010; 42(3): 401-12.

[204] Schweinsburg AD, Schweinsburg BC, Nagel BJ, Eyler LT, Tapert SF. Neural correlates of verbal learning in adolescent alcohol and marijuana users. Addiction 2011; 106(3): 564-73.

[205] Sevy S, Smith GS, Ma Y, *et al*. Cerebral glucose metabolism and D2/D3 receptor availability in young adults with cannabis dependence measured with positron emission tomography. Psychopharmacology (Berl) 2008; 197(4): 549-56.

[206] Silveri MM, Jensen JE, Rosso IM, Sneider JT, Yurgelun-Todd DA. Preliminary evidence for white matter metabolite differences in marijuana-dependent young men using 2D J-resolved magnetic

resonance spectroscopic imaging at 4 Tesla. Psychiatry Res 2011; 191(3): 201-11.
[207] Sneider JT, Pope HG, Jr., Silveri MM, *et al*. Altered regional blood volume in chronic cannabis smokers. Exp Clin Psychopharmacol 2006; 14(4): 422-8.
[208] Stokes PR, Egerton A, Watson B, *et al*. History of cannabis use is not associated with alterations in striatal dopamine D2/D3 receptor availability. J Psychopharmacol 2012; 26(1): 144-9.
[209] Tapert SF, Schweinsburg AD, Drummond SP, *et al*. Functional MRI of inhibitory processing in abstinent adolescent marijuana users. Psychopharmacology (Berl) 2007; 194(2): 173-83.
[210] Vaidya JG, Block RI, O'Leary DS, *et al*. Effects of chronic marijuana use on brain activity during monetary decision-making. Neuropsychopharmacology 2012; 37(3): 618-29.
[211] van Hell HH, Vink M, Ossewaarde L, *et al*. Chronic effects of cannabis use on the human reward system: an fMRI study. Eur Neuropsychopharmacol 2010; 20(3): 153-63.
[212] Volkow ND, Gillespie H, Mullani N, *et al*. Brain glucose metabolism in chronic marijuana users at baseline and during marijuana intoxication. Psychiatry Res 1996; 67(1): 29-38.
[213] Weinstein A, Brickner O, Lerman H, *et al*. Brain imaging study of the acute effects of Delta9-tetrahydrocannabinol (THC) on attention and motor coordination in regular users of marijuana. Psychopharmacology (Berl) 2008; 196(1): 119-31.
[214] Wesley MJ, Hanlon CA, Porrino LJ. Poor decision-making by chronic marijuana users is associated with decreased functional responsiveness to negative consequences. Psychiatry Res 2011; 191(1): 51-9.
[215] Voruganti LN, Slomka P, Zabel P, Mattar A, Awad AG. Cannabis induced dopamine release: an in-vivo SPECT study. Psychiatry Res 2001; 107(3): 173-7.
[216] Wolff V, Lauer V, Rouyer O, *et al*. Cannabis use, ischemic stroke, and multifocal intracranial vasoconstriction: a prospective study in 48 consecutive young patients. Stroke 2011; 42(6): 1778-80.
[217] Volkow ND, Gillespie H, Mullani N, *et al*. Cerebellar metabolic activation by delta-9-tetrahydro-cannabinol in human brain: a study with positron emission tomography and 18F-2-fluoro-2-deoxyglucose. Psychiatry Res 1991; 40(1): 69-78.
[218] Mathew RJ, Wilson WH, Humphreys DF, Lowe JV, Wiethe KE. Regional cerebral blood flow after marijuana smoking. J Cereb Blood Flow Metab 1992; 12(5): 750-8.
[219] Mathew RJ, Wilson WH. Acute changes in cerebral blood flow after smoking marijuana. Life Sci 1993; 52(8): 757-67.
[220] Mathew RJ, Wilson WH, Coleman RE, Turkington TG, Degrado TR. Marijuana intoxication and brain activation in marijuana smokers. Life Sci 1997; 60(23): 2075-89.
[221] Mathew RJ, Wilson WH, Turkington TG, Coleman RE. Cerebellar activity and disturbed time sense after THC. Brain Res 1998; 797(2): 183-9.
[222] Mathew RJ, Wilson WH, Chiu NY, *et al*. Regional cerebral blood flow and depersonalization after tetrahydrocannabinol administration. Acta Psychiatr Scand 1999; 100(1): 67-75.
[223] van Hell HH, Bossong MG, Jager G, *et al*. Evidence for involvement of the insula in the psychotropic effects of THC in humans: a double-blind, randomized pharmacological MRI study. Int J Neuropsychopharmacol 2011; 14(10): 1377-88.
[224] Crippa JA, Zuardi AW, Garrido GE, *et al*. Effects of cannabidiol (CBD) on regional cerebral blood flow. Neuropsychopharmacology 2004; 29(2): 417-26.
[225] O'Leary DS, Block RI, Koeppel JA, *et al*. Effects of smoking marijuana on brain perfusion and cognition. Neuropsychopharmacology 2002; 26(6): 802-16.
[226] O'Leary DS, Block RI, Turner BM, *et al*. Marijuana alters the human cerebellar clock. Neuroreport 2003; 14(8): 1145-51.
[227] O'Leary DS, Block RI, Koeppel JA, *et al*. Effects of smoking marijuana on focal attention and brain blood flow. Hum Psychopharmacol 2007; 22(3): 135-48.
[228] Bossong MG, Jager G, van Hell HH, *et al*. Effects of Delta9-Tetrahydrocannabinol Administration on Human Encoding and Recall Memory Function: A Pharmacological fMRI Study. J Cogn Neurosci 2012; 24(3): 588-99.
[229] Bossong MG, Jansma JM, van Hell HH, *et al*. Effects of delta9-tetrahydrocannabinol on human working memory function. Biol Psychiatry 2012; 71(8): 693-9.
[230] Phan KL, Angstadt M, Golden J, *et al*. Cannabinoid modulation of amygdala reactivity to social signals of threat in humans. J Neurosci 2008; 28(10): 2313-9.
[231] Rabinak CA, Sripada CS, Angstadt M, de WH, Phan KL. Cannabinoid modulation of subgenual anterior cingulate cortex activation during experience of negative affect. J Neural Transm 2012; 119(6): 701-7.
[232] van Hell HH, Jager G, Bossong MG, *et al*. Involvement of the endocannabinoid system in reward processing in the human brain. Psychopharmacology (Berl) 2012; 219(4): 981-90.
[233] Bhattacharyya S, Fusar-Poli P, Borgwardt S, *et al*. 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 2009; 66(4): 442-51.
[234] Bhattacharyya S, Crippa JA, Allen P, *et al*. Induction of psychosis by {delta}9-tetrahydrocannabinol reflects modulation of prefrontal and striatal function during attentional salience processing. Arch Gen Psychiatry 2012; 69(1): 27-36.
[235] Borgwardt SJ, Allen P, Bhattacharyya S, *et al*. Neural basis of Delta-9-tetrahydrocannabinol and cannabidiol: effects during response inhibition. Biol Psychiatry 2008; 64(11): 966-73.
[236] Fusar-Poli P, Crippa JA, Bhattacharyya S, *et al*. Distinct effects of {delta}9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. Arch Gen Psychiatry 2009; 66(1): 95-105.
[237] Winton-Brown TT, Allen P, Bhattacharyya S, *et al*. Modulation of auditory and visual processing by delta-9-tetrahydrocannabinol and cannabidiol: an FMRI study. Neuropsychopharmacology 2011; 36(7): 1340-8.
[238] Fusar-Poli P, Allen P, Bhattacharyya S, *et al*. Modulation of effective connectivity during emotional processing by Delta 9-tetrahydrocannabinol and cannabidiol. Int J Neuropsychopharmacol 2010; 13(4): 421-32.
[239] Bossong MG, van Berckel BN, Boellaard R, *et al*. Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. Neuropsychopharmacology 2009; 34(3): 759-66.
[240] Stokes PR, Mehta MA, Curran HV, Breen G, Grasby PM. Can recreational doses of THC produce significant dopamine release in the human striatum? Neuroimage 2009; 48(1): 186-90.
[241] Barkus E, Morrison PD, Vuletic D, *et al*. Does intravenous Delta9-tetrahydrocannabinol increase dopamine release? A SPET study. J Psychopharmacol 2011; 25(11): 1462-8.
[242] Freedland CS, Whitlow CT, Miller MD, Porrino LJ. Dose-dependent effects of Delta9-tetrahydrocannabinol on rates of local cerebral glucose utilization in rat. Synapse 2002; 45(2): 134-42.
[243] Margulies JE, Hammer RP, Jr. Delta 9-tetrahydrocannabinol alters cerebral metabolism in a biphasic, dose-dependent manner in rat brain. Eur J Pharmacol 1991; 202(3): 373-8.
[244] Pontieri FE, Conti G, Zocchi A, Fieschi C, Orzi F. Metabolic mapping of the effects of WIN 55212-2 intravenous administration in the rat. Neuropsychopharmacology 1999; 21(6): 773-6.
[245] Whitlow CT, Freedland CS, Porrino LJ. Metabolic mapping of the time-dependent effects of delta 9-tetrahydrocannabinol administration in the rat. Psychopharmacology (Berl) 2002; 161(2): 129-36.
[246] Bloom AS, Tershner S, Fuller SA, Stein EA. Cannabinoid-induced alterations in regional cerebral blood flow in the rat. Pharmacol Biochem Behav 1997; 57(4): 625-31.
[247] Goldman H, Dagirmanjian R, Drew WG, Murphy S. delta9-tetrahydrocannabinol alters flow of blood to subcortical areas of the conscious rat brain. Life Sci 1975; 17(3): 477-82.
[248] Stein EA, Fuller SA, Edgemond WS, Campbell WB. Selective effects of the endogenous cannabinoid arachidonylethanolamide (anandamide) on regional cerebral blood flow in the rat. Neuropsychopharmacology 1998; 19(6): 481-91.
[249] Nguyen VH, Verdurand M, Dedeurwaerdere S, *et al*. Increased brain metabolism after acute administration of the synthetic cannabinoid HU210: A small animal PET imaging study with (18)F-FDG. Brain Res Bull 2012; 87(2-3): 172-9.
[250] Castañeda E, Moss DE, Oddie SD, Whishaw IQ. THC does not affect striatal dopamine release: microdialysis in freely moving rats. Pharmacol Biochem Behav 1991; 40(3): 587-91.
[251] Chen J, Paredes W, Lowinson JH, Gardner EL. Delta 9-tetrahydrocannabinol enhances presynaptic dopamine efflux in medial prefrontal cortex. Eur J Pharmacol 1990; 190(1-2): 259-62.

[252] Chen J, Marmur R, Pulles A, Paredes W, Gardner EL. Ventral tegmental microinjection of delta 9-tetrahydrocannabinol enhances ventral tegmental somatodendritic dopamine levels but not forebrain dopamine levels: evidence for local neural action by marijuana's psychoactive ingredient. Brain Res 1993; 621(1): 65-70.

[253] Chen JP, Paredes W, Lowinson JH, Gardner EL. Strain-specific facilitation of dopamine efflux by delta 9-tetrahydrocannabinol in the nucleus accumbens of rat: an *in vivo* microdialysis study. Neurosci Lett 1991; 129(1): 136-80.

[254] Malone DT, Taylor DA. Modulation by fluoxetine of striatal dopamine release following Delta9-tetrahydrocannabinol: a microdialysis study in conscious rats. Br J Pharmacol 1999; 128(1): 21-6.

[255] Murillo-Rodriguez E, Millan-Aldaco D, Palomero-Rivero M, Mechoulam R, Drucker-Colin R. Cannabidiol, a constituent of Cannabis sativa, modulates sleep in rats. FEBS Lett 2006; 580(18): 4337-45.

[256] Solinas M, Justinova Z, Goldberg SR, Tanda G. Anandamide administration alone and after inhibition of fatty acid amide hydrolase (FAAH) increases dopamine levels in the nucleus accumbens shell in rats. J Neurochem 2006; 98(2): 408-19.

[257] Solinas M, Tanda G, Justinova Z, *et al*. The endogenous cannabinoid anandamide produces delta-9-tetrahydrocannabinol-like discriminative and neurochemical effects that are enhanced by inhibition of fatty acid amide hydrolase but not by inhibition of anandamide transport. J Pharmacol Exp Ther 2007; 321(1): 370-80.

[258] Tanda G, Pontieri FE, Di CG. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. Science 1997; 276(5321): 2048-50.

[259] Cheer JF, Wassum KM, Heien ML, Phillips PE, Wightman RM. Cannabinoids enhance subsecond dopamine release in the nucleus accumbens of awake rats. J Neurosci 2004; 24(18): 4393-400.

[260] Diana M, Melis M, Gessa GL. Increase in meso-prefrontal dopaminergic activity after stimulation of CB1 receptors by cannabinoids. Eur J Neurosci 1998; 10(9): 2825-30.

[261] Gessa GL, Melis M, Muntoni AL, Diana M. Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB1 receptors. Eur J Pharmacol 1998; 341(1): 39-44.

[262] Melis M, Gessa GL, Diana M. Different mechanisms for dopaminergic excitation induced by opiates and cannabinoids in the rat midbrain. Prog Neuropsychopharmacol Biol Psychiatry 2000; 24(6): 993-1006.

[263] Corchero J, Manzanares J, Fuentes JA. Cannabinoid/opioid crosstalk in the central nervous system. Crit Rev Neurobiol 2004; 16(1-2): 159-72.

[264] Fattore L, Cossu G, Spano MS, *et al*. Cannabinoids and reward: interactions with the opioid system. Crit Rev Neurobiol 2004; 16(1-2): 147-58.

[265] Fattore L, Deiana S, Spano SM, *et al*. Endocannabinoid system and opioid addiction: behavioural aspects. Pharmacol Biochem Behav 2005; 81(2): 343-59.

[266] Koob GF, Volkow ND. Neurocircuitry of addiction. Neuropsychopharmacology 2010; 35(1): 217-38.

[267] Kapur S, Mizrahi R, Li M. From dopamine to salience to psychosis--linking biology, pharmacology and phenomenology of psychosis. Schizophr Res 2005; 79(1): 59-68.

[268] D'Souza DC, Braley G, Blaise R, *et al*. Effects of haloperidol on the behavioral, subjective, cognitive, motor, and neuroendocrine effects of Delta-9-tetrahydrocannabinol in humans. Psychopharmacology (Berl) 2008; 198(4): 587-603.

Received: May 13, 2013 **Accepted: June 10, 2013**

5

Study 3

Structural and functional imaging studies in chronic cannabis users: a systematic review of adolescent and adult findings

Plos One 2013; 8(2):e55821

Study 3

Summary

Reference

Title: Structural and functional imaging studies in chronic cannabis users: a systematic review of adolescent and adult findings. Authors: Batalla A, Bhattacharyya S, Yücel M, Fusar-Poli P, Crippa JA, Nogué S, Torrens M, Pujol J, Farré M, Martín-Santos R. *PLoS One 2013; 8(2):e55821*. Impact factor 2012: 3.730 (1st quartile multidisciplinary sciences).

Aims

Developing further *hypothesis #*2, a systematic review to assess the evidence of the impact of chronic cannabis use on brain structure and function was conducted. This review included neuroimaging studies performed in chronic cannabis users with a matched control group, considering the studies conducted in adolescents.

Method

Papers published until August 2012 were included from four databases (EMBASE, Medline, PubMed, LILACS) following a comprehensive search strategy and pre-determined set of criteria for article selection based on PRISMA guidelines (96). Only neuroimaging studies involving chronic cannabis users with a matched control group were considered. Chronic cannabis users were defined as subjects who used cannabis several times a week for at least two years. Cannabis users had to be abstinent for at least 12 hours before brain scanning. Studies involving individuals who met criteria for psychiatric disorders or substance use disorders, a part from cannabis, were excluded.

For structural and functional imaging data, the measures of interest were global and regional brain volume, and global and regional brain activity (cerebral blood flow, regional cerebral blood flow or blood dependent signal).

Results

Forty-three studies met the established criteria, of which eight were in adolescent users. Neuroimaging studies provided evidence of morphological brain alterations in adults and adolescents, particularly in medial temporal areas (hippocampus and amygdala) and frontal regions, as well as in the cerebellum. The reported effects were associated with amount of cannabis exposure. Functional neuroimaging studies suggested different patterns of resting global and brain activity during the performance of several cognitive tasks also in both groups, suggesting that compensatory effects in brain activity in response to chronic cannabis exposure might exist.

Conclusion

Chronic cannabis use might potentially alter brain function and structure, especially medial temporal regions, in adults and adolescent users. The amount of exposure to cannabis may be correlated to its detrimental effects. However, the results also pointed out methodological limitations among studies, high heterogeneity in the findings and a lack of studies in young users. As stated in the previous review of acute effects, there is also a strong need for convergent methodology when studying the effects of chronic cannabis exposure. Data-sharing initiatives may prove useful in future research.

OPEN ACCESS Freely available online

PLOS ONE

Structural and Functional Imaging Studies in Chronic Cannabis Users: A Systematic Review of Adolescent and Adult Findings

Albert Batalla[1,2,3], Sagnik Bhattacharyya[4], Murat Yücel[3], Paolo Fusar-Poli[4], Jose Alexandre Crippa[5,6], Santiago Nogué[7], Marta Torrens[8,9], Jesús Pujol[10], Magí Farré[8,9], Rocio Martin-Santos[1,2,6]*

1 Psychiatry, Institute of Neurosciences, Hospital Clínic, IDIBAPS, CIBERSAM, Barcelona, Spain, **2** Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Barcelona, Spain, **3** Melbourne Neuropsychiatry Centre, The University of Melbourne, Melbourne, Victoria, Australia, **4** Department of Psychosis Studies, King's College London, Institute of Psychiatry, London, United Kingdom, **5** Neuroscience and Cognitive Behavior Department, University of Sao Paulo, Ribeirao Preto, Brazil, **6** National Science and Technology Institute for Translational Medicine (INCT-TM, CNPq), Ribeirao Preto, Brazil, **7** Clinical Toxicology Unit, Emergency Department, Hospital Clínic, IDIBAPS, University of Barcelona, Barcelona, Spain, **8** Neuroscience Program, Pharmacology Unit and Drug Addiction Unit, IMIM-INAD-Parc de Salut Mar, Autonomous University of Barcelona, Barcelona, Spain, **9** Red de Trastornos Adictivos (RETIC), IMIM-INAD-Parc de Salut Mar, Barcelona, Spain, **10** Institut d'Alta Tecnologia-PRBB, CRC Mar, Hospital del Mar, Barcelona, Spain

Abstract

Background: The growing concern about cannabis use, the most commonly used illicit drug worldwide, has led to a significant increase in the number of human studies using neuroimaging techniques to determine the effect of cannabis on brain structure and function. We conducted a systematic review to assess the evidence of the impact of chronic cannabis use on brain structure and function in adults and adolescents.

Methods: Papers published until August 2012 were included from EMBASE, Medline, PubMed and LILACS databases following a comprehensive search strategy and pre-determined set of criteria for article selection. Only neuroimaging studies involving chronic cannabis users with a matched control group were considered.

Results: One hundred and forty-two studies were identified, of which 43 met the established criteria. Eight studies were in adolescent population. Neuroimaging studies provide evidence of morphological brain alterations in both population groups, particularly in the medial temporal and frontal cortices, as well as the cerebellum. These effects may be related to the amount of cannabis exposure. Functional neuroimaging studies suggest different patterns of resting global and brain activity during the performance of several cognitive tasks both in adolescents and adults, which may indicate compensatory effects in response to chronic cannabis exposure.

Limitations: However, the results pointed out methodological limitations of the work conducted to date and considerable heterogeneity in the findings.

Conclusion: Chronic cannabis use may alter brain structure and function in adult and adolescent population. Further studies should consider the use of convergent methodology, prospective large samples involving adolescent to adulthood subjects, and data-sharing initiatives.

Citation: Batalla A, Bhattacharyya S, Yücel M, Fusar-Poli P, Crippa JA, et al. (2013) Structural and Functional Imaging Studies in Chronic Cannabis Users: A Systematic Review of Adolescent and Adult Findings. PLoS ONE 8(2): e55821. doi:10.1371/journal.pone.0055821

Editor: Lin Lu, Peking University, China

Received September 12, 2012; **Accepted** January 2, 2013; **Published** February 4, 2013

Funding: This study has been done in part with Spanish grants: Plan Nacional sobre Drogas, Ministerio de Sanidad y Consumo PNSD/2011/050 and PNSD2006/101 (R. MartÂ-n-Santos); and the support of DIUE of Generalitat de Catalunya SGR2009/1435 (R. MartÂ-n-Santos, J.A. Crippa); and the Technology Institute for Translational Medicine (INCT-TM, Conselho Nacional de Desenvolvimiento CientÂ-fico e TecnolÂ2gico (CNPq), Brazil (J.A. Crippa, R. MartÂ-n-Santos); S. Bhattacharyya is supported by a Clinician Scientist award from the National Institute of Health Research, UK; and J.A. Crippa receives a CNPq (Brazil) productivity award (IC). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: rmsantos@clinic.ub.es

Introduction

Cannabis is the illicit drug most widely available and used worldwide [1,2], consumed by between 125 and 203 million people, largely younger age group (15–34 years), which corresponds to an annual prevalence rate of 2.8%–4.5% [1,2]. Despite the fact that many individuals tend to discontinue cannabis use after their initial experimentation with the drug [1] and the percentage of individuals who develop dependence is lower than that associated with alcohol (15%) or tobacco (32%) use, around 9% of cannabis users develop dependence in the long term [3,4]. Cannabis use has been associated with a range of acute and chronic mental health problems, such as anxiety, depression, neurocognitive alterations and deficits as well as increased risk of psychotic symptoms and disorders, the severity of these effects

being dependent on frequency of use, age of onset and genetic vulnerability [5–15]. These effects are probably related to effects on the endocannabinoid system, which can modulate the neuronal activity of other neurotransmitter systems, such as dopamine, through its action on the most abundant cannabinoid receptor in brain, the cannabinoid receptor 1 (CB1) [16,17]. CB1 receptors mature slowly, reaching maximal levels during adolescence [18], and are particularly concentrated in brain regions that are critical for executive functioning, reward processing and memory, such as the prefrontal cortex, anterior cingulate cortex, basal ganglia, medial temporal areas (e.g., hippocampus and amygdala) and cerebellum [19].

Animal studies have consistently demonstrated that delta-9-tetrahydrocannabinol (THC), the main psychoactive component of cannabis [20], is able to disrupt the regulatory role of the endogenous cannabinoid system [21], inducing neurotoxic changes in brain regions rich with cannabinoid receptors that might dramatically affect the process of maturational refinement of cortical neuronal networks [22–24] and lastly promote changes in brain structure and alter emotional and cognitive performance [25], particularly if the exposure has been during the adolescent period [26,27]. In contrast to animal literature, results from human studies investigating chronic cannabis users are often inconsistent. These discrepancies may be due to heterogeneity in socio-demographic characteristics of the population studied, imaging techniques employed, as well as differences in drug usage patterns and psychiatric comorbidities that may not always be apparent or result in contact with mental health services and hence may not be appropriately controlled for in studies where participants are screened for presence of co-morbid psychiatric disorder merely by enquiring about previous contact with mental health services [28–30]. However, overall the results suggest that long-term cannabis use may result in persistent alterations in brain function and morphology that would extend beyond the period of intoxication [28,31], and that earlier onset of use may be associated with greater detrimental effects [32,33].

It is remarkable to note that although the onset of cannabis use is typically during adolescence, a few imaging studies have been conducted with adolescent users [28,34]. Since brain development continues up to young adulthood [35], adolescence may be a critical period during which chronic cannabis exposure may have far-reaching consequences [36]. Although brain size is thought to stabilize around the age of five years [37], important neurodevelopmental processes continue throughout adolescence, including myelinization [38], synaptic refinement [39] and gray matter volume reduction [40]. While the long-term effects of cannabis use may potentially have major implications for social and family life, education and occupational functioning, its effects on brain structure and function have not been well determined.

The growing concern about cannabis use has led to a significant increase in the number of human studies using neuroimaging techniques to determine the effect of the substance on brain structure and function, as well as to several recent reviews examining this topic [28,29,34,41–46]. However, some authors have only reviewed studies investigating the acute effects of cannabis [45,46] or those published over the last decade [41,44], while others did not adequately specify criteria for selecting studies [41,43] or included those studies that investigated only adult population [29,42]. In the present review, we have conducted a systematic literature search to assess and integrate the evidence of the impact of chronic cannabis use on brain structure and function, focusing on studies in the adolescent and adult population. Papers published until August 2012 have been included following a comprehensive search strategy and pre-determined set of criteria for article selection [29].

Methods

Data for this systematic review was collected with an advanced document protocol in accordance with the PRISMA guidelines [47]. This protocol provided a checklist for reporting systematic reviews (see Table S1).

Search strategy

Electronic searches were performed using EMBASE (1980-August 2012), Medline (1966-August 2012), PubMed (1966-August 2012) and LILACS (1982-August 2012) databases. The following key words were used: cannabis; marijuana; marihuana; delta-9-tetrahydrocannabinol; THC; cannabidiol, CBD; neuroimaging; brain imaging; computerized tomography, CT; magnetic resonance, MRI; single photon emission tomography, SPECT; functional magnetic resonance, fMRI; positron emission tomography, PET; diffusion tensor MRI, DTI-MRI; spectroscopy, MRS. All the studies published up to August 2012 were included without language restriction.

Selection criteria

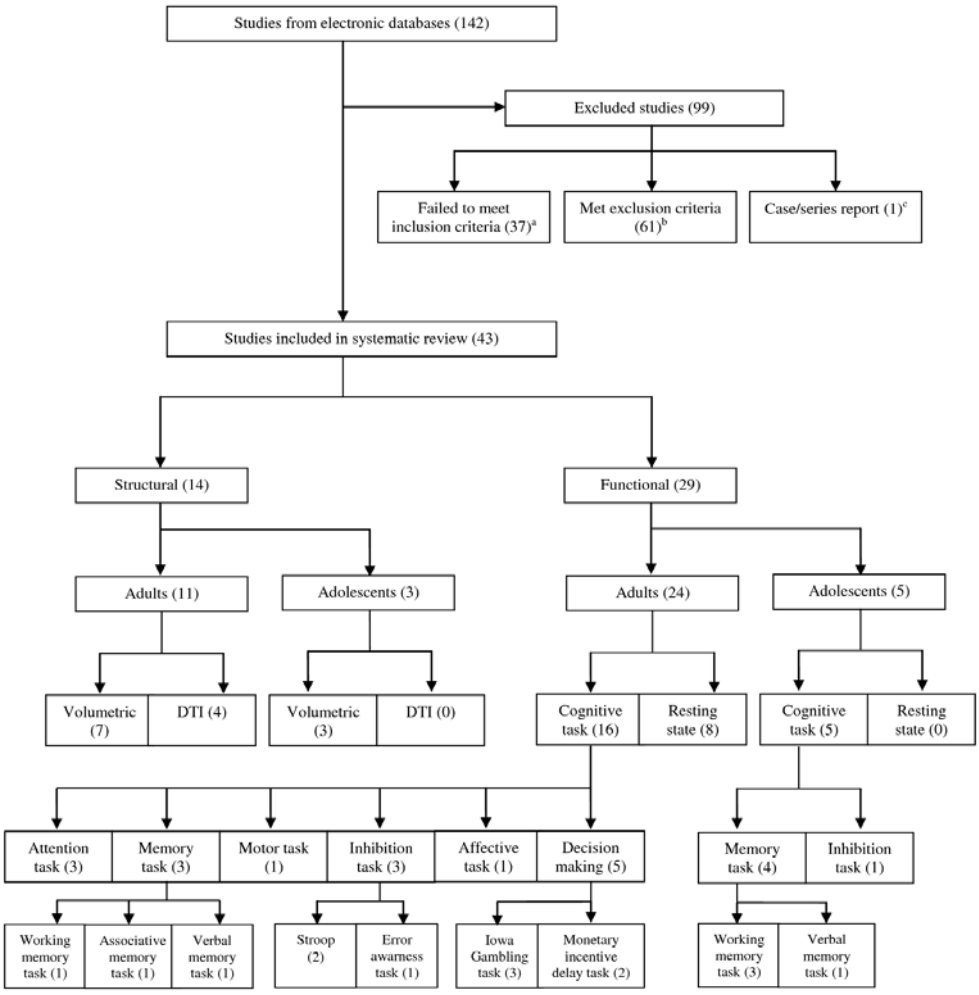
A general review of all neuroimaging studies investigating brain structure or function was initially performed. We obtained a total of 142 published papers (Figure 1). Studies were included or excluded if they expressly stated the following criteria. Inclusion criteria were: (i) use of structural or functional neuroimaging techniques involving chronic cannabis users; (ii) inclusion of a control group of healthy volunteers matched by age, gender and handedness; and (iii) users had to be abstinent for at least 12 hours before brain scanning. Exclusion criteria were: (i) non-neuroimaging studies of cannabis use; (ii) neuroimaging studies that involved participants who had other neurological or psychiatric disorders, or individuals who met criteria for alcohol dependence or other substance use disorders (abuse or dependence) different from cannabis and nicotine, or participants who were not abstinent or who tested positive for drugs other than cannabis on urine screening test; and (iii) neuroimaging studies with recreational or naïve cannabis users.

We defined chronic cannabis users as persons who used cannabis several times a week and who had done so for at least two years. Recreational (or occasional) cannabis users were defined as persons who had used cannabis sporadically (less than four times a month), and naïve users or healthy controls were persons who had used cannabis less than 15 times in their lifetime, according to standardized strict criteria [29,48].

Any publication that reported data using two different neuroimaging techniques from the same subjects (e.g., structural MRI and functional MRI) or a study examining the same subjects with two different cognitive tasks (e.g., verbal working memory and visual attention task) was considered as two studies in this review.

Data extraction

Data was independently extracted by two reviewers. In case of disagreement, opinion from a third senior researcher was sought to assess whether study criteria were fulfilled. From the articles included we recorded names of authors, year of publication, socio-demographic (e.g., sample size, gender, age, handedness) and cannabis use characteristics (e.g., duration, age of onset, frequency of cannabis use), imaging type and design, exclusion criteria (for neurological, psychiatric or drug history), confirmation of absti-



[a]No age, sex or handedness matched: [49–68]. No cannabis abstinence: [69–79]. No healthy control group: [33,80–84]. [b]Psychiatric, other abuse or medical disorder: [12,85–116]. Recreational or naïve cannabis users: [6,30,48,117–141]. [c]Case/series report: [142].

Figure 1. Flow diagram of included neuroimaging studies in chronic cannabis users.
doi:10.1371/journal.pone.0055821.g001

nence from other drugs (whether checked by urine test), rest/active condition (for functional imaging studies), type of cognitive task performed during functional imaging and psychopathological variables assessed (e.g., psychotic or depressive symptoms). With regard to alcohol use, we assessed if subjects met criteria for alcohol abuse or for excessive alcohol consumption (more than 21 or 14 standard alcohol units per week for males or females, respectively) based on the reported data. For structural and functional imaging data, the primary measures of interest were global and regional volume, and global and regional activity [cerebral blood flow (CBF), regional CBF (rCBF) or blood oxygen level dependent signal BOLD)]. The secondary outcome was its correlation with clinical variables. We collected the statistically significant results of each outcome variable, and recorded whether a multiple comparison correction was done to prevent bias towards false positives.

Results

Of the 142 studies identified, thirty-six did not meet the *a priori* selection criteria [33,49–84] and sixty-two met the exclusion criteria [6,12,30,48,85–141] or were case/series reports [142] (for more detailed information, see Figure 1). The remaining 43 studies were classified according to the neuroimaging technique used (structural/functional), age of the participants [adolescents ($\leq$ 18 years) and adults ($>$ 18 years] and testing conditions (resting state/cognitive task) (Figure 1). The studies included comprised: 14 structural neuroimaging studies [11 in adult users and 3 in adolescent users; 10 volumetric studies and 4 diffusion tensor imaging studies (DTI)] and 29 functional neuroimaging studies on the chronic effects of cannabis (24 in adult users and 5 in adolescent users; 8 in the resting state and 21 during a cognitive task).

1. Structural neuroimaging studies in adult chronic cannabis users

We identified 11 structural MRI studies that examined adult chronic cannabis users and met our selection criteria (Table 1). Structural differences were obtained in seven of them in terms of global brain measures [143] or gray/white matter changes [144–149]. Four studies did not find any significant structural alterations when comparing chronic cannabis users with healthy controls [150–153]. The abstinence period for all participants before they underwent the structural MRI was between 12 and 24 hours, apart from two studies [145,152] (for details see Table 1).

1.1. Volumetric studies. Of the seven studies comparing global brain volume measures between chronic cannabis users and healthy controls, there was only one study reporting significant differences [143], namely reduced ventricular cerebral spinal fluid (CSF) in cannabis users. Another study [145] reported total brain volume difference between groups which was no longer significant when the authors covaried for confounding factors such as premorbid intelligence.

Among the six studies employing a whole-brain analysis approach [143,146,148,150–152], two further studies described differences between chronic cannabis users and controls [146,148]. Matochik *et al.* (2005) [148] found lower grey matter density in the right parahippocampus and greater grey matter density in the precentral gyrus and right thalamus in cannabis users, while Cousjin *et al.* (2011) [146] found a larger anterior cerebellum in cannabis users. Matochik *et al.* (2005) [148] also reported differences in white matter density, such as lower density in the left parietal lobe and higher in parahippocampus, fusiform gyrus, lentiform nucleus and pons.

With regard to the three studies that focused on specific regions of interest, all studies reported bilateral volumetric reductions in the hippocampus [145,148,149] and one reported volume reductions in the right amygdala [149]. Some studies have also reported correlations between regional brain volume measures and cannabis use parameters, clinical and neuropsychological measures. For instance, a smaller hippocampal volume has been related to a greater exposure to cannabis [145,146,149], severity of cannabis dependence [146] and more severe positive psychotic symptoms [149]. Ashtari et al. (2011) [145] described a positive association between larger hippocampus volumes and higher verbal learning and memory scores in healthy controls but not in cannabis users [145]. It is remarkable to note that these findings were in patients with an average of 6.7 months of abstinence, which appears to support of the idea that cannabis use may cause long-term brain alterations.

With respect to other brain regions, Cousijn *et al.* (2011) [146] reported a negative correlation between amygdala volume and the amount of cannabis use or dependence, while Matochik *et al.* (2005) [148] found an association between increased white matter density in left precentral gyrus and longer duration of cannabis use.

1.2. Diffusion tensor imaging (DTI) studies. Four studies have used DTI to examine the integrity of white matter tracts in chronic cannabis users [144,147,150,151], of which half have reported positive results [144,147]. Arnone *et al.* (2008) [144] found increased mean diffusivity (MD) in the corpus callosum while Gruber *et al.* (2011) [147] found increased MD in the right genu as well as reductions in left frontal fractional anisotropy (FA). Gruber *et al.* (2011) [147] also reported a positive association between left frontal FA and impulsivity scores, and higher FA and lower MD in the frontal lobes being associated with a later age of initiation of cannabis use.

2. Structural neuroimaging studies in adolescent chronic cannabis users

Three volumetric studies in adolescent chronic cannabis users were included, two of which consist of the same sample [154,155]. As an exception, these two studies [154,155] were included despite involving participants with symptoms of alcohol dependence given the modest number of studies included in this population (for details see Table 1). The MRI scans, focused on specific regions of interest and were obtained following 28 days of abstinence from cannabis use. Medina *et al.* (2009, 2010) [154,155] reported significantly larger volumes of the inferior posterior vermis, as well as a marginal group-by-gender interaction in the prefrontal cortex, in which female and male cannabis users demonstrated, respectively, larger and smaller prefrontal cortex volumes compared to the same-gender controls. McQueeny *et al.* (2011) [156] also described an effect of gender in which female cannabis users but not males, exhibited a larger right amygdala volume.

In terms of correlations, Medina *et al.* (2010) [155] found that larger volumes of the vermis were associated with poorer executive functioning while McQueeny *et al.* (2011) [156] found that larger right amygdala volume was associated with more internalizing symptoms (e.g., anxiety/depression). Lastly, Medina *et al.* (2009) [154] also found that increased volume in the prefrontal cortex was associated with poorer executive functioning among cannabis users while the opposite pattern was observed in controls, suggesting that female users may be at increased risk for cannabis-induced prefrontal abnormalities.

3. Functional neuroimaging studies in adult chronic cannabis users

3.1. Resting state. We included eight case-control studies comparing resting rCBF in adult chronic cannabis users and non cannabis using healthy controls (Table 2). The imaging methods used were as follows: $H2^{15}O$-PET [157], ^{133}Xe-SPECT [158], ^{18}F-FDG-PET [159], [^{11}C]- raclopride-PET [159–162] and [^{18}F]FMPEP-d2 [163]. Functional differences between groups were found in all studies, except for the four [^{11}C]-raclopride-PET studies [159–162]. Abstinence periods ranged from 12 hours to 542 days (for details see Table 2). Block *et al.* (2000) [157] described reduced bilateral rCBF in the posterior cerebellum and ventral prefrontal cortex but also increased rCBF in the anterior cingulate cortex in cannabis users. Lundqvist *et al.* (2001) [158] found a trend of lower global CBF in cannabis users, as well as reduced rCBF in the right prefrontal and superior frontal cortex. Sevy *et al.* (2008) [159] reported lower glucose metabolism in the

Table 1. Structural neuroimaging studies in chronic cannabis users.

Author (yr.)	Method	CU M/F	HCM/F	Mean (SD) age CU/HC	Image analysis	Abstinence (Mean days)	Results*				
ADULTS							Global measures	Regional measures (GM)	Regional measures (WM)	Detailed results	Correlations with clinical variables
							GM-WM CSF-TIV	FL-PL-TL-OL BG-Cb			
Block *et al.* (2000) [143]	MRI 1.5T	9/9	7/6	22.3 (0.5)/ 22.6 (0.5)	Whole brain ROI	≤ 1	○ ○ ● ○	○ ○ ○ ○ ○ ○	○	↓ CSF	
Tzilos *et al.* (2005) [153]	MRI 1.5T	16/6	19/7	38.1 (6.2)/ 29.5 (8.5)	ROI	≤ 1	○ ○ ○ ○	ϴϴ ○ ϴϴϴ	ϴ		
Matochik *et al.* (2005) [148]	MRI 1.5T	11/0	8/0	25.4 (5)/ 29.7 (4.7)	Whole brain ROI‡	≤ 1	ϴ ϴϴ ϴ	● ○ ● ○ ● ○	●	GMd: ↓ R parahippocampus; ↑ precentral gyrus and R thalamus. Hippocampus (ROI): ↓ GMd WMd: ↓ L PL; ↑ parahippocampus and fusiform gyrus, lentiform nucleus and pons	↑ WMd L precentral gyrus with duration of use (yr.)
Gruber *et al.* (2005) [151]	DTI 3T	8/1	8/1	26 (3.6)/ 26.2 (3.1)	Whole brain ROI‡	NE	ϴ ϴ ϴϴ	ϴϴϴϴϴϴ	○		
DeLisi *et al.* (2006)** [150]	DTI 1.5T	9/1	9/1	21.1 (2.9)/ 23 (4.4)	Whole brain ROI	≤ 1	○ ○ ○ ○	ϴϴ ○ ϴϴϴ	○		
Jager *et al.* (2007) [152]	MRI 1.5T	13/7	13/7	24.5 (5.2)/ 23.6 (3.9)	Whole brain ROI‡	7	ϴϴϴϴ	ϴϴ ○ ϴϴϴ	○		
Yücel *et al.* (2008) [149]	MRI 3T	15/0	16/0	38.8 (8.9)/ 36.4 (9.8)	ROI	≤ 1	○ ○ ϴ ○	ϴϴ● ϴϴϴ	ϴ	Hippocampus: ↓ L/R Amygdala: ↓ L/R	↓ L hippocampus with cumulative exposure (yr.) and higher positive psychotic symptoms
Arnone *et al.* (2008) [144]	DTI 1.5T	11/0	11/0	25.0 (2.9)/ 23.3 (2.9)	ROI	≤ 1	ϴϴϴϴ	ϴϴϴϴϴϴ	●	Corpus callosum: ↑ MD	
Ashtari *et al.* (2011)*** [145]	MRI 1.5T	14/0	14/0	19.3 (0.8)/ 18.5 (1.4)	ROI	201	○ ○ ○ ○	ϴϴ● ϴϴϴ	ϴ	Hippocampus: ↓ L/R	↑ hippocampus with verbal learning and memory scores in HC; ↓ hippocampus with amount of cannabis use
Gruber *et al.* (2011) [147]	DTI 3T	14/1	14/1	25.0 (8.7)/ 25.2 (8.4)	ROI	≤ 1	ϴϴϴϴ	ϴϴϴϴϴϴ	●	R Genu: ↑ MD L FL: ↓ FA	↑ FA L frontal with higher BIS total and motor subscale score; ↑ FA and ↓ MD in FL with later age of onset

Table 1. Cont.

