.ANEXO IV

JUSTIFICACION DE PROYECTOS DE INVESTIGACIÓN EN DROGODEPENDENCIAS

MEMORIA CIENTÍFICA DEL PROYECTO DE INVESTIGACIÓN

1ª ANUALIDAD ☐ 2ª ANUALIDAD ☐ 3ª ANUALIDAD ☐ FINAL X ☐

Nº Expediente: 2011/050

Investigador principal: Rocío Martín-Santos Laffon

Otros investigadores: Marta Torrens; Santiago Nogué; Jesús Pujol; Ben Harrison

Título del Proyecto o subproyecto: "Repercusión del inicio precoz del consumo continuado (crónico) de substancias de abuso sobre la red atencional: estudio de conectividad funcional cerebral y modulación genética".

Título del Proyecto Coordinador en el que se integra (sólo en caso de ser un subproyecto):

Organismo: Fundació Clínic per a la Recerca Biomèdica

Centro: Hospital Clínic de Barcelona

Departamento: Psiquiatría

Área temática: N3: Determinantes biológicos y culturales del policonsumo de drogas

Palabras clave: Consumo crónico, cannabis, genética, red atencional, neuroimagen, conectividad cerebral.

RESUMEN: (objetivo, ámbito de estudio, sujetos de estudio, instrumentalización, resultados, conclusiones. Máximo 2000 palabras).

Objetivo
Estudiar la repercusión del inicio precoz del consumo continuado (crónico) de cannabis sobre la red atencional fronto-parieto-cingular mediante el estudio de la conectividad cerebral funcional y sobre la estructura cerebral en consumidores crónicos de cannabis de inicio temprano. En ambos casos se estudió además su posible modulación genética.

Ámbito de estudio
Este estudio se planteó en el marco de un estudio experimental, caso-control, antes y después, en un Hospital Universitario.

Sujetos de estudio
Estudiamos 30 varones consumidores crónicos de cannabis (>14-20 porros/semana/>tres años) de inicio antes de los 16 años. 30 voluntarios sanos, no consumidores, emparejados por edad, sexo, lateralidad, nivel estudios y CI con los casos. Consentimiento informado por escrito. Los criterios de exclusión fueron presencia de diagnóstico psiquiátrico en el eje I con excepción del trastorno de dependencia de cannabis, enfermedades médicas o neurológicas relevantes, alteraciones del aprendizaje, uso de fármacos psicoactivos, uso recreacional previo de cualquier substancias psicoactiva mas allá de 5 veces en la vida con excepción de alcohol o nicotina, criterio de abuso o dependencia de alcohol a lo largo de la vida o consumo actual relevante de alcohol.
El reclutamiento se llevaría a cabo mediante anuncios en pagina web de la institución y distribución de anuncios. Tras solicitud de contacto por email, se realizaría una entrevista telefónica de cribaje, tras lo cual se realizaría una entrevista personal: historia clínica y toxicológica, exploración física, entrevista estructurada para el diagnóstico psiquiátrico en consumidores de substancias psicoactivas, y obtención de muestra de sangre para bioquímica general, y genético y orina para estudio toxicológico.

Instrumentalización
-Variables clínicas: Visita selección: variables sociodemográficas y de consumo, entrevista diagnóstica PRISM-IV-R, ansiedad: STAI-estado, personalidad: STAI-rasgo; batería neuropsicológica estandarizada para evaluar dominios de memoria,velocidad de procesamiento cognitivo, atención y funciones ejecutivas; exploración física; y substancias en orina. Sesión basal y a los 30 días: ansiedad, abstinencia y análisis substancias en orina. Variables genéticas: Muestra de sangre (ADN y estudio polimorfismos genéticos COMT, DAT).- Scanner 1.5 T Signa Exite System (GEMW). -Neuroimagen: a)Resonancia estructural. b)Resonancia magnética funcional en "resting state" basal y a los 30 días de abstinencia. Regiones de interés: prefrontal medial (CPF), cortex cingular anterior (CCA), ambas ínsulas. *Análisis estadístico:* descriptivo, univariado y multivariado. Analisis de correlación voxel a voxel con las variables clínicas y genéticas. SPSS.v15 y SPMS 8 sobre Matlab 7.1.

Resultados principales
Características de la muestra:
Se incluyó en el estudio un total de 28 sujetos varones consumidores de cannabis de inicio temprano antes de los 16 años, con una edad media (DS) de 21 (2) años de edad que fueron comparados con 29 hombres sanos de una edad media de 22 (3) años (p>0,05). Todos los sujetos casos y controles eran diestros.
Todos los sujetos tenían al menos 10 años de educación escolar (media (DS) 14 (2). Sin embargo se observó una diferencia de un año de escolaridad a favor del grupo control (p < 0,32).
Habían consumido cannabis fumado mas de 14 veces a la semana durante al menos los dos últimos dos años, el control urinario era positivo para cannabis y negativo para cualquier otra substancia de abuso

(opiáceos, cocaína, anfetaminas y benzodiacepinas). La media de años de consumo fue de 6,0 (2,5), la media del total de porros consumidos a lo largo de la vida fue de 5.268 (4265), con una media de porros año de 899 (560). Se excluyó un consumidor de cannabis de la muestra original de 29 años porque la adquisición de las imágenes no fue adecuada.

El consumo medio de alcohol fue ambos grupos de sujetos fue de 5,3 (4) unidades de alcohol/semana en los consumidores y de 3,1 (2,6) en el grupo control. Los consumidores fumaron una media de 5,9(5,2) cigarrillos /día y el grupo control una media de 2,4 (5,9). Solo tres de los sujetos consumidores y uno del grupo control fumaban mas de 10 cigarrrillos/día. Todos los participantes fueron evaluados al cabo de un mes de abstinencia controlada. Esta se realizó mediante visita semanal, control de tóxicos en orina y estudio cuantitativo de concentración de cannabinoides en orina a lo largo del mes de seguimiento. Teniendo que ser inexistente o invalorable a las 4 semanas. Todos los sujetos firmaron el consentimiento informado.

Las frecuencias genotípicas del gene COMT fueron: 11 sujetos eran homocigotos para el alelo *met*, 13 eran *val/val* y 33 eran portadores *val/met*. No hubo evidencias de que los datos no estuvieran en equilibrio de Hardy-Weinberg.

A todos los sujetos de estudio se les solicitó que acudieran a la sesión de resonancia (estructural y funcional) tras haber consumido tabaco y cafeína en las últimas 6h y alcohol y cannabis en las12 horas previas a la primera sesión. A la segunda sesión debían acudir tras 28 días de abstinencia al cannabis.

Consumo de cannabis y test conductuales

Los sujetos del grupo consumidor de cannabis mostraron unas puntuaciones de *ansiedad rasgo* mayores que las de los sujetos del grupo control (12,6 (4,3) vs 9,0 (5,6); t: 2,7, p: 0,009). Lo mismo se observó con las puntuaciones de la *ansiedad estado* (12,3 (3,9) vs xx,2 (4,4), t:2,8, p:0,008).

Los sujetos consumidores de cannabis mostraron peores resultados en la memoria verbal que los sujeto del grupo control (6,1 (1,6) vs 7,5 (1,9), t: -2,9, p:0,006). No se observó diferencias en el rendimiento de las pruebas de memoria de aprendizaje (20,6 (6,7) vs 20,9 (1,8), t:-0,2, p:0,858).

Las puntuaciones de recuerdo a los 20 minutos, mostraron de nuevo diferencias entre los sujetos consumidores y los sanos (11,2 (2,7) vs 13,1 (1,8), t:-3,2, p:0,002). El ratio de olvidos fue ligeramente mayor en el grupo consumidor que en el sano (1,9 (1,5) vs 1,1 (1,5),t:2,1, p>0,042) pero tras controlar por la edad de escolaridad desapareció la diferencia (0,069).

Resultados de los mapas de conectividad funcional (Pujol J et al. J Psychiatr Res 2014)

Utilizando la semilla a nivel del córtex prefrontal (CPF) permite identificar los elementos de la red atencional en ambos grupos. Los mapas de conectividad funcional incluyen el CPF/precuneus, giro angular, medial y lateral del córtex frontal, el cíngulo anterior (CCA), y el córtex latero-temporal. Comparado con el grupo control los sujetos consumidores crónicos de cannabis de inicio temprano mostraron un incremento de la conectividad funcional en el área ventral de CPF y una disminución de la conectividad funcional en la zona dorsal del córtex prefrontal y de la unión con el precuneus.

La semilla en la ínsula nos permite identificar la red que incluye la ínsula bilateral y el opérculo, los ganglios de la base, el CCA y las estructuras cerebrales ventrales que envuelven el tronco encefálico y la amígdala derecha en ambos grupos. Los consumidores de cannabis muestran un incremento de la conectividad funcional en la porción anterior de la ínsula izquierda, y el giro supramarginal bilateral respecto a los controles sanos y una conectividad funcional reducida en CCA y el tronco encefálico.

Los consumidores crónicos de cannabis mostraron una asociación negativa intensa entre la conectividad funcional en la ínsula y el estado de ansiedad, y una asociación negativa intensa con el CPF ventral y positiva con el CPF dorsal entre la conectividad funcional y el recuerdo verbal. Se observó una correlación positiva entre la media de porros consumidos por año y la conectividad funcional a nivel CPF (a nivel de tendencia) y de la ínsula.

El análisis de la semilla del hipocampo mostró que los consumidores crónicos de cannabis respecto al grupo control mostraban un área con reducción de la conectividad funcional en el hipocampo derecho, y

una correlación positiva intensa entre la conectividad funcional del área parahipocámpica izquierda y el recuerdo verbal.

A los 28 días de abstinencia se observó una tendencia general a la reducción de magnitud de los cambios observados. Sin embargo, persistían las diferencias entre ambos grupos en el incremento y decremento de la conectividad funcional en el patrón atencional y de la ínsula.

Por ultimo se ha estudiado también la posible modulación genética sobre los cambios en la conectividad funcional observados. Estos resultados están pendientes de acabar de elaborar el manuscrito y enviar para ser publicados.

Resultados de la neuroimagen estructural (Batalla et al. PlosOne 2013; Batalla et al., Addiction Biology 2014; Batalla et al., 2015 submmitted)

La comparación entre ambos grupos de los resultados del volumen global del tejido cerebral (materia gris, materia blanca, LCR, volumen intracraneal) y de las cuatro regiones de interés estudiadas (CPF, CCA, neoestriatum e hipocampo-amigdalar no mostraron diferencias significativas. Los consumidores crónicos de cannabis mostraron un incremento del volumen de materia gris en el giro poscentral del hemisferio izquierdo utilizando un nivel umbral de $p<0,001$ sin corregir.

Al estudiar si existía una interacción entre el genotipo de la COMT y los consumidores crónicos de cannabis observamos diferencias significativas entre grupos en las correlaciones entre volumen de materia gris y genotipo en dos de la cuatro regiones de interés estudiadas. Los consumidores crónicos de cannabis mostraron una correlación negativa entre el volumen de núcleo caudado ventral y el número de alelos *val,* mientras que se observó una asociación inversa en los controles sanos, es decir, a mayor numero de alelos *val,* mayor volumen de la materia gris del caudado ventral. En contraste, también se observó que los consumidores crónicos de cannabis tenían un mayor número de alelos *val* asociados con un aumento significativo del volumen de la amígdala izquierda. Y por otro lado, en el caso del grupo control, lo que ocurría era que a mayor número de alelos *val,* menor volumen de la materia gris de la amígdala izquierda.

También se observó una correlación positiva entre la morfología cerebral y el consumo medio vida de porros a lo largo de la vida con un umbral de $p<0,001$ sin corregir. En concreto la correlación se objetivó entre el volumen del la porción caudal del cortex giro subungeal rectal cingular y el número acumulado de porrros vida. La correlación no se modificaba por el genotipo COMT.

Además hemos estudiado la presencia de influencias epistáticas de variaciones genéticas entre la COMT y el DAT en el volumen hipocampal en los consumidores crónicos. Estos resultados están todavía pendientes de ser publicados.

Conclusión

1. El estudio experimental nos ha permitido identificar un patrón específico de conectividad funcional alterado en los consumidores de cannabis de inicio temprano, antes de los 16 años, comparado con sujetos sanos que afecta áreas cerebrales encargadas conectar y modular redes neuronales importantes para la autoconciencia. Las redes atencionales y de la ínsula por otra parte muestran un solapamiento anatómico importante junto a una fuerte conexión funcional con redes neurales dedicadas al control cognitivo, al almacenamiento de experiencias personales y a la conducta motivacional. Los resultados sugieren que el consumen crónico de inicio temprano interferiría con la redes neurales que intervienen en la experiencia de la autoconciencia.
2. Los resultados sobre la estructura cerebral apoyan resultados recientes de cambio neuroanatómicos asociados al consumo crónico de cannabis y por primera vez muestran que estos cambios pueden estar modulados por variaciones en genes que regulan la dopamina.

ARTÍCULOS PUBLICADOS COMO CONSECUENCIA DE LA ACCIÓN: Se adjuntará una separata de cada uno de ellos y se remitirá una copia en formato digital a pndinvestigacion@msssi.es para el fondo documental de la Delegación

del Gobierno para el Plan Nacional sobre Drogas.

La convocatoria regula en su artículo décimo, punto 3 que la producción científica derivada del proyecto financiado debe ser comunicada a la Delegación del Gobierno para el Plan Nacional sobre Drogas y en cualquier tipo de publicación a que dé lugar, incluso páginas web, **se hará constar expresamente, de forma visible y preferencial que el proyecto se ha realizado con financiación de la Delegación del Gobierno para el Plan Nacional sobre Drogas**.

- Pujol J, Blanco-Hinojo L, Batalla A, López-Solà M, Harrison BJ, Soriano-Mas C, Crippa JA, Fagundo AB, Deus J, de la Torre R, Nogué S, Farré M, Torrens M, Martín-Santos R.
  Funtional connectivity alterations in brain network relevant to self-awareness in chronic cannabis users.
  J Psychiatr Res. 2014 Apr;51:68-78.
  1T Psychiatry FI: 4,09 N citaciones: 2

- Batalla A, Crippa JA, Busatto GF, Guimaraes 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.
  1T Pharmacology and Pharmacy FI: 3,29 N citaciones:

- Batalla A, Soriano-Mas C, López-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 Val158Mwr polymorphism in chronic cannabis users.
  Addiction Biology 2014; 19: 722-32
  1T Substance Abuse FI: 5,91 N citaciones: 4

- Batalla A, Bhattacharyya S, Murat Y, Fusar-Poli P, Crippa, JA, Nogue S, Torrens M, Pujol J, FarréM, Martin-Santos R.
  Structural and Functional Imaging Studies in Chronic Cannabis Users: A Systematic Review of Adolescent and Adult Findings.
  PlosOne 2013; 8:e55821
  1T Multidisciplinary Sciences FI:3,53 N citaciones: 8

- Batalla A, Garcia-Rizo C, Castellví C, Fernandez-Egea E, Yücel M, Parellada C, Kirkpatrick B, Martin-Santos R, Bernardo M. Screening for substance use disorders in first-episode psychosis: Implications for readmission.
  Schizophrenia Research 2013; 146: 125-131.
  1T Psychiatry FI: 4,43 N citaciones: 2

También tenemos en revisión el siguiente articulo:

-Batalla, A, Lorenzetti, V, Yücel, M, Soriano-Mas, Bhattacharyya, S, Torrens, M, Crippa, JA, Martín-Santos, R. Epistatic influence of COMT and DAT gene variations on hippocampal volume in chronic cannabis users: a gene-gene-environment interaction" (enviado para su publicación a revista indexada, actualmente sigue en revisión)

OBJETIVOS

PLANTEADOS: (Transcribir los del proyecto original)

Principal
Estudiar la conectividad cerebral funcional en áreas involucradas en la red de trabajo atencional fronto-parietal-cingular en consumidores crónicos de cannabis de inicio temprano.