Author (yr.)	Method	CU M/F	HCM/F	Mean (SD) age CU/HC	Image analysis	Abstinence (Mean days)	Results*				
Cousijn *et al.* (2011) [146]	MRI 3T	21/12	26/16	21.3 (2.4)/ 21.9 (2.4)	Whole brain ROI‡	≤ 1	○ ○ ○ ○	○ ○ ● ○ ○ ●	○	GM: ↑ anterior cerebellum	↓ R amygdala and ↓ L/R hippocampus with amount of cannabis use (weekly) or severity of cannabis dependence
ADOLESCENTS											
Medina *et al.* (2009)† [154]	MRI 1.5T	12/4	10/6	18.1/ 17.9 (16–18.9)	ROI	28	⊖ ⊖ ⊖ ○	● ⊖ ⊖ ⊖ ⊖ ⊖	○	PFC: ↑ in F CU PFC: ↓ in M CU	↓ PFC in CU and ↑ PFC in HC with better executive functioning
Medina *et al.* (2010)† [155]	MRI 1.5T	12/4	10/6	18 (0.7)/ 18 (0.9)	ROI	28	⊖ ⊖ ⊖ ○	⊖ ⊖ ⊖ ⊖ ⊖ ●	⊖	Cerebellum: ↑ inferior posterior (lobules VIII–X) vermis	↑ Vermis with poorer executive functioning
McQueeny *et al.* (2011) [156]	MRI 3T	27/8	36/11	18.0/ 17.7	ROI	28	⊖ ⊖ ⊖ ○	⊖ ⊖ ● ⊖ ⊖ ⊖	⊖	R amygdala: ↑ in F CU	↑ R amygdala with internalizing symptoms in F CU

Note: Yr. = Years; CU = Cannabis users; HC = Healthy controls; M = Male; F = Female; SD = Standard deviation; GM = Grey matter; GMd = Grey matter density; WM = White matter; WMd = White matter density; CSF = Cerebral spinal fluid; TIV = Total intracranial volume; FL = Frontal lobe; PL = Parietal lobe; TL = Temporal lobe; OL = Occipital lobe; BG = Basal ganglia; Cb = Cerebellum; L = Left hemisphere; R = Right hemisphere; T = Tesla; MRI = Magnetic resonance imaging; DTI = Diffusion tensor imaging; ROI = Region of interest; NE = Not specified; MD = Mean diffusivity; FA = Fractional anisotropy; PFC = Prefrontal cortex; BIS = Barrat Impulsivity scale.
*If not otherwise specified, results are presented in terms of chronic cannabis users.
● = Significant differences; ○ = Non-significant differences; ⊖ = Not examined.
**Two subjects in the marijuana group met criteria for excessive alcohol consumption.
***Five subjects in the marijuana group met criteria for alcohol abuse.
†Subjects with symptoms of alcohol abuse or dependence were included.
‡Multiple comparison correction.
doi:10.1371/journal.pone.0055821.t001

Table 2. Functional neuroimaging studies in chronic cannabis users.

Author (yr.)	Method	CU M/F	HC M/F	Mean (SD) age CU/HC	Image analysis	Condition	Abstinence (Mean days)	Results*		
ADULTS								Brain area	Detailed results	Correlations with clinical variables
Functional (resting state)								FL-PL-TL-OL I-BG-Cb		
Block *et al.* (2000) [157]	$H_2{}^{15}O$-PET	8/9	6/6	22.4 (0.5)/ 22.6 (0.5)	Whole brain	Resting state	≤ 1	● ○ ○ ○ ○ ○ ●	↓ rCBF L/R cerebellum and VMPFC ↑ rCBF R ACC	
Lundqvist *et al.* (2001) [158]	^{133}Xe-SPECT	12/0	14/0	29.8 (5)/ 27.8 (5.2)	Whole brain	Resting state	1.6	● ○ ○ ○ ○ ○ ○	↓ global CBF (trend) ↓ rCBF R superior PFC, superior frontal	
Sevy *et al.* (2008) [159]	^{18}F-FDG-PET	6/0	6/0	20.0 (1.0)/20.0 (1.0)	Whole brain	Resting state	105	● ● ○ ○ ○ ● ○	↓ rCBF R OFC and R posterior parietal cortex and L/R putamen	
Sevy *et al.* (2008) [159]	[^{11}C]-raclopride-PET	6/0	6/0	20.0 (1.0)/20.0 (1.0)	ROI	Resting state	105	ϴ ϴ ϴ ϴ ϴ ○ ϴ		
Hirvonen *et al.* (2011) [163]	[^{18}F]FMPEP-d_2	30/0	28/0	28 (8)/ 32 (10)	Whole brain ROI	Resting state	1 and 26	● ● ● ● ○ ○ ○	1 day: ↓ V_T neocortex and limbic cortex 26 days: ↑ V_T neocortex and limbic cortex except for hippocampus	↓ V_T with longer cannabis exposure (yr.)
Stokes *et al.* (2011) [160]	[^{11}C]-raclopride-PET	6/4	9/1	32.6 (7.7)/ 36.5 (4.5)	ROI	Resting state	542	ϴ ϴ ϴ ϴ ϴ ○ ϴ		
Urban *et al.* (2012) [162]	[^{11}C]-raclopride-PET	15/1	14/2	27.3 (6.1)/ 28.1 (6.9)	ROI	Resting state	30	ϴ ϴ ϴ ϴ ϴ ○ ϴ		
Albrecht *et al.* (2012) [161]	[^{11}C]-raclopride-PET	10/0	8/0	25.1 (4.6)/ 26.4 (5.6)	ROI	Resting state	≤ 1	ϴ ϴ ϴ ϴ ϴ ○ ϴ		↓ BP_{ND} with increase in urine levels of THC-COOH and self-reported recent intake per day
Functional (cognitive task)										
Block et al. (2002) [164]	$H_2{}^{15}O$-PET	18/0	13/0	22.3 (0.5)/ 22.6 (0.5)	Whole brain ROI	Verbal memory	≤ 1	● ○ ● ○ ○ ○ ●	↓ rCBF L/R PFC ↑ rCBF L > R hippocampus in HC ↑ rCBF posterior cerebellum	
Eldreth et al. (2004) [166]	$H_2{}^{15}O$-PET	11/0	11/0	25/29	Whole brain ROI‡	Stroop	25	● ○ ● ● ○ ○ ○	↑ CBF R paracentral lobule and L occipital lobe ↓ CBF R VMPFC and R DLPFC Hippocampus: ↑ rCBF L/R ACC:↓ rCBF L DLPF: ↑ rCBF L/R	

Table 2. Cont.

Author (yr.)	Method	CU M/F	HC M/F	Mean (SD) age CU/HC	Image analysis	Condition	Abstinence (Mean days)	Results*		
Bolla et al. (2005) [165]	$H_2{}^{15}O$-PET	11/0	11/0	26 (21–35)/ 31	Whole brain ROI‡	Iowa Gambling	25	● ○ ● ● ○ ○ ●	↓ CBF OFC and R middle frontal gyrus ↑ CBF L cerebellum, R inferior frontal gyrus and R occipital lobe in moderate CU ↑ CBF L cerebellum in heavy CU Cerebellum: ↑ rCBF L OFC: ↓ rCBF R DLPFC: ↓ rCBF R	↓ rCBF R cerebellum, R orbital gyrus and ↑rCBF R parahippocampus with cannabis exposure
Gruber et al. (2005) [151]	fMRI 3T	8/1	8/1	26 (3.6)/ 26.2 (3.1)	ROI‡	Stroop	NE	● ϴ ϴ ϴ ϴ ϴ	ACC: ↓ BOLD L/R DLPFC: ↑ BOLD R	
Chang et al. (2006) [169]	fMRI 4T	15/9	11/8	27.9 (10.8)/ 30.6 (8.0)	Whole brain ROI‡	Visual attention task	≤ 1 and 1157	● ● ○ ● ○ ○ ●	↓ BOLD L/R PFC, R medial and dorsal parietal cortex and cerebellum ↑ BOLD frontal, parietal and occipital lobules ↑ BOLD cerebellum in current CU	↑ BOLD R PFC and dorsal parietal cortex and ↓ BOLD cerebellum with age of cannabis onset; ↓ BOLD cerebellum with cannabis exposure; ↑ BOLD R PFC and cerebellum (normalization) with duration of cannabis abstinence
Jager et al. (2006) [173]	fMRI 1.5T	7/3	7/3	22.7 (4.2)/ 22.8 (2.9)	Whole brain ROI‡	Working memory	7	○ ● ○ ○ ○ ○ ○	↑ BOLD L superior parietal cortex	
Jager et al. (2007) [152]	fMRI 1.5T	13/7	13/7	24.5 (5.2)/ 23.6 (3.9)	Whole brain ROI‡	Associative memory	7	● ○ ● ○ ○ ○ ○	↓ BOLD R DLPFC and L/R parahippocampus Parahippocampus: ↓ BOLD L/R	
Hester *et al.* (2009) [172]	fMRI 3T	15/1	15/1	24.6 (1.5)/ 25.2 (1.3)	Whole brain ROI‡	Error Awareness Task	≤ 1	● ● ○ ○ ● ○ ○	↓ BOLD ACC, R insula, L/R inferior parietal and middle frontal regions	
Gruber *et al.* (2009) [170]	fMRI 3T	14/1	14/1	25 (8.8)/ 26 (9.0)	ROI	Masked affective stimuli	≤ 1	● ϴ ● ϴ ϴ ϴ ϴ	ACC: ↑ BOLD (masked happy faces) and ↓ BOLD (masked angry stimuli) Amygdala: ↓ BOLD (masked angry stimuli)	↑ BOLD ACC with cannabis exposure (per week) in masked angry faces and with cannabinoid level in masked happy faces; ↑ BOLD amygdala with cannabis exposure (per week) in masked happy faces

Table 2. Cont.

Author (yr.)	Method	CU M/F	HC M/F	Mean (SD) age CU/HC	Image analysis	Condition	Abstinence (Mean days)	Results*		
van Hell *et al.* (2010) [176]	fMRI 1.5T	14/1	11/2	24 (4.4)/ 24 (2.7)	Whole brain ROI‡	Monetary incentive delay task	7	● ○ ● ● ○ ● ○	Compared to non-smoking HC: ↓ BOLD NAcc, caudate nucleus, L putamen, R inferior and medial frontal gyrus, L/R superior frontal gyrus, L cingulate gyrus; ↑ BOLD L/R temporal gyrus, R cuneus and R parahippocampus gyrus Compared to smoking HC: ↓ BOLD caudate nucleus, L/R superior frontal; ↑ BOLD L middle temporal gyrus	
Nestor *et al.* (2010) [175]	fMRI 3T	11/3	12/2	22.1 (1.2)/ 23.1 (1.2)	Whole brain	Monetary incentive delay task	4.5	○ ○ ○ ○ ● ● ○	↑ BOLD R VS (win cue periods) ↓ BOLD L insula (after loss and loss avoidance)	↑ BOLD R VS (win cue periods) with cannabis exposure (yr.)
Abdullaev *et al.* (2010) [168]	fMRI 3T	10/4	10/4	19.5 (0.8)/ 19.7 (1.4)	Whole brain‡	Attention task	2	● ● ○ ○ ○ ○ ○	↑ BOLD R PFC and L/R parietal cortex	
Wesley *et al.* (2011) [177]	fMRI 1.5T	9/7	6/10	26.4 (3.6)/ 26.6 (6.1)	Whole brain	Iowa Gambling	≤ 1	● ● ○ ● ○ ○ ●	↓ BOLD ACC, VMPFC and medial frontal cortex, precuneus, superior parietal, occipital and cerebellum	BOLD activity in ACC, VMPFC and rostral PFC predicted improvement over the task course only in HC
King *et al.* (2011) [174]	fMRI 3T	16/14	16/14	23.7/21.7	ROI	Finger sequencing	≤ 1	● ϴ ϴ ● ϴ ϴ ϴ	Lingual gyrus: ↓ BOLD Superior frontal gyrus: ↑ BOLD	↑ BOLD superior frontal gyrus with cortisol levels in F CU
Vaidya *et al.* (2012) [167]	$H_2{}^{15}O$-PET	28/18	18/16	24.3 (3.9)/ 24.7 (5.3)	Whole brain	Iowa Gambling	≤ 1	● ○ ○ ○ ● ○ ●	↑ rCBF VMPFC and cerebellum Variant IGT: ↑ rCBF VMPFC, cerebellum and anterior insula	↑ rCBF VMPFC with duration of cannabis use
Harding *et al.* (2012) [171]	fMRI 3T	11/10	10/11	36.5 (8.8)/ 31 (11.7)	ROI	MSIT	≤ 1	● ○ ● ● ● ○ ○	↑ connectivity between ACC, L PFC, L/R anterior insula and the L occipitoparietal cortex	↑ connectivity with age of onset and lifetime exposure to cannabis
ADOLESCENTS										
Functional (cognitive task)										
Tapert *et al.* (2007) [183]	fMRI 1.5T	12/4	12/5	18.1 (0.7)/ 17.9 (1.0)	Whole brain	Response inhibition task	28	● ● ○ ● ● ○ ○	↑ BOLD R DLPFC, L/R medial frontal and inferior and superior parietal and R occipital gyrus ↑ BOLD R PFC, insular and parietal cortex during go trials	

Table 2. Cont.

Author (yr.)	Method	CU M/F	HC M/F	Mean (SD) age CU/HC	Image analysis	Condition	Abstinence (Mean days)	Results*		
Padula *et al.* (2007)** [179]	fMRI 1.5T	14/3	12/5	18.1 (0.8)/ 17.9 (1.1)	Whole brain	Spatial working memory	28	●●●○○●○	↑ BOLD R basal ganglia, R precuneus, postcentral gyrus and L/R superior parietal cortex	↑ BOLD L temporal gyrus and L ACC (↓ in HC) and ↓ BOLD R temporal gyrus, R thalamus and pulvinar, L parahippocampus (↑ in HC) with higher score on SWM task
Schweinsburg *et al.* (2008)*** [180]	fMRI 1.5T	11/4	12/5	18.1 (0.7)/ 17.9 (1.0)	Whole brain	Spatial working memory	28	●●○●○○○	↓ BOLD R DLPFC and occipital cortex ↑ BOLD R posterior parietal cortex	
Schweinsburg *et al.* (2010)† [181]	fMRI 1.5T	9/4 – 9/411/7		17.1 (0.5)-17.6 (0.9)/ 17.3 (0.8)	Whole brain‡	Spatial working memory	3 and 38	●○○○●○○	↑ BOLD L superior PFC and L/R anterior insula in recent CU ↑ BOLD R precentral gyrus in abstinent CU	
Schweinsburg *et al.* (2011) [182]	fMRI 3T	4/4	16/6	18.1 (0.9)/ 17.6 (0.8)	Whole brain ROI	Verbal paired associates task	25	○○○○○○○		

Note: Yr. = years; CU = Cannabis users; HC = Healthy controls; M = Male; F = Female; SD = Standard deviation; FL = Frontal lobe; PL = Parietal lobe; TL = Temporal lobe; OL = Occipital lobe; I = Insula; BG = Basal ganglia; Cb = Cerebellum; fMRI = Functional magnetic resonance imaging; SPECT = Single photon emission tomography; PET = Positron emission tomopgraphy; DSC = Dynamic susceptibility contrast; V_T = Distribution volume; BP_{ND} = Non-displaceable binding potential; FDG = Fludeoxyglucose; L = Left hemisphere; R = Right hemisphere; ROI = Region of interest; MSIT = Multi-Source Interference Task; CBF = Global cerebral blood flow; rCBF = Regional cerebral blood flow; BOLD = blood oxygenation-level dependent; NE = Not specified; PFC = Prefrontal cortex; DLPFC = Dorsolateral prefrontal cortex; VMPFC = Ventromedial prefrontal cortex; OFC = Orbitofrontal cortex; ACC = Anterior cingulated cortex; NAcc = Nucleus accumbens; VS = Ventral striatum; STG = Superior temporal gyrus; SWM = Spatial working task; IGT = Iowa Gambling Task.

*If not otherwise specified, results are presented in terms of chronic cannabis users.

● = Significant differences; ○ = Non-significant differences; ⊖ = Not examined.

**Two subjects in the marijuana group met criteria for alcohol abuse.

***Four teens in the chronic cannabis group met criteria for alcohol use disorder, two cases of abuse and two cases of dependence.

†One control, three recent users and two abstinent users met criteria for alcohol use disorders.

‡Multiple comparison correction.

doi:10.1371/journal.pone.0055821.t002

right orbitofrontal cortex, putamen bilaterally and precuneus in chronic cannabis users. However, there were no significant differences between the groups in striatal D2/D3 receptor availability and no correlation between striatal [^{11}C]-raclopride-PET binding potential and glucose metabolism [159]. Consistent with these results, three other [^{11}C]- raclopride-PET studies [160–162] failed to find any differences between groups in dopamine D2/D3 receptor availability in the striatum as a whole or it functional subdivisions. However, while Stokes *et al.* (2012) [160] also failed to find any association between lifetime frequency of cannabis use and binding potential values, Albrecht *et al.* (2012) [161] described a negative correlation with both urine levels of cannabis metabolites and self-report of recent cannabis consumption. Finally, Hirvonen *et al.* (2011) [163] demonstrated a reversible and regionally selective downregulation of CB1 receptors. At baseline, current users had approximately 20% less CB1 receptor density in the neocortex and limbic regions, which was negatively correlated with years of cannabis exposure. After four weeks of abstinence from cannabis use, CB1 receptor density returned to normal levels in all brain regions, except for the hippocampus [163].

3.2. Cognitive paradigms. We identified 16 studies in adult chronic cannabis users that compared regional activation during the performance of a cognitive task with healthy controls (Table 2), four with PET [164–167] and twelve with fMRI [151,152,168–177].

Attention

Chang *et al.* (2006) [169] used fMRI to compare a visual-attention task in current and abstinent cannabis users with healthy controls. Despite all groups showing normal task performance, both active and abstinent chronic cannabis users demonstrated decreased activation in the right prefrontal, medial and dorsal parietal cortices and medial cerebellar regions but greater activation in several smaller regions throughout the frontal, posterior parietal, occipital and cerebellum. An apparent normalization of BOLD signal was described in the right prefrontal and medial cerebellar regions in those with a longer duration of abstinence. In addition, early age of onset and estimated cumulative cannabis lifetime exposure were both associated with reduced activation in the right prefrontal cortex and medial cerebellum. More recently, Abdullaev *et al.* (2010) [168] used two attention tasks [the use generation task and the attention network task (ANT)] to contrast differences between cannabis users and healthy controls. Chronic cannabis users showed poorer performance in the ANT (more errors and longer reaction time), as well as stronger activation within the right prefrontal cortex in both tasks and within the parietal cortices in the ANT, which may indicate a less efficient system for the executive control of attention during conflict resolution tasks. Finally, Harding *et al.* (2012) [171] demonstrated for the first time that long-term heavy cannabis use is associated with increased functional connectivity between several frontal cortex regions and the occipitoparietal cortex using the Multi-Source Interference Task (MSIT). No differences in behavioural performance were evident between groups. The authors suggest that their findings may suggest a compensatory role for these regions in mitigating the effects of abnormal attentional and visual processing following chronic cannabis exposure [171].

Memory

In a H2^{15}O-PET study, Block *et al.* (2002) [164] found that cannabis users performed verbal memory tasks more poorly than controls. This was associated with reduced activation in the prefrontal cortex and greater activation in the posterior cerebellum, as well as with an absence of lateralization of hippocampal activity. Consistent with this, Jager *et al.* (2007) [152] described attenuated activity in the right dorsolateral prefrontal cortex and bilateral (para) hippocampal gyri in cannabis users despite normal performance in an associative memory task. Finally, in a verbal working memory task, Jager *et al.* (2006) [173] found significantly greater activity in the left superior parietal cortex in the cannabis using group despite there being no differences in task performance, which may be consistent with the idea of a compensatory recruitment effect.

Inhibition and impulsivity

Eldreth *et al.* (2004) [166] and Gruber *et al.* (2005) [151] studied the degree of inhibitory control during a Stroop task in current (positive THC urine analysis) and abstinent chronic cannabis users, respectively. Gruber *et al.* (2005) [151] found lower anterior cingulate activity and higher mid-cingulate and bilateral dorsolateral prefrontal cortex activity in current cannabis users relative to healthy controls, who demonstrated focal increased activity within the right dorsolateral prefrontal cortex. Consistently, Eldreth *et al.* (2004) [166] found in abstinent cannabis users a reduced anterior cingulate activation using H2^{15}O-PET during the performance of a modified Stroop test. However, they also reported a reduced dorsolateral prefrontal cortex activation and a greater activation in the hippocampus bilaterally [166]. Lastly, Hester *et al.* (2009) [172] administered a go/no-go response inhibition task to active cannabis users to determine inhibitory control and error awareness compared with healthy controls. Although control performance was equivalent between the two groups, cannabis users displayed a significant deficit in awareness of commission errors, which was associated with decreased a activity in the anterior cingulate cortex and right insula, as well as in the bilateral inferior parietal and middle frontal regions [172].

Decision-making

Bolla *et al.* (2005) [165] and Vaidya *et al.* (2011) [167] using H2^{15}O-PET, and Wesley *et al.* (2011) [177] using fMRI, studied the brain activation pattern in chronic cannabis users compared to healthy controls during the Iowa Gambling Task (IGT). Bolla *et al.* (2005) [165] reported dysfunction during the performance of the task in abstinent cannabis users, demonstrating a lower activation in the right orbitofrontal cortex and dorsolateral prefrontal cortex and greater activation in the left parietal and cerebellar cortices. The number of joints used per week was positively correlated with activation in the right parahippocampal gyrus but inversely correlated with activation in the right cerebellum and orbital gyrus. Wesley *et al.* (2011) [177] also reported a poorer performance on the IGT in active cannabis users. However, there were no differences during the initial strategy development phase, in which cannabis users showed reduced activity in response to losses in anterior cingulate cortex, ventromedial prefrontal cortex, precuneus, superior parietal lobe, occipital lobe and cerebellum compared to controls [177]. Additionally, the functional response to losses in anterior cingulate, ventromedial and rostral prefrontal cortices was positively correlated with improvement over the task course only in the control group, indicating that cannabis users may be less sensitive to negative feedback during the strategy development phase [177]. In contrast, Vaidya *et al.* (2011) [167] did not find differences on the standard IGT performance between active cannabis users and healthy controls. Nevertheless, cannabis users performed significantly worse than controls on a variant version of the same task [178]. Both groups showed increased activity in ventromedial prefrontal cortex on both versions of the

IGT compared to the control task but in contrast to Wesley *et al.* (2011) [177], cannabis users demonstrated greater activity than controls in the ventromedial prefrontal cortex on the standard IGT, as well as in the cerebellum and the anterior insula on both versions of the IGT [167]. Furthermore, duration of cannabis use was associated with greater activity in ventromedial prefrontal cortex [167]. Nestor *et al.* (2010) [175] and van Hell *et al.* (2010) [176] used fMRI to measure brain activity during reward and anticipation of loss with different versions of a monetary reward task. There were no significant behavioural differences between the groups in both studies. Nestor *et al.* (2010) [175] reported a greater right ventral striatum activity in cannabis users during reward anticipation, which was significantly correlated with years of lifetime cannabis use. In addition, response to loss and loss avoidance outcome notification was related with hypoactivity in left insula, and in the post hoc analysis comparing loss and win cues with no-outcome cues, right ventral putamen showed greater BOLD response [175]. Conversely, comparing cannabis users to non tobacco-smoking controls, van Hell *et al.* (2010) [176] demonstrated attenuated activity in the nucleus accumbens and caudate nucleus bilaterally during reward anticipation, as well as left putamen and right inferior and medial frontal gyrus, superior frontal gyrus bilaterally and left cingulate gyrus. Cannabis users showed enhanced reward anticipation activity in the middle temporal gyrus bilaterally, right cuneus and right parahippocampal gyrus. When compared to tobacco-smoking controls, cannabis users also showed reduced anticipation activity in the same areas, with the exception of the nucleus accumbens bilaterally, the right medial frontal gyrus and the left cingulated gyrus, indicating that anticipation activity in these regions may be attenuated by both cannabis and nicotine [176]. In accordance with Nestor *et al.* (2010) [175], response to contrasted outcome notification was associated with greater activity in the putamen bilaterally and the right caudate nucleus compared with non-smoking controls [176]. The putamen was more activated in cannabis users than in non-smokers and tobacco-smoking controls, indicating that changes in this area were mainly due to cannabis use [176].

Motor performance

King *et al.* (2011) [174] reported that chronic cannabis use was associated with slower and less efficient psychomotor function, especially in male users. Cannabis users showed lesser activation in the lingual gyrus and greater activation of the superior frontal gyrus compared to controls while performing a visually paced finger sequencing task, suggesting that the former group shifted from more automated visually-guided responses to more executive or attention control regions of the brain [174].

Affective processing

Gruber *et al.* (2009) [170] examined the BOLD signal changes for two target affective conditions (happy and anger). Region of interest analyses revealed that cannabis users demonstrated relatively lower anterior cingulate and amygdalar activity during the presentation of masked angry stimuli sets relative to the control group, who showed relatively higher activation within these regions. In contrast, cannabis users demonstrated a larger pattern of activation during the presentation of masked happy faces within the cingulate as compared to controls, with no increase in amygdalar activation [170]. Furthermore, the total number of smoking episodes per week was positively associated with cingulate activity during the viewing of masked angry faces and positively associated with amygdalar activity during the viewing of masked happy faces [170]. Finally, overall cannabinoid level was positively related to cingulate activity during the viewing of masked happy faces [170]. The disparate activation patterns showed between groups suggest a different way of processing emotional information between groups [170].

4. Functional neuroimaging studies in adolescent chronic cannabis

We included five case-control fMRI studies in adolescent cannabis users comparing brain activity with healthy controls during a cognitive task performance. As an exception, two of them [180,181] were included despite involving a minor proportion of participants with a co-morbid alcohol dependence given the relatively modest number of studies in this population (for details see Table 2). No resting state studies were identified in the adolescent population.

Memory

Padula *et al.* (2007) [179] and Schweinsburg *et al.* (2008, 2010) [180,181] examined fMRI response during a spatial working memory (SWM) task. In a group of abstinent adolescent cannabis users, Padula *et al.* (2007) [179] described increased activity in the left temporal gyrus and anterior cingulate cortex but lower activity in right temporal gyrus, thalamus, pulvinar and left parahippocampal gyrus related to higher scores on the task, while the reverse pattern was found in the controls. This may suggest that cannabis users employed more of a verbal strategy to achieve the same level of task performance as the controls [179]. Additionally, cannabis users demonstrated greater performance-related activation in the right basal ganglia, precuneus, postcentral gyrus and bilateral superior parietal lobe [179], again suggesting a compensatory neural effort. Consistent with this, Schweinsburg *et al.* (2008) [180] also found a different pattern of activation in abstinent adolescent cannabis users who performed the SWM task similarly to the control group. Thus, cannabis users demonstrated higher activation in the right parietal cortex but also lower activity in the right dorsolateral prefrontal and occipital cortices [180]. Finally, in a cross-sectional study, Schweinsburg *et al.* (2010) [181] compared fMRI responses using the same task among adolescent cannabis users with brief and sustained cannabis abstinence and healthy controls. Although both groups performed at a similar level on the task, recent users showed greater activity in the medial and left superior prefrontal cortices and bilateral insula while abstinent users demonstrated an increased response in the right precentral gyrus [181]. More recently, Schweinsburg *et al.* (2011) [182] compared fMRI response during a verbal paired associates encoding task in 3 groups of participants that included an abstinent cannabis user group, a binge drinker group and a cannabis user group with co-morbid binge-drinking to healthy controls with very limited alcohol or cannabis experience. In general, each group displayed deviations in BOLD response relative to non-using controls, and binge drinking and cannabis use demonstrated independent as well as interactive effects on brain functioning [182].

Inhibition and impulsivity

In a group of abstinent cannabis users, Tapert *et al.* (2007) [183] compared the activation pattern on a go/no-go task during fMRI with seventeen healthy subjects. Despite similar level of task performance, cannabis users showed greater activation during inhibitory trials in the right dorsolateral prefrontal, bilateral medial frontal, bilateral inferior and superior parietal lobules and right occipital gyrus compared to the healthy subjects. During the non-inhibitory trials, differences were located in right prefrontal, insular and parietal cortices, with cannabis users showing greater

activation in these areas compared to the controls. As observed in adults, these results suggest a greater neurocognitive effort during the task in cannabis users, even after the abstinence period.

Discussion

In this systematic review, we identified 43 studies suitable for inclusion regarding the impact of chronic cannabis use on brain structure and functioning, of which eight (19%) were in the adolescent population. Despite the high degree of heterogeneity among the studies reviewed herein, several relatively consistent findings emerged from this review. These findings, discussed in detail below, include: (1) Structural brain abnormalities, mainly in CB_1-rich areas implicated in several cognitive functions, which may be related to the amount of cannabis use; (2) Altered neural activity during resting state and under several different types of cognitive paradigms, that may reflect a different recruitment of brain areas during the tasks, particularly within the prefrontal cortex; and (3) The few studies conducted in adolescents suggest that both structural and functional alterations may appear soon after starting the drug use and may be related to gender.

In terms of structural findings, specific regional brain analyses demonstrated evidence of structural abnormalities when adult chronic cannabis users were compared with healthy controls. The most consistently reported brain alteration was reduced hippocampal volume [145,146,148,149], which was shown to persist even after several months of abstinence in one study [145] and also to be related to the amount of cannabis use [145,146,149]. Other frequently reported morphological brain alterations related to chronic cannabis use were reported in the amygdala [146,149,156], the cerebellum [146,155] and the frontal cortex [148,154]. Lastly, two DTI studies found differences in the mean diffusivity or fractional anisotropy in the corpus callosum and the frontal white matter fibre tract [144,147], suggesting that chronic cannabis exposure may also alter white matter structural integrity, by either affecting demyelination or causing axonal damage or indirectly through delaying normal brain development. With regard to the few structural MRI studies focusing on the effects of cannabis use on brain morphology in adolescents, some discrepancies were reported related to adult population. These inconsistencies may be explained in terms of the disruption of normal pruning during developmental maturation due to early chronic cannabis use, ultimately resulting in larger regional volumes [156]. Notwithstanding, structural results from adolescent population suggest that the effects of chronic cannabis use may appear soon after starting the drug use, persist after a month of abstinence or even be moderated by gender [145,154–156]. In this context, it has been reported that adolescent female cannabis users may be at increased risk for cannabis-induced morphological effects [154,156].

Functional neuroimaging studies that have evaluated the resting state in active and abstinent adult chronic cannabis users suggest that resting global [158], prefrontal cortical [157–159], cerebellar [157] and striatal [159] blood flow may be lower compared with controls. These brain regions correspond to areas with relatively high concentration of CB1 receptors [19]. Hence, it has been hypothesised that the decreased resting state function may represent a down-regulation of CB1 receptors as a result of regular exposure to cannabis [41]. However, it is important to note that not all studies have consistently demonstrated effects in these regions. Furthermore, it has been recently found that, similar to animal studies, down-regulation of CB1 receptors in humans is region-specific and reversible, occurring in the neocortex and limbic cortex but neither in subcortical brain regions nor in the cerebellum [163]. It is also noteworthy that these brain regions correspond to areas that are engaged in the processing of reward [184]. This is also consistent with the evidence of neuropsychological impairments in chronic cannabis users, such as in attention and working memory [185], decision making [186], and psychomotor speed [187]. Also, consistent with experimental animal studies, no differences in striatal D2/D3 receptor availability were found in four studies of chronic cannabis users compared with healthy controls [159–162]. However, in the only study where the chronic cannabis users were not abstinent [161], an inverse correlation between recent cannabis consumption and D2/D3 receptor availability was found, leading the authors to suggest that this effect could be related to a direct effect of cannabis smoking on the expression of striatal DA receptors in heavy cannabis users [161]. Additional studies are needed to better understand the neurochemical basis of this finding.

Functional imaging studies comparing activation in both adult and adolescent chronic cannabis users with healthy controls during the performance of different cognitive tasks indicated that chronic cannabis users would use similar brain areas that engage these cognitive processes but often demonstrating an altered pattern of brain activity [151,152,157,165–177,179,181–183]. However, the level of performance of the cannabis users on the cognitive tasks employed was generally similar to that of controls [164,165,168,171,174,177], or at least within what may be considered a normal range of test performance. Therefore, these findings may be interpreted as reflecting neuroadaptation, perhaps indicating the recruitment of additional regions as a compensatory mechanism to maintain normal cognitive performance in response to chronic cannabis exposure [151,152,164,166,171,172,175,179–181,183], particularly within the prefrontal cortex area [151,166,168,169,171,181,183]. In this regard, the brain seems able to achieve some degree of reorganization, activating brain regions not usually needed to perform the cognitive task in response to an impaired ability of the normally engaged task network. Thus, it is feasible that drug-related compensatory mechanism may work for a period of time until it turns out to be insufficient and differences between groups become apparent. However, the impact of these subtle brain alterations on social, familiar and occupational life as well as its potential relationship with psychiatric disorders remains speculative.

A further important issue emerging out of this review is that few studies have investigated the effects of chronic cannabis use on the brain in adolescence subjects. In light of the popularity of cannabis among teenagers [1,2] and recent data showing the potential neurotoxic effects of chronic cannabis use on the maturational brain [188], investigation of the possible long-term effects on brain structure and function in the adolescent population should be a priority both from the scientific and population health perspective [34,188]. Future studies should consider the need for convergent methodology, replication of known facts with greater methodological rigor, and prospective large samples involving subjects of both genders across the life-span from adolescence to adulthood to delineate the evolution and reversibility of previously reported alterations.

Limitations of the review

Results presented here have pointed out some important methodological differences that limit the generalisation of results and comparison between studies and have doubtless contributed to the slightly disparate array of findings. Despite the use of a strict definition of chronic cannabis user and robust application of inclusion and exclusion criteria in an attempt to avoid excessive heterogeneity between samples, studies often diverged on certain

socio-demographic characteristics and cannabis use parameters, such as gender-bias, age of onset, lifetime use and abstinence period before the acquisition of imaging data. Moreover, it is well known that the THC content of smoked cannabis varies markedly between sources and preparations, with potency reported to have increased substantially over the past ten years [2]. Thus, comparability of earlier to later studies may not always be appropriate [44]. Furthermore, the exclusion of studies involving recreational and naïve cannabis users implies that the question of whether the brains of these subjects are adversely affected by cannabis is not addressed within the framework of the present review. Another important confounding factor is the inclusion of subjects with concurrent use of tobacco, which may affect neural activity as well as potentially interact with the effects of cannabis use [176]. In addition, it is known that co-morbid misuse of alcohol and other illicit drugs, such as cocaine and methamphetamine, may also be associated with significant neurobiological, neurocognitive and psychiatric abnormalities [189]. In the present review, although we excluded studies involving subjects with alcohol dependence, some included subjects with alcohol misuse (abuse [145,179] or excessive consumption [150]), or reported differences in alcohol intake parameters to]despite alcohol consumption was within safe limits [143,144,147,156,157,163,164,169,170]. Moreover, given the relatively modest number of studies in the adolescent population, we included four studies which may involve some participants with co-morbid alcohol dependence [154,155,180,181]. In all these studies, the interaction of alcohol with cannabis use, as well as its contribution to the brain effects cannot be ruled out. On the other hand, the exclusion of those with alcohol dependence, often highly co-morbid with cannabis use, may restrict the generalization of the results to the majority of chronic cannabis users [190].

With regard to other methodological limitations, some studies have reported modest sample sizes, sometimes below the threshold that would be currently regarded as acceptable (for instance, for PET or SPECT studies 10 subjects and for fMRI studies 15 subjects) [29]. In this regard, strategies for expanding data-sharing would be a welcome development in future research (i.e. The Function Biomedical Informatics Research Network [191] or the 1000 Functional Connectomes project [192,193]). However, further obstacles must be addressed to make collaborative analysis efficient, such as between-site differences in scanners and data acquisition parameters, as well as pre- and post-processing schemes. The cross-sectional designs of most of the studies reviewed here complicated the interpretation of results as pre-existing morphological or functional alterations cannot be ruled out. Furthermore, studies that merely compare those subjects exposed to an environmental factor from those that are not, are likely to promote interpretation biases whereby study findings, irrespective of their direction, tend to be interpreted as detrimental. Longitudinal evaluations in larger samples may thus prove particularly useful. With regard to technical limitations, it is remarkable to note that the resting state studies did not control for spontaneous neutral activity and modulation of the BOLD signal, and the functional studies often reported different imaging methods and explored different brain functions using diverse cognitive paradigms, hampering the comparison between the studies. Hence, replication of previous results is critically important. Convergent methodology to sort out the current inconsistencies and controversies among studies would be important for future research in the field.

Supporting Information

Table S1

PRISMA checklist of items to include when reporting a systematic review or meta-analysis.
(DOC)

Author Contributions

Revised the manuscript critically for important intellectual content: SB PFP JAC SN MT JP. Gave final approval of the version to be published: AB SB MY PFP JAC SN MT JP MF RMS. Conceived and designed the experiments: AB MF RMS. Analyzed the data: AB MY RMS. Wrote the paper: AB MY RMS.

References

1. European Monitoring Centre for Drugs and Drug Addiction (2011) The state of the drugs problem in Europe. EMCDDA. Available: http://www.emcdda.europa.eu/publications/annual-report/2011. Accessed 2 February 2012.
2. United Nations Office on Drugs and Crime (2011) World drug report 2011. UNODC, Vienna 2011. Available: http://www.unodc.org/unodc/en/data-and-analysis/WDR-2011.html. Accessed 2 February 2012.
3. Chen CY, O'Brien MS, Anthony JC (2005) Who becomes cannabis dependent soon after onset of use? Epidemiological evidence from the United States: 2000–2001. Drug Alcohol Depend 79: 11–22.
4. Fernandez-Artamendi S, Fernandez-Hermida JR, Secades-Villa R, Garcia-Portilla P (2011) Cannabis and mental health. Actas Esp Psiquiatr 39: 180–190.
5. Bhattacharyya S, Fusar-Poli P, Borgwardt S, Martin-Santos R, Nosarti C, et al. (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.
6. Bhattacharyya S, Crippa JA, Allen P, Martin-Santos R, Borgwardt S, et al. (2012) Induction of psychosis by {delta}9-tetrahydrocannabinol reflects modulation of prefrontal and striatal function during attentional salience processing. Arch Gen Psychiatry 69: 27–36.
7. Bhattacharyya S, Atakan Z, Martin-Santos R, Crippa JA, Kambeitz J, et al. (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 Jan 31.[Epub ahead of print].
8. Hall W, Degenhardt L (2009) Adverse health effects of non-medical cannabis use. Lancet 374: 1383–1391.
9. Moore TH, Zammit S, Lingford-Hughes A, Barnes TR, Jones PB, et al. (2007) Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet 370: 319–328.
10. Morrison PD, Nottage J, Stone JM, Bhattacharyya S, Tunstall N, et al. (2011) Disruption of frontal theta coherence by Delta9-tetrahydrocannabinol is associated with positive psychotic symptoms. Neuropsychopharmacology 36: 827–836.
11. Solowij N, Battisti R (2008) The chronic effects of cannabis on memory in humans: a review. Curr Drug Abuse Rev 1: 81–98.
12. Stone JM, Bhattacharyya S, Barker GJ, McGuire PK (2012) Substance use and regional gray matter volume in individuals at high risk of psychosis. Eur Neuropsychopharmacol 22: 114–122.
13. Stone JM, Morrison PD, Brugger S, Nottage J, Bhattacharyya S, et al. (2012) Communication breakdown: delta-9 tetrahydrocannabinol effects on pre-speech neural coherence. Mol Psychiatry 17: 568–569.
14. Sundram S (2006) Cannabis and neurodevelopment: implications for psychiatric disorders. Hum Psychopharmacol 21: 245–254.
15. 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.
16. Chevaleyre V, Takahashi KA, Castillo PE (2006) Endocannabinoid-mediated synaptic plasticity in the CNS. Annu Rev Neurosci 29: 37–76.
17. Morrison PD, Murray RM (2009) From real-world events to psychosis: the emerging neuropharmacology of delusions. Schizophr Bull 35: 668–674.
18. Belue RC, Howlett AC, Westlake TM, Hutchings DE (1995) The ontogeny of cannabinoid receptors in the brain of postnatal and aging rats. Neurotoxicol Teratol 17: 25–30.
19. Burns HD, Van LK, Sanabria-Bohorquez S, Hamill TG, Bormans G, et al. (2007) [18F]MK-9470, a positron emission tomography (PET) tracer for in vivo human PET brain imaging of the cannabinoid-1 receptor. Proc Natl Acad SciUSA 104: 9800–9805.
20. Gaoni Y, Mechoulam R (1971) The isolation and structure of delta-1-tetrahydrocannabinol and other neutral cannabinoids from hashish. JAm Chem Soc 93: 217–224.

21. Hoffman AF, Oz M, Yang R, Lichtman AH, Lupica CR (2007) Opposing actions of chronic Delta9-tetrahydrocannabinol and cannabinoid antagonists on hippocampal long-term potentiation. Learn Mem 14: 63–74.
22. Landfield PW, Cadwallader LB, Vinsant S (1988) Quantitative changes in hippocampal structure following long-term exposure to delta 9-tetrahydrocannabinol: possible mediation by glucocorticoid systems. Brain Res 443: 47–62.
23. Scallet AC, Uemura E, Andrews A, Ali SF, McMillan DE, et al. (1987) Morphometric studies of the rat hippocampus following chronic delta-9-tetrahydrocannabinol (THC). Brain Res 436: 193–198.
24. Bossong MG, Niesink RJ (2010) Adolescent brain maturation, the endogenous cannabinoid system and the neurobiology of cannabis-induced schizophrenia. Prog Neurobiol 92: 370–385.
25. Adriani W, Laviola G (2004) Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. Behav Pharmacol 15: 341–352.
26. Schneider M, 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.
27. Quinn HR, Matsumoto I, Callaghan PD, Long LE, Arnold JC, et al. (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.
28. Lorenzetti V, Lubman DI, Whittle S, Solowij N, Yucel M (2010) Structural MRI findings in long-term cannabis users: what do we know? Subst Use Misuse 45: 1787–1808.
29. Martin-Santos R, Fagundo AB, Crippa JA, Atakan Z, Bhattacharyya S, et al. (2010) Neuroimaging in cannabis use: a systematic review of the literature. Psychol Med 40: 383–398.
30. Bhattacharyya S, Crippa JA, Martin-Santos R, Winton-Brown T, Fusar-Poli P (2009) Imaging the neural effects of cannabinoids: current status and future opportunities for psychopharmacology. Curr Pharm Des 15: 2603–2614.
31. Solowij N, Pesa N (2010) [Cognitive abnormalities and cannabis use]. Rev Bras Psiquiatr 32 Suppl 1: S31–S40.
32. Pope HG, Jr., Gruber AJ, Hudson JI, Cohane G, Huestis MA, et al. (2003) Early-onset cannabis use and cognitive deficits: what is the nature of the association? Drug Alcohol Depend 69: 303–310.
33. Wilson W, Mathew R, Turkington T, Hawk T, Coleman RE, et al. (2000) Brain morphological changes and early marijuana use: a magnetic resonance and positron emission tomography study. J Addict Dis 19: 1–22.
34. Jager G, Ramsey NF (2008) Long-term consequences of adolescent cannabis exposure on the development of cognition, brain structure and function: an overview of animal and human research. Curr Drug Abuse Rev 1: 114–123.
35. Crews F, He J, Hodge C (2007) Adolescent cortical development: a critical period of vulnerability for addiction. Pharmacol Biochem Behav 86: 189–199.
36. D'Souza DC, Sewell RA, Ranganathan M (2009) Cannabis and psychosis/schizophrenia: human studies. Eur Arch Psychiatry Clin Neurosci 259: 413–431.
37. Durston S, Hulshoff Pol HE, Casey BJ, Giedd JN, Buitelaar JK, et al. (2001) Anatomical MRI of the developing human brain: what have we learned? J Am Acad Child Adolesc Psychiatry 40: 1012–1020.
38. Jernigan TL, Gamst AC (2005) Changes in volume with age--consistency and interpretation of observed effects. Neurobiol Aging 26: 1271–1274.
39. Huttenlocher PR, Dabholkar AS (1997) Regional differences in synaptogenesis in human cerebral cortex. J Comp Neurol 387: 167–178.
40. Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, et al. (2004) Dynamic mapping of human cortical development during childhood through early adulthood. Proc Natl Acad Sci USA 101: 8174–8179.
41. Chang L, Chronicle EP (2007) Functional imaging studies in cannabis users. Neuroscientist 13: 422–432.
42. Crean RD, Crane NA, Mason BJ (2011) An evidence based review of acute and long-term effects of cannabis use on executive cognitive functions. J Addict Med 5: 1–8.
43. Gonzalez R (2007) Acute and non-acute effects of cannabis on brain functioning and neuropsychological performance. Neuropsychol Rev 17: 347–361.
44. Quickfall J, Crockford D (2006) Brain neuroimaging in cannabis use: a review. J Neuropsychiatry Clin Neurosci 18: 318–332.
45. Bhattacharyya S, Sendt KV (2012) Neuroimaging evidence for cannabinoid modulation of cognition and affect in man. Front Behav Neurosci 6: 22.
46. Bhattacharyya S, Atakan Z, Martin-Santos R, Crippa JA, McGuire PK (2012) Neural mechanisms for the cannabinoid modulation of cognition and affect in man: a critical review of neuroimaging studies. Curr Pharm Des 18: 5045–5054.
47. Moher D, Liberati A, Tetzlaff J, Altman DG (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 6: e1000097.
48. Crippa JA, Zuardi AW, Garrido GE, Wichert-Ana L, Guarnieri R, et al. (2004) Effects of cannabidiol (CBD) on regional cerebral blood flow. Neuropsychopharmacology 29: 417–426.
49. Churchwell JC, Lopez-Larson M, Yurgelun-Todd DA (2010) Altered frontal cortical volume and decision making in adolescent cannabis users. Front Psychol 1: 225.
50. Cousijn J, Goudriaan AE, Ridderinkhof KR, van den Brink W, Veltman DJ, et al. (2012) Neural responses associated with cue-reactivity in frequent cannabis users. Addict Biol Jan 20.[Epub ahead of print].
51. Kanayama G, Rogowska J, Pope HG, Gruber SA, Yurgelun-Todd DA (2004) Spatial working memory in heavy cannabis users: a functional magnetic resonance imaging study. Psychopharmacology (Berl) 176: 239–247.
52. Lopez-Larson MP, Bogorodzki P, Rogowska J, McGlade E, King JB, et al. (2011) Altered prefrontal and insular cortical thickness in adolescent marijuana users. Behav Brain Res 220: 164–172.
53. Murphy K, Dixon V, LaGrave K, Kaufman J, Risinger R, et al. (2006) A validation of event-related FMRI comparisons between users of cocaine, nicotine, or cannabis and control subjects. Am J Psychiatry 163: 1245–1251.
54. Prescot AP, Locatelli AE, Renshaw PF, Yurgelun-Todd DA (2011) Neurochemical alterations in adolescent chronic marijuana smokers: a proton MRS study. Neuroimage 57: 69–75.
55. Schneider M (2008) Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure. Addict Biol 13: 253–263.
56. Silveri MM, Jensen JE, Rosso IM, Sneider JT, Yurgelun-Todd DA (2011) Preliminary evidence for white matter metabolite differences in marijuana-dependent young men using 2D J-resolved magnetic resonance spectroscopic imaging at 4 Tesla. Psychiatry Res 191: 201–211.
57. Aasly J, Storsaeter O, Nilsen G, Smevik O, Rinck P (1993) Minor structural brain changes in young drug abusers. A magnetic resonance study. Acta Neurol Scand 87: 210–214.
58. Amen DG, Waugh M (1998) High resolution brain SPECT imaging of marijuana smokers with AD/HD. J Psychoactive Drugs 30: 209–214.
59. Campbell AM, Evans M, Thomson JL, Williams MJ (1971) Cerebral atrophy in young cannabis smokers. Lancet 2: 1219–1224.
60. Co BT, Goodwin DW, Gado M, Mikhael M, Hill SY (1977) Absence of cerebral atrophy in chronic cannabis users. Evaluation by computerized transaxial tomography. JAMA237: 1229–1230.
61. Hannerz J, Hindmarsh T (1983) Neurological and neuroradiological examination of chronic cannabis smokers. Ann Neurol 13: 207–210.
62. Jacobsen LK, Mencl WE, Westerveld M, Pugh KR (2004) Impact of cannabis use on brain function in adolescents. Ann N Y Acad Sci 1021: 384–390.
63. Kuehnle J, Mendelson JH, Davis KR, New PF (1977) Computed tomographic examination of heavy marijuana smokers. JAMA 237: 1231–1232.
64. O'Leary DS, Block RI, Flaum M, Schultz SK, Boles Ponto LL, et al. (2000) Acute marijuana effects on rCBF and cognition: a PET study. Neuroreport 11: 3835–3841.
65. Sneider JT, Pope HG, Jr., Silveri MM, Simpson NS, Gruber SA, et al. (2006) Altered regional blood volume in chronic cannabis smokers. Exp Clin Psychopharmacol 14: 422–428.
66. Ward PB, Solowij N, Peters R, Otton J, Chesher G, et al. (2002) An fMRI study of regional brain volumes in long-term cannabis users. J Psychopharmacol 16(Suppl. 3).
67. Yurgelun-Todd D, Gruber SA, Hanson RA, Baird AA, Renshaw PF, et al. (1998) Residual effects of marijuana use: an fMRI study. In: Problems of drug dependence 1998: Proceedings of the 60th Annual Scientific Meeting, The College on Problems of Drug Dependence. NIDA Research Monograph. pp.78.
68. Zalesky A, Solowij N, Yucel M, Lubman DI, Takagi M, et al. (2012) Effect of long-term cannabis use on axonal fibre connectivity. Brain 135: 2245–2255.
69. Becker B, Wagner D, Gouzoulis-Mayfrank E, Spuentrup E, Daumann J (2010) The impact of early-onset cannabis use on functional brain correlates of working memory. Prog Neuropsychopharmacol Biol Psychiatry 34: 837–845.
70. Demirakca T, Sartorius A, Ende G, Meyer N, Welzel H, et al. (2011) Diminished gray matter in the hippocampus of cannabis users: Possible protective effects of cannabidiol. Drug Alcohol Depend 114: 242–245.
71. Leroy C, Karila L, Martinot JL, Lukasiewicz M, Duchesnay E, et al. (2012) Striatal and extrastriatal dopamine transporter in cannabis and tobacco addiction: a high-resolution PET study. Addict Biol 17:981–990.
72. Mata I, Perez-Iglesias R, Roiz-Santianez R, Tordesillas-Gutierrez D, Pazos A, et al. (2010) Gyrification brain abnormalities associated with adolescence and early-adulthood cannabis use. Brain Res 1317: 297–304.
73. Parkar SR, Ramanathan S, Nair N, Batra SA, Adarkar SA, et al. (2010) Cannabis dependence: Effects of cannabis consumption on inter-regional cerebral metabolic relationships in an Indian population. Indian J Psychiatry 52: 236–242.
74. Smith AM, Longo CA, Fried PA, Hogan MJ, Cameron I (2010) Effects of marijuana on visuospatial working memory: an fMRI study in young adults. Psychopharmacology (Berl) 210: 429–438.
75. Voruganti LN, Slomka P, Zabel P, Mattar A, Awad AG (2001) Cannabis induced dopamine release: an in-vivo SPECT study. Psychiatry Res 107: 173–177.
76. Wiesbeck GA, Taeschner KL (1991) A cerebral computed tomography study of patients with drug-induced psychoses. Eur Arch Psychiatry Clin Neurosci 241: 88–90.
77. Hermann D, Sartorius A, Welzel H, Walter S, Skopp G, et al. (2007) Dorsolateral prefrontal cortex N-acetylaspartate/total creatine (NAA/tCr) loss in male recreational cannabis users. Biol Psychiatry 61: 1281–1289.
78. Nestor L, Roberts G, Garavan H, Hester R (2008) Deficits in learning and memory: parahippocampal hyperactivity and frontocortical hypoactivity in cannabis users. Neuroimage 40: 1328–1339.

79. Weinstein A, Brickner O, Lerman H, Greemland M, Bloch M, et al. (2008) Brain imaging study of the acute effects of Delta9-tetrahydrocannabinol (THC) on attention and motor coordination in regular users of marijuana. Psychopharmacology (Berl) 196: 119–131.
80. Becker B, Wagner D, Gouzoulis-Mayfrank E, Spuentrup E, Daumann J (2010) Altered parahippocampal functioning in cannabis users is related to the frequency of use. Psychopharmacology (Berl) 209: 361–374.
81. Filbey FM, Schacht JP, Myers US, Chavez RS, Hutchison KE (2009) Marijuana craving in the brain. Proc Natl Acad Sci USA 106: 13016–13021.
82. Filbey FM, Schacht JP, Myers US, Chavez RS, Hutchison KE (2010) Individual and additive effects of the CNR1 and FAAH genes on brain response to marijuana cues. Neuropsychopharmacology 35: 967–975.
83. Mathew RJ, Wilson WH, Humphreys DF, Lowe JV, Wiethe KE (1992) Changes in middle cerebral artery velocity after marijuana. Biol Psychiatry 32: 164–169.
84. Volkow ND, Gillespie H, Mullani N, Tancredi L, Grant C, et al. (1991) Cerebellar metabolic activation by delta-9-tetrahydro-cannabinol in human brain: a study with positron emission tomography and 18F-2-fluoro-2-deoxyglucose. Psychiatry Res 40: 69–78.
85. Ashtari M, Cervellione K, Cottone J, Ardekani BA, Sevy S, et al. (2009) Diffusion abnormalities in adolescents and young adults with a history of heavy cannabis use. J Psychiatr Res 43: 189–204.
86. Bava S, Frank LR, McQueeny T, Schweinsburg BC, Schweinsburg AD, et al. (2009) Altered white matter microstructure in adolescent substance users. Psychiatry Res 173: 228–237.
87. Bava S, Jacobus J, Mahmood O, Yang TT, Tapert SF (2010) Neurocognitive correlates of white matter quality in adolescent substance users. Brain Cogn 72: 347–354.
88. Cheetham A, Allen NB, Whittle S, Simmons J, Yucel M, et al. (2012) Orbitofrontal Volumes in Early Adolescence Predict Initiation of Cannabis Use: A 4-Year Longitudinal and Prospective Study. Biol Psychiatry 71:684–692.
89. Chung T, Geier C, Luna B, Pajtek S, Terwilliger R, et al. (2011) Enhancing response inhibition by incentive: comparison of adolescents with and without substance use disorder. Drug Alcohol Depend 115: 43–50.
90. Clark DB, Chung T, Thatcher DL, Pajtek S, Long EC (2012) Psychological dysregulation, white matter disorganization and substance use disorders in adolescence. Addiction 107: 206–214.
91. Cohen M, Rasser PE, Peck G, Carr VJ, Ward PB, et al. (2011) Cerebellar grey-matter deficits, cannabis use and first-episode schizophrenia in adolescents and young adults. Int J Neuropsychopharmacol 1–11.
92. Cornelius JR, Aizenstein HJ, Hariri AR (2010) Amygdala reactivity is inversely related to level of cannabis use in individuals with comorbid cannabis dependence and major depression. Addict Behav 35: 644–646.
93. Cowan RL, Joers JM, Dietrich MS (2009) N-acetylaspartate (NAA) correlates inversely with cannabis use in a frontal language processing region of neocortex in MDMA (Ecstasy) polydrug users: a 3 T magnetic resonance spectroscopy study. Pharmacol Biochem Behav 92: 105–110.
94. Dekker N, Schmitz N, Peters BD, van Amelsvoort TA, Linszen DH, et al. (2010) Cannabis use and callosal white matter structure and integrity in recent-onset schizophrenia. Psychiatry Res 181: 51–56.
95. Habets P, Marcelis M, Gronenschild E, Drukker M, van OJ (2011) Reduced cortical thickness as an outcome of differential sensitivity to environmental risks in schizophrenia. Biol Psychiatry 69: 487–494.
96. Ho BC, Wassink TH, Ziebell S, Andreasen NC (2011) Cannabinoid receptor 1 gene polymorphisms and marijuana misuse interactions on white matter and cognitive deficits in schizophrenia. Schizophr Res 128: 66–75.
97. Jacobus J, McQueeny T, Bava S, Schweinsburg BC, Frank LR, et al. (2009) White matter integrity in adolescents with histories of marijuana use and binge drinking. Neurotoxicol Teratol 31: 349–355.
98. Jager G, Block RI, Luijten M, Ramsey NF (2010) Cannabis use and memory brain function in adolescent boys: a cross-sectional multicenter functional magnetic resonance imaging study. J Am Acad Child Adolesc Psychiatry 49: 561–72, 572.
99. James A, Hough M, James S, Winmill L, Burge L, et al. (2011) Greater white and grey matter changes associated with early cannabis use in adolescent-onset schizophrenia (AOS). Schizophr Res 128: 91–97.
100. Kumra S, Robinson P, Tambyraja R, Jensen D, Schimunek C, et al. (2012) Parietal lobe volume deficits in adolescents with schizophrenia and adolescents with cannabis use disorders. J Am Acad Child Adolesc Psychiatry 51: 171–180.
101. Li CS, Milivojevic V, Constable RT, Sinha R (2005) Recent cannabis abuse decreased stress-induced BOLD signals in the frontal and cingulate cortices of cocaine dependent individuals. Psychiatry Res 140: 271–280.
102. Medina KL, Nagel BJ, Park A, McQueeny T, Tapert SF (2007) Depressive symptoms in adolescents: associations with white matter volume and marijuana use. J Child Psychol Psychiatry 48: 592–600.
103. Parkar SR, Ramanathan S, Nair N, Batra SA, Adarkar SA, et al. (2011) Are the effects of cannabis dependence on glucose metabolism similar to schizophrenia? An FDG PET understanding. Indian J Psychiatry 53: 13–20.
104. Peters BD, de HL, Vlieger EJ, Majoie CB, den Heeten GJ, et al. (2009) Recent-onset schizophrenia and adolescent cannabis use: MRI evidence for structural hyperconnectivity? Psychopharmacol Bull 42: 75–88.
105. Rais M, van Haren NE, Cahn W, Schnack HG, Lepage C, et al. (2010) Cannabis use and progressive cortical thickness loss in areas rich in CB1 receptors during the first five years of schizophrenia. Eur Neuropsychopharmacol 20: 855–865.
106. Roberts GM, Garavan H (2010) Evidence of increased activation underlying cognitive control in ecstasy and cannabis users. Neuroimage 52: 429–435.
107. Safont G, Corripio I, Escarti MJ, Portella MJ, Perez V, et al. (2011) Cannabis use and striatal D2 receptor density in untreated first-episode psychosis: an in vivo SPECT study. Schizophr Res 129: 169–171.
108. Schweinsburg AD, Schweinsburg BC, Cheung EH, Brown GG, Brown SA, et al. (2005) fMRI response to spatial working memory in adolescents with comorbid marijuana and alcohol use disorders. Drug Alcohol Depend 79: 201–210.
109. Solowij N, Yucel M, Respondek C, Whittle S, Lindsay E, et al. (2011) Cerebellar white-matter changes in cannabis users with and without schizophrenia. Psychol Med 41: 2349–2359.
110. Takagi M, Lubman DI, Walterfang M, Barton S, Reutens D, et al. (2011) Corpus callosum size and shape alterations in adolescent inhalant users. Addict Biol Sep 29.[Epub ahead of print].
111. Tanabe J, Thompson L, Claus E, Dalwani M, Hutchison K, et al. (2007) Prefrontal cortex activity is reduced in gambling and nongambling substance users during decision-making. Hum Brain Mapp 28: 1276–1286.
112. Voytek B, Berman SM, Hassid BD, Simon SL, Mandelkern MA, et al. (2005) Differences in regional brain metabolism associated with marijuana abuse in methamphetamine abusers. Synapse 57: 113–115.
113. Welch KA, McIntosh AM, Job DE, Whalley HC, Moorhead TW, et al. (2011) The impact of substance use on brain structure in people at high risk of developing schizophrenia. Schizophr Bull 37: 1066–1076.
114. Welch KA, Stanfield AC, McIntosh AM, Whalley HC, Job DE, et al. (2011) Impact of cannabis use on thalamic volume in people at familial high risk of schizophrenia. Br J Psychiatry 199: 386–390.
115. Wobrock T, Sittinger H, Behrendt B, D'Amelio R, Falkai P (2009) Comorbid substance abuse and brain morphology in recent-onset psychosis. Eur Arch Psychiatry Clin Neurosci 259: 28–36.
116. Yucel M, Zalesky A, Takagi MJ, Bora E, Fornito A, et al. (2010) White-matter abnormalities in adolescents with long-term inhalant and cannabis use: a diffusion magnetic resonance imaging study. J Psychiatry Neurosci 35: 409–412.
117. Barkus E, Morrison PD, Vuletic D, Dickson JC, Ell PJ, et al. (2011) Does intravenous Delta9-tetrahydrocannabinol increase dopamine release? A SPET study. J Psychopharmacol 25: 1462–1468.
118. Borgwardt SJ, Allen P, Bhattacharyya S, Fusar-Poli P, Crippa JA, et al. (2008) Neural basis of Delta-9-tetrahydrocannabinol and cannabidiol: effects during response inhibition. Biol Psychiatry 64: 966–973.
119. Bossong MG, van Berckel BN, Boellaard R, Zuurman L, Schuit RC, et al. (2009) Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. Neuropsychopharmacology 34: 759–766.
120. Bossong MG, Jager G, van Hell HH, Zuurman L, Jansma JM, et al. (2012) Effects of Delta9-Tetrahydrocannabinol Administration on Human Encoding and Recall Memory Function: A Pharmacological fMRI Study. J Cogn Neurosci 24:588–599.
121. Fusar-Poli P, Crippa JA, Bhattacharyya S, Borgwardt SJ, Allen P, et al. (2009) Distinct effects of {delta}9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. Arch Gen Psychiatry 66: 95–105.
122. Fusar-Poli P, Allen P, Bhattacharyya S, Crippa JA, Mechelli A, et al. (2010) Modulation of effective connectivity during emotional processing by Delta 9-tetrahydrocannabinol and cannabidiol. Int J Neuropsychopharmacol 13: 421–432.
123. Mathew RJ, Wilson WH, Tant SR (1989) Acute changes in cerebral blood flow associated with marijuana smoking. Acta Psychiatr Scand 79: 118–128.
124. Mathew RJ, Wilson WH, Humphreys DF, Lowe JV, Wiethe KE (1992) Regional cerebral blood flow after marijuana smoking. J Cereb Blood Flow Metab 12: 750–758.
125. Mathew RJ, Wilson WH (1993) Acute changes in cerebral blood flow after smoking marijuana. Life Sci 52: 757–767.
126. Mathew RJ, Wilson WH, Coleman RE, Turkington TG, Degrado TR (1997) Marijuana intoxication and brain activation in marijuana smokers. Life Sci 60: 2075–2089.
127. Mathew RJ, Wilson WH, Turkington TG, Coleman RE (1998) Cerebellar activity and disturbed time sense after THC. Brain Res 797: 183–189.
128. Mathew RJ, Wilson WH, Chiu NY, Turkington TG, Degrado TR, et al. (1999) Regional cerebral blood flow and depersonalization after tetrahydrocannabinol administration. Acta Psychiatr Scand 100: 67–75.
129. Mathew RJ, Wilson WH, Turkington TG, Hawk TC, Coleman RE, et al. (2002) Time course of tetrahydrocannabinol-induced changes in regional cerebral blood flow measured with positron emission tomography. Psychiatry Res 116: 173–185.
130. O'Leary DS, Block RI, Koeppel JA, Flaum M, Schultz SK, et al. (2002) Effects of smoking marijuana on brain perfusion and cognition. Neuropsychopharmacology 26: 802–816.
131. O'Leary DS, Block RI, Turner BM, Koeppel J, Magnotta VA, et al. (2003) Marijuana alters the human cerebellar clock. Neuroreport 14: 1145–1151.
132. O'Leary DS, Block RI, Koeppel JA, Schultz SK, Magnotta VA, et al. (2007) Effects of smoking marijuana on focal attention and brain blood flow. Hum Psychopharmacol 22: 135–148.

133. Phan KL, Angstadt M, Golden J, Onyewuenyi I, Popovska A, et al. (2008) Cannabinoid modulation of amygdala reactivity to social signals of threat in humans. J Neurosci 28: 2313–2319.
134. Pillay SS, Rogowska J, Kanayama G, Jon DI, Gruber S, et al. (2004) Neurophysiology of motor function following cannabis discontinuation in chronic cannabis smokers: an fMRI study. Drug Alcohol Depend 76: 261–271.
135. Rabinak CA, Sripada CS, Angstadt M, de WH, Phan KL (2012) Cannabinoid modulation of subgenual anterior cingulate cortex activation during experience of negative affect. J Neural Transm 119:701–707.
136. Stokes PR, Mehta MA, Curran HV, Breen G, Grasby PM (2009) Can recreational doses of THC produce significant dopamine release in the human striatum? Neuroimage 48: 186–190.
137. Stokes PR, Egerton A, Watson B, Reid A, Breen G, et al. (2010) Significant decreases in frontal and temporal [11C]-raclopride binding after THC challenge. Neuroimage 52: 1521–1527.
138. van Hell HH, Bossong MG, Jager G, Kristo G, van Osch MJ, et al. (2011) Evidence for involvement of the insula in the psychotropic effects of THC in humans: a double-blind, randomized pharmacological MRI study. Int J Neuropsychopharmacol 14: 1377–1388.
139. van Hell HH, Jager G, Bossong MG, Brouwer A, Jansma JM, et al. (2012) Involvement of the endocannabinoid system in reward processing in the human brain. Psychopharmacology (Berl) 219: 981–990.
140. Volkow ND, Gillespie H, Mullani N, Tancredi L, Grant C, et al. (1996) Brain glucose metabolism in chronic marijuana users at baseline and during marijuana intoxication. Psychiatry Res 67: 29–38.
141. Winton-Brown TT, Allen P, Bhattacharyya S, Borgwardt SJ, Fusar-Poli P, et al. (2011) Modulation of auditory and visual processing by delta-9-tetrahydrocannabinol and cannabidiol: an FMRI study. Neuropsychopharmacology 36: 1340–1348.
142. Wolff V, Lauer V, Rouyer O, Sellal F, Meyer N, et al. (2011) Cannabis use, ischemic stroke, and multifocal intracranial vasoconstriction: a prospective study in 48 consecutive young patients. Stroke 42: 1778–1780.
143. Block RI, O'Leary DS, Ehrhardt JC, Augustinack JC, Ghoneim MM, et al. (2000) Effects of frequent marijuana use on brain tissue volume and composition. Neuroreport 11: 491–496.
144. Arnone D, Barrick TR, Chengappa S, Mackay CE, Clark CA, et al. (2008) Corpus callosum damage in heavy marijuana use: preliminary evidence from diffusion tensor tractography and tract-based spatial statistics. Neuroimage 41: 1067–1074.
145. Ashtari M, Avants B, Cyckowski L, Cervellione KL, Roofeh D, et al. (2011) Medial temporal structures and memory functions in adolescents with heavy cannabis use. J Psychiatr Res 45: 1055–1066.
146. Cousijn J, Wiers RW, Ridderinkhof KR, van den Brink W, Veltman DJ, et al. (2012) Grey matter alterations associated with cannabis use: Results of a VBM study in heavy cannabis users and healthy controls. Neuroimage 59: 3845–3851.
147. Gruber SA, Silveri MM, Dahlgren MK, Yurgelun-Todd D (2011) Why so impulsive? White matter alterations are associated with impulsivity in chronic marijuana smokers. Exp Clin Psychopharmacol 19: 231–242.
148. Matochik JA, Eldreth DA, Cadet JL, Bolla KI (2005) Altered brain tissue composition in heavy marijuana users. Drug Alcohol Depend 77: 23–30.
149. Yucel M, Solowij N, Respondek C, Whittle S, Fornito A, et al. (2008) Regional brain abnormalities associated with long-term heavy cannabis use. Arch Gen Psychiatry 65: 694–701.
150. DeLisi LE, Bertisch HC, Szulc KU, Majcher M, Brown K, et al. (2006) A preliminary DTI study showing no brain structural change associated with adolescent cannabis use. Harm Reduct J 3: 17.
151. Gruber SA, Yurgelun-Todd DA (2005) Neuroimaging of marijuana smokers during inhibitory processing: a pilot investigation. Brain Res Cogn Brain Res 23: 107–118.
152. Jager G, van Hell HH, De Win MM, Kahn RS, van den Brink W, et al. (2007) Effects of frequent cannabis use on hippocampal activity during an associative memory task. Eur Neuropsychopharmacol 17: 289–297.
153. Tzilos GK, Cintron CB, Wood JB, Simpson NS, Young AD, et al. (2005) Lack of hippocampal volume change in long-term heavy cannabis users. Am J Addict 14: 64–72.
154. Medina KL, McQueeny T, Nagel BJ, Hanson KL, Yang TT, et al. (2009) Prefrontal cortex morphometry in abstinent adolescent marijuana users: subtle gender effects. Addict Biol 14: 457–468.
155. Medina KL, Nagel BJ, Tapert SF (2010) Abnormal cerebellar morphometry in abstinent adolescent marijuana users. Psychiatry Res 182: 152–159.
156. McQueeny T, Padula CB, Price J, Medina KL, Logan P, et al. (2011) Gender effects on amygdala morphometry in adolescent marijuana users. Behav Brain Res 224: 128–134.
157. Block RI, O'Leary DS, Hichwa RD, Augustinack JC, Ponto LL, et al. (2000) Cerebellar hypoactivity in frequent marijuana users. Neuroreport 11: 749–753.
158. Lundqvist T, Jonsson S, Warkentin S (2001) Frontal lobe dysfunction in long-term cannabis users. Neurotoxicol Teratol 23: 437–443.
159. Sevy S, Smith GS, Ma Y, Dhawan V, Chaly T, et al. (2008) Cerebral glucose metabolism and D2/D3 receptor availability in young adults with cannabis dependence measured with positron emission tomography. Psychopharmacology (Berl) 197: 549–556.
160. Stokes PR, Egerton A, Watson B, Reid A, Lappin J, et al. (2012) History of cannabis use is not associated with alterations in striatal dopamine D2/D3 receptor availability. J Psychopharmacol 26: 144–149.
161. Albrecht DS, Skosnik PD, Vollmer JM, Brumbaugh MS, Perry KM, et al. (2012) Striatal D(2)/D(3) receptor availability is inversely correlated with cannabis consumption in chronic marijuana users. Drug Alcohol Depend Aug 18.[Epub ahead of print].
162. Urban NB, Slifstein M, Thompson JL, Xu X, Girgis RR, et al. (2012) Dopamine release in chronic cannabis users: a [11c]raclopride positron emission tomography study. Biol Psychiatry 71: 677–683.
163. Hirvonen J, Goodwin RS, Li CT, Terry GE, Zoghbi SS, et al. (2012) Reversible and regionally selective downregulation of brain cannabinoid CB(1) receptors in chronic daily cannabis smokers. Mol Psychiatry 17: 642–649.
164. Block RI, O'Leary DS, Hichwa RD, Augustinack JC, Boles Ponto LL, et al. (2002) Effects of frequent marijuana use on memory-related regional cerebral blood flow. Pharmacol Biochem Behav 72: 237–250.
165. Bolla KI, Eldreth DA, Matochik JA, Cadet JL (2005) Neural substrates of faulty decision-making in abstinent marijuana users. Neuroimage 26: 480–492.
166. Eldreth DA, Matochik JA, Cadet JL, Bolla KI (2004) Abnormal brain activity in prefrontal brain regions in abstinent marijuana users. Neuroimage 23: 914–920.
167. Vaidya JG, Block RI, O'Leary DS, Ponto LB, Ghoneim MM, et al. (2012) Effects of chronic marijuana use on brain activity during monetary decision-making. Neuropsychopharmacology 37: 618–629.
168. Abdullaev Y, Posner MI, Nunnally R, Dishion TJ (2010) Functional MRI evidence for inefficient attentional control in adolescent chronic cannabis abuse. Behav Brain Res 215: 45–57.
169. Chang L, Yakupov R, Cloak C, Ernst T (2006) Marijuana use is associated with a reorganized visual-attention network and cerebellar hypoactivation. Brain 129: 1096–1112.
170. Gruber SA, Rogowska J, Yurgelun-Todd DA (2009) Altered affective response in marijuana smokers: an FMRI study. Drug Alcohol Depend 105: 139–153.
171. Harding IH, Solowij N, Harrison BJ, Takagi M, Lorenzetti V, et al. (2012) Functional connectivity in brain networks underlying cognitive control in chronic cannabis users. Neuropsychopharmacology 37: 1923–1933.
172. Hester R, Nestor L, Garavan H (2009) Impaired error awareness and anterior cingulate cortex hypoactivity in chronic cannabis users. Neuropsychopharmacology 34: 2450–2458.
173. Jager G, Kahn RS, van den Brink W, van Ree JM, Ramsey NF (2006) Long-term effects of frequent cannabis use on working memory and attention: an fMRI study. Psychopharmacology (Berl) 185: 358–368.
174. King GR, Ernst T, Deng W, Stenger A, Gonzales RM, et al. (2011) Altered brain activation during visuomotor integration in chronic active cannabis users: relationship to cortisol levels. J Neurosci 31: 17923–17931.
175. Nestor L, Hester R, Garavan H (2010) Increased ventral striatal BOLD activity during non-drug reward anticipation in cannabis users. Neuroimage 49: 1133–1143.
176. van Hell HH, Vink M, Ossewaarde L, Jager G, Kahn RS, et al. (2010) Chronic effects of cannabis use on the human reward system: an fMRI study. Eur Neuropsychopharmacol 20: 153–163.
177. Wesley MJ, Hanlon CA, Porrino LJ (2011) Poor decision-making by chronic marijuana users is associated with decreased functional responsiveness to negative consequences. Psychiatry Res 191: 51–59.
178. Bechara A, Tranel D, Damasio H (2000) Characterization of the decision-making deficit of patients with ventromedial prefrontal cortex lesions. Brain 123 (Pt 11): 2189–2202.
179. Padula CB, Schweinsburg AD, Tapert SF (2007) Spatial working memory performance and fMRI activation interaction in abstinent adolescent marijuana users. Psychol Addict Behav 21: 478–487.
180. Schweinsburg AD, Nagel BJ, Schweinsburg BC, Park A, Theilmann RJ, et al. (2008) Abstinent adolescent marijuana users show altered fMRI response during spatial working memory. Psychiatry Res 163: 40–51.
181. Schweinsburg AD, Schweinsburg BC, Medina KL, McQueeny T, Brown SA, et al. (2010) The influence of recency of use on fMRI response during spatial working memory in adolescent marijuana users. J Psychoactive Drugs 42: 401–412.
182. Schweinsburg AD, Schweinsburg BC, Nagel BJ, Eyler LT, Tapert SF (2011) Neural correlates of verbal learning in adolescent alcohol and marijuana users. Addiction 106: 564–573.
183. Tapert SF, Schweinsburg AD, Drummond SP, Paulus MP, Brown SA, et al. (2007) Functional MRI of inhibitory processing in abstinent adolescent marijuana users. Psychopharmacology (Berl) 194: 173–183.
184. Koob GF, Volkow ND (2010) Neurocircuitry of addiction. Neuropsychopharmacology 35: 217–238.
185. Solowij N, Stephens RS, Roffman RA, Babor T, Kadden R, et al. (2002) Cognitive functioning of long-term heavy cannabis users seeking treatment. JAMA 287: 1123–1131.
186. Bechara A, Dolan S, Denburg N, Hindes A, Anderson SW, et al. (2001) Decision-making deficits, linked to a dysfunctional ventromedial prefrontal cortex, revealed in alcohol and stimulant abusers. Neuropsychologia 39: 376–389.
187. Bolla KI, Brown K, Eldreth D, Tate K, Cadet JL (2002) Dose-related neurocognitive effects of marijuana use. Neurology 59: 1337–1343.

188. Meier MH, Caspi A, Ambler A, Harrington H, Houts R, et al. (2012) Persistent cannabis users show neuropsychological decline from childhood to midlife. Proc Natl Acad Sci U S A Aug 27. [Epub ahead of print].
189. Licata SC, Renshaw PF (2010) Neurochemistry of drug action: insights from proton magnetic resonance spectroscopic imaging and their relevance to addiction. Ann N Y Acad Sci 1187: 148–171.
190. Okuda M, Hasin DS, Olfson M, Khan SS, Nunes EV, et al. (2010) Generalizability of clinical trials for cannabis dependence to community samples. Drug Alcohol Depend 111: 177–181.
191. Glover GH, Mueller BA, Turner JA, van Erp TG, Liu TT, et al. (2012) Function biomedical informatics research network recommendations for prospective multicenter functional MRI studies. J Magn Reson Imaging 36: 39–54.
192. Biswal BB, Mennes M, Zuo XN, Gohel S, Kelly C, et al. (2010) Toward discovery science of human brain function. Proc Natl Acad Sci USA 107: 4734–4739.
193. Milham MP (2012) Open neuroscience solutions for the connectome-wide association era. Neuron 73: 214–218.

6

Study 4

Modulation of brain structure by catechol-O-methyltransferase *Val*^{158}*Met* polymorphism in chronic cannabis users

Addiction Biology 2013 Jan 14 [Epub ahead of print]

Study 4

Summary

Reference

Title: Modulation of brain structure by catechol-O-methyltransferase *Val(158) Met* polymorphism in chronic cannabis users. Authors: Batalla A, Soriano-Mas C, Lopez-Sola M, Torrens M, Crippa JA, Bhattacharyya S, Blanco-Hinojo L, Fagundo AB, Harrison BJ, Nogué S, de la Torre R, Farré M, Pujol J, Martín-Santos R. *Addiction Biology 2013; [Epub ahead of print]*. Impact factor 2012: 5.914 (1st quartile substance abuse and biochemistry & molecular biology).

Aims

Considering the results of the previous systematic reviews and based on *hypothesis #3*, the aim of the present study was to explore the brain morphology of early-onset chronic cannabis users compared to non-using controls while assessing the influence of the COMT genotype. Based on previous chapters, four brain regions were selected: the prefrontal and anterior cingulate cortex, the neostriatum (caudate-putamen) and the hippocampus-amygdala complex.

Method

Participants were recruited by web page and through advertisements. Inclusion criteria included male gender, age comprised between 18 and 30 years, Caucasian, intelligence quotient (IQ) higher than 90 and fluency in Spanish. Additional criteria for chronic cannabis users were: age of onset of cannabis before the age of 16, smoke between 14 to 28 joints per week during at least two years and continue until entry into the study, no other drug use more than 5 times in life (a part from nicotine and alcohol), positive urine screen for cannabis and negative for any other drug of abuse. Control subjects were included if they reported no more than 15 lifetime experiences

with cannabis (with none in the last month), no previous use of any other drug for more than 5 times lifetime (except nicotine and alcohol) and negative urine drug screen. Exclusion criteria were any lifetime Axis I disorder assessed by a structured psychiatric interview (Psychiatric Research Interview for Substance and Mental Disorders; PRISM) (97), use of psychoactive medication, medical illnesses and left-handedness. The ethical committee CEIC-Parc de Salut Mar and Hospital Clínic approved the study. Written informed consent was obtained from all participants.

Genomic DNA was obtained from peripheral blood leukocytes of all participants using Flexi Gene DNA kit (Qiagen Iberia, SL, Spain) according to the manufacturer's instructions. The COMT *Val*^{158}*Met* single nucleotide polymorphism (SNP) allelic variants were determined using the 5′ exonuclease TaqMan assay with ABI 7900HT Sequence Detection System (Real-Time PCR) supplied by Applied Biosystems, Foster City, CA, USA.

Brain images were acquired with a 1.5 Tesla Signa Excite system (General Electric, Milwaukee, WI, USA). Imaging data were transferred and processed on MATLAB 7.8 (The Math Works Inc, Natick, MA, USA) and Statistical Parametric Mapping software (SPM 8; The Welcome Department of Imaging Neuroscience, London, UK). Image preprocessing was performed with the Voxel Based Morphometry (VBM) toolbox (http://dbm.neuro.uni-jena.de/vbm/).

Global grey matter, white matter, cerebrospinal fluid volumes and total intracranial volume (TIV) were compared between groups with independent samples *t*-tests in SPSS v. 18. Voxel-wise regional volume differences were studied with SPM tools. To study the effects on brain morphology of the interaction of COMT genotype and chronic cannabis use, we used a two-sample *t*-test (chronic cannabis users vs. controls) with age and TIV as nuisance covariates, and modelling the COMT genotype as a quantitative variable (number of *met* alleles: 0, 1, 2) in interaction with group. This analysis was initially restricted to four regions of interest (the prefrontal cortex, the anterior cingulate cortex, neostriatum (caudate and putamen) and the hippocampus-amygdala complex using an anatomical mask. A whole-brain analysis was also performed, assessing between-group differences (irrespective of genotype)

in regional grey matter volumes using a two-sample *t*-test design with age and TIV as nuisance covariates. Exploratory voxel-wise correlation analyses were also performed to test for significant associations between regional volumes and cannabis use parameters (e.g. lifetime cannabis use), adjusting for age and TIV.