Secundarios
1. Comparar la conectividad funcional entre áreas fronto-parieto-cingular en sujetos consumidores crónicos de cannabis respecto al grupo control
2. Estudiar si estas relaciones están moduladas por rasgos de personalidad y la vulnerabilidad genética (polimorfismos COMT (Val156Met), DAT'3 y AKT1 (rs1130233)
3. Estudiar si estas relaciones se asocian en los consumidores crónicos con cambios en la sintomatología y el rendimiento neurológico.
4. Estudiar la presencia de cambios estructurales en los sujetos consumidores crónicos de cannabis respeto al grupo control
5. Estudiar la posible modulación genética de los cambios estructurales

ALCANZADOS: (Ordenar de igual forma que los planteados. En el caso de proyectos coordinados, el coordinador deberá describir además el desarrollo de la coordinación entre subproyectos en este año, y los resultados de dicha coordinación con relación a los objetivos globales del proyecto).

Principal
Se ha realizado el análisis de la conectividad cerebral funcional. Los resultados se han plasmado en un artículo recientemente publicado
Actualmente estamos estudiando la posible modulación genética de las áreas involucradas. Además se están realizando un segundo análisis de conectividad centrada en otras áreas de interés como el núcleo accumbens.

Secundarios
1. Se han comparado los resultados de la conectividad funcional entre ambos grupos: consumidores crónicos de cannabis y grupo control.
2. Se ha estudiando actualmente si estas relaciones están moduladas por rasgos de personalidad y la vulnerabilidad genética (polimorfismos COMT (Val156Met), DAT'3
3. Se ha estudiado la conectividad funcional en relación con sintomatología clínica.
4. Se han estudiado la presencia de cambios estructurales en los consumidores crónicos y su posible modulación genética.

METODOLOGÍA Y PLAN DE TRABAJO

PROYECTADO:
-Creación base de datos clínico y genéticos y validación de la misma.
-Análisis de la conectividad.
-Análisis datos estructurales
-Análisis de correlación vóxel a vóxel con las variables clínicas y genéticas.
-Presentación de datos preliminares a nivel nacional e internacional.
-Realización de tesis doctoral en base al proyecto.
-Reuniones de seguimiento mensuales del estudio.
-Informe de seguimiento anual.

EJECUTADO:

-Se ha creado y validado la base de datos clínicos y genéticos

-Se ha llevado a el análisis de la conectividad y escrito y publicado un primer artículo sobre ello. Se adjunta el pdf. Están pendiente un artículo mas en proceso de elaboración.

-Se han llevado a cabo análisis de resonancia estructural y escrito y publicado artículos sobre ello. Todos los pdf han sido ya enviados en las diferentes memorias. Queda uno pendiente de publicar, actualmente en revisión.

-Se han presentado resultados preliminares en congresos nacionales e internacionales a lo largo de los tres años. Se han adjuntado los comprobantes en las diferentes memorias. Se adjunta en esta solo los de los últimos seis meses. Ver en el apartado actividades proyectadas y realizadas.

-Se ha escrito el trabajo de tesis doctoral: *"Acute and chronic effects of cannabinoids on human brain: gene-environment interaction related to psychiatric disorders"* bajo la dirección del IP del estudio (Rocío Martín-Santos) siendo doctorando el investigador contratado con la ayuda, Albert Batalla Cases en el programa de Doctorado en Medicina de la Universidad de Barcelona (Mención de calidad) (doctorado internacional).

-Se ha realizado reuniones de seguimiento mensual del estudio.

-Se han realizado los informes de seguimiento.

ACTIVIDADES

PROYECTADAS:

En relación al proyecto

-Presentar resultados preliminares en el XVI Congreso Nacional de Psiquiatría: *"Polimorfismo de la COMTVal158Met y morfología cerebral en consumidores crónicos de cannabis"*. Presentación oral. Batalla A, Soriano C, Lopez Sola M, Torrens M, Gafungo B, Harrisson B, Nogue S, Farre M, Pujol J, Martín-Santos R, en Bilbao septiembre de 2012

-Presentación resultados preliminares: *"Repercusión cerebral del consumo crónico de cannabis iniciado antes de los 16 años"* Martín-Santos R, en la VII Jornada de Actualización en Toxicología, Barcelona, enero 2012

-Ponencia invitada: *"Efectos farmacológicos de los cannabinoides en humanos"*en el Symposium científico sobre cannabis Martín-Santos R, Socidrogalcohol, Barcelona, mayo de 2013.

-Presentar resultados en el 21th European Psychiatric Association (EPA): *Structural and functional imaging Studies in chronic cannabis users: a systematic review of adolescente and adult findings*, Batalla A, Martín-Santos R, Niza. abril 2013

-Presentar resultados en el 22th European Psychiatric Association: *COMTVal158Met genotype and neural mechanisms related to response inhibition in chronic cannabis users*. Batalla A, Fagundo A, BlancoHinojo L, Soriano-Mas C, Navines R, Farre M, Udina M, de la Torre R, Bhattacharyya S, Crippa JA, Torrens M, Pujol J, Martín-Santos R, Munich, abril 2014

-Participar como ponente en el Symposium: Alteraciones psiquiátricas asociadas al consumo de alcohol y de cannabis: una aproximación trasnacional en las Jornadas de Patología dual: *"Depresión y cannabis: una* relación compleja" Martin-Santos R, en Valencia, mayo 2014

-Ponencia invitada: *"Cannabinoids and mental health"* in 1st Braziliam Symposium of Neuropsychopharmacology, Ribeirao Preto, Sao Paulo, Brasil, septiembre 2014.

En relación formación investigador contratado

-Inscripción y seguimiento en el programa de doctorado

-Periodo de formación en el procesamiento y análisis de las imágenes

-Participación sesiones formativas del grupo y del servicio

-Solicitud de Travel Grant for oral Communications 22th EPA, Munich abril 2014, Batalla A.

-Realización tesis doctoral en base al proyecto de esta ayuda del PNSD

-Lectura de la tesis doctoral dentro del año 2014

EJECUTADAS:

-Nos aceptaron todas las propuestas presentadas en los congresos nacionales e internacionales.
-Se obtuvo el Travel grant solicitado para el 22thEPA.
-Se leyó la tesis doctoral en la UB obteniendo la calificación Excelente "Cum laude", en julio 2014. Se adjunta en esta memoria el pdf de la tesis realizada y el comprobante de la UB con la calificación obtenida.

APLICABILIDAD Y UTILIDAD PRÁCTICA DE LOS RESULTADOS EN EL ÁREA DE LAS DROGODEPENDENCIAS (solo en caso de memoria final)

- Los resultados finales del proyecto han supuesto un avance en el conocimiento del consumo crónico de cannabis sobre la estructura y la conectividad cerebral y apuntan a una posible modulación genética de los genes que regulan la dopamina. Quisiera señalar que varias de las publicaciones realizadas dentro del proyecto, a pesar de su corta vida publicadas ya han recibido citaciones por la comunidad internacional.

TRANSFERENCIA Y DIFUSIÓN DE RESULTADOS A LA CIUDADANÍA: ACCIONES LLEVADAS A CABO (en caso de memoria de segunda anualidad o de memoria final)

Hemos organizado dos symposiums y un workshop como transferencia y difusión de los resultados del estudio:
- Organización del Symposium sobre "*Brain chronic and acute effects of cannabis*", Chairman: Martín-Santos R en el Congreso Internacional de Patología Dual en Barcelona octubre de 2013
- Organización del Workshop internacional en Barcelona: "*Update on cannabis research*", Martín-Santos R, Torrens M, Nogué S en el Hospital Clinic de Barcelona en octubre de 2013
- Organización del Symposium: *"Neural substrates for the acute and chronic effects of cannabis in man: implications for psychosis"* Chairman: Martín-Santos R, XVI Congreso Mundial de Psiquiatría, Madrid, septiembre 2014

PATENTES U OTROS RESULTADOS EXPLOTABLES COMERCIALMENTE QUE SEAN CONSECUENCIA DEL PROYECTO (solo en caso de memoria final)

OTRAS SUBVENCIONES O RECURSOS (INCLUIDOS FONDOS PROPIOS) QUE FINANCIAN ESTE PROYECTO O PENDIENTES DE RESOLUCIÓN (importe, procedencia y aplicación)

SUBVENCIONES O AYUDAS SOLICITADAS PARA ESTE PROYECTO Y NO CONCEDIDAS (Organismo, convocatoria y cantidad)

OTRAS CONSIDERACIONES QUE SE DESEE HACER CONSTAR

-En el momento de la memoria final queda pendiente un artículo (sometido para su publicación, actualmente en revisión), otro en preparación, y un capítulo de un libro internacional sobre cannabis. En la medida que se vayan publicando serán enviadas al Plan Nacional sobre Drogas.

En esta fecha se remite también por correo electrónico a la dirección pndinvestigacion@msssi.es la presente memoria.

En Barcelona a 15 de .....Enero... de 2015

NOMBRE Y FIRMA DEL INVESTIGADOR PRINCIPAL

ROCÍO MARTIN-SANTOS

Send Reprints Orders on reprints@benthamscience.org

4966 *Current Pharmaceutical Design,* **2012,** *18,* **4966-4979**

Acute Effects of a Single, Oral dose of d9-tetrahydrocannabinol (THC) and Cannabidiol (CBD) Administration in Healthy Volunteers

R. Martín-Santos[1,2,3]*, J.A. Crippa[1, 4], A. Batalla[2], S. Bhattacharyya[1], Z. Atakan[1], S. Borgwardt[1,5], P.Allen[1], M.Seal[1,6], K.Langohr[3,7], M. Farré[3], AW.Zuardi[4] and P.K. McGuire[1]

[1]*Department of Psychosis Studies, Institute of Psychiatry, King's College London, UK;* [2]*Department of Psychiatry, Institut Clínic de Neurociències, Hospital Clínic, IDIBAPS, CIBERSAM, Barcelona and Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Spain;* [3]*Human Pharmacology and Neurosciences, Hospital del Mar Research Institute - IMIM, Barcelona, Spain;* [4]*Department of Neuroscience and Behavior, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil and INCT Translational Medicine, Ribeirão Preto, Brazil;* [5]*Department of Psychiatry, UPK, University of Basel, Switzerland;* [6]*Melbourne Neuropsychiatry Centre, The University of Melbourne, Australia;* [7]*Department of Statistics and Operations Research, Polytechnic University of Catalonia, Barcelona, Spain*

Abstract: *Rationale:* Animal and humans studies suggest that the two main constituents of *cannabis sativa*, delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) have quite different acute effects. However, to date the two compounds have largely been studied separately.

Objective: To evaluate and compare the acute pharmacological effects of both THC and CBD in the same human volunteers.

Methods: A randomised, double-blind, cross-over, placebo controlled trial was conducted in 16 healthy male subjects. Oral THC 10 mg or CBD 600 mg or placebo was administered in three consecutive sessions, at one-month interval. Physiological measures and symptom ratings were assessed before, and at 1, 2 and 3 hours post drug administration. The area under the curve (AUC) between baseline and 3 hours, and the maximum absolute change from baseline at 2 hours were analysed by one-way repeated measures analysis of variance, with drug condition (THC or CBD or placebo) as the factor.

Results: Relative to both placebo and CBD, administration of THC was associated with anxiety, dysphoria, positive psychotic symptoms, physical and mental sedation, subjective intoxication (AUC and effect at 2 hours: $p<0.01$), an increase in heart rate ($p<0.05$). There were no differences between CBD and placebo on any symptomatic, physiological variable.

Conclusions: In healthy volunteers, THC has marked acute behavioural and physiological effects, whereas CBD has proven to be safe and well tolerated.

Keywords: Cannabis, Δ-9-THC-tetrahydrocannabinol, cannabidiol, unique dose, pharmacological acute effects, humans, induced anxiety, induced psychosis, review.

INTRODUCTION

Cannabis sativa preparations (marijuana, hashish, and others) are the illicit drugs most widely used in young people [1]. The plant has around 400 different chemical constituents, but two of its major psychoactive compounds are delta-9-tetrahydrocannabinol (THC) [2] and cannabidiol (CBD) [3,4].

THC acts as a partial agonist at specific endogenous cannabinoid receptors, termed CB1 and CB2, both members of the G-protein coupled receptor class [5]. The CB1 receptors are mainly expressed in the central nervous system, with a high density in the anterior cingulate, prefrontal cortex, medial temporal lobe and other areas [6] and are thought to mediate the majority of the effects of THC in the central nervous system. However, depending on the brain region, and whether the local CB1 receptors are expressed on neurons that release GABA or glutamate, THC can have either inhibitory or excitatory effects [7].

The acute administration of THC is associated with relaxation and enjoyment, but can also lead to unpleasant effects such as anxiety, psychotic symptoms, depression, apathy, and impairment of memory [8]. It has also been associated with impairments in learning, motor coordination, slowed reaction time, impaired concentration during complex tasks, deficits in some executive functions, and impairments in some aspects of verbal processing, such as verbal fluency [9,10]. THC administration can also produce an increase in heart rate and orthostatic hypotension. However, the acute effects of THC and their time of onset are subject to wide inter-individual variation and due to differences in route of administration, rate of absorption, metabolism and the subject's expectation of its effects [11].

In contrast, CBD has a low affinity for CB1 receptors [12] and its molecular mechanism of action remains poorly understood. It may facilitate endocannabinoid signaling by inhibiting the cellular uptake and enzymatic hydrolysis of endocannabinoids [12]. It can also bind to CB1 and to serotonergic (5HT1A) receptors, inhibit adenosine uptake, and can activate vanilloid (TRPV1) receptors at micromolar concentrations [12-16]. CBD is pharmacologically active and can have anticonvulsant, sedative, anxiolytic [3,4,17,18] and antipsychotic effects [4, 19-25]. Unlike THC, CBD does not have acute effects on motor or cognitive performance [26, 27], nor does it have significant effects on pulse rate or blood pressure [28, 29]. Functional neuroimaging studies have confirmed the neurophysiological effects of THC and CBD are distinct and opposite [30-34]. Moreover, co-administration of CBD and THC may alter the pharmacological effect of the THC, in that CBD potentiates some of THC's desirable effects but attenuates some of its negative effects [29, 34-36]. However, it is difficult to establish which

*Address correspondence to this author at the Department of Psychiatry, Institut Clínic de Neurociències, Hospital Clínic, IDIBAPS, CIBERSAM, and Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Villarroel, 170. 08036-Barcelona, Spain; Tel: 34 932275400; Fax: 34 932275538; E-mail: rmsantos@clinic.ub.es

1873-4286/12 $58.00+.00

CBD/THC ratios cause antagonism or potentiation, since other factors could interfere in the effects of these cannabinoids, such as the time between administrations of the two cannabinoids [37, 38]. Recent data showed the absence of significant differences between similar dose of oral THC and Sativex™, a plant extract with a 1: 1 proportion of both compound, on respect to subjective and physiological effects or pharmacokinetic [39, 40].

A better knowledge of the acute pharmacology effects of the two main compounds of the *cannabis sativa* may have implications for future research and therapeutics. We conducted a systematic review to assess the evidence for symptomatic and physiological effects of a single oral dose of THC and CBD in healthy volunteers. We reviewed literature in MEDLINE-PubMed database reporting studies with a cross-over, double-blind, placebo-controlled and randomised design in the last decade (2000-2011) (Table **1**). We found nine studies which met our inclusion criteria in which seven studies compared THC to placebo [41-47], one with Modafinil [46], another with an active placebo (Diazepam) [48], and one in front morphine using an active placebo (Diazepam) [48]. Three of the studies had used cannabis extracts (with small proportion of CBD) [41, 44, 48] and one had compared CBD with placebo [49]. None of the studies had compared both compounds within the same sample.