Results

29 chronic cannabis users and 28 non-using controls matched in terms of age, educational level and IQ were recruited. Genotype frequencies of the COMT gene were in Hardy-Weinberg Equilibrium in both groups. The results showed that the COMT genotype influenced the volume in two out of four regions studied. Variation in the COMT genotype affected the bilateral ventral caudate nucleus in both groups in an opposite direction. That is, more copies of the *val* allele led to lesser volume in chronic cannabis users and more volume in controls (Figure 6.1). The opposite pattern was found in the left amygdala. There were no effects of the COMT genotype on volumes of the whole brain or the other selected regions.

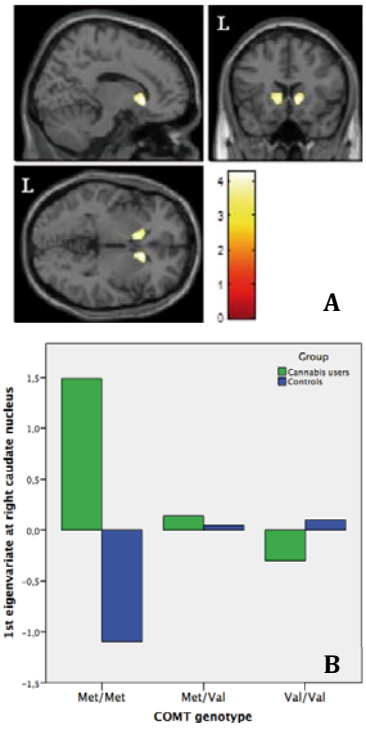


Figure 6.1. Regions of interaction between COMT genotype and brain morphology superimposed on selected slices of a normalized brain (ROI analysis). **(A)** In the right and left ventral caudate nucleus, while grey matter volume was negatively correlated with the number of *val* alleles in chronic cannabis users, the opposite pattern of correlation was observed in control subjects. **(B)** Relationship between grey matter volume in right ventral caudate and COMT genotype. The figure shows a reverse relationship between groups. Voxels with $p < 0.001$ (uncorrected) are displayed. Regional volumes were adjusted to age and TIV. Colour bar represents t value. L indicates left hemisphere.

Conclusion

This study reveals for the first time that the COMT genotype might influence the anatomical brain changes related to chronic cannabis use, consistent with gene-environment interaction models.

Addiction Biology

ORIGINAL ARTICLE

doi:10.1111/adb.12027

Modulation of brain structure by catechol-O-methyltransferase *Val*^{158}*Met* polymorphism in chronic cannabis users

Albert Batalla[1], Carles Soriano-Mas[2,3], Marina López-Solà[2], Marta Torrens[4,5], José A. Crippa[6,7], Sagnik Bhattacharyya[8], Laura Blanco-Hinojo[2], Ana B. Fagundo[4], Ben J. Harrison[2,9], Santiago Nogué[10], Rafael de la Torre[4,11], Magí Farré[4,5], Jesús Pujol[2] & Rocío Martín-Santos[1,4,6]

Department of Psychiatry, Clinical Institute of Neuroscience, Hospital Clínic, IDIBAPS, CIBERSAM, and Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Spain[1], CRC Mar, Hospital del Mar, Spain[2], Department of Psychiatry, Bellvitge University Hospital-IDIBELL, CIBERSAM, Spain[3], Neuroscience Program, IMIM (Hospital del Mar Medical Research Institute)-INAD-Parc de Salut Mar, Autonomous University of Barcelona and Pompeu Fabra University, Spain[4], Red de Trastornos Adictivos (RETIC), IMIM-INAD-Parc de Salut Mar, Spain[5], Neuroscience and Cognitive Behavior Department, University of Sao Paulo, Brazil[6], INCT Translational Medicine (INCT-TM, CNPq), Brazil[7], Department of Psychosis Studies, King's College London, Institute of Psychiatry, UK[8], Melbourne Neuropsychiatry Centre, Department of Psychiatry, The University of Melbourne & Melbourne Health, Australia[9], Clinical Toxicology Unit, Emergency Department, Hospital Clínic, IDIBAPS, University of Barcelona, Spain[10] and CIBEROBN, Spain[11]

ABSTRACT

Neuroimaging studies have shown that chronic consumption of cannabis may result in alterations in brain morphology. Recent work focusing on the relationship between brain structure and the catechol-O-methyltransferase (COMT) gene polymorphism suggests that functional COMT variants may affect brain volume in healthy individuals and in schizophrenia patients. We measured the influence of COMT genotype on the volume of four key regions: the prefrontal cortex, neostriatum (caudate-putamen), anterior cingulate cortex and hippocampus-amygdala complex, in chronic early-onset cannabis users and healthy control subjects. We selected 29 chronic cannabis users who began using cannabis before 16 years of age and matched them to 28 healthy volunteers in terms of age, educational level and IQ. Participants were male, Caucasians aged between 18 and 30 years. All were assessed by a structured psychiatric interview (PRISM) to exclude any lifetime Axis-I disorder according to Diagnostic and Statistical Manual for Mental Disorders-Fourth Edition. COMT genotyping was performed and structural magnetic resonance imaging data was analyzed by voxel-based morphometry. The results showed that the COMT polymorphism influenced the volume of the bilateral ventral caudate nucleus in both groups, but in an opposite direction: more copies of *val* allele led to lesser volume in chronic cannabis users and more volume in controls. The opposite pattern was found in left amygdala. There were no effects of COMT genotype on volumes of the whole brain or the other selected regions. Our findings support recent reports of neuroanatomical changes associated with cannabis use and, for the first time, reveal that these changes may be influenced by the COMT genotype.

Keywords chronic cannabis users, COMT, structural MRI, *Val158Met*, VBM.

Correspondence to: Rocío Martín-Santos, Department of Psychiatry, Instituto Clínico de Neurociencias, Hospital Clinic, University of Barcelona, IDIBAPS, CIBERSAM, Calle Villarroel, 170; 08036 Barcelona, Spain. E-mail: rmsantos@clinic.ub.es

INTRODUCTION

Cannabis is currently the most consumed illicit drug worldwide (Watson, Benson & Joy 2000). Previous structural neuroimaging studies have not reported differences between cannabis users compared with control groups as to global brain measures, and studies based on specific region of interest have reported inconsistent results (Lorenzetti *et al.* 2010; Martin-Santos *et al.* 2010). One explanation for the discrepancies observed in human volumetric studies may be the heterogeneity across study samples in terms of duration and frequency of use, as well as quantity and type of cannabis smoked and demographic characteristics (Lorenzetti *et al.* 2010). Despite these conflicting results, there is evidence that earlier (before the age of 17) onset of cannabis use may be associated with greater detrimental effects on brain morphology compared with onset later on in life (Wilson *et al.* 2000). Additionally, long-term cannabis use may result in persistent alterations in brain function and

morphology, particularly in those areas related with executive functioning, reward circuitry and memory, such as the prefrontal cortex, anterior cingulate cortex (ACC), basal ganglia (e.g. neostriatum) and medial temporal areas (e.g. hippocampus and amygdala) (Lorenzetti *et al.* 2010; Martin-Santos *et al.* 2010), where CB1 receptors are more concentrated (Burns *et al.* 2007). Severity of cannabis use has also been found to be associated with gray matter volume in the prefrontal cortex in a group of subjects at clinical risk for psychosis and healthy controls (Stone *et al.* 2012).

Genetic variation may also play an important role in determining brain morphology. Recent studies focused on the relationship between brain structure and the catechol-O-methyltransferase (COMT) polymorphism suggest that functional COMT variants could affect brain volume in schizophrenia patients (Ohnishi *et al.* 2006), subjects at risk for psychosis (McIntosh *et al.* 2007) and even in healthy individuals (Honea *et al.* 2009), although negative results have also been reported (Barnes *et al.* 2012). In addition, preliminary data of several genes modulating the adverse effects of cannabis on the brain, including COMT polymorphism, have also been reported in long-term chronic cannabis users (Solowij *et al.* 2012). The COMT gene displays a functional polymorphism at codon 158 causing a valine (val) to methionine (met) substitution (*Val*^{158}*Met*, rs4680) resulting in three genotypes (val/val, val/met and met/met). Whereas the met/met variant shows a 40% lower enzymatic activity, which is associated with high levels of extrasynaptic dopamine, the val/val variant implies higher enzymatic activity, which results in low levels of extrasynaptic dopamine (Chen *et al.* 2004). COMT has an important role in clearing dopamine in the prefrontal cortex (Tunbridge, Harrison & Weinberger 2006), in subcortical regions such as basal ganglia and medial temporal lobe, as well as in the cerebellum and the spinal cord (Hong *et al.* 1998; Honea *et al.* 2009). Furthermore, epidemiological as well as experimental studies have shown that *val*-allele carriers may be more sensitive to the longer term effects of cannabis as well as the acute effects of delta-9-tetrahydrocannabinol (THC), the main psychoactive ingredient in cannabis, particularly if there is prior evidence of psychosis liability (Henquet *et al.* 2006; Estrada *et al.* 2011). Nevertheless, to our knowledge, there are no previous studies published that have examined the influence of COMT polymorphism on brain morphology in subjects chronically exposed to cannabis.

The aim of the present study was therefore to explore the influence of COMT *Val*^{158}*Met* functional polymorphism on four key regions: the prefrontal cortex, neostriatum (caudate-putamen), ACC and the hippocampus-amygdala complex, in a group of early-onset chronic cannabis users compared with non-using control subjects using voxel-based morphometry (VBM). VBM has been used successfully in prior research to identify changes in brain morphology related to common genetic polymorphisms, such as COMT (Honea *et al.* 2009) and brain-derived neurotrophic factor (BDNF) (Pezawas *et al.* 2004). We hypothesized that COMT *Val*^{158}*Met* functional polymorphism would be associated with brain morphological deficits in early-onset chronic cannabis users relative to healthy controls, with dose-dependent associations between volume brain variations and *val*-allele dosage.

METHODS

Subjects

Participants were primarily recruited *via* a web page and distribution of flyers and ads. To assess for study eligibility, a comprehensive telephone screening measures was performed (contact and sociodemographic data and a standardized drug use questionnaire). If considered eligible, subjects were required to undergo a detailed medical history check, routine laboratory tests, physical examination, urine and hair toxicology screens and a brief neurological examination. Drug use characteristic were systematically assessed using *ad hoc* questionnaire. The units used were as follows: number of cigarettes for tobacco use per day; standard units of alcohol per week and number of 'joints' for cannabis consumption per day and week.

Inclusion criteria required that participants were male, between 18 and 30 years of age, Caucasian, with IQ > 90 and fluent in Spanish. To be included in the cannabis-user group, the subject had to fulfill the following criteria: onset of cannabis use before the age of 16 years; cannabis use between 14 and 28 'joints'/week during at least the last 2 years and continued until entry into the study; no previous use of any other drug of abuse more than five lifetime except nicotine or alcohol; positive urine drug screen for cannabinoids but negative for opiates, cocaine, amphetamines and benzodiazepines on the day of the assessment, tested using immunometric assay kits. Control subjects had to fulfill the following criteria: no more than 15 lifetime experiences with cannabis (with none in the past month), no previous use of any other drug of abuse more than five lifetime except nicotine or alcohol. All controls had a negative urine drug screen for opiates, cocaine, amphetamines, benzodiazepines and cannabinoids, tested using immunometric assay kits (Instant-View; ASD Inc, Poway, CA, USA). Hair testing was performed in all subjects to verify either repeated cannabis consumption (chronic cannabis users group) or non-consumption (control group).

Exclusion criteria included any lifetime Axis I disorder (substance use disorders and non-substance use

disorders) according to Diagnostic and Statistical Manual for Mental Disorders-Fourth Edition (American Psychiatric Association 2000) except for nicotine use disorder assessed by a structured psychiatric interview (PRISM) (Torrens *et al*. 2004); use of psychoactive medications; history of chronic medical illness or neurological conditions that might affect cognitive function; head trauma with loss of consciousness > 2 minutes; learning disability or mental retardation; left-handedness and non-correctable vision, color blindness or hearing impairments. Subjects also received the vocabulary subscale of WAIS-III, to provide an estimate of verbal intelligence (Wechsler 1997).

Written informed consent was obtained from each subject after they had received a complete description of the study and been given the chance to discuss any questions or issues. Upon completion of the study, all subjects received financial compensation for participation. The study was approved by the Ethical and Clinical Research Committee of our institution (CEIC-Parc de Salut Mar).

Genotyping methods

Genomic DNA was extracted from the peripheral blood leukocytes of all the participants using Flexi Gene DNA kit (Qiagen Iberia, S.L., Spain) according to the manufacturer's instructions. The *COMT Val*^{158}*Met* single nucleotide polymorphism (SNP) allelic variants were determined using the 5′ exonuclease TaqMan assay with ABI 7900HT Sequence Detection System (Real-Time PCR) supplied by Applied Biosystems, Foster City, CA, USA. Primers and fluorescent probes were obtained from Applied Biosystems with TaqMan SNP Genotyping assays (assay ID C_2255335_10). Reaction conditions were those described in the ABI PRISM 7900HT user's guide. Endpoint fluorescent signals were detected on the ABI 7900, and the data were analyzed using Sequence Detector System software, version 2.3 (Applied Biosystems).

Structural image processing and analyses

Images were acquired with a 1.5-T Signa Excite system (General Electric, Milwaukee, WI, USA) equipped with an eight-channel phased-array head coil. A high-resolution T1-weighted anatomical image was obtained for each subject using a three-dimensional fast spoiled gradient inversion-recovery prepared sequence with 130 contiguous slices (TR, 11.8 milliseconds; TE, 4.2 milliseconds; flip angle, 15°; field of view, 30 cm; 256 × 256 pixel matrix; slice thickness, 1.2 mm).

Imaging data were transferred and processed on a Microsoft Windows platform using a technical computing software program (MATLAB 7.8; The MathWorks Inc, Natick, MA, USA) and Statistical Parametric Mapping software (SPM8; The Wellcome Department of Imaging Neuroscience, London, UK). Following inspection for image artifacts, image preprocessing was performed with the VBM toolbox (http://dbm.neuro.uni-jena.de/vbm/). Briefly, native-space magnetic resonance imaging were segmented and normalized to the SPM-T1 template using a high-dimensional DARTEL transformation. In addition, the Jacobian determinants derived from the spatial normalization were used to modulate image voxel values to restore volumetric information (affine and non-linear) (Good *et al*. 2001). Finally, images were smoothed with an 8 mm full width at half maximum isotropic Gaussian kernel.

Statistical analyses

Descriptive results are presented as mean (standard deviation) for continuous variables and frequencies (absolute, relative) for categorical variables.

Global gray matter, white matter and cerebrospinal fluid volumes, as well as total intracranial volume (TIV), were obtained after data pre-processing and compared between groups with independent samples *t*-tests in Statistical Package for the Social Sciences (SPSS, v.18; SPSS Inc., Chicago, IL, USA). Voxel-wise regional volume differences were studied with SPM tools. To study the effects on brain morphology of the interaction of COMT genotype and chronic cannabis use, we used a two-sample *t*-test design (chronic cannabis users versus controls) with age and global gray matter volume as nuisance covariates, and modeling the COMT genotype as a quantitative variable (number of *met* alleles: 0, 1, 2) in interaction with group. This approach allowed the assessment of between-group differences in the correlations of the number of *met* alleles with voxel-wise gray matter values, and we reported results from regions where such between-group differences were statistically significant (i.e. interactions). This analysis was initially restricted to four key regions: the prefrontal cortex, neostriatum (caudate and putamen), ACC and the hippocampus-amygdala complex) using an anatomical mask created with the Wake Forest University pickAtlas (Maldjian *et al*. 2003). Importantly, these masks were used to perform voxel-wise analyses within such regions, allowing a more precise anatomical localization of our findings. However, average volumes were also calculated for each region by adding up modulated voxel values included in the masks (i.e. adding up voxel values previously multiplied by the Jacobian determinants derived from the normalization step). The resulting values were transformed to milliliters and are presented in Table 3 in relation to TIV. In addition, a whole-brain analysis was also performed (see below).

To complement the above analyses, we also assessed for between-group differences (irrespective of genotype)

Table 1 Sociodemographic and drug use characteristics.

	Cannabis users *Mean/n (SD/%)*	*Control* *Mean/n (SD/%)*	$t_{d.f.=57}/\chi^2$	*P*
Age	20.8 (2.1)	22.1 (3.0)	1.87	0.065
Males	29 (100)	28 (100)	—	—
Cannabis use				
Onset of use (age, years)	14.9 (1.1)	16.8 (2.0)	2.96	0.001
Total lifetime cannabis use (number of joints)	5203 (4192)	4.9 (6.1)	6.68	< 0.001
Onset regular use (age, years)	18.1 (2.1)	—	—	—
Duration of use (years)	5.9 (2.4)	—	—	—
Current cannabis use (joints/day)	2.5 (1.5)	—	—	—
Alcohol use				
Age of onset of use	15.0 (1.1)	15.8 (1.5)	2.35	0.023
Duration of use	5.7 (2.3)	6.3 (3.1)	0.87	0.389
Alcohol units per week	5.3 (3.8)	3.1 (3.1)	2.49	0.020
Tobacco use				
Current smokers	27 (93.1)	9 (32.1)	21.8	< 0.001
Age of onset of use	16.3 (1.5)	16.3 (2.2)	0.57	0.955
Duration of use (years)	4.5 (2.7)	4.9 (3.3)	0.34	0.737
Cigarettes per day	6.0 (5.0)	2.4 (5.9)	1.79	0.082

d.f. = degrees of freedom; SD = standard deviation.

in regional gray matter volumes using a two-sample *t*-test design with age and TIV as nuisance covariates. Finally, exploratory voxel-wise correlation analyses were also performed to test, within the cannabis user group, for significant associations between regional volumes and lifetime cannabis consumption (number of 'joints') by introducing this variable as a regressor of interest, as well as age and TIV as nuisance covariates.

Significance thresholds for global brain SPM analyses were set at $P < 0.05$, family-wise error corrected for multiple comparisons across the brain. When the analyses were restricted to a regional anatomical mask (i.e. to study the effects of COMT genotype/cannabis use interaction), the correction for multiple comparison was adjusted to the number of voxels within the mask (i.e. small volume correction). To account for the different number of voxels within each mask, and thus for the different significance threshold set for each region, these analyses were also performed at more lenient significance threshold of $P < 0.001$ uncorrected for multiple comparisons. In addition, to get a better notion of the anatomical extension of the findings, results were always displayed (i.e. in figures) at $P < 0.001$ (uncorrected). For SPSS analyses, the statistical threshold was set at $P < 0.05$.

RESULTS

Sample characteristics

A final sample of 57 subjects was included: 29 early-onset cannabis users and 28 drug-free control subjects. Main demographic and drug use characteristics are described in Table 1. No differences were found in demographic and drug use variables between both groups except for alcohol and tobacco use. None of them met lifetime criteria for abuse or dependence of alcohol. All participants were under the risk dose of 28 unit of alcohol per week. On average, cannabis users smoked no more than seven cigarettes per day (range = 0–20). Only three participants smoked more than 10 cigarettes per day (two cases and one control subject).

Table 2 COMT genotype distribution.

	Cannabis *(n = 29)*	*Control* *(n = 28)*	*P*
COMT *Val*$^{108/158}$ *Met*			0.563
Met/Met	4	7	
Val/Met	18	15	
Val/Val	7	6	

COMT = catechol-O-methyltransferase; met = methionine; val = valine.

Genotype frequencies of the *COMT* gene are presented in Table 2. Genotype frequencies of the *COMT* gene were as follows: 11 subjects were homozygous for the *met* allele, 13 were val/val and 33 were val/met carriers. There was no evidence that these data were not in Hardy-Weinberg Equilibrium.

Global volume measurements and whole-brain between group differences

Global gray matter, white matter and cerebrospinal fluid volumes were related to TIV. Between-group comparisons

Table 3 Global tissues volumes in cannabis users and healthy controls.

		Mean (SD)	$t_{d.f.=55}$	P
Gray matter[a]	Cannabis	49.29 (2.07)	0.77	0.447
	Controls	48.90 (1.84)		
White matter	Cannabis	35.32 (1.61)	−0.54	0.589
	Controls	35.54 (1.49)		
Cerebrospinal fluid	Cannabis	15.39 (1.29)	−0.55	0.586
	Controls	15.56 (1.11)		
Intracranial volume	Cannabis	1488 (137) ml	1.06	0.296
	Controls	1522 (112) ml		
Prefrontal cortex[b]	Cannabis	8.91 (0.57)	0.32	0.747
	Controls	8.86 (0.50)		
Anterior cingulate cortex	Cannabis	0.69 (0.06)	−1.22	0.229
	Controls	0.71 (0.05)		
Neostriatum	Cannabis	0.73 (0.09)	6.46	< 0.001
	Controls	0.60 (0.05)		
Hippocampus-amygdala	Cannabis	0.70 (0.04)	−0.36	0.717
	Controls	0.71 (0.03)		

[a]Global tissue volumes are presented normalized to TIV. [b]Volumes of the four regions of interest are presented normalized to TIV and collapsed across hemispheres. d.f. = degrees of freedom; ml = milliliters; SD = standard deviation.

detected no significant differences for any of these variables. Table 3 presents global tissue volumes normalized to TIV.

Irrespective of genotype, chronic cannabis users showed a gray matter volume increase in the postcentral gyrus of the left hemisphere at a significance threshold of $P < 0.001$ uncorrected (Supporting Information Fig. S1). In a *post hoc* assessment, we observed that the volume of this region was not affected by the genotype or the interaction between group and genotype. Likewise, we did not observe any significant gray matter volume reductions in chronic cannabis users. Finally, we did not observe any significant between-group difference when this analysis was restricted to our four selected regions.

COMT genotype and chronic cannabis use between-group interactions

We found significant between-group differences in the genotype-gray matter volume correlations in two out of our four regions. Specifically, in chronic cannabis users, we found a negative correlation between bilateral ventral caudate nucleus volume and the number of *val* alleles, while the reverse association was observed in healthy controls: the more *val* alleles, the more ventral caudate gray matter volume (Fig. 1). In contrast, we observed that in chronic cannabis users a greater number of *val* alleles were associated with significant increase in left amygdala volume. The opposite was true for controls: the more *val* alleles, the smaller the gray matter volume in left amygdala (Fig. 2).

Importantly, to account for the different number of voxels within each masked region, and thus for the different corrected significance thresholds set for each region, we repeated the interaction analyses at the whole-brain level. While the above findings were also observed at significance level of $P < 0.001$ (uncorrected), no significant findings were observed within the other selected regions (prefrontal cortex and ACC) at this significance threshold.

Lifetime cannabis use

We observed a positive correlation between brain morphology and lifetime cannabis use ('joints') only at a significance threshold of $P < 0.001$ uncorrected. Specifically, this correlation was observed between the volume of the most caudal portion of the rectal gyrus-subgenual cingulate cortex and the accumulated number of joints consumed (Supporting Information Fig. S2). Correlations between regional brain volumes and lifetime cannabis use ('joints') were not affected by COMT genotype.

DISCUSSION

This study provides evidence of the impact of COMT *Val*^{158}*Met* genetic variation on brain structure in a group of early-onset chronic cannabis users compared with healthy controls using VBM. Our results show a significantly influence of the COMT polymorphism in bilateral ventral caudate nucleus volume in both groups but in an opposite direction: more copies of *val* allele was associated with lesser volume in chronic cannabis users and more volume in controls. An opposite pattern was observed for the left amygdala; the greater number of copies of *val*

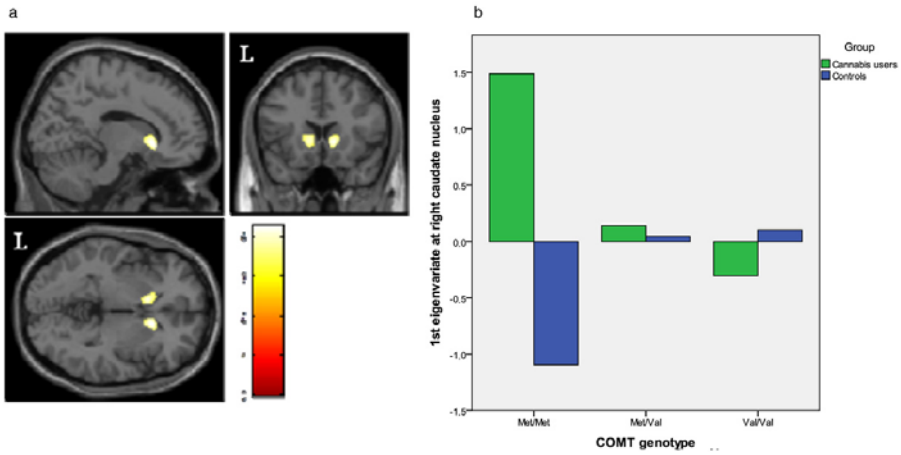


Figure 1 Regions of interaction between catechol-O-methyltransferase (COMT) genotype and brain morphology superimposed on selected slices of a normalized brain (ROI analysis). (a) In the right and left ventral caudate nucleus, while gray matter volume was negatively correlated with the number of *Val* alleles in chronic cannabis users, the opposite pattern of correlation was observed in control subjects (right: peak at x, y, z = 12, 20, –2; $t = 4.07$; $P_{(SVC\text{-}FWE\ corrected)} = 0.034$; left: peak at x, y, z = –11, 15, –0; $t = 4.20$; $P_{(SVC\text{-}FWE\ corrected)} = 0.023$). (b) Relationship between gray matter volume in right ventral caudate and COMT genotype. Figure shows a reverse relationship between groups. Voxels with $P < 0.001$ (uncorrected) are displayed. Regional volumes were adjusted to age and total intracranial volume. Color bar represents *t* value. L indicates left hemisphere

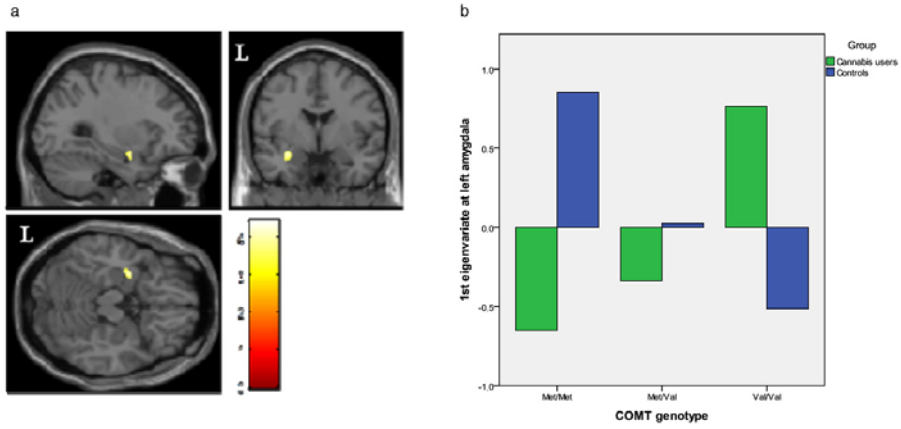


Figure 2 Regions of interaction between catechol-O-methyltransferase (COMT) genotype and brain morphology superimposed on selected slices of a normalized brain (ROI analyses). (a) In the amygdala of the left hemisphere, gray matter volumes were correlated with the number of *Val* alleles in chronic cannabis users, while the opposite pattern of correlation was observed in control subjects (peak at x, y, z = –30, –1, –18; $t = 3.82$; $P_{(SVC\text{-}FWE\ corrected)} = 0.046$). (b) Differences in gray matter volume in left amygdala between *Val* and *Met* alleles. Figure shows a reverse relation between groups. Voxels with $P < 0.001$ (uncorrected) are displayed. Regional volumes were adjusted to age and total intracranial volume. Color bar represents *t* value. L indicates left hemisphere

allele was associated with increased volume in chronic cannabis users and decreased volume in controls. We also identified a significant positive correlation between caudal rectal gyrus-subgenual cingulate cortex volume and the number of joints consumed. Finally, we reported an almost significant gray matter volume increase in the postcentral gyrus of the left hemisphere in chronic cannabis users.

The observed interaction between COMT genotype and chronic cannabis use on brain morphology is a novel and interesting finding, particularly given current models of substance use disorders. For instance, it has been proposed that the transition to addiction may begin with an increased excitability of the mesolimbic dopamine system followed by a cascade of neuroadaptations in areas related to addiction circuitry, such as the ventral striatum, which has a major role in the acute reinforcing effects of drugs of abuse (Koob & Volkow 2010). In this sense, the activation of dopamine, which may be influenced by COMT genotype, contributes to increased excitability of the ventral striatum with decreased glutamatergic activity during withdrawal and increased glutamatergic activity during drug-primed and cue-induced drug seeking (Koob & Volkow 2010). Similar to other drugs of abuse, cannabinoids facilitate the release of dopamine in the nucleus accumbens (Tanda, Pontieri & Di 1997), despite the mechanism by which this occur remaining unknown. On the other hand, several preclinical studies have reported the impact of variation in dopamine neurotransmission, especially extracellular dopamine concentration, on neuronal growth and survival, particularly in striatum (Santiago *et al.* 2000). Animal knockout models with reduction in dopamine signaling show important impairments in neuronal differentiation (Zhou & Palmiter 1995). Chronically elevated extracellular dopamine concentration is neurotoxic (Santiago *et al.* 2000) and alters the expression of the BDNF (Fumagalli *et al.* 2003). Research in animal models suggests that exogenous cannabinoids, like THC, facilitate dopaminergic neurotransmission in several regions of the brain, including the striatum and prefrontal cortex (Maldonado *et al.* 2011). Human neurochemical imaging studies have reported inconsistent results, with only one study reporting a modest increase in dopamine striatal concentrations (Bossong *et al.* 2009). However, there is evidence that cannabis may play a role in modulating striatal function (Bhattacharyya *et al.* 2009b, 2012). Over- and under-stimulation may potentially result in impaired neuronal growth and survival, indicating that an optimum range for extracellular dopamine may exist (Honea *et al.* 2009), which may be region specific and influenced by genetics and environment.

Few studies have described the influence of *Val*^{158}*Met* polymorphism on brain structure in healthy subjects (Ohnishi *et al.* 2006; Zinkstok *et al.* 2006; Honea *et al.* 2009; Ehrlich *et al.* 2010; Barnes *et al.* 2012). In 151 healthy volunteers, subjects carrying the *val* allele had a significantly smaller volume of the hippocampus and parahippocampus gyrus (Honea *et al.* 2009) relative to *met* homozygotes. Conversely, *val*-alleles carriers were also shown to have a non-significant trend-level effect of increased volume in the prefrontal cortex (Honea *et al.* 2009). Consistently, another study also described a linear effect of COMT genotype on medial temporal lobe volumes in 114 healthy individuals (Ehrlich *et al.* 2010). In this study, *val*-allele carriers had decreased volumes in the amygdala bilaterally and in the right hippocampus, with slightly greater effect in the left amygdala (Ehrlich *et al.* 2010). In line with the evidence mentioned above, we also found a decreased volume in the temporal lobe of *val*-allele carrying subjects in the control group, although it was restricted to the left amygdala. The modest size of our sample may have contributed to the relative localized effect of genotype that we have observed. In contrast, one study did not detect a main effect of genotype in the medial temporal lobe in 76 controls (Ohnishi *et al.* 2006), and two other studies found no group differences in regional gray matter density (Zinkstok *et al.* 2006) and volume (Barnes *et al.* 2012) as a function of genotype in 154 and 82 young healthy adults, respectively. It has been suggested that volume measures, as opposed to density measures, may be more sensitive indicators of genotype-related alterations (Zinkstok *et al.* 2006; Honea *et al.* 2009).

To the best of our knowledge, no previous structural or functional imaging study has focused on the influence of COMT genotype in cannabis users. However, it is remarkable to note that the effects of chronic cannabis use on brain structure and integrity are consistent with studies showing similar alterations in patients with schizophrenia (Bhattacharyya *et al.* 2009a). Morphometric studies have consistently reported up to 6% volume reductions in the hippocampus and the amygdala in schizophrenic patients (Honea *et al.* 2005), suggesting that these structural changes could reflect a central pathophysiological process associated with the illness. Furthermore, cannabis use or dependence in schizophrenic patients has been associated with smaller anterior (Szeszko *et al.* 2007) and posterior cingulate cortex (Bangalore *et al.* 2008), and cerebellar white-matter volume reduction (Solowij *et al.* 2011), and those who continue to use cannabis show greater loss of gray matter volume than those who do not (Rais *et al.* 2008). On the other hand, the COMT polymorphism has shown to influence brain structure and function in people at high risk of psychosis and schizophrenia in cingulate, lateral prefrontal cortex and temporal regions (Ohnishi *et al.* 2006; McIntosh *et al.* 2007; Ehrlich *et al.* 2010; Raznahan *et al.* 2011). In particular, the COMT *Met* allele has been associated with larger, and the *val* allele with smaller, medial temporal lobe volumes in schizophrenic patients, suggesting that the *val* allele may contribute, at least in part, to lower medial temporal volumes in these patients (Ehrlich *et al.* 2010). Interestingly, in our chronic cannabis users for whom other schizophrenia risk factors were

exhaustively excluded, we found that the *met* allele was associated with lower, and the *val* allele with higher, left amygdala volume, providing further evidence of how the environment and genetics may interact to influence the brain structure.

We also observed a positive correlation between caudal rectal gyrus-subgenual cingulate cortex volume and the number of 'joints' used (both lifetime and the year before the study), which has not been previously reported (Lorenzetti *et al.* 2010; Cousijn *et al.* 2012). We have found no other correlations, despite an apparent inverse relationship existing between the amounts of cannabis used and (para-) hippocampal and amygdala volumes (Lorenzetti *et al.* 2010). These volumetric discrepancies reported across human studies may be due to differences in imaging methods (e.g. image resolution, used of automated volumetric versus manual methods), cannabis use pattern (age of onset, length of use, frequency, quantity of use, concentration of THC of 'joint'), and demographic characteristics, which easily could lead to non-comparable samples that difficult the interpretation of results (Lorenzetti *et al.* 2010). For instance, samples with greater cannabis exposure (Matochik *et al.* 2005; Yücel *et al.* 2008) have demonstrated reductions in medial temporal brain regions, while samples with a relatively lower quantity of smoked cannabis, more similar to our sample, have exhibited no morphological changes (Wilson *et al.* 2000; Lorenzetti *et al.* 2010; Cousijn *et al.* 2012). Furthermore, our results support that additional factor, such as the genetic influence may also be determinant on brain morphology.

Animal studies have consistently demonstrated that THC induces dose-dependent neurotoxic changes in brain regions that are rich with cannabinoid receptors (Landfield, Cadwallader & Vinsant 1988), such as hippocampus, septum, amygdala and cerebral cortex (Heath *et al.* 1980; Lawston *et al.* 2000; Downer *et al.* 2001). In contrast, human imaging studies that have examined regular cannabis users present contradictory findings (Lorenzetti *et al.* 2010), insomuch as both positive (Yücel *et al.* 2008) and negative (Jager *et al.* 2007) influences on brain structure have been noted. In line with other published studies and recent reviews (Lorenzetti *et al.* 2010; Martin-Santos *et al.* 2010), we found no differences between groups in terms of global measures, but we reported a trend-level increase in gray matter volume of the left postcentral gyrus in chronic cannabis users. The only other VBM study in chronic cannabis users also showed cannabis users to have greater gray matter tissue density in the left pre and postcentral gyrus (Matochik *et al.* 2005). Interestingly, recent data from animal studies suggest that sensorimotor cortex may be especially vulnerable to cannabis abuse during adolescence due to the different developmental trajectories of CB1 expression (Heng *et al.* 2011). Thus, while in medial prefrontal and in limbic/associative regions seems to be a pronounced and progressive decrease in CB1 expression, major changes in sensorimotor cortices occurred only after the adolescence period, suggesting that cannabis abuse during adolescence may have a relatively more impact on sensorimotor functions (Heng *et al.* 2011). Exogenous cannabinoid administration may alter astrocyte functioning, which play a critical role in eliminating weaker connections (Bindukumar *et al.* 2008). By interfering with these processes, cannabis exposure during adolescence may impair typical pruning and ultimately result in larger regional volumes in specific brain areas. The mentioned VBM study also reported other structural differences that we have not observed despite having a greater sample size, such as a greater gray matter tissue density in right sensorimotor area, right thalamus and white-matter tissue density differences in parietal lobule, fusiform gyrus, lentiform nucleus and pons (Matochik *et al.* 2005). Discrepancies could be explained by differences in cannabis use parameters (such as pattern of cannabis use, early onset), sociodemographic features (we included only Caucasian subjects that were on average 5 years younger) and sample characteristics (i.e. sample size).

No other structural differences between the chronic cannabis users and healthy controls were found using our VBM approach, but it has been described both positive and negative results when studies investigated specific regions, such as hippocampus, parahippocampus, amygdala and cerebellum [for review see (Lorenzetti *et al.* 2010; Martin-Santos *et al.* 2010)].

Our study has some limitations. Firstly, we use a relatively small sample size for a structural neuroimaging study; however, the strength of our observed findings instills confidence in their validity. The results cannot be generalized to all chronic cannabis users as our sample was comprised of a group of male early-onset regular cannabis users without the confounding effect of other drug use and neurological or other psychiatric illnesses. The cross-sectional design does not allow us to address the question whether cannabis abuse alters brain morphology although its impact on normal neurodevelopment or if the observed structural differences are preexistent, causing individuals to be more prone to develop cannabis dependence (Cheetham *et al.* 2012). Overall, despite methodological differences across previous structural studies, findings appears to support of the idea that regular cannabis use may have a modulatory structural effect on specific brain regions, and that the $Val^{158}Met$ polymorphism may play a particular role in the sensitivity of these effects of cannabis on brain morphology.

In summary, our findings support recent reports of neuroanatomical changes associated with cannabis use

and, for the first time, reveal that these changes may be influenced by the COMT genotype. Further prospective, longitudinal research is needed to examine the gene-environment influence and the mechanisms of long-term cannabis related brain impairment.

Acknowledgements

This paper has been partially supported by grants: Ministerio de Sanidad y Consumo, PNSD: PI101/2006 and PNSD: PI041731/2011 (R.M-S.), PNSD: SOC/3386/2004 (M.F.), Fondo de Investigación Sanitaria, ISCIII-FEDER, RTA:RD06/0001/1009 (M.T.). DIUE de la Generalitat de Catalunya (2009 SGR 718 (R.D.T., M.F.) and SGR 1435 (R.M.-S., J.A.C.); SGR 2009/719 (R.D.T., M.F.); C.S-M. is funded by a Miguel Servet contract from the Carlos III Health Institute (CP10/00604). B.J.H. is supported by a National Health and Medical Research Council of Australia (NHMRC) Clinical Career Development Award (I.D. 628509). J.A.C receives a CNPq (Brazil) productivity award (IC). None of the grants had further role in the study design, collection, analysis and interpretation of data, writing of the manuscript and decision to submit it for publication. The authors disclose no competing financial interests.

Authors Contribution

RM-S, CS-M, MF and JP were responsible for the study design. ABF, ML-S and LBH contributed to the acquisition of the clinical and neuroimaging data. CS-M, MLS, LBH and AB performed the neuroimaging and statistical analysis. AB, CS-M and RM-S drafted the manuscript. SB, JP, BJH and JAC provided critical revision of the manuscript for important intellectual content. All the authors contributed and critically reviewed the content and have approved the final version of the manuscript.

References

American Psychiatric Association (2000) Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), 4th edn. Washington, DC: American Psychiatic Association.

Bangalore SS, Prasad KM, Montrose DM, Goradia DD, Diwadkar VA, Keshavan MS (2008) Cannabis use and brain structural alterations in first episode schizophrenia—a region of interest, voxel based morphometric study. Schizophr Res 99: 1–6.

Barnes A, Isohanni M, Barnett JH, Pietilainen O, Veijola J, Miettunen J, Paunio T, Tanskanen P, Ridler K, Suckling J, Bullmore ET, Murray GK, Jones PB (2012) No association of COMT (Val158Met) genotype with brain structure differences between men and women. PLoS ONE 7:e33964.

Bhattacharyya S, Crippa JA, Allen P, Martin-Santos R, Borgwardt S, Fusar-Poli P, Rubia K, Kambeitz J, O'Carroll C, Seal ML, Giampietro V, Brammer M, Zuardi AW, Atakan Z, McGuire PK (2012) Induction of psychosis by {delta}9-tetrahydrocannabinol reflects modulation of prefrontal and striatal function during attentional salience processing. Arch Gen Psychiatry 69:27–36.

Bhattacharyya S, Crippa JA, Martin-Santos R, Winton-Brown T, Fusar-Poli P (2009a) Imaging the neural effects of cannabinoids: current status and future opportunities for psychopharmacology. Curr Pharm Des 15:2603–2614.