Therefore we aimed to carry out a study with the objective of evaluating the acute effects of THC and CBD in the same group of healthy volunteers. Subjects were studied after a single dose of THC, CBD or placebo in three consecutive sessions separated by an interval of one month. Given the findings from previous studies [29, 50], our main hypothesis was that THC and CBD would have distinct effects on symptoms and physiological measures.

MATERIAL AND METHODS

Subjects

The study was conducted in accordance with the Declaration of Helsinki, approved by the local research committee (The Joint South London and Maudsley Trust and Institute of Psychiatry NHS Research Ethics Committee). All participants signed an informed consent form after full explanation of the study was given and were paid for their participation. Thirty right-handed, English-speaking healthy male volunteers, aged 18 to 42 years, were recruited through advertisement in local newspapers, posters and word-of-mouth referrals. Alcohol and illicit drug use was assessed in detail using a semi-structured questionnaire [51], and used to screen potential participants. Only individuals who had used cannabis less than 15 times in their lifetime and had not experienced any undesirable effects after use, such as anxiety and/or psychotic symptoms were included. They were also required not to have used cannabis in the previous month and abstain from using cannabis over the study duration. Exclusion criteria included those who had used any other psychotropic drug on a regular basis or drank more than 21 units of alcohol per week or had any psychiatric, neurological or severe medical illness history. Those with a family history of a psychotic illness were also excluded.

Sixteen right-handed male volunteers, with a mean (SD) age of 26.4 (5.3) years (range 20-42) were selected for the study. They had completed a mean (SD) of 16.46 (3.9) years of education. Nine subjects (56.3%) reported having used cannabis less than 5 times in their lifetime, while 7 (43.8%) reported having used cannabis on between 5-14 occasions. None had a history of substance abuse or dependence defined according to DSM-IV criteria, except for nicotine dependence. Seven subjects were current smokers, but only two subjects smoked more than 10 cigarettes/day. All subjects had Reading scores on the WRAT-R test [52] within the normal range (mean (SD) = 98.67 (7.078); range 79-108).

Participants remained under close clinical observation in the research centre for at least 3 hours after each administration, with this period extended if they had not yet completely recovered. All participants agreed not to drive or use any machinery until the following day. A taxi was provided to take them home after each session.

Drugs

THC and CBD (approximately 99.6% and 99.9% pure, respectively) were supplied by THC-Pharm (Frankfurt, Germany) and STI Pharmaceuticals Ltd, (Brentwood, UK), and prepared by the Pharmacy Department of the Maudsley Hospital as identically appearing opaque capsules. The three drug conditions in the study were as follows: 10 mg THC, 600 mg CBD and placebo (flour). The doses of THC and CBD were selected on the basis of previous research [37,54-56] to produce a neurocognitive effect without provoking severe toxic, psychiatric or physical symptoms, which might confound interpretation of physiological and neuro-psychological data, or lead to the subject being unable to co-operate with the assessment.

Study Design

A crossover, double-blind, repeated measures design was used to compare the effects of THC, CBD and placebo. Participants were tested on three occasions at one-month intervals. The order of drug administration was pseudo-randomised to control for order effects. During the initial screening process, potential participants were familiarized with the testing procedures and questionnaires.

On each study day, subjects arrived at the research centre 1 hour before starting, having slept at least 6 hours and having had a standardised light breakfast. At each session, and before starting each assessment, urine samples were collected for screening for opiates, cocaine, amphetamines, benzodiazepines and THC using immunometric assay kits. None of the participants tested positive on any of the sessions. An indwelling intravenous catheter was then inserted into a subcutaneous vein in the forearm of the non-dominant arm. Thereafter, subjects remained seated in a quiet room throughout the session. Each drug was administered approximately after one hour of basal assessment.

Symptomatic Effects

Symptoms were evaluated at baseline and at 1, 2 and 3 hours after drug administration, using the Positive and Negative Psychotic Syndrome Scale (PANSS) [57], assessed by an experienced psychiatrist, and using a set of self-administered scales (below). The PANNS [57] a 30-item rating instrument was used to assess psychotic symptoms, with ratings based on a semi-structured clinical interview. Scores for each item range from 0 (absent) to 7 (extreme), and yield sub-scores for positive, negative, and general psychopathology domains. The self-administered scales comprised a 16-item version of the Visual Analogue Mood Scale (VAMS) [58], with four subscales: mental sedation or intellectual impairment, physical sedation or bodily impairments, anxiety effects and other types of feelings or attitudes. We also used the Addiction Research Centre Inventory (ARCI 49 item short form), a standardised measure of drug effects developed by Martin *et al* (1971) [59], comprising 49 true/false statements describing the subjective effects of various classes of substances. It has five empirically derived scales, measuring drug-induced euphoria (morphine-benzedrine group: MBG), stimulant-like effects (amphetamine group: A), intellectual efficiency and energy (benzedrine group: BG) and sedation (phenobarbital-chlorpromazine, alcohol group: PCAG), and dysphoria and somatic effects (lysergic acid: LSD). The Spielberger State Anxiety Inventory (STAI-T/S) [60] was used to assess state anxiety at hourly intervals, with subjects completing 20 items on current feelings and 20 on feelings in general.

Physiological Measures

Non-invasive systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate were recorded at 1 hour before administration, immediately before drug administration (time 0, base-

Table 1. Systematic review (MEDLINE-PubMED, 2000-2011) of cross-over, double-blind, placebo-controlled, randomized studies of subjective and physiological effects of a single oral dose, THC, CBD, administration in healthy volunteers*.

Author (year)	Inclusion (In) /Exclusion (Ex)** criteria	M/F	M (SD) range	Drugs administered	Dose mg	Measures hours	Clinical tools	Symptomatic effects		Physiological effects	Plasma concentrations ng/mL (Mean (SD)
								Increased	Decreased		
Sugarman *et al.* (2011) [46]	In: Healthy occasional volunteers THC+urine Ex: Any abuse/depend. Current psychiatric disorder Physical illness	11/1	33.7 (7.7)	THC (dronabinol) +Placebo Modafinil +Placebo THC+Modafinil Placebo	15 +400 15+400	Basal, ½, 1, 1½, 2½, 3, 3½, 4, 4½ & 5h	ARCI DEQ POMS BP HR	*THC* ARCI (sedation, dysphoria) DEQ ("feel high", "feel sedated", & "feel the drug strength")	*THC* POMS (vigor, depression) *THC +Modafinil* ARCI (euphoria)	*THC* HR increase Systolic BP low *THC +Modafinil* HR>increase	NA
Roser *et al.* (2008, 2009; Nadulski *et al.*, 2005 a,b) [41, 67-69]	In: Healthy occasional volunteers Ex: Any abuse/depend. Current/past psychiatric disorder Positive urine analysis Pregnancy	12/12	27.9 (2.9) 18-45	THC Cannabis extract Placebo	10 THC:10 CBD:5.4	Basal, ½, 1, 1½, 2, 4, 7, 9 & 24h	AIR FTA NA	*THC* AIR (subjective level of intoxication) *Cannabis ext* AIR Both similar	-	-	*THC* peak at 2h slightly > in F Similarly results with *Cannabis ext.* (THC and CBD).
Menetrey *et al.* (2005) Favrat *et al.* (2005) [42,70]	In: Healthy occasional volunteers Ex: Any abuse/depend. Current/past psychiatric disorder Physical illness	8/0	22-30	THC (dronabinol) Milk decoction Placebo	20 THC:16.5 THC:45.7 THC:1% CBD:0.4%	Basal, 1, 1½, 4, 5½, 7, 10 & 24h	VAS BP HR Conjuntival reddening	*THC & decoction* VAS (strong feeling of high intoxication) > after the highest dose *Decoction of 45.7 mg* > Nausea and vomiting *Two subjects excluded for anxiety (decoction 16.5 mg) and psychotic symptoms (dronabinol)*	-	*THC & decoction* HR slight/ moderate increased & conjunctival reddening	The highest mean THC was after ingestion the highest milk decoction.
Crippa *et al.* (2004) [49]	In: Healthy occasional volunteers Ex: Any abuse/depend. Personal/family current/past psychiatric disorder Physical illness Positive urine analysis	10/0	29.8 (5.1) 25-42	CBD Placebo	400	-½ (basal), 0, 1, & 1¼ h	VAMS NA	*CBD* VAMS (mental sedation)	*CBD* VAMS (subjective anxiety)	-	NA

(Table 1) Contd....

Author (year)	Inclusion (In) /Exclusion (Ex)** criteria	M/F	M (SD) range	Drugs administered	Dose mg	Measures hours	Clinical tools	Symptomatic effects		Physiological effects	Plasma concentrations ng/mL (Mean (SD)
								Increased	Decreased		
McDonald *et al*. (2003) [43]	In: Healthy occasional volunteers Ex: Any abuse/depend. Current/past psychiatric disorder Physical illness Low level education BMI: out of 19–26 kg/m^2 Positive urine analysis Pregnancy	18/19	23 (4.5) 18-45	THC (dronabinol) Placebo	7 15	Basal, 1/3, 11/3 & 21/3h	DEQ ARCI POMS BP HR	*THC* ARCI (stimulant-effects, marijuana-like effects, dysphoria, euphoria, somatic effects & sedation) DEG dose-dependently ("feel drug," "feel high", & "want more") POMS dose-dependently (anxiety, fatigue, anger, & confusion)	*THC* ARCI (intellectual efficiency and energy)	*THC* HR increase dose dependently BP was not affected	NA
Wachtel *et al*. (2002) [44]	In: Healthy occasional volunteers Ex: Any abuse/depend. Current/past psychiatric disorder Physical illness Low level education BMI: out of 19–26 kg/m^2 Pregnancy	7/5	23 (4) 18-31	THC Whole-plant marijuana, Placebo	8.4 16.9 8.4 16.9	Basal, ½, 1, 1½, 2, 2½, 3, 4 & 5h	VAS DEQ POMS BP HR RR BT	*THC dose-dependent* DEQ, ARCI (marijuana subscale & sedation) > marijuana group *THC-High condition* ARCI (stimulant effect, dysphoria & euphoria) > marijuana group *Marijuana* DEQ & ARCI (marijuana scores and sedation) dose-dependently *Marijuana-High condition* VAS (sedated, drowsy and tired)	-	Any relevant physiological effect	*THC* increases dose dependent 1h after *11-OH-THC* after 1.5h *THC-High condition* > levels than marijuana-High condition
Curran *et al*. (2002) [45]	In: Healthy occasional volunteers Ex: Any abuse/depend Current psychiatric disorder Physical illness Any drug use Positive urine analysis	15/0	24.2 (2.1) 18-30	THC (dronabinol) Placebo	7.5 15	Basal, 1, 2, 4, 6, 8, 24 & 48h	VAMS VAS NA	*THC* VAMS (drowsiness, anxiety) VAS (dizziness, dry mouth, palpitation and stoned feeling) No residual effects were found at 24h and 48h	*THC* VAMS (memory, concentration)	*THC* HR increase on the high dose	*THC* peak at 2h after both high and low dose. *11-OH-THC* levels same pattern. Levels at 24 & 48h were below limit detection

(Table 1) Contd....

Author (year)	Inclusion (In) /Exclusion (Ex)** criteria	M/F	M (SD) range	Drugs administered	Dose mg	Measures hours	Clinical tools	Symptomatic effects		Physiological effects	Plasma concentrations ng/mL (Mean (SD)
								Increased	Decreased		
Kauf-mann *et al.* *** (2010; Kraft *et al.* 2008) [48, 66]	In: Healthy cannabis and BDZ naïve volunteers Ex: Any abuse/depend. Current or past psychiatric disorder Physical/pain illness Any drug use Positive urine analysis Pregnancy	0/16	23.6 (2.7) 19-29	Cannabis extract Active placebo (diazepam)	THC:20 THC:CBD: 2:1 Other can.<5% 5	Basal, every hour up to 8h	VAS BPRS BP HR BT PO	*THC* VAS (tiredness, dizziness drowsiness, feeling high) max. after 2h *One subject excluded for severe acute psychotic symptoms*	*THC* BPRS (emotional withdrawal, motor retardation, poor affective response and disturbance of orientation) after 3h	*THC* HR increase from baseline & placebo	*THC & CBD* peak were found between 2h and 4h Low levels of THC and high levels of metabolites. Intersubject variability for both cannabinoids
Naef *et al.* *** (2003) [48]	In: Healthy naïve volunteers Ex: Any abuse/depend. Current/past psychiatric disorder Physical illness Positive urine analysis Pregnancy Hypersensitivity to cannabinoids/ opioids,	6/6	M:27 (11) F: 25 (7)	THC (dronabinol), Morphine THC + morphine,	20 30 20+30	Basal, every hour up to 8h	VAS for pain BP HR PO	*THC* VAS (transient sleepiness, confusion, alt. perception, anxiety & aggression) VAS (pain) *THC + morphine* VAS (hyperalgesia effect was reversed) compared to morphine session	*THC+morphine* (euphorigenic & hallucinogenic effects) compared to THC session Nausea and vomiting > morphine session	*THC* HR increase *THC+morph* BP (systolic & diastolic) PO decrease	*THC* peak at 1-2h *11-OH-THC* peak at 2h and *THC-COOH* at 2-4h Low levels of *THC* and high levels of metabolites *THC+ morphine* Levels of THC were > than THC alone. *THC* plasma levels correlated with side effects

* The MEDLINE-PubMed database (2000-2011) was searched to locate articles using the keywords cross-over, placebo-controlled, randomized studies, single oral dose, healthy, physiological effects, subjective effects, delta-9-tetrahydroccaninol, THC, cannabidiol, CBD, and Boolean operators. Initially we found 20 studies. We excluded five studies for methodological aspects: Not cross-over design (Bergamaschi *et al*., 2011), open design (Ploner *et al*., 2002), no randomized design (Leweke *et al*., 2000), healthy volunteers with cannabis use more than 15-20 times (Stokes *et al*., 2010, 2009). When the data from a single subject sample were reported in separate publications, these were treated as a single study with multiple independent variables (Kraft *et al*., 2008, Roser *et al*., 2009, Nadulski *et al*., 2005a,b).

** Smoking tobacco was allowed in almost all studies.

*** These studies included cannabis naïve subjects because the objective was to evaluate analgesic properties in experimental pain models.

M/F= Male /Female. Symptomatology rating scales: AIR = Analogue Intoxication Rating Scale; ARCI = Addiction Research Centre Inventory; DEQ = Drug Effects Questionnaire; POMS = Profile of Mood States; VAMS = Visual Analogue Mood Scale; VAS = Visual Analogue Scale; STAI = State-Trait Anxiety Inventory; ASI = Addiction Severity Index; BPRS = Brief Psychiatric Rating Scale. Physiological measures: BP = blood pressure; BT = body temperature; HR = heart rate; min. = minute; PO = pulse oxymetry; RR = respiration rate.

line) and at 1, 2 and 3 hours after administration of drug. Blood pressure was measured when the subject had been sitting for at least 15 minutes. Heart rate and blood preassure were monitorised through a digital recorder and an automated arm cuff.

THC Concentrations

Blood samples for determination of THC, 11-hydroxy-delta 9-THC (11-OH-THC), and 11-nor-delta-9-tetrahydrocannabinol (THC-COOH) whole blood concentration were collected during each experimental session at baseline, and at 1, 2 and 3 hours after drug administration. THC is converted by microsomal hydroxylation to 11-OH-THC, which is both a key intermediate for further metabolism to THC-COOH by liver alcohol-dehydrogenase enzymes and a potent psychoactive metabolite [61,62]. Whole blood THC, 11-OH-THC, and THC-COOH concentrations (ng/mL) were measured by immunoassay. Positives were confirmed by gas chromatography-mass spectrometry (GC/MS) or GC/MS/MS.