Bhattacharyya S, Fusar-Poli P, Borgwardt S, Martin-Santos R, Nosarti C, O'Carroll C , Allen P, Seal ML, Fletcher PC, Crippa JA, Giampietro V, Mechelli A, Atakan Z, McGuire P (2009b) 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.

Bindukumar B, Mahajan SD, Reynolds JL, Hu Z, Sykes DE, Aalinkeel R, Schwartz SA (2008) Genomic and proteomic analysis of the effects of cannabinoids on normal human astrocytes. Brain Res 1191:1–11.

Bossong MG, van Berckel BN, Boellaard R, Zuurman L, Schuit RC, Windhorst AD, van Gerven JM, Ramsey NF, Lammertsma AA, Kahn RS (2009) Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. Neuropsychopharmacology 34:759–766.

Burns HD, Van Leare K, Sanabria-Bohorquez S, Hamill TG, Bormans G, Eng WS, Gibson R, Ryan C, Connolly B, Patel S, Krause S, Vanko A, Van Hecken A, Dupont P, De Lepeleire I, Rothenberg P, Stoch SA, Cote J, Hagmann WK, Jewell JP, Lin LS, Liu P, Goulet MT, Gottesdiener K, Wagner JA, de Hoon J, Mortelmans L, Fong TM, Hargreaves RJ (2007) [18F]MK-9470, a positron emission tomography (PET) tracer for in vivo human PET brain imaging of the cannabinoid-1 receptor. Proc Natl Acad Sci U S A 104:9800–9805.

Cheetham A, Allen NB, Whittle S, Simmons JG, Yücel M, Lubman DI (2012) Orbitofrontal volumes in early adolescence predict initiation of cannabis use: a 4-year longitudinal and prospective study. Biol Psychiatry 71:684–692.

Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, Kolachana BS, Hyde TM, Herman MM, Apud J, Egan MF, Kleinman JE, Weinberger DR (2004) Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet 75:807–821.

Cousijn J, Wiers RW, Ridderinkhof KR, van den Brink W, Veltman DJ, Goudriaan AE (2012) Grey matter alterations associated with cannabis use: results of a VBM study in heavy cannabis users and healthy controls. Neuroimage 59:3845–3851.

Downer E, Boland B, Fogarty M, Campbell V (2001) Delta 9-tetrahydrocannabinol induces the apoptotic pathway in cultured cortical neurones via activation of the CB1 receptor. Neuroreport 12:3973–3978.

Ehrlich S, Morrow EM, Roffman JL, Wallace SR, Naylor M, Bockholt HJ, Lundquist A, Yendiki A, Ho BC, White T, Manoach DS, Clark VP, Calhoun VD, Gollub RL, Holt DJ (2010) The COMT Val108/158Met polymorphism and medial temporal lobe volumetry in patients with schizophrenia and healthy adults. Neuroimage 53:992–1000.

Estrada G, Fatjo-Vilas M, Munoz MJ, Pulido G, Minano MJ, Toledo E, Illa JM, Martin M, Miralles ML, Miret S, Campanera S, Bernabeu C, Navarro ME, Fananas L (2011) Cannabis use and age at onset of psychosis: further evidence of interaction with COMT Val158Met polymorphism. Acta Psychiatr Scand 123:485–492.

Fumagalli F, Racagni G, Colombo E, Riva MA (2003) BDNF gene expression is reduced in the frontal cortex of dopamine transporter knockout mice. Mol Psychiatry 8:898–899.

Good CD, Johnsrude IS, Ashburner J, Henson RN, Friston KJ, Frackowiak RS (2001) A voxel-based morphometric study of ageing in 465 normal adult human brains. Neuroimage 14:21–36.

Heath RG, Fitzjarrell AT, Fontana CJ, Garey RE (1980) *Cannabis sativa*: effects on brain function and ultrastructure in rhesus monkeys. Biol Psychiatry 15:657–690.

Heng L, Beverley JA, Steiner H, Tseng KY (2011) Differential developmental trajectories for CB1 cannabinoid receptor expression in limbic/associative and sensorimotor cortical areas. Synapse 65:278–286.

Henquet C, Rosa A, Krabbendam L, Papiol S, Fananas L, Drukker M, Ramaekers JG, van Os 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.

Honea R, Crow TJ, Passingham D, Mackay CE (2005) Regional deficits in brain volume in schizophrenia: a meta-analysis of voxel-based morphometry studies. Am J Psychiatry 162: 2233–2245.

Honea R, Verchinski BA, Pezawas L, Kolachana BS, Callicott JH, Mattay VS, Weinberger DR, Meyer-Lindenberg A (2009) Impact of interacting functional variants in COMT on regional gray matter volume in human brain. Neuroimage 45:44–51.

Hong J, Shu-Leong H, Tao X, Lap-Ping Y (1998) Distribution of catechol-O-methyltransferase expression in human central nervous system. Neuroreport 9:2861–2864.

Jager G, van Hell HH, de Win MM, Kahn RS, van den Brink W, van Ree JM, Ramsey NF (2007) Effects of frequent cannabis use on hippocampal activity during an associative memory task. Eur Neuropsychopharmacol 17:289–297.

Koob GF, Volkow ND (2010) Neurocircuitry of addiction. Neuropsychopharmacology 35:217–238.

Landfield PW, Cadwallader LB, Vinsant S (1988) Quantitative changes in hippocampal structure following long-term exposure to delta 9-tetrahydrocannabinol: possible mediation by glucocorticoid systems. Brain Res 443:47–62.

Lawston J, Borella A, Robinson JK, Whitaker-Azmitia PM (2000) Changes in hippocampal morphology following chronic treatment with the synthetic cannabinoid WIN 55,212-2. Brain Res 877:407–410.

Lorenzetti V, Lubman DI, Whittle S, Solowij N, Yücel M (2010) Structural MRI findings in long-term cannabis users: what do we know? Subst Use Misuse 45:1787–1808.

Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH (2003) An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage 19:1233–1239.

Maldonado R, Berrendero F, Ozaita A, Robledo P (2011) Neurochemical basis of cannabis addiction. Neuroscience 181:1–17.

Martin-Santos R, Fagundo AB, Crippa JA, Atakan Z, Bhattacharyya S, Allen P, Fusar-Poli P, Borgwardt S, Seal M, Busatto GF, McGuire P (2010) Neuroimaging in cannabis use: a systematic review of the literature. Psychol Med 40:383–398.

Matochik JA, Eldreth DA, Cadet JL, Bolla KI (2005) Altered brain tissue composition in heavy marijuana users. Drug Alcohol Depend 77:23–30.

McIntosh AM, Baig BJ, Hall J, Job D, Whalley HC, Lymer GK, Moorhead TW, Owens DG, Miller P, Porteous D, Lawrie SM, Johnstone EC (2007) Relationship of catechol-O-methyltransferase variants to brain structure and function in a population at high risk of psychosis. Biol Psychiatry 61: 1127–1134.

Ohnishi T, Hashimoto R, Mori T, Nemoto K, Moriguchi Y, Iida H, Noguchi H, Nakabayashi T, Hori H, Ohmori M, Tsukue R, Anami K, Hirabayashi N, Harada S, Arima K, Saitoh O, Kunugi H (2006) The association between the Val158Met polymorphism of the catechol-O-methyl transferase gene and morphological abnormalities of the brain in chronic schizophrenia. Brain 129:399–410.

Pezawas L, Verchinski BA, Mattay VS, Callicott JH, Kolachana BS, Straub RE, Egan MF, Meyer-Lindenberg A, Weinberger DR (2004) The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. J Neurosci 24:10099–10102.

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

Raznahan A, Greenstein D, Lee Y, Long R, Clasen L, Gochman P, Addington A, Giedd JN, Rapoport JL, Gogtay N (2011) Catechol-o-methyltransferase (COMT) val158met polymorphism and adolescent cortical development in patients with childhood-onset schizophrenia, their non-psychotic siblings, and healthy controls. Neuroimage 57:1517–1523.

Santiago M, Matarredona ER, Granero L, Cano J, Machado A (2000) Neurotoxic relationship between dopamine and iron in the striatal dopaminergic nerve terminals. Brain Res 858:26–32.

Solowij N, Fernandez F, Murray R, Yücel M (2012) Genetic modulation of the long-term effects of cannabis on brain structure, function and symptomatology. Schizophr Res 136 (Suppl 1):S134.

Solowij N, Yücel M, Respondek C, Whittle S, Lindsay E, Pantelis C, Lubman DI (2011) Cerebellar white-matter changes in cannabis users with and without schizophrenia. Psychol Med 41: 2349–2359.

Stone JM, Bhattacharyya S, Barker GJ, McGuire PK (2012) Substance use and regional gray matter volume in individuals at high risk of psychosis. Eur Neuropsychopharmacol 22:114–122.

Szeszko PR, Robinson DG, Sevy S, Kumra S, Rupp CI, Betensky JD, Lencz T, Ashtari M, Kane JM, Malhotra AK, Gunduz-Bruce H, Napolitano B, Bilder RM (2007) Anterior cingulate grey-matter deficits and cannabis use in first-episode schizophrenia. Br J Psychiatry 190:230–236.

Tanda G, Pontieri FE, Di CG (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. Science 276:2048–2050.

Torrens M, Serrano D, Astals M, Perez-Dominguez G, Martin-Santos R (2004) Diagnosing comorbid psychiatric disorders in substance abusers: validity of the Spanish versions of the Psychiatric Research Interview for Substance and Mental Disorders and the Structured Clinical Interview for DSM-IV. Am J Psychiatry 161:1231–1237.

Tunbridge EM, Harrison PJ, Weinberger DR (2006) Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond. Biol Psychiatry 60:141–151.

Watson SJ, Benson JA, Jr, Joy JE (2000) Marijuana and medicine: assessing the science base: a summary of the 1999 Institute of Medicine report. Arch Gen Psychiatry 57:547–552.

Wechsler D (1997) WAIS-III & WMS-III Technical Manual. San Antonio, TX: The Psychological Corporation.

Wilson W, Mathew R, Turkington T, Hawk T, Coleman RE, Provenzale J (2000) Brain morphological changes and early marijuana use: a magnetic resonance and positron emission tomography study. J Addict Dis 19:1–22.

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

Zhou QY, Palmiter RD (1995) Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. Cell 83:1197–1209.

Zinkstok J, Schmitz N, van Amelsvoort T, de Win M, van den Brink W, Baas F, Linszen D (2006) The COMT val158met polymorphism and brain morphometry in healthy young adults. Neurosci Lett 405:34–39.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 Regions of gray matter volume change in cannabis users superimposed on selected slices of a normalized brain. Cannabis users showed a gray matter volume increase in the postcentral gyrus (peak at x, y, z = −48, −36, 54; $t = 4.60$; $P_{(\text{uncorrected})} < 0.001$). Voxels with $P < 0.001$(uncorrected) are displayed. Regional volumes were adjusted to age and total intracranial volume. Color bar represents t value. L indicates left hemisphere

Figure S2 Correlation in chronic cannabis users of gray matter volume with lifetime cannabis use (log transformed) superimposed on selected slices of a normalized brain. (a) The figure shows the cluster of correlation between regional gray matter volume and log [lifetime cannabis use (joints)] located in the most caudal portion of the rectal gyrus (peak at x, y, z = 11, 11, −23; $t = 3.94$; r = 0.502). (b) Plot depicting the correlation between gray matter volume in the subgenual cingulate cortex and log [lifetime cannabis use ('joints')]. Voxels with $P < 0.001$ (uncorrected) are displayed. Regional volumes were adjusted to age and total intracranial volume. Color bar represents t value. L indicates left hemisphere

7

Study 5

Epistatic influence of COMT and DAT1 gene variations on hippocampal volume in chronic cannabis users: a gene-gene environment interaction

Under review

Study 5

Summary

Reference

Title: Epistatic influence of COMT and DAT1 gene variations on hippocampal volume in chronic cannabis users: a gene-gene-environment interaction. Authors: Batalla A, Lorenzetti V, Yücel M, Soriano-Mas C, Bhattacharyya S, Torrens M, Crippa JA, Martín-Santos R. *Under review*.

Aims

Moving forward in *hypothesis* #3 and based on the results of the previous study, in the present work we aimed to explore whether variation in the COMT and DAT1 genes interact to moderate individual differences in brain volume in the hippocampus, an area particularly vulnerable to heavy cannabis exposure.

Method

Recruitment of participants, assessments, inclusion and exclusion criteria and determination of the COMT polymorphism are analogous to the previous study (98).

DAT1 VNTR genotyping was performed using polymerase chain reaction (PCR). Primers used were Forward 5'- FAM- TGTGGTGTAGGGAACGGCCTGAG, reverse 5'-CTTCCTGGAGGTCACGGCTCAAGG. Amplification conditions were 35 cycles of 30 s at 95ºC, 40 s at 58ºC, 45 s at 72ºC and 5 min at 72ºC, with an initial denaturation step of 5 min at 95ºC. A 10 µl total reaction volume was used and, after PCR, the products of allelic-specific amplifications (allele 9R, 450 bp; allele 10R, 480 bp) were detected on an automatic ABI 3730XL capillary sequencer and analysed by GeneMapper Software v3.5 (Applied Biosystems).

After genotype determination, the sample was divided in subgroups based on COMT, DAT1 and COMT-DAT1 genotypes. COMT genotype participants were grouped into *val* homozygote and *met*-allele carriers (i.e., val/met and *met* homozygous), and DAT1 genotype into 9-repeat and 10-repeat allele.

MRI acquisition and image preprocessing were also analogous to the previous study (98). However, the hippocampus was manually delineated using Analyze software (Analyze Version 9.0, Mayo Clinic, Rochester, MN). Hippocampi were traced by the same investigator (Albert Batalla), while being blind to group membership. Intraclass correlation coeficients (ICC, absolute agreement) were higher than 90% for intra-rater reliability and inter-rater reliability against an experienced hippocampus tracer (Valentina Lorenzetti). Manual tracing of the hippocampus was performed based on a previously validated protocol (99, 100).

A series of repeated measures ANCOVAs were performed to examine the impact of cannabis and genetic polymorphisms on the hippocampus using group (i.e., cannabis users vs. controls) and genetic polymorphism (i.e., val/val vs. *met*-carriers; and 9-repeat carriers vs. 10-repeat homozygous) as between-group factors, hippocampal volumes as the dependent variable, and hemisphere (i.e., left and right) as a repeated measure to investigate the effects of interest across both hemispheres. TIV was retained as a covariate.

To examine the impact of the interaction between cannabis use and COMT and DAT1 genotypes on the hippocampus, we utilized a cross-product of COMT and DAT1 values (i.e., val/val 10-repeat homozygous, *met*-carriers 10-repeat homozygous, val/val 9-repeat homozygous, *met*-carriers 9-repeat homozygous). Due to the small sample size of the val/val 9-repeat homozygous in chronic cannabis users (n=1), we analysed the data by combining this group with the *met*-carriers 10-repeat homozygous (n=9). Post hoc comparisons were made to confirm the patterns of interaction.

A series of partial correlations to explore the association between hippocampal volume and cannabis use patterns was also performed, while retaining TIV as a covariate

Results

30 chronic cannabis users and 29 non-using controls matched in terms of age, educational level and IQ were included in the present work. Genotype frequencies of the COMT and DAT1 genes were in Hardy-Weinberg Equilibrium in both groups. Our results showed that the interaction between COMT and DAT1 polymorphisms significantly affected hippocampal volumes depending on the individuals' exposure to cannabis. In controls, hippocampal volumes were largest in DAT1 10-repeat homozygous and val/val carriers, and smallest in DAT1 9-repeat carriers and *met* carriers. In contrast, both conjunctions were associated with the largest hippocampal volumes in chronic cannabis users (Figure 7.1). The association between these functional genotypes and hippocampal volumes suggests a linear relationship with dopamine availability in controls, which was not observed in chronic cannabis users. In addition, hippocampal volumes were smaller in cannabis users compared to controls, and the magnitude of volumetric reduction was associated with lifetime cannabis exposure.

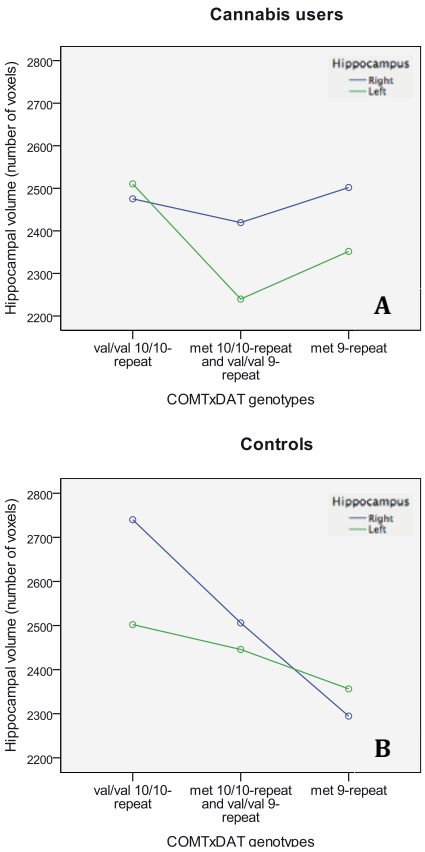


Figure 7.1. Interaction between cannabis use and COMTxDAT1 genotypes on hippocampal volume in **(A)** chronic cannabis users and **(B)** non-using controls.

Conclusion

This is the first study showing preliminary data that gene-gene interactions may mediate hippocampal volumetric alterations in chronic cannabis users, while replicating previous evidence on hippocampal morphology alterations.

Total word count: 4610

Abstract word count: 244

Number of tables: 2

Number of figures: 3

Supplementary material: 0

Epistatic influence of COMT and DAT1 gene variations on hippocampal volume in chronic cannabis users: a gene-gene-environment interaction

Running head: Epistasis between COMT and DAT1 on hippocampus

Albert Batalla, MD,[a] Valentina Lorenzetti, PhD,[b,c] Murat Yücel, PhD,[b,c] Carles Soriano-Mas, PhD,[d] Sagnik Bhattacharyya, MD, PhD,[e] Marta Torrens, MD, PhD,[f] José A. S. Crippa, MD, PhD,[g,h] Rocío Martín-Santos, MD, PhD.[a,f]

a.Department of Psychiatry, Clinical Institute of Neuroscience, Hospital Clínic, IDIBAPS, CIBERSAM, and Department of Psychiatry and Clinical Psychobiology, University of Barcelona, 08036, Spain.
b.Melbourne Neuropsychiatry Centre, Department of Psychiatry, The University of Melbourne & Melbourne Health, Melbourne, Victoria 3053, Australia.
c.Monash Clinical and Imaging Neuroscience Laboratory, School of Psychology and Psychiatry & Monash Biomedical Imaging, Monash University, Clayton Campus, Melbourne, Victoria 3800, Australia.
d.Department of Psychiatry, Bellvitge University Hospital-IDIBELL, CIBERSAM, Barcelona, 08907 Spain.
e.Department of Psychosis Studies, King's College London, Institute of Psychiatry, London, WC2R 2LS, UK.
f.Neuroscience Program, Human Pharmacology and Clinical Neuroscience and Disorders by Use of Substance, research groups, IMIM (Hospital del Mar Medical Research Institute) INAD-Parc de Salut Mar, Autonomous University of Barcelona, 08003, Spain.
g.Neuroscience and Behavior Department, University of Sao Paulo, Ribeirão Preto, Brazil.
h.INCT Translational Medicine (INCT-TM, CNPq), Brazil.

Corresponding author:

Rocío Martín-Santos, MD, PhD. Department of Psychiatry. Instituto Clínico de Neurociencias, Hospital Clinic, University of Barcelona, IDIBAPS, CIBERSAM, Calle Villarroel, 170; 08036-Barcelona. Spain. Phone number: (34) 93 227 55 47. Fax number: (34) 93 227 55 48. Email: rmsantos@clinic.ub.es

Abstract

Genetic variations in dopaminergic candidate genes and environmental factors including regular exposure to cannabis are known to be associated with structural alterations in the hippocampus. Here, we examine the interaction between the functional polymorphisms of catechol-O-methyltransferase (COMT) and dopamine transporter (DAT1) genes and how these interactions affect hippocampal volume in chronic cannabis users compared to non cannabis-using controls. The sample consisted of 59 male Caucasians aged between 18-30 years, 30 of which were chronic and early-onset cannabis users (all initiated cannabis use prior to age 16) and 29 age-, education- and intelligence-matched controls. We performed COMT and DAT1 genotyping and computed hippocampal volumes via well-validated manual tracing methods. Our results showed that the interaction between COMT and DAT1 polymorphisms significantly affected hippocampal volumes depending on the individuals' exposure to cannabis. In controls, hippocampal volumes were largest in DAT1 10-repeat homozygous and val/val carriers, and smallest in DAT1 9-repeat carriers and *met* carriers. In contrast, both conjunctions were associated with the largest hippocampal volumes in chronic cannabis users. The association between these functional genotypes and hippocampal volumes suggests a linear relationship with dopamine availability in controls which was not observed in chronic cannabis users. Furthermore, hippocampal volumes were smaller in cannabis users compared to controls, and the magnitude of volumetric reduction was associated with lifetime cannabis exposure. This is the first study showing preliminary data that gene-gene interactions may mediate hippocampal volumetric alterations in chronic cannabis users, while replicating previous evidence on hippocampal morphology alterations.

1. Introduction

Cannabis is currently the most widely available and used illicit drug worldwide, with the majority of users commencing cannabis consumption during adolescence (European Monitoring Centre for Drugs and Drug Addiction, 2012). Animal studies (Adriani and Laviola, 2004; Landfield et al., 1988) and later human studies (Cousijn et al., 2012; Stone et al., 2012; Yucel et al., 2008) have provided evidence that brain anatomical alterations occur in association with chronic cannabis exposure. These alterations implicate most consistently regions where CB_1 receptors are highly concentrated, including the prefrontal cortex, basal ganglia, medial temporal areas (e.g., hippocampus and amygdala) and cerebellum (Burns et al., 2007). Of these brain areas, the hippocampus appears to be particularly vulnerable to heavy cannabis exposure. There have been multiple reports of reduced hippocampal volume in chronic cannabis users (Batalla et al., 2013a; Lorenzetti et al., 2013), with some evidence that these effects may be dose-dependent (Ashtari et al., 2011; Cousijn et al., 2012; Yucel et al., 2008) and persist beyond prolonged abstinence (Ashtari et al., 2011). This is consistent with evidence from a number of studies that acute administration of cannabis ingredients affect hippocampal function measured using functional MRI independent of the specific cognitive paradigm tested (Bhattacharyya et al., 2012b). Notably, brain alterations related to early and chronic cannabis exposure may result in persistent neuropsychological, emotional and motivational impairment (Meier et al., 2012). Identifying the factors determining brain volumetric alterations in chronic cannabis users, which may include genetic influences (Batalla et al., 2013b), would improve our understanding of which vulnerability factors might mediate persistent adverse outcomes observed in chronic cannabis users.

Variations in the expression of genes implicated in the regulation of neurotransmitters may play an important role in determining individual variability in brain morphology (Durston et al., 2005; Honea et al., 2009; Kambeitz et al., 2012). Dopaminergic function has been shown to influence brain structure and plasticity (Scheepers et al., 2001), hence variation in dopaminergic candidate genes might be related to volumetric variations in the brain. Dopamine inactivation from the extracellular space involves both catechol-O-methyltransferase (COMT) and the

dopamine transporter (DAT1), both of which are expressed in the hippocampus, particularly in the dentate gyrus (Matsumoto et al., 2003). The COMT (*Val*^{158}*Met*, rs4680) gene displays a single-nucleotid polymorphism, which results in three genotypes (val/val, val/met, and met/met) (Chen et al., 2004). Whereas the met/met variant shows a 40% lower enzymatic activity, which is associated with high levels of extrasynaptic dopamine, the val/val variant implies higher enzymatic activity, which results in low levels of extrasynaptic dopamine (Chen et al., 2004). The DAT1 gene displays a polymorphic 40-base pair (bp) variable number of tandem repeats (VNTR) in an untranslated region (UTR). This polymorphism consists of a repetition of 40 bp that leads to several alleles, 9- and 10-repeat alleles being the most common (Vandenbergh et al., 1992). The 10-repeat allele has been associated with increased gene expression both in vitro and in vivo (Heinz et al., 2000).

These dopamine-regulating genes have shown to influence cognitive function (Bertolino et al., 2006; Prata et al., 2009a) and even interact with each other in the modulation of cortical activity in several brain regions (Bertolino et al., 2006; Bertolino et al., 2008; Prata et al., 2009b), including the hippocampus (Bertolino et al., 2008). In addition, it has been reported that these functional polymorphisms may affect brain volume in healthy individuals (Durston et al., 2005; Honea et al., 2009), as well as in some psychiatric disorders such as schizophrenia (Ohnishi et al., 2006), attention deficit hyperactivity disorder (Durston et al., 2005; Shook et al., 2011), and subjects at risk for psychosis (McIntosh et al., 2007).

Current evidence suggests that cannabis exposure is an environmental factor that may affect brain structure in interaction with genetic polymorphisms (Batalla et al., 2013b). Using an automated approach, we recently demonstrated an opposite pattern of influence of the COMT polymorphism on subcortical and medial temporal volumes in a group of early-onset chronic cannabis users compared with non-using controls (Batalla et al., 2013b). Notably, the presence of more copies of the *val* allele was associated with reduced volume of the ventral caudate nucleus in chronic cannabis users and increased volume in the same region in controls, while the opposite pattern was found in regard to the left amygdala (Batalla et al., 2013b). In a previous study, we also reported evidence of modulation of the acute effect of the main psychoactive

ingredient of cannabis on hippocampal function during the encoding and recall of new information (Bhattacharyya et al., 2012a). In the current study, we used high-resolution structural magnetic resonance imaging (MRI) to assess volumetric changes in the hippocampus, the most susceptible region to the neurotoxic effects of cannabis exposure. To our knowledge, the potential epistasis between COMT and DAT1 polymorphisms on hippocampal volume in subjects chronically exposed to cannabis has never been studied.

The aim of the present study was to: (i) investigate whether variation in the COMT and DAT1 genes interact to moderate individual differences in hippocampal volume; and (ii) whether the nature of this association depends on previous exposure to cannabis. On the basis of evidence that variation in dopaminergic genes may modulate brain morphology, and that cannabis is an environmental factor that may alter brain structure by interacting with those genes, we hypothesized that COMT and DAT1 functional polymorphisms would have an epistatic interaction between their effects on hippocampal volume that would be differentially influenced by cannabis use. We further tested the hypothesis that both polymorphisms would have significant associations with hippocampal volume separately, and that this association would occur in opposite direction depending on whether there was previous exposure to cannabis or not. Finally, we hypothesized that cannabis users would have reduced hippocampal volumes relative to controls.

2. Experimental Procedures

2.1. Subjects

Participants were recruited by advertisement in the general community (web page and distribution of flyers) and screened in a comprehensive telephone interview examining socio-demographic data and level of substance exposure to determine study eligibility. Participants included in the study underwent a detailed medical history check, routine laboratory tests, physical/neurological examinations, and urine and hair toxicology screens via immunometric assay kits (Instant-View; ASD Inc,

Poway, California). Hair testing was performed to corroborate repeated cannabis consumption reported in the group of chronic cannabis users and non-consumption in controls. Lifetime exposure to substances was systematically assessed using an *ad-hoc* questionnaire. We utilised standardized units to quantify substance exposure in the sample: number of daily cigarettes for tobacco use; number of standard units of alcohol/week for ethanol exposure; and number of daily and weekly "joints" for cannabis use.

Inclusion criteria for all participants were: male gender, age between 18 and 30 years, Caucasian ethnicity, IQ score > 90, lifetime exposure to psychoactive substances other than cannabis, nicotine or alcohol inferior to 5 occasions. Cannabis users were included if they met the following criteria: onset of cannabis use before 16 years of age; consumption of 14 to 28 weekly "joints" during at least the last two years and continued until entry into the study; outcome from urine toxicology screen resulting positive for cannabinoids but negative for opiates, cocaine, amphetamines and benzodiazepines on the day of the assessment. Control subjects were included if their exposure to cannabis did not exceed 15 lifetime episodes of cannabis use and if they had not used in the past month. All controls had a negative test on urine drug screen for opiates, cocaine, amphetamines, benzodiazepines and cannabinoids.

Exclusion criteria for all participants were: any lifetime Axis I disorder (substance and non-substance use disorders) according to the Diagnostic and Statistical Manual for Mental Disorders - Fourth Edition (DSM-IV) except for nicotine use disorder, which was assessed with a structured psychiatric interview (PRISM) (Torrens et al., 2004); use of psychoactive medications; history of chronic medical illness or neurological conditions that might affect cognitive function; head trauma with loss of consciousness >2 min; learning disability or mental retardation; left-handedness; and uncorrected visual impairment; colour-blindness; or hearing impairment. Subjects also completed the vocabulary subscale of the WAIS-III to provide an estimate of verbal intelligence (Wechsler, 1997).

All participants provided written informed consent after receiving a complete description of the study and having discussed questions or issues, if any. Upon

completion of the study all subjects received a financial compensation for any costs incurred during participation. The study was approved by the Ethical and Clinical Research Committee of our institution (CEIC-Parc de Salut Mar and Hospital Clínic).

2.2. Genotyping Methods

Genomic DNA was extracted from peripheral blood leukocytes of all the participants by using Flexi Gene DNA kit (Qiagen Iberia, S.L., Spain) according to the manufacturer's instructions. We determined the *COMT Val^{158}Met* single nucleotide polymorphism (SNP) allelic variants by using the 5' exonuclease TaqMan assay with ABI 7900HT Sequence Detection System (Real Time PCR) supplied by Applied Biosystems. Primers and fluorescent probes were obtained from Applied Biosystems with TaqMan SNP Genotyping assays (assay ID C_2255335_10). Reaction conditions were those described in the ABI PRISM 7900HT user's guide. Endpoint fluorescent signals were detected on the ABI 7900, and the data analyses were performed with the software Sequence Detector System (version 2.3, Applied Biosystems). DAT1 VNTR genotyping was performed using polymerase chain reaction (PCR) as described previously by Vandenbergh and colleagues (1992). Briefly, primers used were Forward 5'- FAM- TGTGGTGTAGGGAACGGCCTGAG, reverse 5'- CTTCCTGGAGGTCACGGCTCAAGG. Each reaction mixture contained: 1x PCR amplification buffer and 0.3x PCR enhancer solution (Invitrogen, Carlsbad, CA), 3 mM $MgSO_4$, 200 mM dNTPs, 0.2 mM l of each primer, 1U of Taq DNA polymerase (Invitrogen) and 50 ng of genomic DNA as template. Amplification conditions were 35 cycles of 30 s at 95ºC, 40 s at 58ºC, 45 s at 72ºC and 5 min at 72ºC, with an initial denaturation step of 5 min at 95ºC. A 10 µl total reaction volume was used and, after PCR, the products of allelic-specific amplifications (allele 9R, 450 bp; allele 10R, 480 bp) were detected on an automatic ABI 3730XL capillary sequencer and analysed by GeneMapper Software v3.5 (Applied Biosystems).

After genotype determination, the sample was divided in subgroups based on COMT, DAT1 and COMT-DAT1 genotypes, consistently with a series of earlier studies assessing the implication of these polymorphisms for the human brain structure and

function (Bertolino et al., 2008; Prata et al., 2009b; Wetherill et al., 2012). COMT genotype participants were grouped into *val* homozygote and *met*-allele carriers (i.e., val/met and *met* homozygous), and DAT1 genotype into 9-repeat and 10-repeat allele.

2.3. Image data acquisition

MRI images were acquired with a 1.5T Signa Excite system (General Electric, Milwaukee, Wisconsin) equipped with an 8-channel phased-array head coil. High-resolution T1-weighted anatomical images were obtained by using a three-dimensional fast spoiled gradient inversion-recovery prepared sequence with 130 contiguous slices (TR, 11.8 milliseconds; TE, 4.2 milliseconds; flip angle, 15°; field of view, 30 cm; 256x256 pixel matrix; slice thickness, 1.2 mm).

2.4. Preprocessing

After acquisition, images were transferred to a Linux workstation for data processing. All MRI data was aligned to the Montréal Neurological Institute standard template using FSL's brain extraction technique (www.fmrib.ox.ac.uk/fsl), to enable a standardized application of the brain tracing protocol across all participants.

2.5. Manual volumetry and reliability values

Manual delineation of the hippocampus was then performed using Analyze software (Analyze Version 9.0, Mayo Clinic, Rochester, MN). Hippocampi were traced by the same investigator (AB), while being blind to group membership and based on a previously validated protocol (Velakoulis et al., 1999; Velakoulis et al., 2006). Intra-class correlation coefficients (ICC, absolute agreement), as computed based on ten randomly selected images, were for right and left hemisphere, 0.96 and 0.95 respectively for intra-rater reliability; and 0.94 and 0.90 for inter-rater reliability against an experienced hippocampus tracer (VL).

Manual tracing of the hippocampus was performed on coronally displayed MRI slices and proceeded on a caudal to rostral direction. Hippocampal volumes were computed by summing the number of voxels included in the traced hippocampus across MRI slices. The protocol developed by Watson and colleagues (1992) was applied to separate the hippocampus from the amygdala (Watson et al., 1992).

The CA-1 through CA-4 sectors of the hippocampus proper were included in the tracing, while the subiculum was excluded. Key hippocampal boundaries were determined as follows: medially, by the cerebral spinal fluid (CSF) of the uncal cistern; laterally, by the CSF in the temporal horn of the lateral ventricle; inferiorly by the parahippocampal white matter running medially from the temporal horn of the lateral ventricle.

Intracranial volumes were obtained after processing imaging data with the VBM toolbox (http://dbm.neuro.uni-jena.de/vbm/). Specifically, native-space magnetic resonance images were segmented and normalized to the SPM-T1 template using a high-dimensional DARTEL transformation and the Jacobian determinants derived from the spatial normalization to modulate image voxel values and thus restore volumetric information (affine and non-linear) (Good et al., 2001). Subsequently, global grey matter, white matter and cerebrospinal fluid volumes were calculated by integrating the voxel values from the corresponding image segments (using an in-house MATLAB code). The volumes were then added up to obtain the TIV of each participant.

2.6. Statistical analyses

Descriptive results are presented as means (standard deviation) for continuous variables and frequencies (absolute, relative) for categorical variables. For all the statistical analyses, the threshold of significance was set at $p<0.05$.

A series of repeated measures ANCOVAs were performed to examine the impact of cannabis and genetic polymorphisms on the hippocampus using group (i.e., cannabis users vs. controls) and genetic polymorphism (i.e., val/val vs. *met*-carriers; and 9-

repeat carriers vs. 10-repeat homozygous) as between-group factors, hippocampal volumes as the dependent variable, and hemisphere (i.e., left and right) as a repeated measure to investigate the effects of interest across both hemispheres. TIV was retained as a covariate, given the positive association between regional brain volumes and head size (Mathalon et al., 1993).

To examine the impact of the interaction between cannabis use and COMT and DAT1 genotypes on the hippocampus, we utilized a cross-product of COMT and DAT1 values (i.e., val/val 10-repeat homozygous, *met*-carriers 10-repeat homozygous, val/val 9-repeat homozygous, *met*-carriers 9-repeat homozygous). Due to the small sample size of the val/val 9-repeat homozygous in chronic cannabis users (n=1), we analysed the data by combining this group with the *met*-carriers 10-repeat homozygous (n=9). Post hoc comparisons were made to confirm the patterns of interaction.

We performed a series of partial correlations to explore the association between hippocampal volume and cannabis use patterns (i.e. cumulative number of joints past month, past year and over lifetime), while retaining TIV as a covariate.

3. Results

3.1. Sample characteristics

The final sample included 59 subjects: 30 early-onset cannabis users and 29 drug-free control subjects. Main demographic and drug use characteristics are described in Table 1. No differences were found in demographic and drug use variables between groups except for alcohol and tobacco use. However, none of the participants in either group met lifetime criteria for alcohol abuse or dependence and weekly use was always below the risk dose of 28 standard units of alcohol. On average, cannabis users smoked no more than seven regular cigarettes per day (range = 0-20). Only three participants smoked more than 10 regular cigarettes per day (two cases and one control subject).

Table 1. Sociodemographic and drug use characteristics.

	Cannabis users Mean/N (SD/%)	**Controls** Mean/N (SD/%)	$t_{df=58}/\chi^2$	***p***
Age	21.0 (2.3)	22.4 (3.3)	1.84	0.071
Males	30 (100)	29 (100)	-	-
Cannabis use				
Onset of use (age, years)	15.0 (1.1)	16.7 (2.0)	2.96	0.01
Total lifetime cannabis use (number of joints)	5203 (4192)	5.1 (11.3)	6.68	< 0.001
Onset regular use (age, years)	18.1 (2.0)	-	-	-
Duration of use (years)	5.7 (2.4)	-	-	-
Current cannabis use (joints/day)	2.5 (1.5)	-	-	-
Alcohol use				
Age of onset of use	15.0 (1.1)	15.7 (1.5)	2.20	0.032
Duration of use	5.7 (2.3)	6.2 (3.1)	0.68	0.498
Alcohol units per week	5.3 (3.8)	3.2 (2.6)	2.35	0.023
Tobacco use				
Current smokers	27 (90.0)	9 (31.0)	21.6	< 0.001
Age of onset of use	16.3 (1.5)	16.3 (2.2)	0. 57	0.955
Duration of use (years)	4.5 (2.7)	4.9 (3.3)	0.34	0.737
Cigarettes per day	6.0 (5.0)	2.4 (5.9)	1.79	0.082

Table 2. COMT and DAT1 genotype distribution.

	Cannabis users (n = 30)	**Controls** (n = 29)	***p***
COMT Val108/158 Met			0.942
met-carriers	23	22	
val/val	7	7	
DAT1 3' UTR VNTR			0.509
9-repeat carriers	15	13	
10/10-repeat	13	16	
COMTxDAT1			0.291
val/val 10/10-repeat	4	3	
met 10/10-repeat & val/val 9-repeat	10	17	
met 9-repeat	14	9	

Genotype frequencies in groups COMT, DAT1 and COMT-DAT1 are presented in Table 2. Control participants included: 9-repeat carriers- 13 (of which four were val/val and nine *met*-carriers); 10-repeat homozygotes- 16 (3 val/val and 13 *met*-carriers). Chronic cannabis users included: 9-repeat carriers- 15 (1 val/val and 14 *met*-carriers); 10-repeat homozygotes- 13 (four val/val and nine *met*-carriers). The allelic distribution of both genes was in Hardy-Weinberg Equilibrium.

3.2. COMT x DAT1 and chronic cannabis use between-group interactions

We found a significant interaction between COMT and DAT1 functional polymorphisms in affecting bilateral hippocampi, which was mediated by cannabis use (F=3.62; p=.034). Specifically, the association between genetic functional

polymorphisms and hippocampal volumes was linear in controls but not in cannabis users (Figure 1). While the conjunctions 'DAT1 10-repeat homozygous and val/val' and 'DAT1 9-repeat carriers and *met*-carriers' were associated with the largest and the smallest volumes in controls respectively, both conjunctions were associated with the largest volumes in cannabis users.

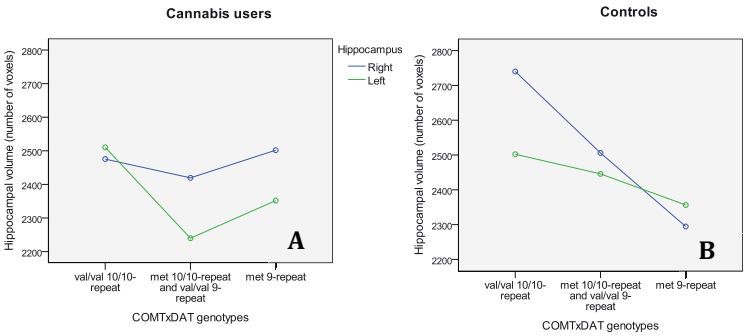


Figure 1. Interaction between cannabis use and COMTxDAT1 genotypes on hippocampal volume in **(A)** chronic cannabis users and **(B)** non-using controls.

Post hoc analyses showed that *met* 9-repeat carriers had smaller right hippocampal volume compared to val/val 10-repeat homozygous (p=.011) and intermediate genotypes (*met* 10/10-repeat and val/val 9-repeat) (p=.027) in the control group. Within the group of cannabis users, the val/val 10-repeat homozygous had larger left hippocampal volumes compared to intermediate genotypes (p=.049). We did not find any other volumetric differences between genotypes.

3.3. Individual effects of DAT1 and COMT genotypes

When DAT1 and COMT were studied separately, we found a trend for an association between the DAT1 polymorphism and bilateral hippocampal volumes in

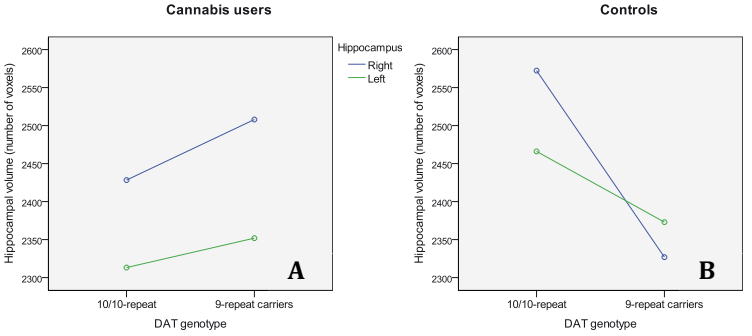


Figure 2. DAT1 genotype influence on hippocampal volume in **(A)** chronic cannabis users and **(B)** non-using controls.

both groups, in opposite directions (F=3.17; p=.081). In particular, we found that DAT1 10-repeat homozygous were associated with larger hippocampal volumes compared to 9-repeat carriers in controls, while the reverse pattern was observed in chronic cannabis users (Figure 2). No association was found between COMT and hippocampal volume.

3.4. Hippocampal volume and lifetime cannabis use

Irrespective of genotype, chronic cannabis users tended to have a smaller hippocampus than controls in the left hemisphere (p=.068). In addition, we observed a negative correlation between hippocampal volume and lifetime cannabis exposure (r=-0.38; p=.046) (Figure 3).

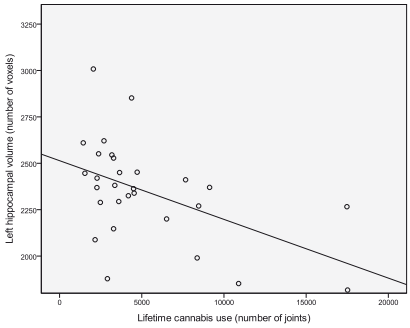


Figure 3. Relationship between left hippocampal volume and lifetime cannabis use.

4. Discussion

This study provides preliminary evidence of the influence of COMT *Val*^{158}*Met* and DAT1 3' UTR VNTR functional polymorphisms on hippocampal volume in a group of early-onset chronic cannabis users compared with non-using controls. There was an interaction between COMT and DAT1 functional polymorphisms that influenced hippocampal volumes bilaterally. Importantly, this association occurred in opposite directions depending on whether or not the individual had been regularly exposed to cannabis. Controls showed a linear relationship between dopamine availability and hippocampal volume that was not observed in cannabis users. In addition, the left hippocampus was reduced in cannabis users and there was a negative association between hippocampal volume and lifetime cannabis exposure.

The results indicate a significant interaction between COMT and DAT1 genotypes dependent on chronic cannabis use affecting bilateral hippocampal volume. Animal and human studies have demonstrated that the neurotoxic effects induced by cannabis are particularly prominent within the hippocampus (Batalla et al., 2013a; Landfield et al., 1988; Lorenzetti et al., 2013), a region with a high density of CB_1 receptors (Burns et al., 2007). The precise mechanisms underlying hippocampal morphological changes remain unclear, but may be related to the modulatory effect of cannabis and the endocannabinoid system on dopaminergic neurotransmission (Bhattacharyya et al., 2012a; Bhattacharyya et al., 2009; Bloomfield et al., 2013).

Several preclinical studies have reported the impact of variations in dopamine neurotransmission, especially extracellular dopamine concentrations, on neuronal growth and survival (Santiago et al., 2000). Chronically elevated extracellular dopamine concentrations are neurotoxic (Santiago et al., 2000), and animal knockout models with reduced dopamine signaling show important impairments in neuronal differentiation (Zhou and Palmiter, 1995). COMT and DAT1 genotypes have a crucial role in determining the extracellular concentration of dopamine. The combination of the two genotypes associated with the most active forms of COMT and DAT1 (val/val and 10-repeat) would be associated with maximal dopamine removal and the lowest dopamine levels. Conversely, the *met* 9-repeat combination would be associated with the least effective dopamine removal and maximal cortical dopamine levels (Prata et al., 2009b). Interestingly, we found that these latter combinations had a linear relationship in controls, with greater dopamine availability being associated with smaller bilateral hippocampal volumes. In contrast, chronic cannabis users did not show this pattern, as these combinations were associated with the largest hippocampal volumes. Subjects under chronic cannabis exposure apparently showed a U-shaped relationship between cortical volume and dopamine availability, such that the combination of genotypes associated with intermediate levels of dopamine was associated with smaller hippocampal volumes. Post hoc analysis pointed out the linear relationship in the right hippocampus of controls, but did not confirm the U-shaped relationship in chronic cannabis users. However, chronic cannabis users showed an opposite pattern compared to controls in the left hippocampus, as

genotypes related to intermediate levels of dopamine were associated with smaller hippocampal volumes compared to those associated with the lowest dopamine levels.

These results are consistent with our previous study, in which we demonstrated that the COMT polymorphism influenced the volume of the bilateral caudate nucleus and the left amygdala in opposite directions between chronic cannabis users and controls (Batalla et al., 2013b). Overall, these results suggest that the possible influence of the interaction between dopaminergic genes on brain development may be modulated by cannabis use. Individual differences in the expression of COMT and DAT1 genes may imply different liability to present volumetric alterations under chronic cannabis exposure.

To the best of our knowledge, no previous studies have reported the influence of the COMT x DAT1 interaction in terms of structural neuroimaging. Only one study explored the epistasis between two dopamine-related genes in mediating the caudate volume (Bertolino et al., 2009). In that study, which involved healthy adults, Bertolino and colleagues (2009) found that caudate volume was reduced in 10-repeat homozygous relative to 9-repeat carriers carrying the GT variant of the D_2 receptor genotype (DRD_2), which is associated with reduced presynaptic expression. Although the opposite pattern (10/10 > 9-repeat carriers) was true for the GG variant, the effect of DAT1 was mostly evident in the context of the DRD_2 GT genotype (Bertolino et al., 2009).

Interactive effects of COMT and DAT1 genotypes have been mainly described in functional neuroimaging studies involving healthy subjects (Bertolino et al., 2006; Bertolino et al., 2008; Caldu et al., 2007; Prata et al., 2009b). For instance, additive effects of the COMT and DAT1 genotypes on activation in the precentral, anterior cingulate (Bertolino et al., 2006), and dorsolateral prefrontal cortex have been reported during working memory tasks (Bertolino et al., 2006; Caldu et al., 2007); with the *val* allele in combination with the 9-repeat being associated with the greatest activation, whereas the *met* allele combined with the 10-repeat being associated with the lowest activation in these regions. However, non-additive effects have also been described in the left parietal cortex during an overt verbal fluency task (Prata et al.,

2009b), and in the prefrontal cortex and hippocampus during a working memory task (Bertolino et al., 2008). In these studies, both combinations of *met* and 10-repeat alleles and of the *val* and 9-repeat alleles were associated with reduced brain activity (Bertolino et al., 2008; Prata et al., 2009b). These findings are consistent with the notion that cortical function may become inefficient when local dopamine activity is either unusually low or unusually high (Prata et al., 2009b).

The few studies on single polymorphisms in healthy subjects have revealed associations with the volume of brain structures. Overall, the studies based on COMT genotype reported that subjects carrying the *val* allele had smaller hippocampal volumes relative to *met* carriers (Ehrlich et al., 2010; Honea et al., 2009), although negative results have also been reported (Barnes et al., 2012; Ohnishi et al., 2006). Consistently with our previous results using VBM (Batalla et al., 2013b), we failed to identify an independent effect of COMT polymorphisms on hippocampal volume, despite the fact that our manual tracing methodology is likely to have increased the sensitivity to detect subtle alterations in hippocampal morphology. On the other hand, we described for the first time that the 10-repeat allele of the DAT1 genotype tended to be associated with larger hippocampal volume in non-using controls. In contrast, previous studies exploring the association between the DAT1 genotype and brain volume have shown that the 10-repeat allele was associated with smaller manually-traced caudate volume (Honea et al., 2009; Shook et al., 2011). However, comparisons are limited because the mentioned studies involved children and the analysis was confined to the caudate. Interestingly, subjects characterised with a chronic exposure to cannabis showed an inverse pattern, as there was a tendency toward an association between the 10-repeat allele and smaller hippocampal volumes, which is consistent with the COMT x DAT1 interaction and with our previous reports concerning the COMT genotype (Batalla et al., 2013b). Overall, these results support the notion that dopamine-regulating genes may contribute to determine brain morphology, and that this effect may be region-specific and simultaneously influenced by both genetics and environmental factors (Batalla et al., 2013b).

These data are also consistent with previous findings of reduced hippocampal volume among chronic cannabis users compared to controls (Batalla et al., 2013a;

Lorenzetti et al., 2013). While the left hippocampus was markedly reduced in the group of cannabis users, this failed to reach statistical significance, which may be due to the fact that this was a relatively young group, hence characterized by a relatively reduced duration of exposure. Samples with longer exposure to cannabis (Yucel et al., 2008) have more often demonstrated volume reductions in medial temporal brain regions compared to samples with a relatively lower quantity of smoked cannabis, more similar to our sample (Cousijn et al., 2012). To this end, there was a negative association between hippocampal volume and lifetime cannabis exposure, suggesting that with continued use this group would indeed have differences in hippocampal volumes (Ashtari et al., 2011; Cousijn et al., 2012; Yucel et al., 2008). Importantly, our results indicate that the interaction of the dopamine-regulating genes may be determinant on brain morphology and be modified by cannabis exposure; this, in turn, suggests that the combination of particular genotypes may be associated with increased liability to morphological alterations in chronic cannabis users. In particular, subjects chronically exposed to cannabis carrying the genotypes associated with intermediate extracellular dopamine levels may be more prone to present reduced hippocampal volume. This is of major importance given that reduced hippocampal volume may underlie a variety of symptoms of chronic cannabis use, including verbal learning and spatial working memory deficits (Ashtari et al., 2011; Rubino et al., 2009), and contribute to the reported neuropsychological decline (Meier et al., 2012).

Some potential limitations of the present study must be discussed at this point. We acknowledge that the relatively small number of subjects may have affected the sensitivity of our measures and prevented the detection of effects that the COMT genotype alone might have on hippocampal plasticity. Our results cannot be generalized to all chronic cannabis users, as our sample consisted of a group of male early-onset regular cannabis users without the confounding effects of other drugs and neurological or other psychiatric illnesses. The cross-sectional design does not allow us to address the question of whether cannabis abuse alters brain morphology through its impact on normal neurodevelopment or if the observed structural differences are preexistent, causing individuals to be more prone to develop cannabis

dependence (Cheetham et al., 2012). Also, additional genetic factors not covered here are also likely to contribute in determining hippocampal volume. For instance, Schacht and colleagues (2012) reported that the cannabis receptor-1 gene (CNR1) rs2023239 variation predisposes to smaller hippocampal volumes in chronic cannabis users compared to non-using controls (Schacht et al., 2012). Furthermore, homozygote *val* carriers of the Brain Derived Neurotrophic Factor (BDNF)-gene (a gene involved in reducing the amount of naturally occurring neuronal cell death), were found to have increased hippocampal volume compared to *met* carriers (Bueller et al., 2006; Szeszko et al., 2005). As we did not control for these genotypes, their contribution in determining hippocampal volume cannot be ruled out.

5. Conclusion

Overall, these preliminary findings support the notions that 1) regular cannabis use has structural modulatory effects on the hippocampus, and dopamine-regulating genes may play a particular role in the sensitivity to the effects of cannabis on brain morphology; and 2) that single genetic factors are unlikely to explain the intricate interactions between cannabis and complex phenotypes, such as brain volume or psychiatric disorders. Therefore, other genetic and non-genetic variants should be considered for inclusion in gene-environmental interaction models. Such approaches are likely to provide further insights into the mechanisms of cannabis-related brain impairment and genetic vulnerability.

References

Adriani, W., Laviola, G., 2004. Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. Behav. Pharmacol. 15, 341-352.

Ashtari, M., Avants, B., Cyckowski, L., Cervellione, K.L., Roofeh, D., Cook, P., Gee, J., Sevy, S., Kumra, S., 2011. Medial temporal structures and memory functions in adolescents with heavy cannabis use. J. Psychiatr. Res. 45, 1055-1066.

Barnes, A., Isohanni, M., Barnett, J.H., Pietilainen, O., Veijola, J., Miettunen, J., Paunio, T., Tanskanen, P., Ridler, K., Suckling, J., Bullmore, E.T., Murray, G.K., Jones, P.B., 2012. No association of COMT (Val158Met) genotype with brain structure differences between men and women. PLoS One. 7, e33964.

Batalla, A., Bhattacharyya, S., Yucel, M., Fusar-Poli, P., Crippa, J.A., Nogue, S., Torrens, M., Pujol, J., Farre, M., Martin-Santos, R., 2013a. Structural and functional imaging studies in chronic cannabis users: a systematic review of adolescent and adult findings. PLoS One. 8, e55821.

Batalla, A., Soriano-Mas, C., Lopez-Sola, M., Torrens, M., Crippa, J.A., Bhattacharyya, S., Blanco-Hinojo, L., Fagundo, A.B., Harrison, B.J., Nogue, S., de la Torre, R., Farre, M., Pujol, J., Martin-Santos, R., 2013b. Modulation of brain structure by catechol-O-methyltransferase Val(158) Met polymorphism in chronic cannabis users. Addict. Biol. Jan 14. [Epub ahead of print].

Bertolino, A., Blasi, G., Latorre, V., Rubino, V., Rampino, A., Sinibaldi, L., Caforio, G., Petruzzella, V., Pizzuti, A., Scarabino, T., Nardini, M., Weinberger, D.R., Dallapiccola, B., 2006. Additive effects of genetic variation in dopamine regulating genes on working memory cortical activity in human brain. J. Neurosci. 26, 3918-3922.

Bertolino, A., Di, G.A., Blasi, G., Sambataro, F., Caforio, G., Sinibaldi, L., Latorre, V., Rampino, A., Taurisano, P., Fazio, L., Romano, R., Douzgou, S., Popolizio, T., Kolachana, B., Nardini, M., Weinberger, D.R., Dallapiccola, B., 2008. Epistasis between dopamine regulating genes identifies a nonlinear response of the human hippocampus during memory tasks. Biol. Psychiatry. 64, 226-234.

Bertolino, A., Fazio, L., Di, G.A., Blasi, G., Romano, R., Taurisano, P., Caforio, G., Sinibaldi, L., Ursini, G., Popolizio, T., Tirotta, E., Papp, A., Dallapiccola, B., Borrelli, E., Sadee, W., 2009. Genetically determined interaction between the dopamine transporter and the D2 receptor on prefronto-striatal activity and volume in humans. J. Neurosci. 29, 1224-1234.

Bhattacharyya, S., Atakan, Z., Martin-Santos, R., Crippa, J.A., Kambeitz, J., Prata, D., Williams, S., Brammer, M., Collier, D.A., 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. 17, 1152-5.

Bhattacharyya, S., Atakan, Z., Martin-Santos, R., Crippa, J.A., McGuire, P.K., 2012b. Neural mechanisms for the cannabinoid modulation of cognition and affect in man: a critical review of neuroimaging studies. Curr. Pharm. Des. 18, 5045-5054.

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

Bloomfield MA, Morgan CJ, Egerton A, Kapur S, Curran HV, Howes OD., 2014 Dopaminergic function in cannabis users and its relationship to cannabis-induced psychotic symptoms. Biol. Psychiatry. 75, 470-8.

Bueller, J.A., Aftab, M., Sen, S., Gomez-Hassan, D., Burmeister, M., Zubieta, J.K., 2006. BDNF Val66Met allele is associated with reduced hippocampal volume in healthy subjects. Biol. Psychiatry. 59, 812-815.

Burns, H.D., Van, L.K., Sanabria-Bohorquez, S., Hamill, T.G., Bormans, G., Eng, W.S., Gibson, R., Ryan, C., Connolly, B., Patel, S., Krause, S., Vanko, A., Van, H.A., Dupont, P., De, L., I, Rothenberg, P., Stoch, S.A., Cote, J., Hagmann, W.K., Jewell, J.P., Lin, L.S., Liu, P., Goulet, M.T., Gottesdiener, K., Wagner, J.A., de, H.J., Mortelmans, L., Fong, T.M., Hargreaves, R.J., 2007. [18F]MK-9470, a positron emission tomography (PET) tracer for in vivo human PET brain imaging of the cannabinoid-1 receptor. Proc. Natl. Acad. Sci. U.S.A. 104, 9800-9805.

Caldu, X., Vendrell, P., Bartres-Faz, D., Clemente, I., Bargallo, N., Jurado, M.A., Serra-Grabulosa, J.M., Junque, C., 2007. Impact of the COMT Val108/158 Met and DAT genotypes on prefrontal function in healthy subjects. Neuroimage. 37, 1437-1444.

Cousijn, J., Wiers, R.W., Ridderinkhof, K.R., van den Brink, W., Veltman, D.J., Goudriaan, A.E., 2012. Grey matter alterations associated with cannabis use: Results of a VBM study in heavy cannabis users and healthy controls. Neuroimage. 59, 3845-3851.

Cheetham, A., Allen, N.B., Whittle, S., Simmons, J., Yucel, M., Lubman, D.I., 2012. Orbitofrontal volumes in early adolescence predict initiation of cannabis use: a 4-year longitudinal and prospective study. Biol. Psychiatry. 71, 684-692.

Chen, J., Lipska, B.K., Halim, N., Ma, Q.D., Matsumoto, M., Melhem, S., Kolachana, B.S., Hyde, T.M., Herman, M.M., Apud, J., Egan, M.F., Kleinman, J.E., Weinberger, D.R., 2004. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am. J. Hum. Genet. 75, 807-821.

Durston, S., Fossella, J.A., Casey, B.J., Hulshoff Pol, H.E., Galvan, A., Schnack, H.G., Steenhuis, M.P., Minderaa, R.B., Buitelaar, J.K., Kahn, R.S., van, E.H., 2005. Differential effects of DRD4 and DAT1 genotype on fronto-striatal gray matter volumes in a sample of subjects with attention deficit hyperactivity disorder, their unaffected siblings, and controls. Mol. Psychiatry. 10, 678-685.

Ehrlich, S., Morrow, E.M., Roffman, J.L., Wallace, S.R., Naylor, M., Bockholt, H.J., Lundquist, A., Yendiki, A., Ho, B.C., White, T., Manoach, D.S., Clark, V.P., Calhoun, V.D., Gollub, R.L., Holt, D.J., 2010. The COMT Val108/158Met polymorphism and medial temporal lobe volumetry in patients with schizophrenia and healthy adults. Neuroimage. 53, 992-1000.

European Monitoring Centre for Drugs and Drug Addiction, 2012. The state of the drugs problem in Europe. Lisbon: EMCCDA.

http://www.emcdda.europa.eu/publications/annual-report/2012. Accessed 29 January 2013.

Good, C.D., Johnsrude, I.S., Ashburner, J., Henson, R.N., Friston, K.J., Frackowiak, R.S., 2001. A voxel-based morphometric study of ageing in 465 normal adult human brains. Neuroimage. 14, 21-36.

Heinz, A., Goldman, D., Jones, D.W., Palmour, R., Hommer, D., Gorey, J.G., Lee, K.S., Linnoila, M., Weinberger, D.R., 2000. Genotype influences in vivo dopamine transporter availability in human striatum. Neuropsychopharmacology. 22, 133-139.

Honea, R., Verchinski, B.A., Pezawas, L., Kolachana, B.S., Callicott, J.H., Mattay, V.S., Weinberger, D.R., Meyer-Lindenberg, A., 2009. Impact of interacting functional variants in COMT on regional gray matter volume in human brain. Neuroimage. 45, 44-51.

Kambeitz, J.P., Bhattacharyya, S., Kambeitz-Ilankovic, L.M., Valli, I., Collier, D.A., McGuire, P., 2012. Effect of BDNF val(66)met polymorphism on declarative memory and its neural substrate: a meta-analysis. Neurosci. Biobehav. Rev. 36, 2165-2177.

Landfield, P.W., Cadwallader, L.B., Vinsant, S., 1988. Quantitative changes in hippocampal structure following long-term exposure to delta 9-tetrahydrocannabinol: possible mediation by glucocorticoid systems. Brain Res. 443, 47-62.

Lorenzetti, V., Solowij, N., Fornito, A., Lubman, D., Yucel, M., 2013. The Association between Regular Cannabis Exposure and Alterations of Human Brain Morphology: An Updated Review of the Literature. Curr. Pharm. Des. Jun 14. [Epub ahead of print].

Mathalon, D.H., Sullivan, E.V., Rawles, J.M., Pfefferbaum, A., 1993. Correction for head size in brain-imaging measurements. Psychiatry Res. 50, 121-139.

Matsumoto, M., Weickert, C.S., Akil, M., Lipska, B.K., Hyde, T.M., Herman, M.M., Kleinman, J.E., Weinberger, D.R., 2003. Catechol O-methyltransferase mRNA expression in human and rat brain: evidence for a role in cortical neuronal function. Neuroscience. 116, 127-137.

McIntosh, A.M., Baig, B.J., Hall, J., Job, D., Whalley, H.C., Lymer, G.K., Moorhead, T.W., Owens, D.G., Miller, P., Porteous, D., Lawrie, S.M., Johnstone, E.C., 2007. Relationship of catechol-O-methyltransferase variants to brain structure and function in a population at high risk of psychosis. Biol. Psychiatry. 61, 1127-1134.

Meier, M.H., Caspi, A., Ambler, A., Harrington, H., Houts, R., Keefe, R.S., McDonald, K., Ward, A., Poulton, R., Moffitt, T.E., 2012. Persistent cannabis users show neuropsychological decline from childhood to midlife. Proc. Natl. Acad. Sci. U.S.A. 109, E2657-64.

Ohnishi, T., Hashimoto, R., Mori, T., Nemoto, K., Moriguchi, Y., Iida, H., Noguchi, H., Nakabayashi, T., Hori, H., Ohmori, M., Tsukue, R., Anami, K., Hirabayashi, N., Harada, S., Arima, K., Saitoh, O., Kunugi, H., 2006. The association between the Val158Met polymorphism of the catechol-O-methyl transferase gene and morphological abnormalities of the brain in chronic schizophrenia. Brain. 129, 399-410.

Prata, D.P., Mechelli, A., Fu, C.H., Picchioni, M., Kane, F., Kalidindi, S., McDonald, C., Howes, O., Kravariti, E., Demjaha, A., Toulopoulou, T., Diforti, M., Murray, R.M., Collier, D.A., McGuire, P.K., 2009a. Opposite effects of catechol-O-methyltransferase

Val158Met on cortical function in healthy subjects and patients with schizophrenia. Biol.Psychiatry. 65, 473-480.

Prata, D.P., Mechelli, A., Fu, C.H., Picchioni, M., Toulopoulou, T., Bramon, E., Walshe, M., Murray, R.M., Collier, D.A., McGuire, P., 2009b. Epistasis between the DAT 3' UTR VNTR and the COMT Val158Met SNP on cortical function in healthy subjects and patients with schizophrenia. Proc. Natl. Acad. Sci. U.S.A. 106, 13600-13605.

Rubino, T., Realini, N., Braida, D., Guidi, S., Capurro, V., Vigano, D., Guidali, C., Pinter, M., Sala, M., Bartesaghi, R., Parolaro, D., 2009. Changes in hippocampal morphology and neuroplasticity induced by adolescent THC treatment are associated with cognitive impairment in adulthood. Hippocampus. 19, 763-772.

Santiago, M., Matarredona, E.R., Granero, L., Cano, J., Machado, A., 2000. Neurotoxic relationship between dopamine and iron in the striatal dopaminergic nerve terminals. Brain Res. 858, 26-32.

Schacht, J.P., Hutchison, K.E., Filbey, F.M., 2012. Associations between cannabinoid receptor-1 (CNR1) variation and hippocampus and amygdala volumes in heavy cannabis users. Neuropsychopharmacology. 37, 2368-2376.

Scheepers, F.E., de Wied, C.C., Hulshoff Pol, H.E., van de Flier, W., van der Linden, J.A., Kahn, R.S., 2001. The effect of clozapine on caudate nucleus volume in schizophrenic patients previously treated with typical antipsychotics. Neuropsychopharmacology. 24, 47-54.

Shook, D., Brady, C., Lee, P.S., Kenealy, L., Murphy, E.R., Gaillard, W.D., VanMeter, J.W., Cook, E.H., Jr., Stein, M., Vaidya, C.J., 2011. Effect of dopamine transporter genotype on caudate volume in childhood ADHD and controls. Am. J. Med. Genet. B Neuropsychiatr. Genet. 156B, 28-35.

Stone, J.M., Bhattacharyya, S., Barker, G.J., McGuire, P.K., 2012. Substance use and regional gray matter volume in individuals at high risk of psychosis. Eur.Neuropsychopharmacol. 22, 114-122.

Szeszko, P.R., Lipsky, R., Mentschel, C., Robinson, D., Gunduz-Bruce, H., Sevy, S., Ashtari, M., Napolitano, B., Bilder, R.M., Kane, J.M., Goldman, D., Malhotra, A.K., 2005. Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation. Mol. Psychiatry. 10, 631-636.

Torrens, M., Serrano, D., Astals, M., Perez-Dominguez, G., Martin-Santos, R., 2004. Diagnosing comorbid psychiatric disorders in substance abusers: validity of the Spanish versions of the Psychiatric Research Interview for Substance and Mental Disorders and the Structured Clinical Interview for DSM-IV. Am. J. Psychiatry. 161, 1231-1237.

Vandenbergh, D.J., Persico, A.M., Hawkins, A.L., Griffin, C.A., Li, X., Jabs, E.W., Uhl, G.R., 1992. Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR. Genomics. 14, 1104-1106.

Velakoulis, D., Pantelis, C., McGorry, P.D., Dudgeon, P., Brewer, W., Cook, M., Desmond, P., Bridle, N., Tierney, P., Murrie, V., Singh, B., Copolov, D., 1999. Hippocampal volume in first-episode psychoses and chronic schizophrenia: a high-resolution magnetic resonance imaging study. Arch. Gen. Psychiatry. 56, 133-141.

Velakoulis, D., Wood, S.J., Wong, M.T., McGorry, P.D., Yung, A., Phillips, L., Smith, D., Brewer, W., Proffitt, T., Desmond, P., Pantelis, C., 2006. Hippocampal and amygdala volumes according to psychosis stage and diagnosis: a magnetic resonance imaging study of chronic schizophrenia, first-episode psychosis, and ultra-high-risk individuals. Arch.Gen.Psychiatry. 63, 139-149.

Watson, C., Andermann, F., Gloor, P., Jones-Gotman, M., Peters, T., Evans, A., Olivier, A., Melanson, D., Leroux, G., 1992. Anatomic basis of amygdaloid and hippocampal volume measurement by magnetic resonance imaging. Neurology. 42, 1743-1750.

Wechsler, D., 1997. WAIS-III & WMS-III technical manual. The Psychological Corporation, San Antonio.

Wetherill, R.R., Jagannathan, K., Lohoff, F.W., Ehrman, R., O'Brien, C.P., Childress, A.R., Franklin, T.R., 2012. Neural correlates of attentional bias for smoking cues: modulation by variance in the dopamine transporter gene. Addict. Biol. Oct 12. [Epub ahead of print].

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

Zhou, Q.Y., Palmiter, R.D., 1995. Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. Cell. 83, 1197-1209.

8

General discussion

Discussion

Main findings

The present thesis improves current knowledge of the effects of drug use on psychotic disorders, and the consequences of acute and chronic use of cannabinoids on brain structure and function while assessing gene-environment interactions that are relevant for psychiatric disorders.

Based on *hypothesis* #1, **Chapter 3** provides data of the influence of drug use on readmission risk in a first-episode psychosis sample. The results show that a screening scale for cannabis and cocaine use disorders and urinalysis for cannabis are predictors for readmission, especially during the first years of the illness. Therefore, early intervention might potentially have a great impact on long-term outcomes.

Moving towards *hypothesis* #2, **Chapter 4** systematically reviews the acute effects of cannabinoids on the brain, considering the possible pathways that may lead to psychosis. The results show that acute administration of cannabinoids modulate resting state activity and alter neural activity during performance of several cognitive tasks in areas related with reward and psychiatric disorders. In contrast to animal studies, the few neurochemical studies performed in humans show inconsistencies regarding the increased dopaminergic activity that might be related to THC-induced psychosis. **Chapter 5** systematically reviews the evidence of the impact of chronic cannabis use on brain structure and function in adult and adolescent population. The results show that chronic cannabis use is associated with alterations in brain function and structure especially in medial temporal regions both in adults and adolescents, and that the amount of exposure may be related to its harmful effect.

Finally, bearing in mind *hypothesis* #3, the last two chapters provide evidence of how proneness to cannabis-induced brain impairment may also rely on aspects related to individual's genetic background. Based on gene-interaction models, **Chapter 6** shows how chronic cannabis use is associated with morphologic alterations in brain areas mentioned in the previous reviews and how variation in the COMT genotype results in diverse liability to experience brain impairment. This data

is expanded in **Chapter 7** by showing preliminary data of a gene-gene-environment interaction; that is, how dopamine-regulating genes may interact with each other to moderate individual differences in areas particularly vulnerable to heavy cannabis exposure, such as the hippocampus.

Consequences of drug use in first-episode psychosis

According to the results of **Chapter 3**, nearly two thirds of our first-episode psychosis sample reported having taken at least one substance of abuse (different from tobacco) in the last three months. Consistently with other European studies in first-episode psychosis (101-105), cannabis was the most frequently reported substance of abuse. In fact, the DALI cannabis/cocaine subscale found 50% of individuals with first-episode psychosis at risk of a cannabis and/or cocaine use disorder. Urinary analyses underdetected drug use compared with self-report, which supports the validity of self-report data among this group of patients.

Both DALI cannabis/cocaine subscale and urinalysis for cannabis were predictors of readmission among adults with first-episode psychosis, together with younger age, male gender and higher scores on the PANSS positive subscale. After controlling for confounding factors, only the DALI cannabis/cocaine subscale remained as a predictor of readmission, supporting the utility of this screening test over laboratory parameters. Our results suggest an overall 4.5 fold increase in risk of readmission, which is in line with other studies (106-108). Furthermore, the DALI cannabis/cocaine subscale showed good psychometric properties for predicting readmission. In fact, compared to urinalysis, the subscale showed a greater AUC due its higher sensitivity. Therefore, in addition to is significant reduction in costs, a positive result on this screening scale may be more reliable for detecting current use and misuse, and even for predicting readmission, than a urine sample.

It is worth noting that survival plots showed the greatest differences in readmission rates during the first five years of follow-up. Relapse prevention during the first years of the illness may have a critical impact on life-long outcomes in

schizophrenia. It is well known that comorbid diagnosis of a drug use disorder may enhance the risk of relapse (106, 108, 109) and that abstaining from use after the first psychotic episode may contribute to a better outcome (107, 110-113). Therefore, avoidance of this modifiable risk factor should be considered a priority for clinicians and intervention programs.

Overall, results of this study have direct clinical implications for preventing readmission during the early course of psychotic illness, when intervention may have a great impact on long-term outcome. After patients are screened, they can be referred to dual diagnosis integrative care programs, which may prevent readmission and improve outcome by decreasing cannabis use.

Effects of cannabinoids on brain function and structure

Detrimental consequences of cannabis use reported in **Chapter 3** are probably mediated by disturbances of the endocannabinoid system. CB_1 receptors are abundantly distributed throughout the brain, notably in brain areas implicated in psychosis such as the prefrontal cortex, basal ganglia, medial temporal areas (hippocampus and amygdala) and cerebellum (114).

Acute effects of cannabinoids on cognitive function

Despite the substantial degree of methodological differences in the imaging studies included in **Chapter 4**, the results of this systematic review have provided several consistent findings regarding the acute effects of cannabinoids on brain function in cannabinoid-naïve subjects and animals. Acute administration of cannabinoids has demonstrated to increase cerebral blood flow in CB_1-rich brain areas during baseline brain perfusion. Such areas are implicated in reward processes and several cognitive functions. Actually, challenging studies also showed alterations in neural activity during performance of several cognitive tasks, such as memory, attention, emotion and salience processing. Interestingly, opposite effects between the two main

constituents of cannabis, THC and CBD, have also been described, which is consistent with their opposite clinical effects. For instance, Bhattacharyya et al. (2012) (115) examined acute THC and CBD effects on salience processing, as aberrant salience processes have been linked with presence of psychotic symptoms (116). Opposite effects of THC and CBD relative to placebo were present on prefrontal, left caudate and hippocampal activation during visual oddball salience processing. Moreover, THC-related activation in the caudate was negatively correlated with severity of drug-induced psychotic symptoms (115). This data supports the notion that cannabinoid modulation of activity of dopaminergic projections from the brain stem to the striatum may play a role in the pathogenesis of cannabis-induced psychosis (117).

Acute and chronic effects of cannabinoids on dopamine release: paths to psychosis

Chapter 4 provides data about animal and human studies regarding cannabis influence on striatal dopaminergic activity. Animal studies clearly showed that administering cannabinoids alters the balance of excitation and inhibition of dopamine cells, most frequently causing an increase in firing with attendant elevations of dopamine release in the striatum. This is likely to be attributable to activation of CB_1 receptors on GABAergic interneurons that synapse with dopamine neurons (117). However, human studies have reported mixed results, with only one out of three studies showing a moderate increase of endogenous dopamine release in the striatum. Therefore, it is feasible that the psychotropic effects of THC arise from direct actions in glutamate and GABAergic terminals rather than exclusively via dopamine signalling. Kuepper et al. (2013) (118) suggests that although THC may not induce a significant increase in striatal dopamine in healthy subjects, it might do so in patients with schizophrenia and their relatives. Thus, some families would transmit a vulnerability to react in an amplified manner to cannabinoids.

It is also noteworthy that converging human data show that chronic cannabis use might be associated with reduced dopamine synthesis capacity (119, 120), which is in line with the general evidence from studies of substance abusers that dependence is associated with decreased striatal dopamine. Although animal and human studies

have not found differences in striatal D_2/D_3 receptor availability after chronic cannabis exposure (**Chapter 5**), experimental animal studies have reported that chronic administration of THC induces sensitization of the D_2/D_3 receptor in the striatum, which supports the notion that chronic THC exposure may induce a hypodopaminergic state (121). This would be congruent with an alteration of the mechanisms controlling dopamine synthesis and release, with the concurrent development of postsynaptic dopamine receptor supersensitivity, which might potentially contribute to the development of psychosis or other cannabis-related brain impairment in vulnerable subjects (121-123) (**Chapters 5-7**).

Chronic effects of cannabinoids on brain function and structure

In contrast with the acute effects, resting state neuroimaging studies in adult chronic cannabis users have reported decreased cerebral blood flow in brain regions rich in CB_1 receptors (**Chapter 5**). It has been suggested that the decreased resting state activity may represent a down-regulation of these receptors as a result of chronic exposure to exogenous cannabinoids (124). Similar to animal studies, this down-regulation may be region-specific (e.g. neocortex and limbic areas) and reversible (125). On the other hand, similar to challenging studies (**Chapter 4**), functional neuroimaging studies comparing brain activation in both adult and adolescent chronic users during the performance of several cognitive tasks have shown altered patterns of brain activity, with the level of performance on the task generally within the normal limits. Overall, these findings may be interpreted in terms of neuroadaptation, possibly indicating the recruitment of additional regions, mainly within the prefrontal cortex, as a compensatory mechanism to maintain normal cognitive performance in response to chronic cannabis exposure. It is possible that this drug-regulatory mechanism works until it turns out to be insufficient and between-group differences arise.

Chapter 5 also shows that chronic cannabis use is related to decreases in grey matter volume, particularly in medial temporal regions, such as the hippocampus and the amygdala. These regions are known to be functionally associated with memory,

executive and affective processing. Therefore, together with functional alterations, these changes may be related with reports of neuropsychological decline observed in chronic cannabis users (16). Importantly, grey matter volume reductions have been related to age of onset of cannabis use and amount of exposure, with recent data showing that these two key factors may occur independently (126). That is, significant grey matter atrophy in temporal brain regions would occur either with chronic cannabis use independent of the age of onset or with recreational consumption that started during the adolescence (before the age of 18) (126). These two factors may interplay together with genetic vulnerability to determine the extent of brain impairment related to chronic cannabis use (**Chapters 6 and 7**).

Despite the few neuroimaging studies involving adolescents, these studies have revealed that functional and structural alterations are similar to those observed in adults. Therefore, consequences may appear soon after starting the drug use (**Chapter 5**). Although still nascent, current data also provides preliminary evidence of gender differences in the impact of chronic cannabis use in structural impairment, with data showing that female cannabis users may be at increased risk (127, 128). Disparity in risk may reflect differences in neurodevelopment (38, 129), hormones and CB_1 receptor densities during maturation (130). Given that endocannabinoid signalling plays a crucial role in establishing normal gender differences in the brain (37), it is not unexpected that disruption of this system may produce gender-specific differences in neurocognitive, functional and structural outcomes. The studies described in **Chapters 6 and 7** only included male subjects in order to avoid this potential confounding factor.

Genetic vulnerability to cannabis-related brain impairment

The results of **Chapters 6 and 7** provide multimodal evidence of the impact of COMT and DAT1 genetic variations on brain structure in a group of early-onset chronic cannabis users compared to age-, education- and intelligence-matched non-using controls. In **Chapter 6**, VBM analysis showed that the COMT genotype significantly influenced two out of four studied areas: the bilateral caudate nucleus

and the left amygdala. **Chapter 7** expanded previous results by showing the influence of variation of COMT and DAT1 genotypes and its interaction in manual-traced hippocampi.

According to the results of **Chapter 6**, the COMT genotype influenced the bilateral ventral caudate nucleus in an opposite direction depending whether the subject had been chronically exposed to cannabis or not. That is, more copies of *val* allele were associated with smaller volumes in chronic cannabis users and greater volume in controls. Converging findings in the addiction literature implicate the striatum as being important to the impact that cannabis may have on the brain. For instance, the ventral striatum is known to play a major role in the reinforcing effects of drugs of abuse (131). The activation of dopamine, which is influenced by COMT genotype, may contribute to the increased excitability of the ventral striatum with decreased glutamatergic activity during withdrawal and increased glutamatergic activity during drug-primed and cue-induced drug seeking (131). On the other hand, preclinical studies have reported the impact of variation in dopamine neurotransmission on neural growth and survival, particularly within the striatum (132). Although the precise mechanisms underlying morphological changes remain unclear, they might be related to the modulatory effect of cannabis and the endocannabinoid system on dopaminergic neurotransmission (**Chapter 4**). Over- and under stimulation may potentially result in impaired neuronal viability, indicating that an optimum range for extracellular dopamine may exist (75), which may be region-specific and influenced by genetics and environmental factors.

The COMT genotype also influenced the amygdala volume differently in cannabis users and non-using controls (**Chapter 6**). The amygdala has also been implicated in the reward effects of drugs (133, 134) and functional and structural brain alterations have been widely described under acute and chronic cannabis exposure (**Chapters 4 and 5**). It is interesting to note that the effects of chronic cannabis use on brain structure are consistent with studies showing similar alterations in patients with schizophrenia. Brain imaging studies have consistently reported up to 6% volume reductions in the hippocampus and the amygdala in schizophrenic patients (135, 136), particularly during the first years of the illness (137). This highlights the

importance of early intervention of drug misuse (**Chapter 3**) and suggests that these structural changes may reflect a central pathophysiological process associated with the illness. In addition, cannabis misuse in schizophrenia has been associated with more pronounced loss of grey matter volume compared to those patients who do not use cannabis (138), suggesting that this group might be particularly vulnerable to cannabis exposure (**Chapter 3**).

These results are in line with previous studies exploring the influence of the COMT genotype on brain structure in healthy subjects. In general, such studies have described smaller volumes in medial temporal regions in *val* carriers (75, 90), although negative results have also been reported (139). The same influence has been observed in non-using schizophrenia patients and subjects at high risk of psychosis (88, 90, 140). That is, the COMT met allele has been associated with larger, and the *val* allele with smaller, medial temporal volumes, suggesting that the *val* allele may play a role, at least in part, to lower the volume of these brain regions (90). However, subjects chronically exposed to cannabis showed exactly the opposite pattern. Therefore, this finding provides further evidence of how environmental factors and genetics may interact to determine complex phenotypes, such as brain volume.

Finally, **Chapter 7** brought preliminary data of the interaction of COMT and DAT1 genotypes on bilateral hippocampi volumes by using manual tracing. We focused specifically on this brain region as both animal and human literature have provided extensive evidence that cannabis exposure might be particularly neurotoxic in this brain area (**Chapter 5**). COMT and DAT1 genotypes interacted to moderate hippocampal volume, and once again this association occurred in opposite directions depending on whether or not there was previous exposure to cannabis, consistently with the results exposed in **Chapter 6**. Interestingly, non-using controls showed a linear relationship between dopamine availability and hippocampal volume that was not present in cannabis users. Early-onset chronic cannabis users apparently showed a U-shaped relationship between cortical volume and dopamine availability, such that the combination of genotypes associated with intermediate levels of dopamine was associated with smaller hippocampal volumes.