Data Analysis

Statistical analyses of these measures were carried out using SPPS (v.15) by two of the researchers (RMS and KL) blind to the drug conditions. The various measures obtained from the experimental sessions (symptomatic, physiological, and drug level data) were transformed to permit analysis of the differences in each variable relative to baseline. For each variable, the area under the curve (AUC) between baseline and 3 hours was calculated using the trapezoidal rule. The maximum absolute change from baseline at 2 hours was also determined. The AUC and the effect at 2 hours were analysed using a one-way repeated measures analysis of variance with drug condition (THC or CBD or placebo) as factor. When ANOVA showed significant effects for drug condition, post-hoc multiple comparisons were performed, using the Tukey's test for repeated measures. Correlations between whole blood levels of the drugs and its metabolites and statistical significant symptomatic effects, and physiological measures were analysed using Spearman's correlation coefficient. Differences associated with P-values lower than 0.05 were considered to be statistically significant. When necessary, Bonferroni multiple testing correction test was used.

RESULTS

Symptomatic Effects

Table **2** shows that there were highly significant differences between the effects of the THC in comparison to CBD and placebo. THC produced changes on positive and negative psychotic symptoms, and general psychopathology (PANSS), anxiety (STAI-S), dysphoria (ARCI), sedation (VAMS, ARCI), and the level of subjective intoxication (ASI, ARCI), as indexed by both the AUC and by the effect at 2 hours ($p<0.001$). There was also difference on the VAMS anxiety ratings, which was significant at 2 hours ($p<0.03$) between THC and CBD, but not in the AUC analysis. Some volunteers, 5 (33%) showed severe effects and became markedly paranoid and anxious, but there was a wide inter-subject variability, with a wide range of scores on the PANSS positive scale. Pair-wise comparisons revealed significant differences between the effects of THC relative to both placebo, and to CBD (Table **2**). In contrast, there were no significant differences between the effects of CBD and placebo on any variable. The transient psychotic symptoms observed had resolved spontaneously within two hours. No psychopathological symptoms were reported on follow-up at next day, 1 and 3 weeks later.

(Figs. **1**, **2**, **3** and **4**) show the effects of the drugs on each measure (ASI, STAI-S, VAMS, ARCI, and PANNS) at 1, 2, and 3 hours post administration.

PHYSIOLOGICAL EFFECTS AND PLASMATIC CONCENTRATIONS OF THC AND CBD

Physiological Parameters

There were significant differences between drug effects on heart rate (Table **3**; Fig. **5**). Pair-wise comparisons showed that this reflected an increase in heart rate with THC relative to both placebo, and to CBD (placebo vs. THC: $p=0.0491$; THC vs. CBD: $p=0.0133$; placebo vs. CBD: $p=0.8596$). There was also a trend ($p<0.07$) towards difference in the drug effects on diastolic blood pressure at 2 hours (Table **3**).

Blood Levels

Mean (SD) whole blood levels of THC at 1, 2 and 3 hours after administration were 0.5 (0.8) ng/mL and 0.67 (0.66) ng/mL, and 0.44 (0.40) ng/mL, respectively. Mean (SD) whole blood levels of CBD at the same time points were 0.36 (0.64) ng/mL, 1.62 (2.98) ng/mL and 3.4 (6.42) ng/mL, respectively. Levels of 11-OH-THC and THC-COOH were elevated after administration of THC (but not CBD or placebo) and followed a similar time course (Fig. **6**).

Relationship between Blood Levels and Acute Symptomatic Effects

Both the level of subjective intoxication (ASI) and the PANSS total score (PANSS-TS) were directly correlated with THC-COOH levels at 1 hour post drug administration ($rho=0.665$; $p=0.009$; $rho=0.687$; $p=0.007$), and with THC levels at 3 hours post drug administration ($rho=0.760$; $p=0.002$; $rho=0.731$; $p=0.003$). Negative symptom levels (PANSS-N) also showed a positive correlation with both THC and 11-OH-THC levels at 3 hours post drug administration ($rho=0.813$; $p<0.001$; $rho=0.727$; $p=0.003$). We did not find significant correlation between heart rate and neither THC, 11-OH-THC nor THC-COOH whole blood levels.

DISCUSSION

Acute Symptomatic Effects

The administration of a single oral dose of THC produced the typical transient effects previously described for this substance in an experimental laboratory setting: feelings of anxiety, euphoria, dysphoria and subjective intoxication. Positive and negative psychotic symptoms were also evident in some, but not all subjects, again consistent with previous studies [63-65]. In the review done, seven of nine studies described "feel high", dysphoria, and subjective intoxication [41,42-44,46,48,66-69] (Table **1**). The intensity of symptomatology appeared to be dose-dependent [42, 44]. Moreover, from the 146 subjects involved in the review, 3 (2.1%) were excluded because they presented severe acute psychotic symptomatology during the study [42, 58, 70]. In our study, 5 (33%) subjects presented transient psychotic symptomatology in the THC session, which resolved spontaneously in two hours. This variabilility probably reflects differences in individual, or genetic susceptibility to THC proness to psychosis [71, 72].

Although studies in both experimental animals [73-77] and healthy volunteers [18, 29, 34, 49,78,79] have shown that CBD has anxiolytic properties, there were remarkably few differences between the effects of CBD and placebo on anxiety [17], save for a reduction in the VAMS anxiety scale at 2 hours post administration. However, in such previous human studies, the anxiolytic effect of CBD has only been evident in subjects in whom anxiety had already been induced experimentally, in contrast to the subjects in the present study. In addition, in animal models, the effect of CBD on anxiety appears to follow an inverted U-shaped dose-response curve [4, 75]. The dose of CBD used in the present study was higher than in previous human anxiety experimental studies (60-300 mg/day), [18, 29, 34, 49,78] and so may have exceeded the dose associated with a clear anxiolytic effect. Unlike THC, CBD had no effects on sedation, intoxication, mood or psychotic symptoms. These data suggest that CBD alone has remarkably few symptomatic effects in non-anxious healthy subjects, which is important in relation to the potential therapeutic utility of CBD in neurology, psychiatry and other fields of medicine [4, 24]. Recently, a double-blind, randomised study showed that CBD reduces anxiety induced by a simulation public speaking test in a group of patients with generalized social anxiety disorder to a similar response as healthy controls [79].

Physiological Measures

THC increased the heart rate as observed in other studies [42, 43, 45-48], but did not produce an increased systolic and diastolic blood pressure or an orthostatic hypotension, although there was a tendency for an effect on diastolic blood pressure [11]. This may reflect an effect of the THC mediated by sympathetic activation and cholinergic inhibition [80]. As expected from previous investigations [28, 29], CBD did not have any significant physiological effects.

Table 2. Results of Symptomatic Effects Comparisons after a Single Oral dose of Placebo, THC, CBD and Placebo Administration with Respect to the Area Under the Curve (AUC) and Effect at 2 Hours

	AUC				Effect at 2 hours			
	F	**p**		**p***	**F**	**p**		**p***
Symptomatic effects								
ASI	7.81	0.002	1	<0.001	14.33	<0.001	1	<0.001
			2	0.929			2	0.778
			3	0.003			3	<0.001
STAI-S	6.20	0.006	1	0.002	10.50	0.001	1	<0.001
			2	0.455			2	0.354
			3	0.055			3	0.005
VAMS								
Anxiety	2.46	0.105			3.97	0.03	1	0.179
							2	0.634
							3	0.020
Mental sedation	4.67	0.018	1	0.010	6.89	0.004	1	0.001
			2	0.517			2	0.739
			3	0.166			3	0.015
Physical sedation	3.67	0.039	1	0.019	6.18	0.006	1	0.002
			2	0.374			2	0.417
			3	0.358			3	0.084
Other feelings	0.45	0.64			0.20	0.816		
ARCI								
Stimulant-like effects-A	2.42	0.111			2.86	0.076		
Euphoria-MBG	2.22	0.314			2.73	0.084		
Dysphoria-LSD	9.16	0.001	1	0.001	15.03	0.001	1	<0.001
			2	0.963			2	0.535
			3	<0.001			3	<0.001
Intellectual efficiency-BG	4.76	0.019	1	0.024	2.85	0.077		
			2	0.996				
			3	0.023				
Sedation-PCAG	8.33	0.002	1	<0.001	11.32	<0.001	1	<0.001
			2	0.928			2	0.845
			3	0.003			3	<0.001
PANNS								
General psychopathology	9.10	<0.001	1	<0.001	10.71	<0.001	1	<0.001
			2	0.668			2	0.91
			3	0.003			3	<0.001
Positive symptoms	9.14	0.001	1	<0.001	5.37	0.010	1	0.010
			2	0.966			2	0.975
			3	<0.001			3	0.019
Negative symptoms	5.65	0.008	1	0.002	5.73	<0.001	1	0.002
			2	0.359			2	0.317
			3	0.109			3	0.131

ASI= Subjective level of intoxication; STAI-S=Spielberger State Anxiety Inventory; VAMS= Visual Analogue Mood Scale; ARCI= Addiction Research Center Inventory; PANNS= Positive and Negative Psychotic Symptomatology Scale

*Pair wise comparisons: 1) placebo vs. THC, 2) placebo vs. CBD, and 3) THC vs. CBD

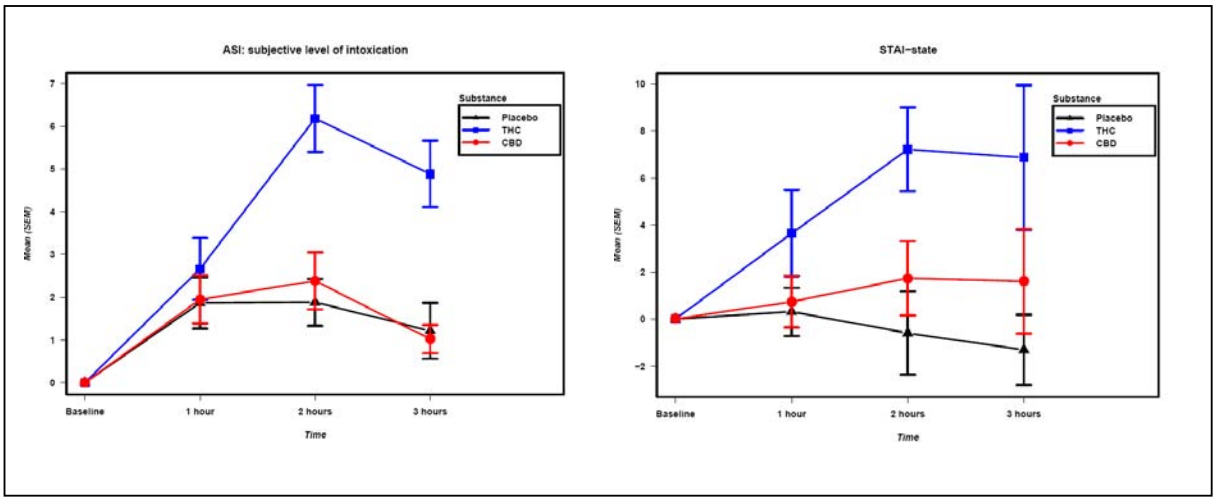


Fig. (1). Changes from baseline over time in the level of subjective intoxication (ASI) score and the level of anxiety (STAI-S) after oral administration of 10 mg THC, 600 mg CBD, and placebo. The figure shows mean (±SEM) values.

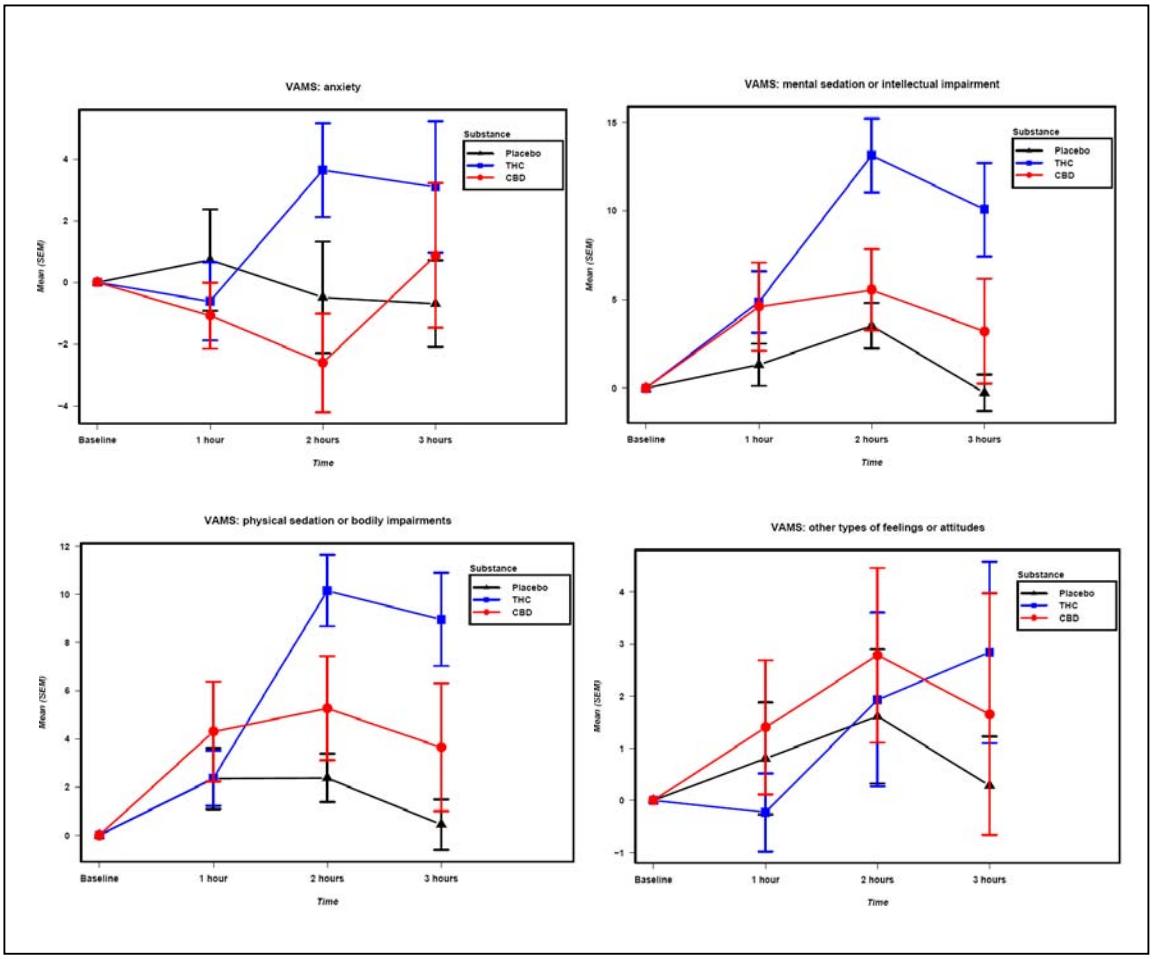


Fig. (2). Changes from baseline over time in anxiety level, mental and physical sedation and other feelings (VAMS) after administration of 10 mg THC, 600 mg CBD, and placebo. The figure shows mean (±SEM) values.

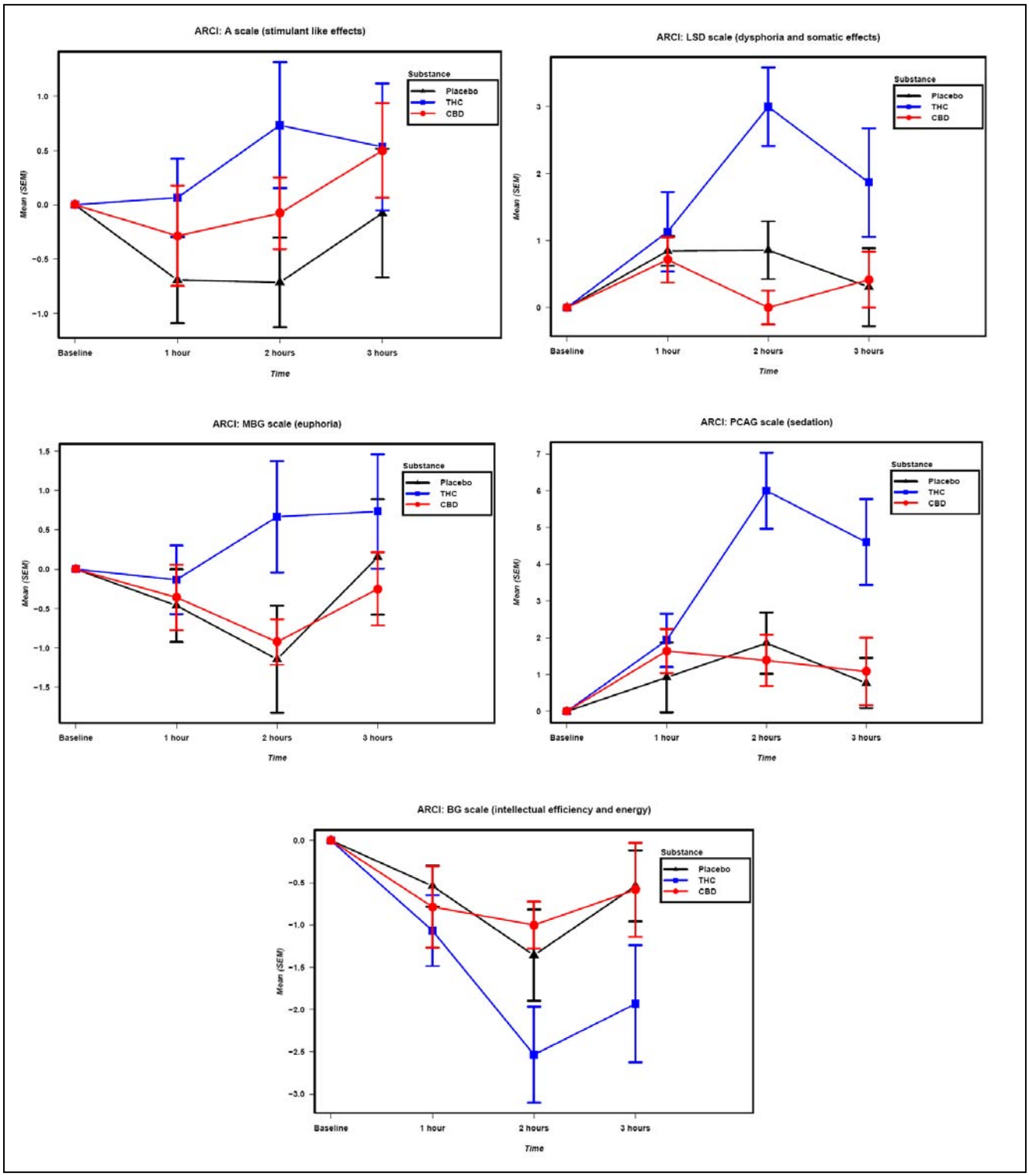


Fig. (3). Changes in subjective symptomatology related to drug intoxication: stimulant effects, induced euphoria, dysphoria, intellectual efficiency and sedation (ARCI) scores after administration of 10 mg THC, 600 mg CBD, and placebo. The figure shows mean (±SEM) values

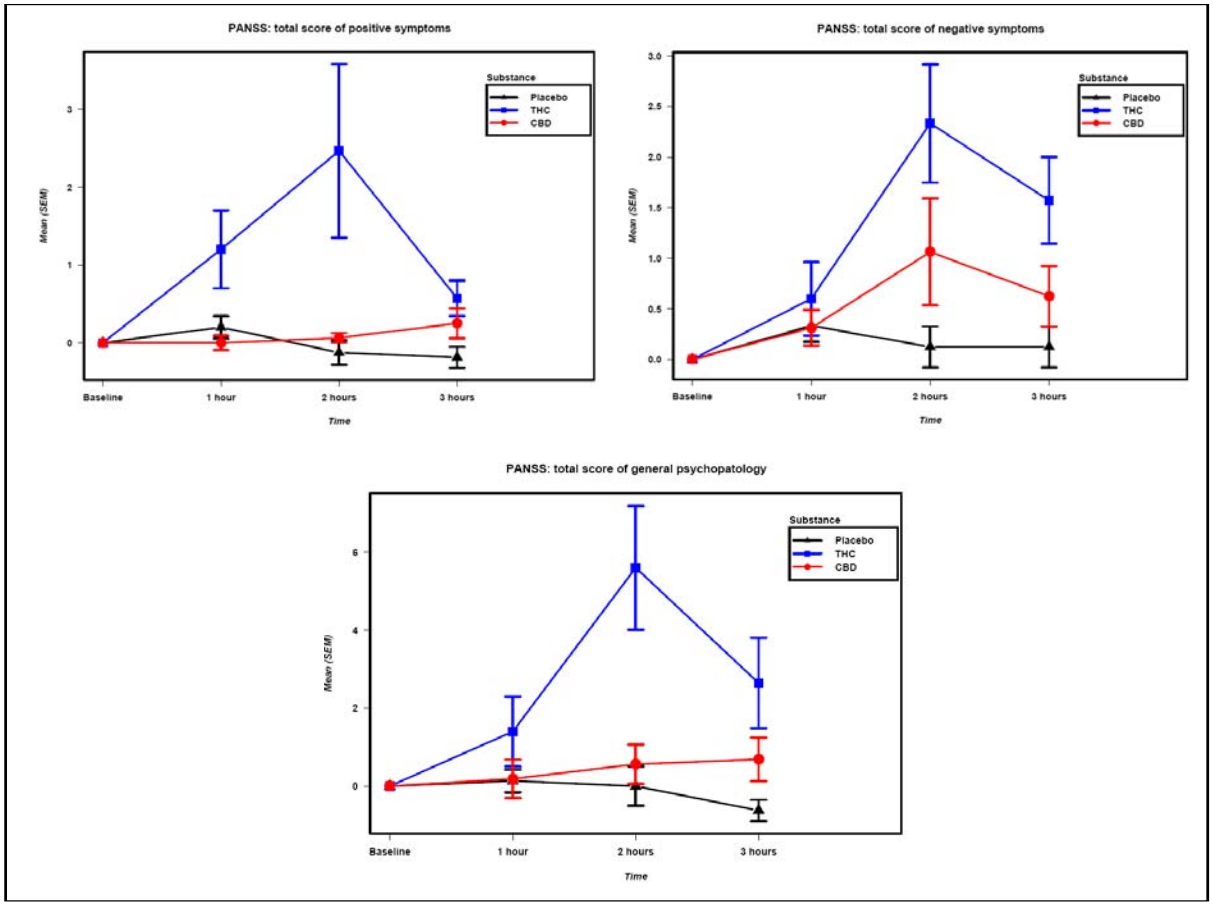


Fig. (4). Changes from baseline over time in positive and negative psychotic symtomatology and total score of general psychopatology of PANNS after administration of THC, CBD, and placebo. The figure shows mean (±SEM) values.

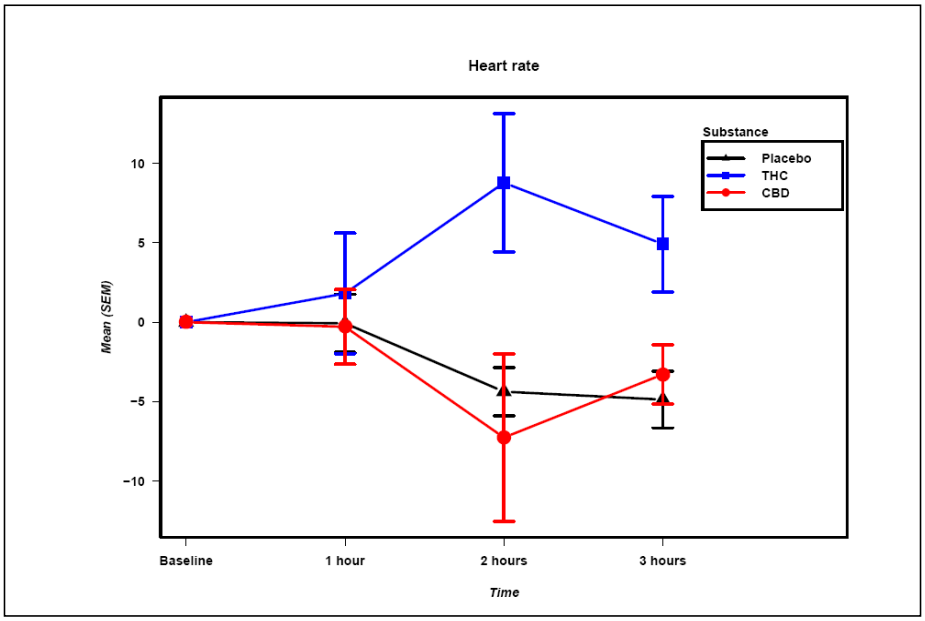


Fig. (5). Changes from baseline over time in heart rate after oral administration of 10 mg of THC, 600 mg of CBD, and placebo. Figure shows mean (+SEM) values.

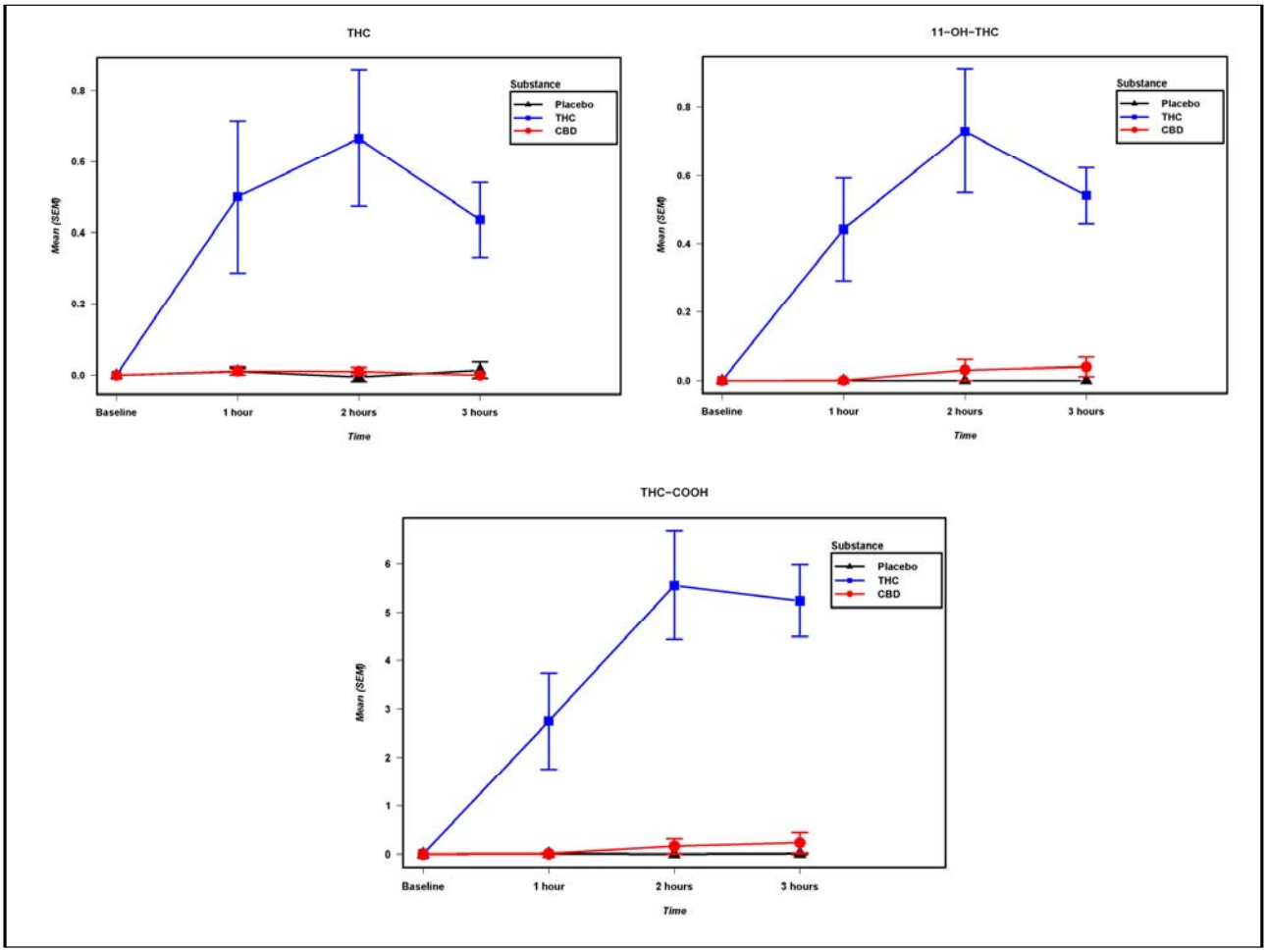


Fig. (6). Time course of THC, 11-OH-THC and THC-COOH whole blood levels after oral administration of 10 mg of THC, 600 mg of CBD, and placebo. Figure shows mean (±SEM) values.

Table 3. Results of Physiological Effects Comparisons after a Single Oral dose of Placebo, THC, CBD and Placebo Administration with Respect to the Area Under the Curve (AUC) and Effect at 2 Hours

	AUC				Effect at 2 hours			
Physiological parameters	**F**	**p**		**p***	**F**	**p**		**p***
Systolic blood pressure	0.96	0.397			1.17	0.327		
Diastolic blood pressure	0.27	0.769			2.44	0.07		
Heart rate	4.72	0.019	1	0.010	4.83	0.016	1	0.049
			2	0.924			2	0.859
			3	0.037			3	0.013

*Pair wise comparisons: 1) placebo vs. THC, 2) placebo vs. CBD, and 3) THC vs. CBD

Whole Blood Drug Concentration Levels

Although some previous studies have reported that THC plasma concentrations were out of phase with its behavioural, cognitive or endocrine effects [61,62, 81, 82], we found that the level of subjective intoxication (ASI) and the severity of positive and negative total score (PANSS-TS) correlated with whole blood levels of 11-OH-THC at 1 hour post drug administration, and with the levels of THC at 3 hours post drug administration.

Limitations

Some methodological limitations of this study need to be noted. First, we used a within-subject cross-over design, which minimised the confounding of effects of inter-subject differences, but was logistically demanding, limited the total number of participants that could be studied. In an effort to minimise the potentially confounding effects of previous substance use, we restricted inclusion to volunteers who has taken cannabis less than 15 times in their life-

time, with none in the last month. However, for ethical reasons, it was not possible to study participants who were completely cannabis naïve. The subjective effects of cannabis may be greater at the first time of use [11, 17], so we might have observed different results in a sample with more experience with cannabis. In the systematic review we observed that one of the three subjects, a women, who presented acute psychotic symptoms was from a study in naïve subjects [48] (Table **1**). The dose of THC chosen for this study (10mg) was designed to be comparable to that delivered from a typical cannabis cigarette, and it is possible that had we used a higher dose, effects on cognitive performance may have been more evident.

In summary, the data from the present study suggest that a single dose of THC, comparable to that delivered form a cannabis cigarette, had significant acute symptomatic and physiological effects in healthy volunteers. Moreover, CBD has confirmed to be safe and well-tolerated in humans as previously observed [25].