Irrespective of genotype, results of **Chapter 7** are also in line with previous studies showing reduced hippocampal volume among chronic cannabis users compared to non-using controls (**Chapter 5**), even though results failed to reach statistical significance. This may be due to the fact that our chronic cannabis users had a relatively reduced duration of exposure compared with other studies (66). However, the negative association between hippocampal volume and lifetime cannabis exposure supports the notion that with continued use this group would finally have differences in hippocampal volumes.

Overall, these results support that the possible influence of the interaction between dopaminergic genes on brain development may be modulated by cannabis use. Individual differences in the expression of these genes may imply different liability to present volumetric alterations under chronic cannabis exposure. This is of major importance given that reduced hippocampal volume may underlie a variety of symptoms of chronic cannabis use, and contribute to the neuropsychological decline observed among subjects chronically exposed to cannabis (16).

Limitations

Several limitations have to be considered when interpreting the results of the studies included in this thesis. Drug assessment on **Chapter 3** was limited to the last three months before admission and was based on self-reported information, which may raise concerns about non-disclosure. However, studies in patients with severe mental illness generally rely on self-reports (105) and our results actually favoured the use of self-reports over laboratory tests. As the drug assessment was conducted only at baseline, we could not obtain a clear description of the temporal relationship between substance misuse and readmission during the follow-up. Longitudinal studies with periodical assessments would overcome such limitation. Finally, as medication adherence was not assessed, the influence of this well-known factor associated with readmission (141) could not be ruled out.

Both systematic reviews in **Chapters 4 and 5** pointed out important methodological differences among the studies included. This often limited the generalisation of results and hampered comparisons among them. PRISMA guidelines, strict definitions (e.g. naïve, recreational/occasional and chronic cannabis user) and the use of pre-determined criteria for article selection were determinant to diminish heterogeneity. Unfortunately, differences in socio-demographic characteristics (e.g. gender, age) and cannabis use patterns (e.g. age of onset, frequency of use, lifetime use) were common, and may help to explain some discrepancies in the results. In addition, the authors used diverse units to measure cannabis exposure (e.g. joints, grams) so comparisons were not always appropriate. These facts indicate that convergent methodology should be a priority, and there is a need for a standardisation of quantification of cannabis use (142). Furthermore, the content and ratio of THC and CBD in smoked cannabis vary widely between sources, preparations and countries, with potency reported to have increased substantially in the last years (1). Increases in potency, which means increases in the THC/CBD ratio, may have clinical (7) and neuroanatomical consequences (64). Therefore, comparability between earlier and later studies may also be limited. Furthermore, a considerable overlap between cannabis and other drug use may have played a confounding role, as comorbid drug misuse may be associated with significant neurobiological and neurocognitive abnormalities (143).

With regard to the case-control study (**Chapters 6 and 7**), the relatively small sample size for a structural imaging study may have limited our results. However, the strength of our findings across two brain imaging modalities instils confidence in our data. Because this is a cross-sectional study, causation cannot be determined, although cannabis lifetime use parametrically correlated with structural differences, which suggests the possibility of causation. Though few studies have taken a longitudinal approach whilst investigating the relation between cannabis use and structural abnormalities, there is data suggesting that some structural abnormalities could predate the onset of cannabis use (144). The reported results cannot be generalised to all chronic cannabis users, as our sample included Caucasian male early-onset cannabis users without other drug use or psychiatric illnesses. In addition,

the potential effect in brain morphology of other neurotransmitters, such as glutamate (145), and other non-genetic and genetic factors not considered in the analyses, such as the CNR1 (146) and BDNF genes (147, 148), cannot be dismissed.

Future perspectives

The emergent indication that cannabis use may modify the course of psychiatric illness, and that the endocannabinoid system plays a critical role in brain functions and structure implicated in psychiatric disorders, encourages further research. First, longitudinal studies with periodical assessments of drug use using validated and brief screening tools would help to better describe the influence of cannabis use in the course of the psychotic illness, as well as to evaluate treatment strategies designed to improve long-term outcomes. Second, there is a great need for replication of the neuroimaging studies exploring the effects of cannabinoids on brain. Future studies should consider the use of convergent methodology and standardised measures of cannabis use in order to enable comparisons among them. Convergent methodology would also result in less inconsistent findings. Third, the endocannabinoid system is a promising target in the treatment of symptoms of psychiatric disorders, such as schizophrenia. Currently, the most promising cannabinoid compound for development as an antipsychotic drug is CBD. Further neuroimaging studies could compare the effects of CBD administration between schizophrenia or subjects at high risk for psychosis and healthy controls, while examining behavioural symptoms, striatal dopamine function or brain activity patterns.

The involvement of genes in the individual susceptibility to cannabis-related impairment also inspires further research. It is likely that variation across several genes might explain differential sensitivity to the effects of long-term cannabis use. Assessing this potential vulnerability by using multimodal imaging, such as brain function, spectroscopy and connectivity analysis, might strengthen the findings. Multimodal approaches may demonstrate certain abnormalities more detectable using one modality than another due to different etiological sensitivities between neuroimaging techniques. In addition, it would be of great interest to perform similar

analysis in patients with schizophrenia with and without chronic cannabis use, in order to better understand the mechanisms underlying gene-environment interactions in psychiatric patients. Finally, the inclusion of other genes related to schizophrenia, dopamine metabolism and neurodevelopmental processes, such as the BDNF, CNR1, NRG1 (neuregulin-1) and AKT1 (V-akt murine thymoma viral oncogene homolog 1), should also be considered in future gene-environmental models in order to explore its individual effects and potential interactions.

Considering that normal variation in brain function and structure is likely to be partially determined by genetic variation in neurotransmitter pathways, further studies of these genes on brain function and structure in healthy subjects is a necessary step towards understanding the basis of abnormal function. In addition, as large samples are required to detect subtle and interactive effects of genes, strategies for expanding data sharing are highly advisable. For instance, initiatives such as the ENIGMA (Enhancing Neuro Imaging Genetics through Meta-Analysis) Consortium (149), that our group joined recently (ENIGMA-Addiction), offer platforms to bring together neuroimaging and genetic data of multiple sites around the world. However, further obstacles must be addressed to make collaborative analysis efficient, such as between-site differences in scanners and data acquisition parameters, as well as pre- and post-processing schemes.

9

Conclusion

Conclusion

» First-episode psychosis patients at risk of cannabis and cocaine misuse measured by a screening drug scale have a 4.5-fold increased risk for readmission, especially during the first five years of follow-up. A screening self-report scale is more useful than urinary analysis for predicting that risk.

» The acute effects of cannabinoids on brain function have demonstrated to:

a. Modulate resting state activity, with increases mainly in CB1-rich areas implicated in several cognitive functions and in the reward circuitry.
b. Alter neural activity during performance of different types of cognitive paradigms, possibly reflecting a different recruitment of brain areas during the task.
c. THC and CBD showed opposite neurophysiological properties.
d. While the psychotomimetic effects of THC in humans are likely to arise from direct actions at CB1 receptors, it is unclear whether this occurs mainly through a modulatory effect on dopamine signalling.

» The chronic effects of cannabinoids on brain structure and function have been related to:

a. Structural brain abnormalities, mostly in CB1-rich areas and particularly within the hippocampus, which may be related to the amount of cannabis used.
b. Altered neural activity during resting state and under several cognitive paradigms, which may reflect neuroadaptation by increasing recruitment of brain areas during the tasks, particularly within the prefrontal cortex.
c. Studies conducted in adolescents suggest that both structural and functional alterations may appear soon after starting the drug use and may be related to gender.

» The dopamine-regulating genes COMT and DAT1 play a role in determining brain morphology, and its influence may be modified by chronic cannabis exposure.

a. The COMT genotype influenced the volume of the bilateral caudate nucleus and the left amygdala in early-onset chronic cannabis users and healthy controls in an opposite direction.
b. Consistently, the interaction between COMT and DAT1 polymorphisms significantly affected hippocampal volumes in an opposite direction depending on whether there was previous exposure to cannabis or not.
c. Irrespective of genotype, hippocampal volumes were smaller in cannabis users compared to controls, and the magnitude of volumetric reduction was associated with lifetime cannabis exposure.

» Regular cannabis use has structural modulatory effects on the hippocampus, and the dopamine-regulating genes may play a particular role in the sensitivity to the effects of cannabis on brain morphology.

» Single genetic factors are unlikely to explain the intricate interactions between cannabis and complex phenotypes, such as brain volume or psychiatric disorders. Other genetic and non-genetic variants should be considered for inclusion in gene-environmental interaction models.

Resum en català

Resum en català

Introducció

Donat que el cànnabis és la droga il·legal més consumida al món (1), hi ha un creixent interès en estudiar els seus efectes sobre la salut mental. El principal constituent psicoactiu del cànnabis, el THC, pot comportar l'aparició de tot un seguit de problemes psiquiàtrics tant aguts com crònics en alguns individus, com son la dependència (8), trastorns d'ansietat i de l'estat d'ànim (18), psicosis (21) i dèficits neuropsicològics (16). A més, l'ús de cànnabis pot empitjorar el curs i el pronòstic de trastorns psiquiàtrics establerts. L'abús d'aquesta substància entre els pacients amb diagnòstic psiquiàtric, especialment entre els trastorns psicòtics, és major que en la població general (25). L'ús de cànnabis, especialment en les fases inicials de la malaltia, pot alterar el curs, la fenomenologia i el pronòstic, tot avançant l'edat d'inici (7), empitjorant l'adherència al tractament i incrementant les recaigudes i les hospitalitzacions (26). Per tant, avaluar l'ús de trastorns relacionats amb el consum de substàncies d'abús durant fases precoces de la malaltia és crucial, donat que les potencials intervencions podrien tenir un gran impacte en el pronòstic a llarg termini (**Capítol 3**).

El THC exerceix el seu efecte a través del sistema endocannabinoid endogen, el qual juga un paper clau en el neurodesenvolupament (35-37) i regulant l'activitat neuronal d'altres neurotransmissors (27). Pertorbacions d'aquest sistema per l'efecte de cannabinoids exògens podrien ser responsables de l'aparició d'efectes aguts i a llarg termini, com trastorns psiquiàtrics o alteracions en la funció i estructura cerebral. Donat que les xarxes neuronals alterades durant l'ús de cànnabis són similars a aquelles observades durant els estats psicòtics i pre-psicòtics (42-46), resulta d'interès investigar els efectes aguts dels cannabinoids en el cervell i explorar les possibles vies que podrien conduir cap a la síndrome psicòtica (**Capítol 4**).

El fet que no tots els individus presentin alteracions cerebrals o trastorns psiquiàtrics després d'una exposició perllongada al cànnabis suggereix que l'eventualitat de patir problemes relacionats amb el consum crònic podria recaure en

un seguit de factors clau, els quals podrien tenir un paper amplificador. Aquests factors estarien relacionats amb l'edat d'inici, els paràmetres de consum (per exemple, quantitat, freqüència i duració) i amb fenòmens de vulnerabilitat genètica individual.

Diversos estudis en animals suggereixen que iniciar el consum de cànnabis de forma precoç, per exemple durant l'adolescència, podria tenir pitjors conseqüències en diversos processos cognitius que un inici més tardà (47-49). A més, el THC és capaç d'induir toxicitat dosi-depenent i canvis estructurals en regions cerebrals riques en receptors cannabinoids endògens, particularment en regions temporals medials com l'hipocamp i l'amígdala (50, 59-63). Els estudis en humans sobre els danys estructurals associats a l'ús crònic de cànnabis però han proporcionat resultats menys consistents, amb pocs estudis duts a terme en població adolescent (58). Així doncs, una qüestió d'interès és conèixer si l'ús crònic de cànnabis s'associa a alteracions en la funció i estructura cerebral, i si aquestes són precoces i similars a les observades en els estudis duts a terme en població adulta (**Capítol 5**).

Un altre explicació de per què només alguns individus pateixen dany cerebral associat al consum crònic de cànnabis podria ser que certes persones presenten una vulnerabilitat genètica particular. La variació en l'expressió de gens implicats en la regulació de neurotransmissors, com per exemple la dopamina, podria jugar un paper clau en la determinació de la vulnerabilitat individual en diversos àmbits, ja siguin clínics [per exemple, risc de psicosis (68-70), modulació de funcions cognitives (71)], d'activitat neuronal (72, 73) o del volum cerebral (74-76). Així les coses, considerant el potencial efecte nociu del cànnabis en la morfologia cerebral, especialment en els consumidors d'inici temprà, és rellevant investigar si l'ús crònic de cànnabis es relaciona amb alteracions volumètriques, i si les variacions en els gens reguladors de la dopamina poden resultar en diferent risc a patir dany cerebral (**Capítol 6**). A més, és notable conèixer si aquests gens interactuen entre sí per moderar diferències individuals en zones particularment vulnerables a l'exposició a cànnabis, com l'hipocamp, així com esbrinar si la naturalesa d'aquesta interacció depèn també de l'exposició prèvia a aquesta substància (**Capítol 7**).

Objectius i hipòtesi

Aquesta tesi té com a objectiu eixamplar el coneixement actual sobre els efectes aguts i crònics dels cannabinoids, tot avaluant interaccions gen-ambient que són rellevants pels trastorns psiquiàtrics. L'objectiu s'assoleix a través de l'estudi de les conseqüències de l'ús de drogues d'abús en primers episodis psicòtics, i posteriorment per mitjà de l'estudi de la influència dels gens reguladors de la dopamina en l'estructura cerebral de consumidors crònics de cànnabis d'inici temprà comparats amb controls sans aparellats no consumidors, en base a les següents hipòtesis:

- L'ús de cànnabis en primers episodis psicòtics s'associaria a un pitjor curs evolutiu en relació a la taxa de reingressos, ja sigui mesurat per mitjà d'una escala de cribatge o través d'anàlisis d'orina (**Capítol 3**).
- L'ús agut i crònic de cànnabis s'associaria a alteracions de l'estructura i funció cerebral en regions clau relacionades amb trastorns psiquiàtrics, i aquestes alteracions estarien presents en població adolescent (**Capítols 4 i 5**).
- Els consumidors crònics de cànnabis d'inici temprà presentarien alteracions en l'estructura cerebral comparats amb els controls no consumidors, i la variació en els gens reguladors de la dopamina resultaria en diferent probabilitat de presentar dany cerebral induït per cànnabis (**Capítols 6 i 7**).

Mètodes

Estudi d'una cohort de 58 primers episodis psicòtics ingressats consecutivament en la unitat d'hospitalització d'un hospital general. Tots els pacients foren avaluats per mitjà de la entrevista semiestructurada SCID-I (93), l'escala PANSS (94) y l'escala DALI (95), la qual es centra en la detecció de trastorns d´ús de drogues en població amb patologia mental severa. La principal mesura de resultat fou el temps fins que el pacient era reingressat a la unitat d'hospitalització. A tots els participants se'ls va recollir mostres de sang i d'orina dins les primeres 48 hores de l'ingrés per la detecció de substàncies d'abús. Es va fer servir Kaplan-Meier per estimar les corbes de

supervivència, emprant el temps fins el primer reingrés com a variable depenent. També es va realitzar un anàlisi multivariant. Els paràmetres de validesa i les corbes ROC es varen calcular i relacionar amb futurs reingressos. Els anàlisis es van realitzar amb el programa SPSS versió 19 (**Capítol 3**).

A més, es van dur a terme dues revisions sistemàtiques de la literatura a partir de quatre bases de dades (EMBASE, Medline, PubMed, LILACS), seguint una estratègia de cerca exhaustiva i un protocol predefinit segons les directrius indicades a les guies PRISMA (96). En el **Capítol 4** es van incloure 43 estudis de neuroimatge sobre l'administració experimental de cannabinoids en animals no tractats prèviament i consumidors puntuals/ocasionals de cànnabis. El **Capítol 5** recull 45 estudis de neuroimatge en consumidors crònics de cànnabis i un grup control aparellat.

Per últim, es va realitzar un estudi cas-control de neuroimatge en homes caucàsics. 30 consumidors crònics de cànnabis d'inici temprà van ser aparellats en edat, educació i intel·ligència amb 29 controls no consumidors. Tots els participants van ser avaluats per mitjà d'una entrevista estructurada (PRISM) (97) per tal d'excloure qualsevol trastorn psiquiàtric de l'eix I segons els DSM-IV. Es van genotipar la catecol-O-metiltransferasa (COMT *Val*^{158}*Met*, rs4680) i el transportador de la dopamina (DAT1 VNTR). Les dades de neuroimatge es van analitzar mitjançant VBM (**Capítol 6**) i el traçat manual de l'hipocamp, tot seguint una metodologia validada (99, 100) (**Capítol 7**).

Resultats

Capítol 3

Dels 58 pacients ingressats consecutivament amb un primer episodi psicòtic, es varen trobar substàncies psicoactives en sang o orina (excloent benzodiazepines) en 25 (43%). El cànnabis fou la substància trobada amb més freqüència tant en els controls d'orina (38%) com notificada pels propis pacients (50%). La subescala cànnabis/cocaïna de la DALI va classificar a 29 pacients (50%) com d'alt risc de

presentar un trastorn per ús de cànnabis i/o cocaïna, i 11 (19%) com d'alt risc de presentar un trastorn per ús d'alcohol.

L'anàlisi bivariat va mostrar que tant puntuar per risc de trastorn en la subescala cànnabis/cocaïna de la DALI (p=0.002) com la positivitat en l'anàlisi d'orina per cànnabis (p=0.02) estaven relacionats amb un major risc de reingrés. Una menor edat (p=0.03), el gènere masculí (p=0.04) i una alta puntuació en la subescala positiva de la PANSS, també es van associar amb una major probabilitat de reingrés durant el període de seguiment de l'estudi. Per contra, l'ús d'alcohol [tant la positivitat en mostres de sang/orina (p=0.77) com la subescala DALI per alcohol (p=0.33)] no es va associar a reingrés.

En l'anàlisi multivariant, la subescala cànnabis/cocaïna de la DALI es va mantenir com a predictora de reingrés [HR = 4.5; 95% CI = 1.1 to 18.7; p=0.036] després de controlar per potencials variables de confusió (edat, gènere, duració de psicosis sense tractar i puntuació en la subescala de símptomes positius de la PANSS), mentre que la positivitat en orina per cànnabis no (HR=2.9; 95% CI=0.7 to 5.7; p=0.20).

Les corbes ROC van mostrar una major àrea sota la corba per la subescala cànnabis/cocaïna de la DALI (0.716; 95% CI=0.572 to 0.860) respecte la positivitat per cànnabis en l'anàlisi d'orina (0.626; 95% CI=0.462 to 0.791).

Capítols 4 i 5

Dels quaranta-cinc estudis inclosos en el **Capítol 4**, vint-i-quatre van ser duts a terme en humans i vint-i-un en animals. Malgrat el considerable grau d'heterogeneïtat metodològica, els estudis van mostrar que l'administració aguda de cannabinoids és capaç de a) modular l'activitat cerebral basal, en concret causar increments sobretot en regions cerebrals riques en receptors CB_1 implicades en processos cognitius i de recompensa, i b) alterar l'activitat neural durant la realització de diverses tasques cognitives, tot reflectint un reclutament neural diferent respecte el grup control. A més, els components del cànnabis THC i CBD van mostrar efectes neurofisiològics oposats. En contrast amb els estudis realitzats en animals, els escassos estudis

neuroquímics duts a terme en humans van evidenciar inconsistències en relació a l'increment de l'activitat dopaminèrigca que podria estar relacionada amb la psicosi induïda per cànnabis.

Quaranta-tres estudis van ser inclosos en el **Capítol 5**, dels quals vuit van ser duts a terme en adolescents. Els estudis de neuroimatge estructural en consumidors crònics de cànnabis van aportar evidència d'alteracions morfològiques en ambdós grups, particularment en regions temporals medials (hipocamp i amígdala). Aquests efectes es van relacionar amb la quantitat d'exposició a cànnabis. Els estudis de neuroimatge funcional van evocar diferències respecte al grup control tant en relació als patrons d'activitat cerebral basal com durant la realització de diversos paradigmes cognitius, suggerint que podrien existir efectes compensatoris en l'activitat cerebral en resposta a l'exposició crònica a cànnabis.

Capítols 6 i 7

En l'estudi cas-control, els consumidors crònics de cànnabis d'inici temprà van presentar alteracions morfològiques en les regions senyalades en els **Capítols 4 i 5**, les quals van ser influenciades de forma diferent pels genotips de la COMT i el DAT1 segons si l'individu havia estat exposat regularment a cànnabis o no. En concret, el genotip la COMT va modular el volum de dues de les quatre regions explorades per mitjà de VBM (**Capítol 6**). La variació del genotip de la COMT va afectar al nucli caudat ventral bilateral en ambdós grups en una direcció oposada. Això és, més còpies de l'al·lel *val* es van relacionar amb un menor volum en els consumidors crònics de cànnabis però amb un major volum en els controls. També es va observar un patró invers en l'amígdala esquerra.

El **Capítol 7** va ampliar aquests resultats tot mostrant que els gens de la COMT i DAT1 interactuen per moderar diferències individuals en el volum de l'hipocamp. L'associació entre aquests polimorfismes funcionals i els volums hipocampals suggerien una relació lineal amb la disponibilitat de dopamina en els controls que no es va observar en els consumidors crònics de cànnabis. Els volums de l'hipocamp

foren menors en els consumidors crònics en comparació amb els controls, i la magnitud de la reducció volumètrica es va associar amb l'exposició a cànnabis al llarg de la vida.

Discussió

Conseqüències de l'ús de drogues en primers episodis psicòtics

Segons els resultats del **Capítol 3**, i en consonància amb d'altres estudis europeus (101-105), el cànnabis fou la substància més consumida entre els pacients amb primer episodi psicòtic. De fet, la subescala cànnabis/cocaïna de la DALI va classificar al 50% de la mostra com d'alt risc de presentar un trastorn d'ús de cànnabis i/o cocaïna. Tant la subescala per cànnabis/cocaïna de la DALI com els anàlisis d'orina van ser predictors de reingrés, juntament amb una menor edat, el gènere masculí i una alta puntuació en la subescala de símptomes positius de la PANSS. Després de controlar els potencials factors de confusió, només la subescala cànnabis/cocaïna de la DALI va romandre com a predictora de reingrés, indicant un increment global en el risc de 4.5 vegades (106-108). A més, aquesta subescala va demostrar unes bones propietats psicomètriques per predir el reingrés, presentant una major sensibilitat que el control positiu d'orina. Per tant, a més de la reducció associada en els costos, un resultat positiu en aquesta subescala pot ser més fiable per la detecció d'abús de substàncies, i fins i tot per predir el reingrés, que una mostra d'orina. D'altre banda, les corbes de supervivència van mostrar les majors diferències en les taxes de reingrés durant els primers cinc anys de seguiment. La prevenció de recaigudes en aquest període té un impacte crític en el pronòstic a llarg termini de l'esquizofrènia. És ben conegut que aturar el consum de substàncies d'abús després d'un primer episodi psicòtic contribueix a un millor pronòstic (107, 110-113). Així doncs, la prevenció d'aquest factor de risc modificable s'hauria de considerar una prioritat. Els resultats d'aquests estudi tenen aplicació clínica directa en la prevenció de recaigudes durant les fases inicials de l'esquizofrènia. Un cop cribats, els pacients poden ser derivats a unitats integrals de diagnòstic dual per tal de millorar el curs del trastorn tot reduint el consum de cànnabis.

Efectes dels cannabinoids sobre la funció i estructura cerebral

Efectes aguts dels cannabinoids sobre la funció cognitiva

Malgrat l'alt grau de discrepàncies metodològiques entre els estudis de neuroimatge inclosos en el **Capítol 4**, els resultats han proporcionat diverses troballes consistents respecte l'efecte agut dels cannabinoids en la funció cerebral d'individus i animals no exposats prèviament a cànnabis. L'administració aguda de cannabinoids ha demostrat incrementar la perfusió cerebral basal en regions riques en receptors CB_1, i alterar l'activitat neural durant la realització de diverses tasques cognitives. A més, els dos principals components del cànnabis, THC i CBD, han demostrat efectes neurofisiològics oposats, que són congruents amb els seus efectes clínics també oposats. Per exemple, Bhattacharyya et al. (2012) (115) descriu aquests efectes oposats en regions prefrontals, caudat esquerra i hipocamp durant el processament d'estímuls visuals inesperats (saliència). Les alteracions en el processament de la saliència s'han associat amb la presència de símptomes psicòtics (116). L'activació del caudat després de l'administració de THC va correlacionar negativament amb la severitat dels símptomes psicòtics induïts per la droga, recolzant la idea de què la modulació de l'activitat dopaminèrgica a l'estriat pot tenir un paper rellevant en la patogènesi de la psicosi induïda per cànnabis (117).

Efectes aguts i crònics dels cannabinoids sobre l'alliberació de dopamina

El **Capítol 4** també recull dades d'estudis en animals i humans sobre la influència del cànnabis en l'activitat dopaminèrgica en el cos estriat. Mentre que els estudis en animals demostren clarament que l'administració aguda de cannabinoids comporta un increment en l'alliberació de dopamina, només un dels tres estudis en humans observa un moderat increment. Això podria indicar que els efectes psicotròpics del THC podrien sorgir de l'acció directa sobre terminals glutamatèrgiques i GABAèrgiques enlloc d'exclusivament a través de la via dopaminèrgica. Kuepper et al. (2013) (118) suggereix que l'increment en l'alliberació de dopamina podria donar-se únicament en pacients amb esquizofrènia i parents propers, de manera que algunes

famílies transmetrien una vulnerabilitat particular a reaccionar de manera exagerada als cannabinoids.

Cal també destacar que hi ha cada cop més dades que indiquen que l'ús crònic de cànnabis podria estar associat a una reducció de la capacitat de síntesi de dopamina (119, 120). Malgrat que no s'han trobat diferències en la disponibilitat de receptors D_2/D_3 estriatals després d'exposicions còniques a cànnabis (**Capítol 5**), hi ha estudis que han trobat indicis de què l'exposició crònica de THC pot induir un estat d'hipodopaminergia (121). Aquest fet seria congruent amb alteracions dels mecanismes responsables del control de la síntesi i alliberació de la dopamina, junt amb l'emergència simultània d'hipersensibilitat del receptor postsinàptic de la dopamina. Aquests canvis potencialment podrien contribuir al desenvolupament de psicosi o d'altres trastorns cerebrals relacionats amb el consum de cànnabis en persones vulnerables (121-123) (**Capítols 5-7**).

Efectes crònics dels cannabinoids sobre la funció i estructura cerebral

En contrast amb els efectes aguts, l'exposició crònica a cànnabis s'ha associat amb un descens en l'activitat cerebral basal en regions riques en receptors CB_1 (**Capítol 5**), el que podria indicar una regulació a la baixa d'aquestes receptors secundaria a l'exposició crònica a cannabinoids exògens (124). D'altre banda, seguint la línia dels estudis presentats en el **Capítol 4**, els estudis de neuroimatge funcional tant en adults com en adolescents han mostrat alteracions en els patrons d'activitat neural durant la realització de tasques cognitives, tot mantenint uns resultats en la realització de la tasca dins d'uns límits normals. En conjunt, aquestes dades poden ser interpretades en termes de neuroadaptació, possiblement indicant que els consumidors crònics de cànnabis necessiten reclutar àries cerebrals addicionals, sobretot en el còrtex prefrontal, per tal de mantenir un rendiment cognitiu normal.

El **Capítol 5** també mostra que l'ús crònic de cànnabis s'associa a disminucions de la substància gris cerebral, sobretot en regions temporals medials. Aquestes regions son conegudes per estar funcionalment associades a processos de memòria, funció

executiva i emoció. Per tant, juntament amb les alteracions funcionals, aquests canvis podrien estar relacionats amb la davallada neuropsicològica observada en els consumidors crònics de cànnabis (16). És important també destacar que les pèrdues de volum s'han relacionat amb l'edat d'inici de consum i la quantitat d'exposició, amb dades recents que indiquen que ambdos factors podrien actuar de forma independent (126). Així doncs, aquests dos factors podrien interactuar juntament amb la vulnerabilitat genètica individual determinant el grau de dany cerebral relacionat amb el consum crònic de cànnabis (**Capítols 6 i 7**).

Malgrat els escassos estudis realitzats en població adolescent, aquests han revelat que les alteracions funcionals i estructurals son similars a les observades en població adulta. Aquest fet indica doncs que les conseqüències derivades de l'ús crònic de cànnabis poden aparèixer poc després de començar-ne l'ús (**Capítol 5**). A més, l'evidència apunta a què l'impacte del consum crònic podria ser diferent segons el gènere de l'usuari, havent-hi dades preliminars que indiquen que les dones podrien presentar un major risc (127, 128).

Vulnerabilitat genètica al dany cerebral induït per cànnabis

Els resultats dels **Capítols 6 i 7** aporten evidència multimodal sobre l'impacte de les variacions dels gens reguladors de la dopamina COMT i DAT1 en l'estructura cerebral d'un grup de consumidors crònics de cànnabis que iniciaren el consum de forma precoç, abans dels 16 anys d'edat.

Segons els resultats del **Capítol 6**, el genotip de la COMT va influenciar el volum d'ambos nuclis caudats ventrals d'una manera oposada segons si l'individu havia estat exposat crònicament a cànnabis o no. D'aquesta manera, posseir més còpies de l'al·lel *val* es va associar amb volums menors en els consumidors crònics de cànnabis però volums majors en els controls. L'estriat és una estructura implicada en el circuit de recompensa, doncs l'activitat dopaminèrgica juga un paper clau en els efectes de reforç associat a l'ús de drogues (131). D'altre banda, estudis preclínics han demostrat que la variació en l'activitat de la dopamina està relacionada amb el creixement i la

supervivència neuronal (132). Malgrat que el mecanisme subjacent a aquests canvis morfològics es desconeix, és possible que estigui relacionat amb l'efecte modulador que exerceixen el cànnabis i el sistema endocannabinoid en la neurotransmissió dopaminèrgica. Tant la sobre- com la infraestimulació poden potencialment resultar en alteracions de la viabilitat neuronal, indicant que possiblement existeix un interval òptim de dopamina (75), el qual podria ser específic per cada regió i estar influenciat per factors genètics i ambientals.

El genotip de la COMT també va modular el volum de l'amígdala esquerra de manera oposada entre consumidors crònics i controls (**Capítol 6**). L'amígdala és una estructura cerebral també implicada en el circuit de recompensa (133, 134), i la seva afectació ha estat àmpliament descrita sota els efectes aguts i crònics de l'exposició a cannabinoids (**Capítols 4 i 5**). És destacable notar que els efectes del consum crònic de cànnabis sobre l'estructura cerebral són similars a aquells descrits en pacients amb esquizofrènia. Estudis de neuroimatge han descrit reduccions volumètriques de fins el 6% en l'hipocamp i l'amígdala de pacients amb esquizofrènia (135, 136), els quals podrien ser particularment vulnerables als efectes del cànnabis (138). Aquest fet remarca la importància de la intervenció precoç davant la sospita d'abús de substàncies nocives en pacients (**Capítol 3**), i suggereix que aquests canvis estructurals podrien reflectir un procés fisiopatològic central associat amb la malaltia. Els resultats d'aquest estudi coincideixen amb els resultats d'altres estudis sobre la influència del genotip de la COMT en individus sans (75, 90) i en pacients amb esquizofrènia (88, 90, 140). En general, aquests estudis descriuen menor volums temporals medials en els portadors de l'al·lel *val*. Resulta interessant que en els nostres individus exposats crònicament a cànnabis s'observi exactament el patró oposat. Aquesta troballa aporta nova evidència sobre com els factors ambientals i genètics poder interactuar per determinar fenotips complexos, com ara el volum cerebral.

Per últim, el **Capítol 7** aporta dades preliminars sobre la interacció dels genotips COMT i DAT1 en el volum de l'hipocamp, l'estructura més vulnerable als efectes neurotòxics del cànnabis (**Capítol 5**). Ambdós genotips van interaccionar moderant el volum de l'hipocamp, i altre cop aquesta associació va ocórrer de forma diferent en

funció si els individus havien estat exposats prèviament a cànnabis, el que és consistent amb els resultats exposats al **Capítol 6**. Independentment dels genotips, els consumidors crònics de cànnabis van presentar un menor volum de l'hipocamp comparat amb els controls no consumidors. Les diferències no van arribar a assolir la significació estadística probablement degut a la relativa curta exposició dels nostres consumidors en comparació amb mostres d'altres estudis (66). No obstant, la correlació negativa entre el volum de l'hipocamp i l'exposició a cànnabis al llarg de la vida dóna suport a la idea de què amb el consum continuat les diferències esdevindrien finalment significatives.

Conclusió

En conjunt, aquests resultats donen suport a la participació del sistema endocannabinoid en el curs dels trastorns mentals, així com en el control de diverses funcions cognitives, modulació de dopamina i volum cerebral, apareixent de forma primerenca les alteracions derivades del seu ús crònic. Els resultats també demostren que els gens reguladors de la dopamina poden tenir un paper rellevant en la sensibilitat als efectes del cànnabis en la morfologia cerebral, proporcionant nous coneixements sobre el mecanismes subjacents al dany cerebral induït per cànnabis i sobre aspectes de vulnerabilitat genètica.

References

References

1. European Monitoring Centre for Drugs and Drug Addiction. European Drug Report 2013: Trends and developments. Lisbon: EMCDDA 2013. http://www.emcdda.europa.eu/publications/edr/trends-developments/2013. Accessed 23 April 2014.

2. Mechoulam R, Gaoni Y. Recent advances in the chemistry of hashish. Fortschr Chem Org Naturst. 1967;25:175-213.

3. Mechoulam R, Parker LA. The Endocannabinoid System and the Brain. Annu Rev Psychol. 2013;64:21-47.

4. Crippa JA, Derenusson GN, Ferrari TB, Wichert-Ana L, Duran FL, Martin-Santos R, et al. Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. J Psychopharmacol. 2011;25(1):121-30.

5. Zuardi AW, Crippa JA, Hallak JE, Bhattacharyya S, Atakan Z, Martin-Santos R, et al. A critical review of the antipsychotic effects of Cannabidiol: 30 years of a translational investigation. Curr Pharm Des. 2012;18(32):5131-40.

6. Di Forti M, Morgan C, Dazzan P, Pariante C, Mondelli V, Marques TR, et al. High-potency cannabis and the risk of psychosis. Br J Psychiatry. 2009;195(6):488-91.

7. Di Forti M, Sallis H, Allegri F, Trotta A, Ferraro L, Stilo SA, et al. Daily Use, Especially of High-Potency Cannabis, Drives the Earlier Onset of Psychosis in Cannabis Users. Schizophr Bull. 2014; Mar 19 [Epub ahead of print].

8. Chen CY, O'Brien MS, Anthony JC. Who becomes cannabis dependent soon after onset of use? Epidemiological evidence from the United States: 2000-2001. Drug Alcohol Depend. 2005;79(1):11-22.

9. Budney AJ, Moore BA, Rocha HL, Higgins ST. Clinical trial of abstinence-based vouchers and cognitive-behavioral therapy for cannabis dependence. J Consult Clin Psychol. 2006;74(2):307-16.

10. Fernandez-Artamendi S, Fernandez-Hermida JR, Secades-Villa R, Garcia-Portilla P. Cannabis and mental health. Actas Esp Psiquiatr. 2011;39(3):180-90.

11. Crean RD, Crane NA, Mason BJ. An evidence based review of acute and long-term effects of cannabis use on executive cognitive functions. J Addict Med. 2011;5(1):1-8.

12. Grant I, Gonzalez R, Carey CL, Natarajan L, Wolfson T. Non-acute (residual) neurocognitive effects of cannabis use: a meta-analytic study. J Int Neuropsychol Soc. 2003;9(5):679-89.

13. Schreiner AM, Dunn ME. Residual effects of cannabis use on neurocognitive performance after prolonged abstinence: a meta-analysis. Exp Clin Psychopharmacol. 2012;20(5):420-9.

14. Bolla KI, Brown K, Eldreth D, Tate K, Cadet JL. Dose-related neurocognitive effects of marijuana use. Neurology. 2002;59(9):1337-43.

15. Pope HG, Jr., Gruber AJ, Hudson JI, Cohane G, Huestis MA, Yurgelun-Todd D. Early-onset cannabis use and cognitive deficits: what is the nature of the association? Drug Alcohol Depend. 2003;69(3):303-10.

16. Meier MH, Caspi A, Ambler A, Harrington H, Houts R, Keefe RS, et al. Persistent cannabis users show neuropsychological decline from childhood to midlife. Proc Natl Acad Sci USA. 2012;109(40):E2657-64.

17. Crippa JA, Derenusson GN, Chagas MH, Atakan Z, Martin-Santos R, Zuardi AW, et al. Pharmacological interventions in the treatment of the acute effects of cannabis: a systematic review of literature. Harm Reduct J. 2012;9(1):7.

18. Arendt M, Rosenberg R, Foldager L, Perto G, Munk-Jorgensen P. Psychopathology among cannabis-dependent treatment seekers and association with later substance abuse treatment. J Subst Abuse Treat. 2007;32(2):113-9.

19. Bovasso GB. Cannabis abuse as a risk factor for depressive symptoms. Am J Psychiatry. 2001;158(12):2033-7.

20. van Os J, Bak M, Hanssen M, Bijl RV, de Graaf R, Verdoux H. Cannabis use and psychosis: a longitudinal population-based study. Am J Epidemiol. 2002;156(4):319-27.

21. Moore TH, Zammit S, Lingford-Hughes A, Barnes TR, Jones PB, Burke M, et al. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet. 2007;370(9584):319-28.

22. Thomas H. A community survey of adverse effects of cannabis use. Drug Alcohol Depend. 1996;42(3):201-7.

23. Arseneault L, Cannon M, Poulton R, Murray R, Caspi A, Moffitt TE. Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. BMJ. 2002;325(7374):1212-3.

24. D'Souza DC, Abi-Saab WM, Madonick S, Forselius-Bielen K, Doersch A, Braley G, et al. Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. Biol Psychiatry. 2005;57(6):594-608.

25. Margolese HC, Malchy L, Negrete JC, Tempier R, Gill K. Drug and alcohol use among patients with schizophrenia and related psychoses: levels and consequences. Schizophr Res. 2004;67(2-3):157-66.

26. Zammit S, Moore TH, Lingford-Hughes A, Barnes TR, Jones PB, Burke M, et al. Effects of cannabis use on outcomes of psychotic disorders: systematic review. Br J Psychiatry. 2008;193(5):357-63.

27. Maldonado R, Berrendero F, Ozaita A, Robledo P. Neurochemical basis of cannabis addiction. Neuroscience. 2011;181:1-17.

28. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science. 1992;258(5090):1946-9.

29. Stella N, Schweitzer P, Piomelli D. A second endogenous cannabinoid that modulates long-term potentiation. Nature. 1997;388(6644):773-8.

30. Wilson RI, Nicoll RA. Endocannabinoid signaling in the brain. Science. 2002;296(5568):678-82.

31. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature. 1990;346(6284):561-4.

32. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature. 1993;365(6441):61-5.