CONFLICTS OF INTEREST

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

ACKNOWLEDGEMENTS

This study was supported by grants from the Psychiatry Research Trust, UK and the Ministerio de Sanidad y Consumo: Plan Nacional sobre Drogas: 2006I101, and PI041731/2011, and the Generalitat de Catalunya: SGR2009/1435, Spain. JAC and AWZ are recipients of a CNPq Productivity fellowship (Brazil). SB has received support from the Medical Research Council, UK (Joint MRC/Priory Clinical research training fellowship; G0501775) and is currently supported by a NIHR Clinician Scientist Award (NIHR CS-11-001).

REFERENCES

[1] European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) Annual report 2011: the state of the drugs problem in Europe. Luxembourg: Publications Office of the European Union 2011.

[2] Gaoni Y, Menchoulam R. The isolation of delta-1-tetrahydocannabinol and other neutral cannabinoids from hashish. J Am Chem Soc 1971; 93: 217-24.

[3] Mechoulam R, Hanus L Cannabidiol: an overview of some chemical and pharmacological aspects. Part I: chemical aspects. Chem Phys Lipids 2002; 121: 35-43.

[4] Crippa JAS, Zuardi AW, Hallak JEC. Therapeutical use of the cannabinoids in psychiatry. Rev Bra Psiquiatri 2012; 32 (Suppl 1): 556-65.

[5] Grotenhermen F. Cannabinoids. Curr drug targets CNS Neurol Disor 2005; 4: 507-30.

[6] Iversen L. Cannabis and the brain. Brain 2003; 126: 1252-70.

[7] Pertwee RG. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Delta9-tetrahydrocannabinol, cannabidiol and Delta-9-tetrahydrocannabivarin Br J Pharmacol 2008; 153: 199–215

[8] Devane WA, Dysarz FA, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 1988; 34: 605-13.

[9] Solowij N. Cannabis and cognitive functioning. Cambridge: Cambridge University Press 1998.

[10] Kelleher LM, Stough C, Sergejew AA, Rolfe T. The effects of cannabis on information-processing speed. Addict Behav 2004; 29: 1213-9.

[11] Gorelick DA, Heishman SJ. Methods for clinical research involving cannabis administration. Methods Mol Med 2006; 123: 235-53.

[12] Bisogno T, Hanus L, De Petrocellis L, *et al* . Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br J Pharmacol 2001; 134: 845-52.

[13] Russo EB, Burnett A, Hall B, Parker KK. Agonistic properties of cannabidiol at 5-HT1a receptors. Neurochem Res 2005; 30: 1037-43

[14] Carrier EJ, Auchampach JA, Hillard CJ. Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. Proc Natl Acad Sci U S A 2006; 103: 7895-900.

[15] Thomas R, Chesher G. The pharmacology of marijuana. Med J Aust 1973; 2: 229-37.

[16] Soares VDEP, Campos AC, Bortoli VC, Zangrossi H Jr, Huimaraes FS, Zuardi AW. Intra-dorsal periqueductal gray administration of cannabidiol blocks panic-like response by activating 5-HT1A receptors. Behav Brain Res 2010; 213: 225-9.

[17] Crippa JA, Zuardi AW, Martin-Santos R, *et al*. Cannabis and anxiety: a critical review of the evidence. Hum Psycopharmacol 2009; 24: 513-23.

[18] Crippa JA, Darenusson 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: 121-30.

[19] Leweke FM, Schneider U, Radwan M, Schmidt E, Emrich HM. Different effects of nabilone and cannabidiol on binocular depth inversion in Man. Pharmacol Biochem Behav 2000; 66: 175-81.

[20] Krystal JH, D'Souza DC, Madonick S, Petrakis IL. Toward a rational pharmacotherapy of comorbid substance abuse in schizophrenic patients. Schizophr Res 2000; 35 S35-49.

[21] Zuardi AW, Morais SL, Guimaraes FS, Menchoulam R. Antipsychotic effect of cannabidiol. J Clin Psychiatry 1995; 56: 485-6.

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

[23] Zuardi AW, Hallak JE, Dursun SM, *et al*. Cannabidiol monotherapy for treatment-resistant schizophrenia. J Psychopharmacol **2006(b)**; 20: 683-6.

[24] Zuardi AW. Cannabidiol: from an inactive cannabinoid to a drug with wide spectrum of action. Rev Bras Psiquiatr 2008; 30: 271-80.

[25] Bergamaschi MM, Queiroz RH, Zuardi AW, Crippa JA. Safety and Side Effects of Cannabidiol, a Cannabis sativa Constituent. Curr Drug Saf 2011; 6: 237-49.

[26] Consroe P, Carlini EA, Zwicker AP, Lacerda LA. Interaction of cannabidiol and alcohol in humans. Psychopharmacology (Berl) 1979; 66: 45-50.

[27] Belgrave BE, Bird KD, Chesher GB, *et al*. The effect of cannabidiol, alone and in combination with ethanol, on human performance. Psychopharmacology (Berl) 1979; 64: 243-6.

[28] Karniol IG, Carlini EA. Pharmacological interaction between cannabidiol and delta 9-tetrahydrocannabinol. Psychopharmacologia 1973; 33: 53-70.

[29] Zuardi AW, Shirakawa I, Finkelfarb E, Karniol IG. Action of cannabidiol on the anxiety and other effects produced by delta 9-THC in normal subjects. Psychopharmacology (Berl) 1982; 76: 245.

[30] 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: 95-105.

[31] Fusar-Poli P, Allen P, Bhattacharyya S, *et al*. Modulation of effective connectivity during emotional processing by Delta9-tetrahydrocannabinol and cannabidiol. Int J Neuropsychopharmacol 2010; 13: 421-32.

[32] 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: 764-74.

[33] Borgwardt SJ, Allen P, Bhattacharyya S, *et al. et al*. Neural basis of Delta-9-tetrahydrocannabinol and cannabidiol: effects during response inhibition. Biol Psychiatry 2008; 64: 966-73.

[34] Karniol IG, Shirakawa I, Kasinski N, Pfeferman A, Carlini EA. Cannabidiol interferes with the effects of delta 9 - tetrahydrocannabinol in man. Eur J Pharmacol 1974; 28: 172-7.

[35] Dalton WS, Martz R, Lemberger L, Rodda BE, Forney RB. Influence of cannabidiol on delta-9-tetrahydrocannabinol effects. Clin Pharmacol Ther 1976; 19: 300-9.

[36] Russo E, Guy GW. A tale of two cannabinoides: the therapeutic rationales for combining tetrahydrocannabinol and cannabidiol. Med Hypotheses 2006; 66: 234-46.

[37] Zuardi AW, Crippa JA, Hallak JE. Cannabis sativa: the plant that can induce unwanted effects and also treat them]. Rev Bras Psiquiatr 2011; 32 Suppl 1: S1-2.

[38] Zuardi AW, Hallak JEC, Crippa JAS. Interaction between cannabidiol (CBD and delta-9-tetrahydrocannabinol (THC): influence of administration interval and dose ratio between the cannabinoids. Psychopharmacology 2012; 219: 247-9.

[39] Karschner EL, Darwin WD, McMahon RP, *et al*. Subjective and physiological effects after controlled Sativex and oral THC administration.Clin Pharmacol Ther 2011; 89: 400-7.

[40] Karschner EL, Darwin WD, Goodwin RS, Wright S, Huestis MA. Plasma cannabinoid pharmacokinetics following controlled oral delta9-tetrahydrocannabinol and oromucosal cannabis extract administration. Clin Chem 2011; 57: 66-75.

[41] Roser P, Juckel G, Rentzsch J, Nadulski T, Gallinat J, Stadelmann AM. Effects of acute oral Delta9-tetrahydrocannabinol and standardised cannabis extract on the auditory P300 event-related potential in healthy volunteers. Eur Neuropsychopharmacol 2008; 18: 569-77.

[42] Ménetrey A, Augsburger M, Favrat B, *et al*. Assessment of driving capability through the use of clinical and psychomotor tests in relation to blood cannabinoids levels following oral administration of 20 mg dronabinol or of a cannabis decoction made with 20 or 60 mg Delta9-THC. J Anal Toxicol 2005; 29: 327-38.

[43] McDonald J, Schleifer L, Richards JB, de Wit H. Effects of THC on behavioral measures of impulsivity in humans. Neuropsychopharmacology 2003; 28: 1356-65.

[44] Wachtel SR, ElSohly MA, Ross SA, Ambre J, de Witt H. Comparison of the subjective effects of Delta(9)-tetrahydrocannabinol and marijuana in humans. Psychopharmacology (Berl) 2002; 161: 331-39.

[45] Curran HV, Brignell C, Fletcher S, Middleton P, Henry J. Cognitive and subjective dose-response effects of acute oral Delta 9-tetrahydrocannabinol (THC) in infrequent cannabis users. Psychopharmacology (Berl) 2002; 164: 61-70.

[46] Sugarman, DE, Poling J, Sofuoglu M. The safety of modafinil in combination with oral 9-tetrahydrocannabinol in humans. Pharmacol Biochem Behav 2011; 98: 94-100.

[47] Kaufmann RM, Kraft B, Frey R, *et al*. Acute psychotropic effects of oral cannabis extract with a defined content of Delta9-tetrahydrocannabinol (THC) in healthy volunteers. Pharmacopsychiatry 2010; 43: 24-32.

[48] Naef M, Curatolo M, Petersen-Feliz S, Arendt-Nielsen L, Zbinden A, Brenneisen R. The analgesic effect of oral delta-9-tetrahydrocannabinol (THC), morphine, and a THC-morphine combination in healthy subjects under experimental pain conditions. Pain 2003; 105: 79-88.

[49] Crippa JA, Zuardi AW, Garrido GE, *et al*. Effects of cannabidiol (CBD) on regional cerebral blood flow. Neuropsychopharmacology 2004; 29: 417-26.

[50] Morgan CJ, Curran HV. Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. Br J Psychiatry 2008; 192: 306-7.

[51] McLellan AT, Kushner H, Metzger D, *et al*. The Fifth Edition of the Addiction Severity Index. J Subst Abuse Treat 1992; 9: 199-13.

[52] Nelson HE. National Adult Reading Test (NART). Test Manual. NFER-Nelson: Windsor, Berkshire, England 1982.

[53] Agurell S, Carlsson S, Lindgren JE, Ohlsson A, Gillespie H, Hollister L. Interactions of delta 9-tetrahydrocannabinol with cannabinol and cannabidiol following oral administration in man. Assay of cannabinol and cannabidiol by mass fragmentography. Experientia 1981; 37: 1090-2.

[54] Chesher GB, Bird KD, Jackson DM, Perrignon A, Starmer GA. The effects of orally administered delta 9-tetrahydrocannabinol in man on mood and performance measures: a dose response study. Pharmacol Biochem Behav 1990; 35: 861-4.

[55] Leweke FM, Schneider U, Thies M, Munte TF, Emrich HM. Effects of synthetic delta9 tetrahydrocannabinol on binocular depth inversion of natural and artificial objects in man. Psychopharmacology (Berl) 1999; 142: 230-5.

[56] Koethe D, Gerth CW, Neatby MA, *et al*. Disturbances of visual information processing in early states of psychosis and experimental delta-9-tetrahydrocannabinol altered states of consciousness. Schizophr Res 2006; 88: 142-50.

[57] Kay SR, Fiszbein A, Opler LA. The Positive and Negative Syndrome Scale (PANSS) for schizophrenia. Schizophr Bull 1987; 13: 261-76.

[58] Folstein MF, Luria R. Reliability, validity, and clinical application of the Visual Analogue Mood Scale. Psychol Med 1973; 3: 479-86.

[59] Martin WR, Sloan JW, Sapira JD, Jasinski DR. Physiologic, subjective, and behavioral effects of amphetamine, methamphetamine, ephedrine, phenmetrazine, and methylphenidate in man. Clin Pharmacol Ther 1971; 12: 245–58.

[60] Spielberger C. Manual of the State-Trait Anxiety Inventory. CA Consulting. Palo Alto: Psychologists Press Inc. 1983.

[61] Huestis MA, Henningfield JE, Cone EJ. Blood cannabinoids. II. Models for the prediction of time of marijuana exposure from plasma concentrations of delta 9-tetrahydrocannabinol (THC) and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THCCOOH). J Anal Toxicol 1992; 16: 283-90.

[62] Huestis MA. Human cannabinoid pharmacokinetics. Chem Biodivers 2007; 4: 1770-804.

[63] D'Souza DC, Perry E, MacDougall L, *et al*. The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. Neuropsychopharmacology 2004; 29: 1558-72.

[64] Morrison PD, Zois V, McKeown DA, *et al*. The acute effects of synthetic intravenous D9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. Psychol Med 2009; 1: 1-10.

[65] Sewell RA, Skosnik PD, Garcia-Sosa I, Ranganathan M, D'Souza DC. Behavioral, cognitive and psychophysiological effects of cannabinoids: relevance to psychosis and schizophrenia. Rev Bras Psiquiatr 2011; 32 (Suppl 1): S15-30.

[66] Kraft B, Frickey NA, Kaufmann RM, *et al*. Lack of analgesia by oral standardised cannabis extract on acute inflammatory pain and hyperalgesia in volunteers. Anesthesiology 2008; 109: 101-10.

[67] Roser P, Gallinat J, Weinberg G, Juckel G, Gorynia I, Stadelman AM. Psychomotor performance in relation to acute oral administration of Delta9-tetrahydrocannabinol and standardised cannabis extract in healthy human subjects. Eur Arch Psychiatry Clin Neurosci 2009; 259: 284-92.

[68] Nadulski T, Sporkert F, Schnelle M, *et al*. Simultaneous and sensitive analysis of THC, 11-OH-THC, THC-COOH, CBD, and CBD by GS-MS in plasma after oral application of small doses of THC and cannabis extract. J Anal Toxicol 2005; 29: 782-9.

[69] Nadulski T, Pragst F, Weinberg G, *et al*. Randomised, double-blind, placebo-controlled study about the effects of cannabidiol (CBD) on the pharmacokinetics of delta-9-tetrahydrocannabinol (THC) after oral application of THC verse standardised cannabis extract. Ther Drug Monit 2005; 27: 799-810.

[70] Favrat B, Menetry A, Augsburger M, *et al*. Two cases of cannabis acute psychosis following the administration of oral cannabis. BMC Psychiatry 2005; 5: 17.

[71] Luzi S, Morrison PD, Powell J, di Forti M, Murray RM. What is the mechanism whereby cannabis use increases risk of psychosis? Neurotox Res 2008; 14: 105-12.

[72] Bhattacharyya S, Atakan Z, Martín-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; doi: 10.1038/mp.2011.187.

[73] Zuardi AW, Karniol IG. Changes in the conditioned emotional response of rats induced by delta9-THC, CBD and mixture of the two cannabinoids. Arq Biol Tecnol 1983; 26: 391-7.

[74] Musty RE, Conti LH, Mechoulam R. Anxiolytic properties of cannabidiol. In: Harvey DJ Ed. Marihuana '84. Proceedings of the Oxford Symposium on Cannabis. Oxford, UK: IRL Press Limited, 1984: pp. 713-9.

[75] Guimarães FS, Chiaretti TM, Graeff FG, Zuardi AW. Antianxiety effect of cannabidiol in the elevated plus-maze. Psychopharmacology 1990; 100: 558-9.

[76] Onaivi ES, Green MR, Martin BR. Pharmacological characterization of cannabinoids in the elevated plus maze. J Pharmacol Exp Ther 1990; 253: 1002-9.

[77] Moreira FA, Aguiar DC, Guimarães FS. Anxiolytic-like effect of cannabidiol in the rat Vogel conflict test. Prog Neuropsychopharmacol Biol Psychiatry 2006; 30: 1466-71.

[78] Zuardi AW, Cosme RA, Graeff FG, Guimarães FS. Effects of ipsapirone and cannabidiol on human experimental anxiety. J Psychopharmacol 1993; 7: 82-8.

[79] Bergamaschi MM, Costa Queiroz RH, Hortes M, *et al*. Cannabidiol reduces the anxiety induced by simulated public speaking in treatment.naïve social phobia patients. Neuropsychopharmacology 2011; 36: 1219-26.

[80] Jones RT. Cardiovascular system effects of marijuana. J Clin Pharmacol 2002; 42: 585-635.

[81] Cocchetto DM, Owens SM, Perez-Reyes M, DiGuiseppi S, Miller LL. Relationship between plasma delta-9-tetrahydrocannabinol concentration and pharmacologic effects in man. Psychopharmacology (Berl) 1981; 75: 158-64.

[82] Cone EJ, Huestis MA. Relating blood concentrations of tetrahydrocannabinol and metabolites to pharmacologic effects and time of marijuana usage. Ther Drug Monit 1993; 15: 527-32.

Received: March 30, 2012 **Accepted: April 10, 2012**

XVI WORLD CONGRESS OF PSYCHIATRY
"FOCUSING ON ACCESS, QUALITY AND HUMANE CARE"
MADRID, SPAIN | September 14-18, 2014

WORLD PSYCHIATRIC ASSOCIATION

Certificate of Participation

Rocío Martín-Santos

has participated as

CHAIRPERSON

at the XVI World Congress of the World Psychiatry Association
which took place in Madrid - Spain,
from September 14th to September 18th, 2014.

Pedro Ruiz, M.D.
President
World Psychiatric Association (2011 - 2014)

EUROPEAN PSYCHIATRIC ASSOCIATION
21st EUROPEAN
CONGRESS OF PSYCHIATRY
Nice, France 6-9 April 2013

Poster Presentation Certificate

This is to certify that the abstract entitled

STRUCTURAL AND FUNCTIONAL IMAGING STUDIES IN CHRONIC CANNABIS USERS: A SYSTEMATIC REVIEW OF ADOLESCENT AND ADULT FINDINGS

Was presented by

A. Batalla, S. R. Martín-Santos

21st European Congress of Psychiatry
6-9 April 2013
Nice, France

D. Wasserman

Danuta Wasserman
EPA President

Philippe Courtet
Local Organising Committee Chair

CERTIFICATE OF PARTICIPATION

Rocío Martín-Santos

has participated as

CHAIR

during the III International Congress Dual Disorders
Addictions and other Mental Disorders

held in Barcelona from October 23rd to October 26th, 2013.

Prof. Miguel Casas
Congress Chairman

Dr. Carlos Roncero
Scientific Committee Coordinator

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. MartÃ-n-Santos); and the support of DIUE of Generalitat de Catalunya SGR2009/1435 (R. MartÃ-n-Santos, J.A. Crippa); and the Technology Institute for Translational Medicine (INCT-TM, Conselho Nacional de Desenvolvimiento CientÃ-fico e TecnolÃ2gico (CNPq), Brazil (J.A. Crippa, R. MartÃ-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 predetermined 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

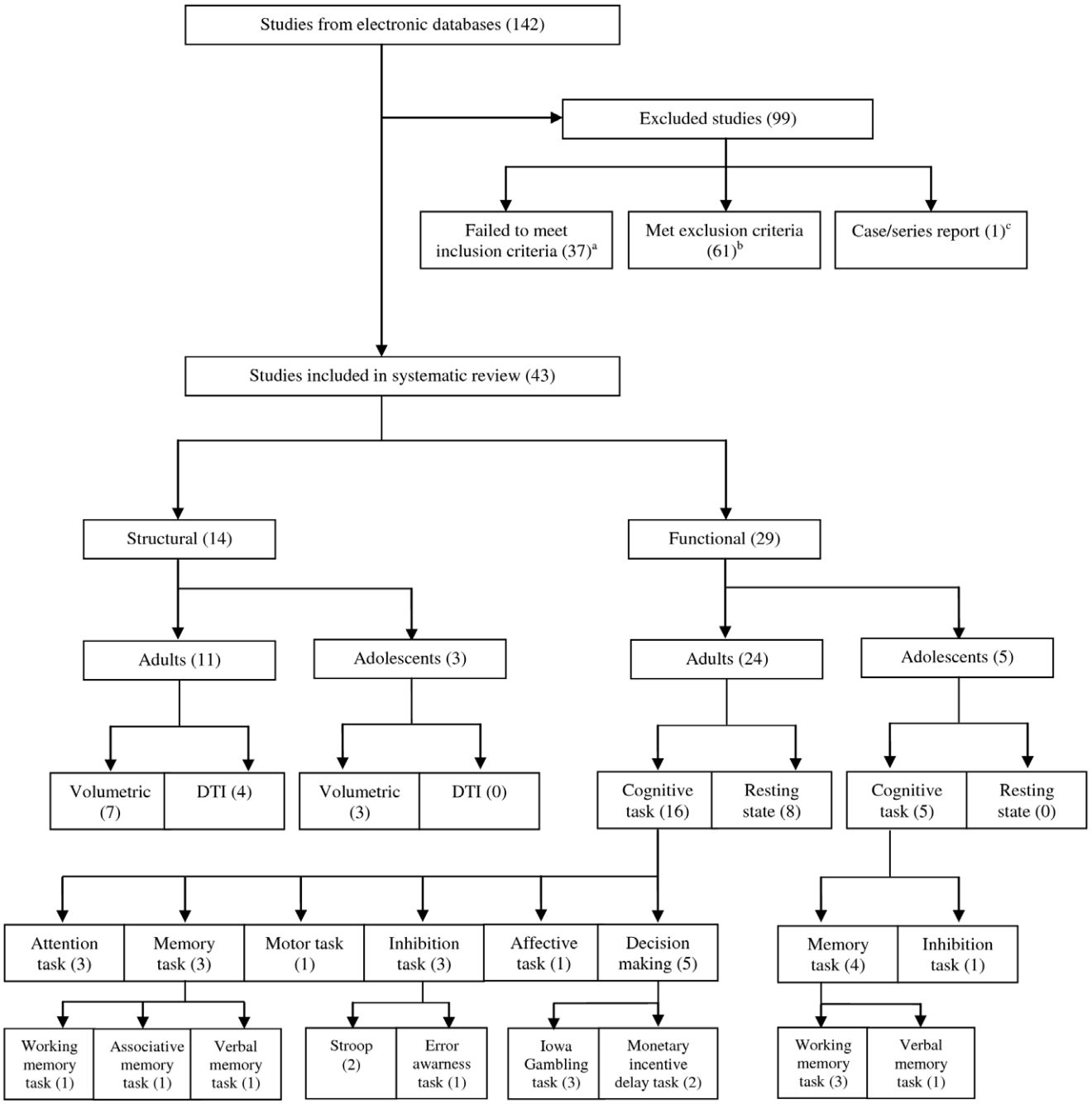
A general review of all neuroimaging studies investigating brain structure or function was initially performed. We obtained a total of 142 published papers (Figure 1). Studies were included or excluded if they expressly stated the following criteria. Inclusion criteria were: (i) use of structural or 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	○ ○ ○ ○	Θ Θ ○ Θ Θ Θ	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 Θ Θ 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 Θ Θ	Θ Θ Θ Θ Θ Θ	○		
DeLisi *et al.* (2006)** [150]	DTI 1.5T	9/1	9/1	21.1 (2.9)/ 23 (4.4)	Whole brain ROI	≤ 1	○ ○ ○ ○	Θ Θ ○ Θ Θ Θ	○		
Jager *et al.* (2007) [152]	MRI 1.5T	13/7	13/7	24.5 (5.2)/ 23.6 (3.9)	Whole brain ROI‡	7	Θ Θ Θ Θ	Θ Θ ○ Θ Θ Θ	○		
Yücel *et al.* (2008) [149]	MRI 3T	15/0	16/0	38.8 (8.9)/ 36.4 (9.8)	ROI	≤ 1	○ ○ Θ ○	Θ Θ ● Θ Θ Θ	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	Θ Θ Θ Θ	Θ Θ Θ Θ Θ Θ	●	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	○ ○ ○ ○	Θ Θ ● Θ Θ Θ	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	Θ Θ Θ Θ	Θ Θ Θ Θ Θ Θ	●	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	Θ Θ Θ ○	● Θ Θ Θ Θ Θ	○	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	Θ Θ Θ ○	Θ Θ Θ Θ Θ ●	Θ	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	Θ Θ Θ ○	Θ Θ ● Θ Θ Θ	Θ	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; Θ = 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	●ΘΘΘΘΘΘ	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 *el 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●ΘΘΘΘ	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.

BARCELONA
16/17 mayo
2013

CANNABIS SYMPOSIUM CIENTÍFICO

CERTIFICA
Que

D/Dª. ROCIO MARTIN-SANTOS LAFFON

ha impartido la ponencia titulada:
EFECTOS FARMACOLÓGICOS DE LOS CANNABINOIDES EN HUMANOS

dentro de la mesa **Mecanismos bioquímicos de la adicción**

desarrollada en el **Symposium Científico Cannabis** celebrado **en el Hotel Petit Palace de Barcelona** los días **16 y 17 de mayo de 2013**

Barcelona, 17 de mayo de 2013

Francisco Pascual Pastor
Coordinador del Symposium

Julio Bobes Garcia
Presidente Socidrogalcohol

SOCIDROGALCOHOL
Sociedad Científica Española
de Estudios sobre el Alcohol,
el Alcoholismo y las otras Toxicomanías

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

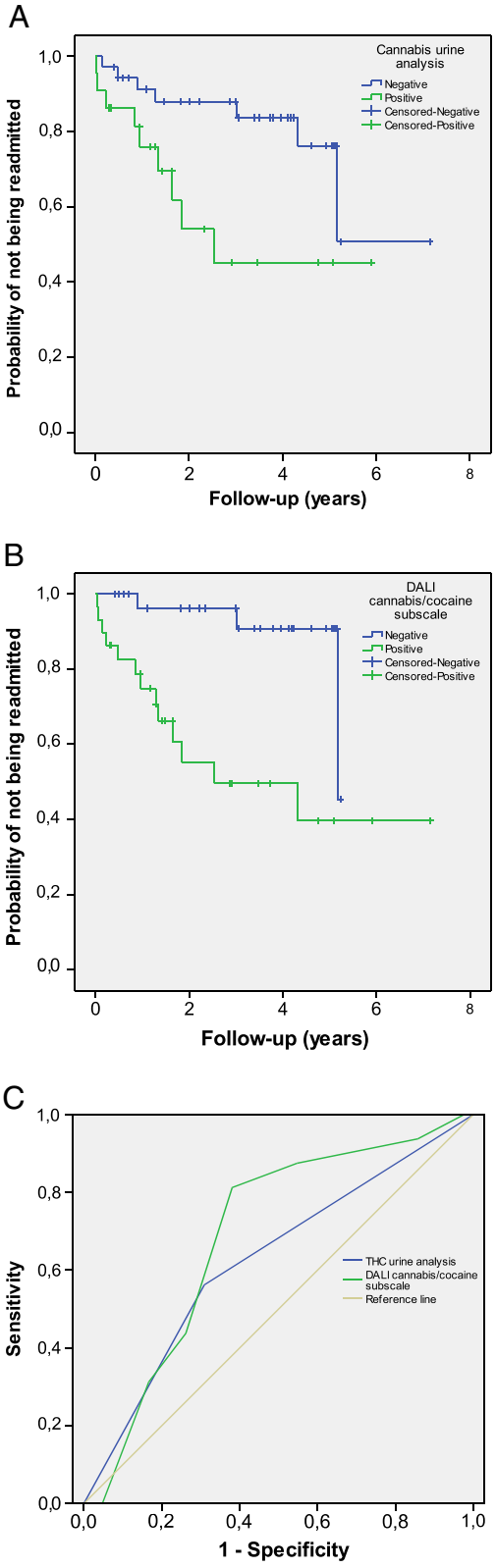


Fig. 1. (A) & (B) Survival plot of cannabis urine analysis and DALI cannabis/cocaine subscale, respectively. (C) ROC curves of DALI cannabis/cocaine subscale compared with positive urine analysis for cannabis for readmission during the whole study period.

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.

RV: Final Sessions' Details

RMSANTOS@clinic.ub.es [RMSANTOS@clinic.ub.es]

Enviado el: jueves, 28 de agosto de 2014 14:07
Para: MARTIN-SANTOS LAFFON, ROCIO
Datos adjuntos: CONFLICT OF INTERESTS_POSI~1.PPT (98 KB) [Abrir como página web]; ISTRUCTIONS FOR CHAIRPERSO~1.PDF (291 KB) [Abrir como página web]

De: XVI World Congress of Psychiatry. Madrid 2014 [mailto:abstracts@wpamadrid2014.com]
Enviado el: miércoles, 13 de agosto de 2014 13:17
Para: MARTIN SANTOS, ROCIO (ICN)
Asunto: Final Sessions' Details

Final Sessions' Details **DATE: 13 August 2014**

Ref: 4919
Dr. Rocio Martin-Santos
rmsantos@clinic.ub.es

Dear Dr. Martin-Santos,

Please take note of the Final Details regarding your participation into the XVI World Congress of Psychiatry and please **read carefully** the Instructions attached in this email.

IMPORTANT: All presenters **must include ONE** of the attached "Conflict of Interest's" slides at the beginning of their Power Point Presentation.

Audiovisual material (Powr Point or others) should be previously delivered in the Speakers Preview Room at least 4 hours before your session begins. For sessions beginning at 9:00 hours, please deliver it the afternoon before your presentation date (previous day before your session).

Detailed below, you will find your **Sessions' Details**.

Presentation Details	
Session:	**437 Depression, addiction and hepatitis C infection: a gender approach**
Title of your presentation:	**Depression and HCV treatment in females**
Type of presentation:	Speaker of Regular Symposium
Session Details:	**Date:** 16/09/2014 **Hour:** 1200 - 1330 h.
Room:	**Room 6**
Session:	**416 "NEURAL SUBSTRATES FOR THE ACUTE AND CHRONIC EFFECTS OF CANNABIS IN MAN: IMPLICATIONS FOR PSYCHOSIS"**
Title of your presentation:	**NEURAL SUBSTRATES FOR THE ACUTE AND CHRONIC EFFECTS OF CANNABIS IN MAN: IMPLICATIONS FOR PSYCHOSIS**
Type of presentation:	Chair of Regular Workshop
Session Details:	**Date:** 16/09/2014 **Hour:** 1630 - 1715 h.
Room:	**Room 9**
Session:	**097 Born this Way: Finding Solutions for Global Challenges in Perinatal Mental Health**

Title of your presentation:	**Personality traits and childhood trauma as risk factors for postpartum depression in Spain**
Type of presentation:	**Speaker of WPA Section Symposium**
Session Details:	**Date:** 16/09/2014 **Hour:** 0900 - 1030 h.
Room:	**Room 19**

Registration

Are you already registered??? **IF NOT,** please remember ALL PRESENTERS must be registered in order to present their work at the Congress.

http://www.wpamadrid2014.com/registration/register-now/

Program at a Glance and Preliminary Program available now on the Congress's website!