33. Glass M, Dragunow M, Faull RL. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neuroscience. 1997;77(2):299-318.

34. Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M. Endocannabinoid-mediated control of synaptic transmission. Physiol Rev. 2009;89(1):309-80.
35. Berghuis P, Rajnicek AM, Morozov YM, Ross RA, Mulder J, Urban GM, et al. Hardwiring the brain: endocannabinoids shape neuronal connectivity. Science. 2007;316(5828):1212-6.
36. Mulder J, Aguado T, Keimpema E, Barabas K, Ballester Rosado CJ, Nguyen L, et al. Endocannabinoid signaling controls pyramidal cell specification and long-range axon patterning. Proc Natl Acad Sci USA. 2008;105(25):8760-5.
37. Viveros MP, Llorente R, Suarez J, Llorente-Berzal A, Lopez-Gallardo M, de Fonseca FR. The endocannabinoid system in critical neurodevelopmental periods: sex differences and neuropsychiatric implications. J Psychopharmacol. 2012;26(1):164-76.
38. Giedd JN. Structural magnetic resonance imaging of the adolescent brain. Ann N Y Acad Sci. 2004;1021:77-85.
39. Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, Vaituzis AC, et al. Dynamic mapping of human cortical development during childhood through early adulthood. Proc Natl Acad Sci USA. 2004;101(21):8174-9.
40. Fusar-Poli P, Borgwardt S, Bechdolf A, Addington J, Riecher-Rossler A, Schultze-Lutter F, et al. The psychosis high-risk state: a comprehensive state-of-the-art review. JAMA Psychiatry. 2013;70(1):107-20.
41. Paus T, Keshavan M, Giedd JN. Why do many psychiatric disorders emerge during adolescence? Nat Rev Neurosci. 2008;9(12):947-57.
42. Fusar-Poli P, Borgwardt S, Crescini A, Deste G, Kempton MJ, Lawrie S, et al. Neuroanatomy of vulnerability to psychosis: a voxel-based meta-analysis. Neurosci Biobehav Rev. 2011;35(5):1175-85.
43. Fusar-Poli P, Howes OD, Allen P, Broome M, Valli I, Asselin MC, et al. Abnormal frontostriatal interactions in people with prodromal signs of psychosis: a multimodal imaging study. Arch Gen Psychiatry. 2010;67(7):683-91.
44. Fusar-Poli P, Howes OD, Allen P, Broome M, Valli I, Asselin MC, et al. Abnormal prefrontal activation directly related to pre-synaptic striatal dopamine dysfunction in people at clinical high risk for psychosis. Mol Psychiatry. 2011;16(1):67-75.
45. Fusar-Poli P, Meyer-Lindenberg A. Striatal presynaptic dopamine in schizophrenia, part II: meta-analysis of [(18)F/(11)C]-DOPA PET studies. Schizophr Bull. 2013;39(1):33-42.
46. Fusar-Poli P, Radua J, McGuire P, Borgwardt S. Neuroanatomical maps of psychosis onset: voxel-wise meta-analysis of antipsychotic-naive VBM studies. Schizophr Bull. 2012;38(6):1297-307.
47. O'Shea M, Singh ME, McGregor IS, Mallet PE. Chronic cannabinoid exposure produces lasting memory impairment and increased anxiety in adolescent but not adult rats. J Psychopharmacol. 2004;18(4):502-8.
48. Rubino T, Realini N, Braida D, Alberio T, Capurro V, Vigano D, et al. The depressive phenotype induced in adult female rats by adolescent exposure to THC is associated with cognitive impairment and altered neuroplasticity in the prefrontal cortex. Neurotox Res. 2009;15(4):291-302.

49. Rubino T, Realini N, Braida D, Guidi S, Capurro V, Vigano D, et al. Changes in hippocampal morphology and neuroplasticity induced by adolescent THC treatment are associated with cognitive impairment in adulthood. Hippocampus. 2009;19(8):763-72.

50. Quinn HR, Matsumoto I, Callaghan PD, Long LE, Arnold JC, Gunasekaran N, et al. 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. 2008;33(5):1113-26.

51. Harte LC, Dow-Edwards D. Sexually dimorphic alterations in locomotion and reversal learning after adolescent tetrahydrocannabinol exposure in the rat. Neurotoxicol Teratol. 2010;32(5):515-24.

52. Battisti RA, Roodenrys S, Johnstone SJ, Pesa N, Hermens DF, Solowij N. Chronic cannabis users show altered neurophysiological functioning on Stroop task conflict resolution. Psychopharmacology (Berl). 2010;212(4):613-24.

53. Ehrenreich H, Rinn T, Kunert HJ, Moeller MR, Poser W, Schilling L, et al. Specific attentional dysfunction in adults following early start of cannabis use. Psychopharmacology (Berl). 1999;142(3):295-301.

54. Fontes MA, Bolla KI, Cunha PJ, Almeida PP, Jungerman F, Laranjeira RR, et al. Cannabis use before age 15 and subsequent executive functioning. Br J Psychiatry. 2011;198(6):442-7.

55. Gruber SA, Sagar KA, Dahlgren MK, Racine M, Lukas SE. Age of onset of marijuana use and executive function. Psychol Addict Behav. 2012;26(3):496-506.

56. Solowij N, Jones KA, Rozman ME, Davis SM, Ciarrochi J, Heaven PC, et al. Verbal learning and memory in adolescent cannabis users, alcohol users and non-users. Psychopharmacology (Berl). 2011;216(1):131-44.

57. Solowij N, Jones KA, Rozman ME, Davis SM, Ciarrochi J, Heaven PC, et al. Reflection impulsivity in adolescent cannabis users: a comparison with alcohol-using and non-substance-using adolescents. Psychopharmacology (Berl). 2012;219(2):575-86.

58. Lorenzetti V, Solowij N, Fornito A, Lubman D, Yucel M. The Association between Regular Cannabis Exposure and Alterations of Human Brain Morphology: An Updated Review of the Literature. Curr Pharm Des. 2013; Jun 14 [Epub ahead of print].

59. Adriani W, Laviola G. Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. Behav Pharmacol. 2004;15(5-6):341-52.

60. Bossong MG, Niesink RJ. Adolescent brain maturation, the endogenous cannabinoid system and the neurobiology of cannabis-induced schizophrenia. Prog Neurobiol. 2010;92(3):370-85.

61. Landfield PW, Cadwallader LB, Vinsant S. Quantitative changes in hippocampal structure following long-term exposure to delta 9-tetrahydrocannabinol: possible mediation by glucocorticoid systems. Brain Res. 1988;443(1-2):47-62.

62. Scallet AC, Uemura E, Andrews A, Ali SF, McMillan DE, Paule MG, et al. Morphometric studies of the rat hippocampus following chronic delta-9-tetrahydrocannabinol (THC). Brain Res. 1987;436(1):193-8.

63. Schneider M, Koch M. 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. 2003;28(10):1760-9.
64. Demirakca T, Sartorius A, Ende G, Meyer N, Welzel H, Skopp G, et al. Diminished gray matter in the hippocampus of cannabis users: Possible protective effects of cannabidiol. Drug Alcohol Depend. 2011;114(2-3):242-5.
65. Matochik JA, Eldreth DA, Cadet JL, Bolla KI. Altered brain tissue composition in heavy marijuana users. Drug Alcohol Depend. 2005;77(1):23-30.
66. Yucel M, Solowij N, Respondek C, Whittle S, Fornito A, Pantelis C, et al. Regional brain abnormalities associated with long-term heavy cannabis use. Arch Gen Psychiatry. 2008;65(6):694-701.
67. Zalesky A, Solowij N, Yucel M, Lubman DI, Takagi M, Harding IH, et al. Effect of long-term cannabis use on axonal fibre connectivity. Brain. 2012;135(Pt 7):2245-55.
68. Estrada G, Fatjo-Vilas M, Munoz MJ, Pulido G, Minano MJ, Toledo E, et al. Cannabis use and age at onset of psychosis: further evidence of interaction with COMT Val158Met polymorphism. Acta Psychiatr Scand. 2011;123(6):485-92.
69. Henquet C, Di Forti M, Morrison P, Kuepper R, Murray RM. Gene-environment interplay between cannabis and psychosis. Schizophr Bull. 2008;34(6):1111-21.
70. Henquet C, Rosa A, Krabbendam L, Papiol S, Fananas L, Drukker M, et al. An experimental study of catechol-o-methyltransferase Val158Met moderation of delta-9-tetrahydrocannabinol-induced effects on psychosis and cognition. Neuropsychopharmacology. 2006;31(12):2748-57.
71. Barnett JH, Jones PB, Robbins TW, Muller U. Effects of the catechol-O-methyltransferase Val158Met polymorphism on executive function: a meta-analysis of the Wisconsin Card Sort Test in schizophrenia and healthy controls. Mol Psychiatry. 2007;12(5):502-9.
72. Bertolino A, Blasi G, Latorre V, Rubino V, Rampino A, Sinibaldi L, et al. Additive effects of genetic variation in dopamine regulating genes on working memory cortical activity in human brain. J Neurosci. 2006;26(15):3918-22.
73. Prata DP, Mechelli A, Fu CH, Picchioni M, Kane F, Kalidindi S, et al. Opposite effects of catechol-O-methyltransferase Val158Met on cortical function in healthy subjects and patients with schizophrenia. Biol Psychiatry. 2009;65(6):473-80.
74. Durston S, Fossella JA, Casey BJ, Hulshoff Pol HE, Galvan A, Schnack HG, et al. Differential effects of DRD4 and DAT1 genotype on fronto-striatal gray matter volumes in a sample of subjects with attention deficit hyperactivity disorder, their unaffected siblings, and controls. Mol Psychiatry. 2005;10(7):678-85.
75. Honea R, Verchinski BA, Pezawas L, Kolachana BS, Callicott JH, Mattay VS, et al. Impact of interacting functional variants in COMT on regional gray matter volume in human brain. Neuroimage. 2009;45(1):44-51.
76. Kambeitz JP, Bhattacharyya S, Kambeitz-Ilankovic LM, Valli I, Collier DA, McGuire P. Effect of BDNF val(66)met polymorphism on declarative memory and its neural substrate: a meta-analysis. Neurosci Biobehav Rev. 2012;36(9):2165-77.
77. Matsumoto M, Weickert CS, Akil M, Lipska BK, Hyde TM, Herman MM, et al. Catechol O-methyltransferase mRNA expression in human and rat brain: evidence for a role in cortical neuronal function. Neuroscience. 2003;116(1):127-37.
78. Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, et al. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on

mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet. 2004;75(5):807-21.

79. Vandenbergh DJ, Persico AM, Hawkins AL, Griffin CA, Li X, Jabs EW, et al. Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR. Genomics. 1992;14(4):1104-6.

80. Heinz A, Goldman D, Jones DW, Palmour R, Hommer D, Gorey JG, et al. Genotype influences in vivo dopamine transporter availability in human striatum. Neuropsychopharmacology. 2000;22(2):133-9.

81. Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, et al. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci U S A. 2001;98(12):6917-22.

82. Goldberg TE, Egan MF, Gscheidle T, Coppola R, Weickert T, Kolachana BS, et al. Executive subprocesses in working memory: relationship to catechol-O-methyltransferase Val158Met genotype and schizophrenia. Arch Gen Psychiatry. 2003;60(9):889-96.

83. Bilder RM, Volavka J, Lachman HM, Grace AA. The catechol-O-methyltransferase polymorphism: relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes. Neuropsychopharmacology. 2004;29(11):1943-61.

84. Kapur S. Psychosis as a state of aberrant salience: a framework linking biology, phenomenology, and pharmacology in schizophrenia. Am J Psychiatry. 2003;160(1):13-23.

85. Jaspar M, Genon S, Muto V, Meyer C, Manard M, Dideberg V, et al. Modulating effect of COMT genotype on the brain regions underlying proactive control process during inhibition. Cortex. 2014;50:148-61.

86. Bertolino A, Di GA, Blasi G, Sambataro F, Caforio G, Sinibaldi L, et al. Epistasis between dopamine regulating genes identifies a nonlinear response of the human hippocampus during memory tasks. Biol Psychiatry. 2008;64(3):226-34.

87. Prata DP, Mechelli A, Fu CH, Picchioni M, Toulopoulou T, Bramon E, et al. Epistasis between the DAT 3' UTR VNTR and the COMT Val158Met SNP on cortical function in healthy subjects and patients with schizophrenia. Proc Natl Acad Sci USA. 2009;106(32):13600-5.

88. McIntosh AM, Baig BJ, Hall J, Job D, Whalley HC, Lymer GK, et al. Relationship of catechol-O-methyltransferase variants to brain structure and function in a population at high risk of psychosis. Biol Psychiatry. 2007;61(10):1127-34.

89. Ohnishi T, Hashimoto R, Mori T, Nemoto K, Moriguchi Y, Iida H, et al. The association between the Val158Met polymorphism of the catechol-O-methyl transferase gene and morphological abnormalities of the brain in chronic schizophrenia. Brain. 2006;129(Pt 2):399-410.

90. Ehrlich S, Morrow EM, Roffman JL, Wallace SR, Naylor M, Bockholt HJ, et al. The COMT Val108/158Met polymorphism and medial temporal lobe volumetry in patients with schizophrenia and healthy adults. Neuroimage. 2010;53(3):992-1000.

91. Fernandez-Egea E, Bernardo M, Donner T, Conget I, Parellada E, Justicia A, et al. Metabolic profile of antipsychotic-naive individuals with non-affective psychosis. Br J Psychiatry. 2009;194(5):434-8.

92. Garcia-Rizo C, Fernandez-Egea E, Oliveira C, Justicia A, Parellada E, Bernardo M, et al. Prolactin concentrations in newly diagnosed, antipsychotic-naive patients with nonaffective psychosis. Schizophr Res. 2012;134(1):16-9.
93. First M, Spitzer R. SCID-I Structured Clinical Interview for the DSM-IV Axis I Disorders [Spanish] (trans. J Blanch, I Andreu). Masson ed. 1999.
94. Peralta V, Cuesta MJ. Psychometric properties of the positive and negative syndrome scale (PANSS) in schizophrenia. Psychiatry Res. 1994;53(1):31-40.
95. Rosenberg SD, Drake RE, Wolford GL, Mueser KT, Oxman TE, Vidaver RM, et al. Dartmouth Assessment of Lifestyle Instrument (DALI): a substance use disorder screen for people with severe mental illness. Am J Psychiatry. 1998;155(2):232-8.
96. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoSMed. 2009;6(7):e1000097.
97. Torrens M, Serrano D, Astals M, Perez-Dominguez G, Martin-Santos R. Diagnosing comorbid psychiatric disorders in substance abusers: validity of the Spanish versions of the Psychiatric Research Interview for Substance and Mental Disorders and the Structured Clinical Interview for DSM-IV. Am J Psychiatry. 2004;161(7):1231-7.
98. Batalla A, Soriano-Mas C, Lopez-Sola M, Torrens M, Crippa JA, Bhattacharyya S, et al. Modulation of brain structure by catechol-O-methyltransferase Val(158) Met polymorphism in chronic cannabis users. Addict Biol. 2013; Jan 14 [Epub ahead of print].
99. Velakoulis D, Pantelis C, McGorry PD, Dudgeon P, Brewer W, Cook M, et al. Hippocampal volume in first-episode psychoses and chronic schizophrenia: a high-resolution magnetic resonance imaging study. Arch Gen Psychiatry. 1999;56(2):133-41.
100. Velakoulis D, Wood SJ, Wong MT, McGorry PD, Yung A, Phillips L, et al. Hippocampal and amygdala volumes according to psychosis stage and diagnosis: a magnetic resonance imaging study of chronic schizophrenia, first-episode psychosis, and ultra-high-risk individuals. Arch Gen Psychiatry. 2006;63(2):139-49.
101. Barnes TR, Mutsatsa SH, Hutton SB, Watt HC, Joyce EM. Comorbid substance use and age at onset of schizophrenia. Br J Psychiatry. 2006;188:237-42.
102. Cantwell R, Brewin J, Glazebrook C, Dalkin T, Fox R, Medley I, et al. Prevalence of substance misuse in first-episode psychosis. Br J Psychiatry. 1999;174:150-3.
103. Kamali M, McTigue O, Whitty P, Gervin M, Clarke M, Browne S, et al. Lifetime history of substance misuse in first-episode psychosis: prevalence and its influence on psychopathology and onset of psychotic symptoms. Early Interv Psychiatry. 2009;3(3):198-203.
104. Larsen TK, Melle I, Auestad B, Friis S, Haahr U, Johannessen JO, et al. Substance abuse in first-episode non-affective psychosis. Schizophr Res. 2006;88(1-3):55-62.
105. Van Dorn RA, Desmarais SL, Scott YM, Sellers BG, Swartz MS. Assessing illicit drug use among adults with schizophrenia. Psychiatry Res. 2012;200(2-3):228-36.
106. Malla A, Norman R, Bechard-Evans L, Schmitz N, Manchanda R, Cassidy C. Factors influencing relapse during a 2-year follow-up of first-episode psychosis in a specialized early intervention service. Psychol Med. 2008;38(11):1585-93.
107. Turkington A, Mulholland CC, Rushe TM, Anderson R, McCaul R, Barrett SL, et al. Impact of persistent substance misuse on 1-year outcome in first-episode psychosis. Br J Psychiatry. 2009;195(3):242-8.

108. Wade D, Harrigan S, Edwards J, Burgess PM, Whelan G, McGorry PD. Substance misuse in first-episode psychosis: 15-month prospective follow-up study. Br J Psychiatry. 2006;189:229-34.

109. Hides L, Dawe S, Kavanagh DJ, Young RM. Psychotic symptom and cannabis relapse in recent-onset psychosis. Prospective study. Br J Psychiatry. 2006;189:137-43.

110. Baeza I, Graell M, Moreno D, Castro-Fornieles J, Parellada M, Gonzalez-Pinto A, et al. Cannabis use in children and adolescents with first episode psychosis: influence on psychopathology and short-term outcome (CAFEPS study). Schizophr Res. 2009;113(2-3):129-37.

111. Gonzalez-Pinto A, Alberich S, Barbeito S, Gutierrez M, Vega P, Ibanez B, et al. Cannabis and first-episode psychosis: different long-term outcomes depending on continued or discontinued use. Schizophr Bull. 2011;37(3):631-9.

112. Grech A, van OJ, Jones PB, Lewis SW, Murray RM. Cannabis use and outcome of recent onset psychosis. Eur Psychiatry. 2005;20(4):349-53.

113. Sorbara F, Liraud F, Assens F, Abalan F, Verdoux H. Substance use and the course of early psychosis: a 2-year follow-up of first-admitted subjects. Eur Psychiatry. 2003;18(3):133-6.

114. Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. Physiol Rev. 2003;83(3):1017-66.

115. Bhattacharyya S, Crippa JA, Allen P, Martin-Santos R, Borgwardt S, Fusar-Poli P, et al. Induction of psychosis by {delta}9-tetrahydrocannabinol reflects modulation of prefrontal and striatal function during attentional salience processing. Arch Gen Psychiatry. 2012;69(1):27-36.

116. Murray RM, Lappin J, Di Forti M. Schizophrenia: from developmental deviance to dopamine dysregulation. Eur Neuropsychopharmacol. 2008;18 Suppl 3:S129-34.

117. Morrison PD, Murray RM. From real-world events to psychosis: the emerging neuropharmacology of delusions. Schizophr Bull. 2009;35(4):668-74.

118. Kuepper R, Ceccarini J, Lataster J, van Os J, van Kroonenburgh M, van Gerven JM, et al. Delta-9-tetrahydrocannabinol-induced dopamine release as a function of psychosis risk: 18F-fallypride positron emission tomography study. PLoS One. 2013;8(7):e70378.

119. Bloomfield MA, Morgan CJ, Egerton A, Kapur S, Curran HV, Howes OD. Dopaminergic function in cannabis users and its relationship to cannabis-induced psychotic symptoms. Biol Psychiatry. 2014;75(6):470-8.

120. Thompson JL, Urban N, Slifstein M, Xu X, Kegeles LS, Girgis RR, et al. Striatal dopamine release in schizophrenia comorbid with substance dependence. Mol Psychiatry. 2013;18(8):909-15.

121. Ginovart N, Tournier BB, Moulin-Sallanon M, Steimer T, Ibanez V, Millet P. Chronic Delta(9)-tetrahydrocannabinol exposure induces a sensitization of dopamine D(2)/(3) receptors in the mesoaccumbens and nigrostriatal systems. Neuropsychopharmacology. 2012;37(11):2355-67.

122. Murray RM, Mehta M, Di Forti M. Different dopaminergic abnormalities underlie cannabis dependence and cannabis-induced psychosis. Biol Psychiatry. 2014;75(6):430-1.

123. Seeman MV, Seeman P. Is schizophrenia a dopamine supersensitivity psychotic reaction? Prog Neuropsychopharmacol Biol Psychiatry. 2014;48:155-60.
124. Chang L, Chronicle EP. Functional imaging studies in cannabis users. Neuroscientist. 2007;13(5):422-32.
125. Hirvonen J, Goodwin RS, Li CT, Terry GE, Zoghbi SS, Morse C, et al. Reversible and regionally selective downregulation of brain cannabinoid CB(1) receptors in chronic daily cannabis smokers. Mol Psychiatry. 2012;17(6):642-9.
126. Battistella G, Fornari E, Annoni JM, Chtioui H, Dao K, Fabritius M, et al. Long-Term Effects of Cannabis on Brain Structure. Neuropsychopharmacology. 2014;Mar 17 [Epub ahead of print].
127. McQueeny T, Padula CB, Price J, Medina KL, Logan P, Tapert SF. Gender effects on amygdala morphometry in adolescent marijuana users. Behav Brain Res. 2011;224(1):128-34.
128. Medina KL, McQueeny T, Nagel BJ, Hanson KL, Yang TT, Tapert SF. Prefrontal cortex morphometry in abstinent adolescent marijuana users: subtle gender effects. Addict Biol. 2009;14(4):457-68.
129. Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, et al. Brain development during childhood and adolescence: a longitudinal MRI study. Nat Neurosci. 1999;2(10):861-3.
130. Van Laere K, Goffin K, Casteels C, Dupont P, Mortelmans L, de Hoon J, et al. Gender-dependent increases with healthy aging of the human cerebral cannabinoid-type 1 receptor binding using [(18)F]MK-9470 PET. Neuroimage. 2008;39(4):1533-41.
131. Koob GF, Volkow ND. Neurocircuitry of addiction. Neuropsychopharmacology. 2010;35(1):217-38.
132. Santiago M, Matarredona ER, Granero L, Cano J, Machado A. Neurotoxic relationship between dopamine and iron in the striatal dopaminergic nerve terminals. Brain Res. 2000;858(1):26-32.
133. Gilman JM, Kuster JK, Lee S, Lee MJ, Kim BW, Makris N, et al. Cannabis use is quantitatively associated with nucleus accumbens and amygdala abnormalities in young adult recreational users. J Neurosci. 2014;34(16):5529-38.
134. Gilman JM, Ramchandani VA, Davis MB, Bjork JM, Hommer DW. Why we like to drink: a functional magnetic resonance imaging study of the rewarding and anxiolytic effects of alcohol. J Neurosci. 2008;28(18):4583-91.
135. Honea R, Crow TJ, Passingham D, Mackay CE. Regional deficits in brain volume in schizophrenia: a meta-analysis of voxel-based morphometry studies. Am J Psychiatry. 2005;162(12):2233-45.
136. Yoshida T, McCarley RW, Nakamura M, Lee K, Koo MS, Bouix S, et al. A prospective longitudinal volumetric MRI study of superior temporal gyrus gray matter and amygdala-hippocampal complex in chronic schizophrenia. Schizophr Res. 2009;113(1):84-94.
137. Andreasen NC, Nopoulos P, Magnotta V, Pierson R, Ziebell S, Ho BC. Progressive brain change in schizophrenia: a prospective longitudinal study of first-episode schizophrenia. Biol Psychiatry. 2011;70(7):672-9.

138. Rais M, van Haren NE, Cahn W, Schnack HG, Lepage C, Collins L, et al. Cannabis use and progressive cortical thickness loss in areas rich in CB1 receptors during the first five years of schizophrenia. Eur Neuropsychopharmacol. 2010;20(12):855-65.

139. Barnes A, Isohanni M, Barnett JH, Pietilainen O, Veijola J, Miettunen J, et al. No association of COMT (Val158Met) genotype with brain structure differences between men and women. PLoS One. 2012;7(3):e33964.

140. Raznahan A, Greenstein D, Lee Y, Long R, Clasen L, Gochman P, et al. Catechol-o-methyl transferase (COMT) val158met polymorphism and adolescent cortical development in patients with childhood-onset schizophrenia, their non-psychotic siblings, and healthy controls. Neuroimage. 2011;57(4):1517-23.

141. Alvarez-Jimenez M, Priede A, Hetrick SE, Bendall S, Killackey E, Parker AG, et al. Risk factors for relapse following treatment for first episode psychosis: A systematic review and meta-analysis of longitudinal studies. Schizophr Res. 2012;139(1-3):116-28.

142. Lopez-Pelayo H, Batalla A, Balcells M, Colom J, Gual A. Assessment of cannabis use disorders: a systematic review of screening and diagnostic instruments. Psychol Med. 2014; Accepted.

143. Licata SC, Renshaw PF. Neurochemistry of drug action: insights from proton magnetic resonance spectroscopic imaging and their relevance to addiction. Ann NY Acad Sci. 2010;1187:148-71.

144. Cheetham A, Allen NB, Whittle S, Simmons J, Yucel M, Lubman DI. Orbitofrontal volumes in early adolescence predict initiation of cannabis use: a 4-year longitudinal and prospective study. Biol Psychiatry. 2012;71(8):684-92.

145. Hermann D, Schneider M. Potential protective effects of cannabidiol on neuroanatomical alterations in cannabis users and psychosis: a critical review. Curr Pharm Des. 2012;18(32):4897-905.

146. Schacht JP, Hutchison KE, Filbey FM. Associations between cannabinoid receptor-1 (CNR1) variation and hippocampus and amygdala volumes in heavy cannabis users. Neuropsychopharmacology. 2012;37(11):2368-76.

147. Bueller JA, Aftab M, Sen S, Gomez-Hassan D, Burmeister M, Zubieta JK. BDNF Val66Met allele is associated with reduced hippocampal volume in healthy subjects. Biol Psychiatry. 2006;59(9):812-5.

148. Szeszko PR, Lipsky R, Mentschel C, Robinson D, Gunduz-Bruce H, Sevy S, et al. Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation. Mol Psychiatry. 2005;10(7):631-6.

149. Thompson PM, Stein JL, Medland SE, Hibar DP, Vasquez AA, Renteria ME, et al. The ENIGMA Consortium: large-scale collaborative analyses of neuroimaging and genetic data. Brain Imaging Behav. 2014;8(2):153-82.

List of abbreviations

List of abbreviations

2-AG	2-arachidonylglycerol
2-DG	2-deoxyglucose
11-OH-THC	11-hydroxy-tetrahydrocannabinol
ACC	anterior cingulate cortex
AEA	anandamide
AIS	analog intoxication scale
AKT1	v-akt murine thymoma viral oncogene homolog 1
ANCOVA	analysis of covariance
ANT	attention network task
ASL	arterial spin labelling
AUC	area under the curve
BD	basal ganglia and diencephalon
BDNF	brain derived neurotrophic factor
BIS	Barrat impulsivity scale
BOLD	blood oxygen level dependent signal
BP_{ND}	non-displaceable binding potential
BS	between-subjects
Cb	cerebellum
CB_1	cannabinoid receptor-1
CB_2	cannabinoid receptor-2
CBD	cannabidiol
CBF	cerebral blood flow
CG	control group
CI	confidence interval
CNR1	cannabis receptor-1 gene
CU	cannabis users
COMT	catechol-O-methyltransferase
CSF	cerebral spinal fluid
CT	computerised tomography
DA	dopamine
DALI	Dartmouth assessment of lifestyle inventory
DAT1	dopamine transporter gene
DB	double-blinded
DEQ	drug effects questionnaire
d.f.	degrees of freedom
DLPFC	dorsolateral prefrontal cortex

DPI	depersonalization inventory
DRD_2	D_2 receptor genotype
DSC	dynamic susceptibility contrast
DSM-IV	diagnostic and statistical manual for mental disorders-fourth edition
DTI	diffusion tensor imaging
DUP	duration of untrated psychosis
EC	electrochemical
eCB	endocannabinoid
EG	experimental group
ENIGMA	enhancing neuroimaging genetics through meta-analysis
F	female
FA	fractional anisotropy
FAAH	fatty acid amide hydrolase
FDG	fludeoxyglucose
FL	frontal lobe
fMRI	functional magnetic resonance imaging
G	gavage
GABA	gamma-aminobutyric acid
GM	grey matter
GMd	grey matter density
HC	healthy controls
HR	hazard ratio
I	insula
IAP	iodoantipyrine
Inh	inhaled
IBZM	iodobenzamide
ICC	intra-class correlation coefficients
ICV	intracerebroventricular
IGT	Iowa gambling task
IP	intraperitoneally
IQ	intelligence quotient
IV	intravenous
kg	kilogram
L	left hemisphere
LCGU	local cerebral glucose utilisation
LE	long evans
LSD	lysergic acid diethylamide
M	male

MAEA	methaanandamide
MC	marijuana cigarette
MD	mean diffusivity
Met	methionine
mg	milligram
MI	microingection
ml	millilitre
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MSTI	multi-source interference task
NAcc	nucleus accumbens
NB	non-blinded
NC	non-controlled
NE	not specified
NR	non-randomized
NRG1	neuregulin-1
NS	non-significant
NPV	negative predictive value
O	oral
OFC	orbitofrontal cortex
OL	occipital lobe
PANSS	positive and negative syndrome scale
PC	placebo-controlled
PCR	polymerase chain reaction
PET	positron emission tomography
PFC	prefrontal cortex
PL	parietal lobe
PPV	positive predictive value
PRISM	psychiatric research interview for substance and mental disorders
PRISMA	preferred reporting Items for systematic reviews and meta-analyses
R	right hemisphere
Random	randomized
rCBF	regional cerebral blood flow
ROI	region of interest
ROC	receiver operating characteristic
S	smoking
SB	single-blinded
SC	subcutaneously

SCID-I	structured clinical interview for DSM-IV axis I disorders
SCR	skin conductance response
SD	standard deviation
SDw	Sprague Dawley
Se	sensitivity
SN	substantia nigra
SNP	single nucleotide polymorphism
Sp	specificity
SPECT	single photon emission tomography
SPM	statistical parametric mapping
SPSS	statistical package for the social sciences
STG	superior temporal gyrus
SWM	spatial working memory
Tc-ECD	ethyl-cisteinate-dimer labelled with technetium-99
T	tesla
TDI	temporal disintegration inventory
TE	echo time
TIV	total intracranial volume
THC	Δ9-tetrahydrocannabinol
TL	temporal lobe
tSNR	temporal signal-to-noise ratio
TTX	tetrodotoxin
UnR	unrestrained
UTR	untranslated region
Val	valine
VBM	voxel based morphometry
VMFPC	ventromedial prefrontal cortex
VNTR	variable number of tandem repeats
VS	ventral striatum
V_T	distribution volume
VTA	ventral tegmental area
WAIS	Wechsler adult intelligence scale
WM	white matter
WMd	white matter density
WS	within-subjects
yr	year

Acknowledgments

Acknowledgments

There are a lot of people who I would like to thank the work developed herein. First of all, I thank the collaboration of all the participants and their families for their selfless help. Science would never go forward without your help.

Rocío, me gustaría darte las gracias por toda la confianza que siempre has depositado en mi, y por haberme dado la oportunidad de trabajar contigo en un tema del que compartimos (y siempre compartiremos) muchas ideas y un gran interés.

Sagnik, I would like to thank you for your kindness, closeness and patience. I hope I will continue learning from your great ideas, passion and motivation. Your comments, suggestions and help have always been greatly enlightening.

Vale and Murat, my Australian teachers and friends, thanks for spreading your knowledge and for growing up in me (even further) the passion for neuroimaging. Vale, thanks a lot for everything but especially for your helpful advices (not in music though!). Murat, thank you very much for sharing your brilliance and eloquence (even though the Dutch is still a matter to discuss). També he de donar les gràcies a altres mestres que han contribuït enormement en el meu coneixement -encara bàsic- en neuroimatge: Carles Soriano, Esther Via (mil gràcies per tota l'ajuda i suport, especialment en terres llunyanes), Jesús Pujol i Laura Blanco (per totes les tardes i matins que t'he envaït el despatx; tan de bo puguis complir aviat el teu somni-utopia).

BCSU (antes conocido como PEC). Eduard i Dr. Pons, gràcies per la vostra confiança i ensenyança en tot lo referent al trastorn mental més apassionant de la psiquiatria. Clem, por querer comprar mi moto sin éxito. A tots dos Miquels, per compartir una passió comú. Bibi i Cristina, perquè l'esquiada Clínic no hauria estat el mateix sense vosaltres.

Emili, per ensenyar-me, entre moltes d'altres coses, que la distància no és obstacle per treballar. Has estat un referent constant, des de la distància, des que era R1.

Pere, infinites gràcies per tota l'estadística que m'has ensenyat i motivat a aprendre, i sobretot per haver pogut comptar amb tu sempre que he necessitat un cop de mà.

També voldria agrair el suport que sempre he tingut de tots els membres de la Unitat d'Addiccions del Clínic, i en particular de l'Anna Lligoña (proveïdora oficial de feines) i de la Mercè Balcells, oferint motivació constant des de que ens vam conèixer per l'Hospital més bell de Barcelona. Vora el mar també hi treballen altres professors, com la Marta Torrens i el Magí Farré, als que també els hi dec la meva gratitud. I també és de reconèixer l'amable col·laboració de l'admirable toxicòleg del Clínic, Santi Nogué.

Spider (Spa). Gràcies per tots el moments de glòria viscuts. Ha estat un honor el temps compartit i aprendre de la teva sapiència. Sempre recordaré les nostres aventures com a membres d'honor del TT (cafès, cançons, i mil històries inexplicables), el qual vam ampliar posteriorment amb un altre membre honorífic, la Cynthia, rebatejada per ella mateixa com 'Dora la exploradora'.

A mis co-R, núcleo de la generación *lover*. Ana, por enseñarme el sentido *ucru* de la vida. Mo, por cuidar tan bien de mis pacientes. Armando, por haberme guiado en Australia en la toma de la correcta decisión.

A mis R mayores, *lover*-Itzi (también parte clave del núcleo *lover*), Gis y Guille, por todo lo que me habéis enseñado y por haberme dirigido por el buen camino desde mis inicios en la psiquiatría.

A tots i cadascun dels residents, a la generació que em va alimentar amb galetes, als fans de les addiccions, a los perros interconsultores, a tots els petits que m'heu ensenyat a ensenyar, i aquells ja no tan petits dels que tant he après (Iria G, Juanito, Laura R, Dr. Roig). Ah! I a la Carme, vigilant incansable de la 9.6.

Per suposat, aquesta tesi tampoc hauria estat possible sense el suport incondicional de tota la meva família (el pare, ma mare, sempre amb un somriure i tot a punt, tiets (i en particular, la tieta Mayte), cosins, ¡viva los Cases!) i tots els amics dels que ens hem acomiadat, pel moment, amb un 'a reveure'.

I per últim, Lisa, per ser com ets, per estimar-me tant, i per ser la raó per la que canviaria de país.

List of publications

List of publications

Manuscripts in preparation

Batalla A, Blanco-Hinojo L, Crippa JA, Navinés R, Nogué S, Harrinson BJ, Torrens M, Pujol J, Martin-Santos R. Genetic effects on functional connectivity brain networks related to reward in chronic cannabis users.

Batalla A, Bargalló N, Gassó P, Molina O, Pareto D, Mas S, Roca JM, Bernardo M, Lafuente A, Parellada E. Apoptotic markers in cultured fibroblasts correlate with brain metabolites and brain volume in antipsychotic-naïve first-episode schizophrenia and healthy controls.

Submitted for publication

Batalla A, Lorenzetti V, Yücel M, Soriano-Mas C, Bhattacharyya S, Torrens M, Crippa JA, Martín-Santos R. Epistatic influence of COMT and DAT1 gene variations on hippocampal volume in chronic cannabis users: a gene-gene-environment interaction.

Martín-Santos R*, Batalla A*, Fagundo AB*, Blanco-Hinojo L, Soriano-Mas C, López-Solà M, Navinés R, Torrens M, de la Torre R, Crippa JA, Bhattacharyya S, Harrison BJ, Pujol J, Farre M. Catechol O-methyltransferase Val158Met genotype and neural mechanisms related to response inhibition in chronic cannabis users.
* Authors contributed equally.

Binelli C, Subirà S, Batalla A, Muñiz A, Sugranyes G, Crippa JA, Farre M, Pérez-Jurado L, Martín-Santos R. Common and distinct neural correlates in facial emotion processing in social anxiety disorder (SAD) and Williams syndrome.

Binelli C, Muñiz A, Batalla A, Subirà S, López M, Pérez-García D, Crippa JA, Farré M, Pérez-Jurado L, Pujol J, Martín-Santos R. Facial emotion processing in Social Anxiety Disorder and Williams Syndrome: an fMRI study.

Binelli C, Muñiz A, Sanches S, Ortiz A, Udina M, Batalla A, López-Solà C, Crippa JA, Subirà S, Martín-Santos R. New evidence of heterogeneity in social anxiety disorder: defining two distinct personality profiles taking into account clinical, environmental and genetic risk factors.

Dominguez I, Bruguera P, Balcells-Oliveró M, Batalla A. Neurobiology of gamma-hydroxybutyrate (GHB) in depression: a case report.

Peer reviewed papers

López-Pelayo H, Batalla A, Balcells-Oliveró M, Colom J, Gual A. Assessment of cannabis use disorders: a systematic review of screening and diagnostic instruments. *Psychol Med. Accepted*.

Pujol J, Blanco-Hinojo L, Batalla A, López-Solà M, Harrison BJ, Soriano-Mas C, Crippa JA, Fagundo B, Deus J, de la Torre R, Nogué S, Farré M, Torrens M, Martín-Santos R. Functional connectivity alterations in brain networks relevant to self-awareness in chronic cannabis users. *J Psychiatr Res. 2014; 51:68-78*.

Batalla A, Crippa JA, Busatto GF, Guimarães FS, Zuardi AW, Valverde O, Atakan Z, McGuire PK, Bhattacharyya S, Martín-Santos R. Neuroimaging Studies of Acute Effects of THC and CBD in humans and animals: a Systematic Review. *Curr Pharm Des. 2014; 20(13):2168-85*.

Barrio P, Batalla A, Castellvi P, Hidalgo D, Garcia M, Ortiz A, Grande I, Pons A, Parellada E. Effectiveness of long-acting injectable risperidone versus oral antipsychotics in the treatment of recent-onset schizophrenia: a case-control study. *Int Clin Psychopharmacol. 2013; 28(4):164-70*.

Batalla A, Garcia-Rizo C, Castellvi P, Fernandez-Egea E, Yücel M, Parellada E, Kirkpatrick B, Martín-Santos R, Bernardo M. Screening for substance use disorders in first-episode psychosis: Implications for readmission. *Schizophr Res. 2013; 146(1-3):125-131*.

Batalla A, Bhattacharyya S, Yücel M, Fusar-Poli P, Crippa JA, Nogué S, Torrens M, Pujol J, Farré M, Martín-Santos R. Structural and functional imaging studies in chronic cannabis users: a systematic review of adolescent and adult findings. *PLoS One. 2013; 8(2):e55821*.

Batalla A, Soriano-Mas C, Lopez-Sola M, Torrens M, Crippa JA, Bhattacharyya S, Blanco-Hinojo L, Fagundo AB, Harrison BJ, Nogué S, de la Torre R, Farré M, Pujol J, Martín-Santos R. Modulation of brain structure by catechol-O-methyltransferase Val(158) Met polymorphism in chronic cannabis users. *Addict Biol. 2013; [Epub ahead of print]*.

Martín-Santos R, Crippa JA, Batalla A, Bhattacharyya S, Atakan Z, Borgwardt S, Allen P, Seal M, Langohr K, Farré M, Zuardi AW, McGuire PK. Acute Effects of a Single, Oral dose of d9-tetrahydrocannabinol (THC) and Cannabidiol (CBD) Administration in Healthy Volunteers. *Curr Pharm Des. 2012; 18 (32):4966-4979*.

Batalla A, Ilzarbe D, González J, Soler V. Withdrawal syndrome from abusive consumption of liquid ecstasy precursors. *Med Clinc (Barc). 2012; 139(4):181-182*.

Pons A, Undurraga J, Batalla A, Bernardo M. Clozapine and agranulocitosis in Spain: do we have a safer population? A 5-year hematologic follow-up. *Rev Psiquiatr Salud Ment. 2012; 5(1):37-42*.

Batalla A, Vera M, Torra M, Parellada E. Antipsychotic treatment in a patient with schizophrenia undergoing hemodialysis. *J Clin Psychopharmacol. 2010; 30(1):92-94*.

Curriculum vitae

Curriculum vitae

Albert Batalla was born on the 1st of August in 1983, in Barcelona. He graduated from secondary school (Montseny College, Barcelona) in 2001. In that same year he started his studies of Medicine at the Autonomous University of Barcelona, becoming progressively attracted to the field of Psychiatry. In June 2007 he completed the Bachelor of Medicine and Surgery (BMBS), being granted with the Extraordinary Award of Bachelor. In January 2008 he performed the MIR exam and started the residency in Psychiatry at Hospital Clínic de Barcelona, where he became interested in the neurobiology of cannabis use and its relation to psychotic disorders. In 2011 he started his PhD at the Department of Psychiatry and Clinical Psychobiology, University of Barcelona, under the supervision of Dr. Martín-Santos. In 2012 he performed a four-month traineeship in the neuroimaging lab of Melbourne Neuropsychiatry Centre, University of Melbourne, Australia. That same year he completed the residency of Psychiatry and started working as a researcher at the Fundació Clínic per a la Recerca Biomédica (grant: PNSD 2011, PI: R. Martín-Santos). The results of his research are described in this thesis. In addition, we worked partial time as a psychiatrist in an outpatient unit in Barcelona, in charge of the Dual Disorders Program. In 2014 he moved to the Netherlands, where he obtained a position in an Addiction Psychiatry Centre (Tactus) combined with a research fellowship at the Radboud University Nijmegen.