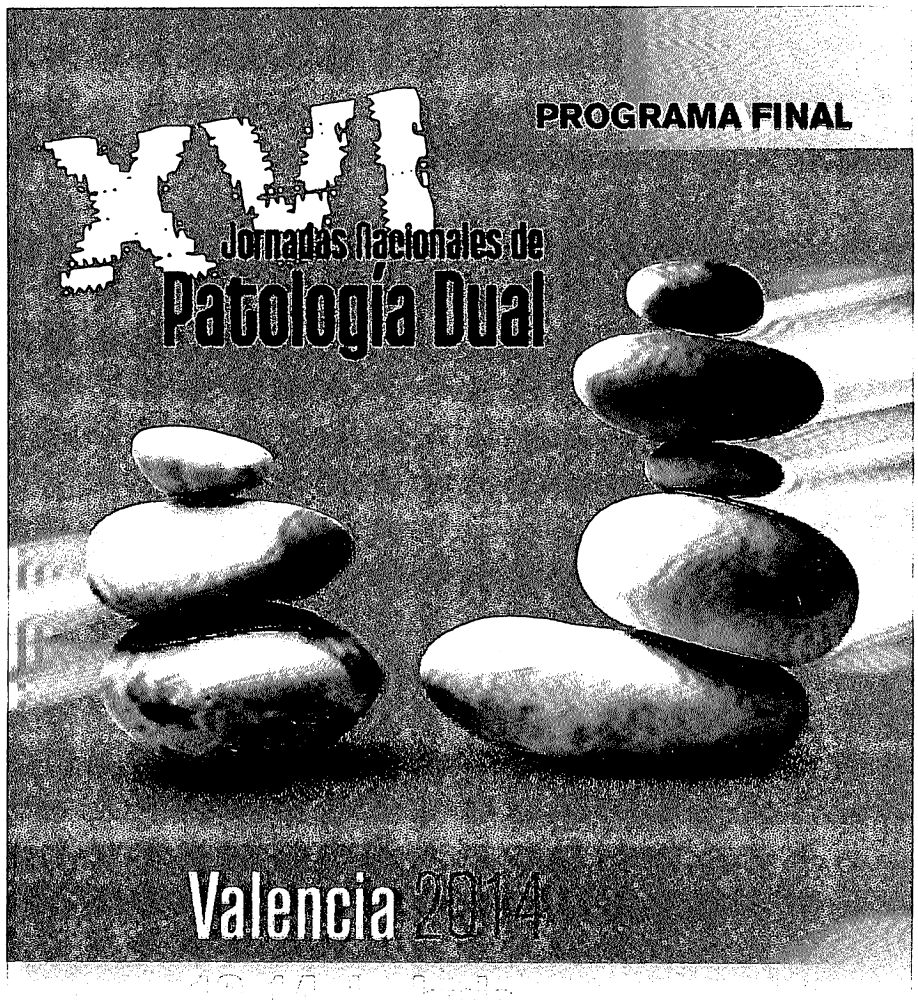
http://www.wpamadrid2014.com/scientific-information/preliminary-timetable/

For any doubt or question, do not hesitate to contact us.

See you in Madrid!

Kind regards,
Congress Secretariat
Tilesa OPC
C/ Londres 17, 28028, Madrid
+34 91 361 2600
abstracts@wpamadrid2014.com

Conectado a Microsoft Exchange



ORGANIZADO POR:

Sociedad Española de Patología Dual
SEPD
Adicciones y otros Trastornos Mentales

ORGANIZACIONES CO-PATROCINADORAS

NIDA

ciber

CON LA COLABORACIÓN DE:

MINISTERIO DE SANIDAD, SERVICIOS SOCIALES E IGUALDAD

SECRETARÍA DE ESTADO DE SERVICIOS SOCIALES E IGUALDAD

DELEGACIÓN DEL GOBIERNO PARA EL PLAN NACIONAL SOBRE DROGAS

SALA VALENTIA A Track: Abordaje Multidisciplinar y Patología Dual

16:30-18:00 h. Simposio – SY04

EXPERIENCIA DE ATENCIÓN INTEGRAL DE LA PATOLOGÍA DUAL A NIVEL AMBULATORIO EN EL ÁREA DE LES CORTS (BARCELONA): RESULTADOS DE TRES AÑOS DE EXPERIENCIA DEL PROGRAMA JOVE DE PATOLOGÍA DUAL DE LES CORTS (PJPD-LC)

MODERADORA:

Maite San Emeterio. *Centre d'Higiene Mental LesCorts. Barcelona.*

PONENTES:

PRESENTACIÓN DEL PROGRAMA JOVE DE PATOLOGÍA DUAL DE LES CORTS (PJPD-LC)

Maite San Emeterio. *Centre d'Higiene Mental LesCorts. Barcelona.*

FUNCIÓN DEL GESTOR DE CASOS EN EL PJPD-LC

Inés Casamada. *Centre d'Higiene Mental LesCorts. Barcelona.*

Yolanda Lozano. *Centre d'Higiene Mental LesCorts. Barcelona.*

DATOS Y REFLEXIONES DE LA EXPERIENCIA DE TRES AÑOS EN DEL PROGRAMA JOVE DE PATOLOGÍA DUAL DE LES CORTS (PJPD-LC)

Carolina Franco. *Centre d'Higiene Mental LesCorts. Barcelona.*

SALA VALENTIA C Track: Investigación y Patología Dual

16:30-18:00 h. Simposio – SY05

ALTERACIONES PSIQUIÁTRICAS ASOCIADAS CON EL CONSUMO DE ALCOHOL Y DE CANNABIS: UNA APROXIMACIÓN TRASLACIONAL

MODERADORA:

María Paz Viveros. *Universidad Complutense de Madrid. Madrid.*

PONENTES:

DEPRESIÓN Y CONSUMO DE ALCOHOL: NUEVOS RETOS TERAPÉUTICOS

Marta Torrens. *Instituto de Neuropsiquiatría y Addicciones - Parc de Salut Mar, Universitat Autònoma de Barcelona. Barcelona.*

DEPRESIÓN Y CANNABIS, UNA COMPLEJA RELACIÓN

Rocío Martín-Santos. *Hospital Clínic de Barcelona y Universidad de Barcelona. Barcelona.*

INVESTIGACIÓN BÁSICA Y PATOLOGÍA DUAL: MODELOS DE COMORBILIDAD ASOCIADA A CANNABIS Y ALCOHOL

María Paz Viveros. *Universidad Complutense de Madrid. Madrid.*

BRAZILIAN SYMPOSIUM OF NEUROPSYCOPHARMACOLOGY

Certificate of Oral Presentation

This is to certify that

Rocío Martín-Santos

presented the talk entitled "Cannabinoids and mental health"

on September 12th during the 1st Brazilian Symposium of Neuropsycopharmacology (SBNP 2014), which was held at the School of Medicine of Ribeirão Preto, in Ribeirão Preto, São Paulo, Brazil, September 11-14th 2014.

Sâmia Regiane Lourenço Joca
Assistant Prof. Pharmacology
School of Pharmaceutical Sciences of Ribeirão Preto
University of São Paulo (USP)

Francisco Silveira Guimarães
Prof. Pharmacology
School of Medicine of Ribeirão Preto
University of São Paulo (USP)

Journal of Psychiatric Research 51 (2014) 68–78

Contents lists available at ScienceDirect

Journal of Psychiatric Research

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

Functional connectivity alterations in brain networks relevant to self-awareness in chronic cannabis users

Jesus Pujol [a,*], Laura Blanco-Hinojo [a,b], Albert Batalla [c,d], Marina López-Solà [a,e], Ben J. Harrison [f], Carles Soriano-Mas [g,h], Jose A. Crippa [i,j], Ana B. Fagundo [b], Joan Deus [a,k], Rafael de la Torre [b,l], Santiago Nogué [m], Magí Farré [b,n], Marta Torrens [b,n], Rocío Martín-Santos [c,d,j]

[a] MRI Research Unit, CRC Mar, Hospital del Mar, Barcelona, Spain
[b] Human Pharmacology and Neurosciences, Institute of Neuropsychiatry and Addiction, Hospital del Mar Research Institute, Barcelona, Spain
[c] Clinical Institute of Neuroscience, Hospital Clínic, IDIBAPS, CIBERSAM, University of Barcelona, Barcelona, Spain
[d] Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Barcelona, Spain
[e] Department of Psychology and Neuroscience, University of Colorado, Boulder, CO, USA
[f] Melbourne Neuropsychiatry Centre, Department of Psychiatry, The University of Melbourne, Melbourne, Australia
[g] Department of Psychiatry, Bellvitge University Hospital-IDIBELL, Barcelona, Spain
[h] Carlos III Health Institute, Ministry of Science and Innovation, CIBERSAM, Spain
[i] Department of Neuroscience and Behavior, RibeirãoPreto Medical School, University of São Paulo, Brazil
[j] INCT Translational Medicine (CNPq), Brazil
[k] Department of Clinical and Health Psychology, Autonomous University of Barcelona, Spain
[l] Red-Fisiopatología de la Obesidad y Nutrición (CIBERobn), Santiago de Compostela, Spain
[m] Section of Clinical Toxicology, Emergency Service, Hospital Clínic, IDIBAPS, University of Barcelona, Barcelona, Spain
[n] School of Medicine, Autonomous University of Barcelona, Red de Trastornos Adictivos (RETIC), Barcelona, Spain

ARTICLE INFO

Article history:
Received 16 June 2013
Received in revised form
15 November 2013
Accepted 17 December 2013

Keywords:
Anxiety
Memory
Cannabis
fMRI
Resting state
Self-awareness

ABSTRACT

Background: Recreational drugs are generally used to intentionally alter conscious experience. Long-lasting cannabis users frequently seek this effect as a means to relieve negative affect states. As with conventional anxiolytic drugs, however, changes in subjective feelings may be associated with memory impairment. We have tested whether the use of cannabis, as a psychoactive compound, is associated with alterations in spontaneous activity in brain networks relevant to self-awareness, and whether such potential changes are related to perceived anxiety and memory performance.
Methods: Functional connectivity was assessed in the Default and Insula networks during resting state using fMRI in 28 heavy cannabis users and 29 control subjects. Imaging assessments were conducted during cannabis use in the unintoxicated state and repeated after one month of controlled abstinence.
Results: Cannabis users showed increased functional connectivity in the core of the Default and Insula networks and selective enhancement of functional anticorrelation between both. Reduced functional connectivity was observed in areas overlapping with other brain networks. Observed alterations were associated with behavioral measurements in a direction suggesting anxiety score reduction and interference with memory performance. Alterations were also related to the amount of cannabis used and partially persisted after one month of abstinence.
Conclusions: Chronic cannabis use was associated with significant effects on the tuning and coupling of brain networks relevant to self-awareness, which in turn are integrated into brain systems supporting the storage of personal experience and motivated behavior. The results suggest potential mechanisms for recreational drugs to interfere with higher-order network interactions generating conscious experience.

1. Introduction

Individuals may use recreational drugs altering conscious experience because of a variety of reasons including being adventurous and curious, and peer pressure. Nevertheless, the most common

* Corresponding author. Department of Magnetic Resonance, CRC-Mar, Hospital del Mar, Passeig Marítim 25-29, 08003 Barcelona, Spain. Tel.: +34 93 221 21 80; fax: +34 93 221 21 81.
E-mail addresses: jpujol@crccorp.es, 21404jpn@comb.cat (J. Pujol).

0022-3956/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jpsychires.2013.12.008

reason given for long-lasting cannabis use is relief from tension or to attenuate negative affect states such as anxiety (Buckner et al., 2007; Crippa et al., 2009; Ogborne et al., 2000; Reilly et al., 1998). Like other psychoactive drugs, however, cannabis has potential side effects. Apart from the possibility of generating drug dependence and the potentially deleterious effect in subjects at risk of developing psychosis (Large et al., 2011), continued cannabis use may impair cognition. Memory is the cognitive domain that has been most consistently reported as impaired in cannabis users (Solowij and Battisti, 2008), although such impairment tends to be mild when no other substances of abuse are implicated (Hall and Solowij, 1998; Solowij and Battisti, 2008).

Neuroimaging research is contributing uniquely to understand the biological bases of mental states. Functional magnetic resonance imaging (fMRI) of spontaneous brain activity permits the identification of a range of functional networks on the basis of region synchrony, defined as functional connectivity. Of relevance are recent studies suggesting the contribution of particular networks to conscious awareness of self. The "Default" network is perhaps the network most extensively investigated. Its main elements are the posterior cingulate cortex (PCC) and adjacent precuneus, angular gyri and medial frontal cortex (Buckner et al., 2008; Harrison et al., 2008). Default network contribution to self-referential mental processes is thought to be related to awareness of the (somatic) body and its relationship to the external environment (Buckner and Carroll, 2007; Buckner et al., 2008; Shulman et al., 1997; Small et al., 2003). In the temporal dimension, the Default network may assist autobiographical memory retrieval, but may also modulate working memory processes (Bluhm et al., 2011; Buckner et al., 2008; Leech et al., 2011). On the other hand, the insula cortex and functionally connected regions are known to be relevant for interoceptive awareness (Caseras et al., 2011; Craig, 2009; Critchley et al., 2004). Activity in the Insula network is associated with conscious perception of the physiological conditions of the (visceral) body (e.g., cardiovascular, airway, gut and sexual sensations) that jointly give rise to an internal representation of oneself, and provide a foundation for subjective feeling states that color emotional experience (Craig, 2002, 2009; Critchley et al., 2004).

Although the Default and Insula networks underlie distinct aspects of self-awareness, there is relevant function overlap, which concerns the contribution of Default network anterior areas to the cognitive control of interoception (Bishop et al., 2004; Ochsner and Gross, 2005; Sylvester et al., 2012), and Insula network posterior areas to the somatic representation of the body (Augustine, 1996; Eickhoff et al., 2006; Ruben et al., 2001). Moreover, activity in these networks seems to be closely coordinated, as their fMRI signal fluctuations show a strong negative correlation during resting state, with periods of high activity in one network often corresponding to low activity in the other network (Fox et al., 2005; Harrison et al., 2011). These functionally "anticorrelated" networks, however, may synchronically deactivate during highly-demanding goal-directed behavior suggesting the attenuation of both somatic and visceral awareness when attention is focused on external targets (Harrison et al., 2011).

In the current study, we have assessed spontaneous activity in the Default and Insula networks in chronic cannabis users. Our hypothesis was that cannabis, as a psychoactive compound, would modulate activity in networks relevant to self-awareness and that this effect would be related to both anxiety levels and cognitive performance. Specifically, we have investigated whether resting-state functional connectivity alterations exist in early onset and heavy cannabis users without comorbid psychiatric disorders compared with control subjects, and whether such potential alterations are associated with variations in anxiety and memory measurements. Resting-state fMRI was initially acquired during cannabis chronic use in the unintoxicated state. The assessment was repeated after one month of abstinence with the prediction that functional alterations may show long-lasting effects, as suggested in a recent review by our group (Batalla et al., 2013).

2. Methods

2.1. Participants

A total of 28 chronic cannabis user men (mean ± SD age, 21 ± 2 years) were assessed and compared with a reference control group of 29 men (age, 22 ± 3 years, ns). One cannabis user was excluded from an original sample of 29 subjects due to non-optimal data acquisition. All participants were followed-up during one month of controlled abstinence, and 27 cannabis users and 28 control subjects were available to repeat fMRI with identical procedures. Written informed consent was obtained from all participants. The study was approved by the local ethics committee (CEIC-IMAS, Barcelona) and was in compliance with the Declaration of Helsinki.

Participants were recruited via a web page and distribution of flyers and ads. To evaluate study eligibility, a comprehensive telephone screening was carried out. When eligible, participants were assessed using a detailed medical history, physical examination, a structured psychiatric interview (PRISM; Torrens et al., 2004), blood biochemical analyses and urine toxicology analyses. To facilitate open disclosure, confidentiality was guaranteed within ethical and legal limits.

Inclusion to the cannabis group required participants to be male, aged between 18 and 30 years, with at least 10 years of education (mean ± SD, 14 ± 2 years), cannabis use onset before age 16, cannabis consumption (smoking) more than 14 times a week at the time of selection and during at least 2 years prior to the study, positive urine test for cannabinoids and negative for opiates, cocaine, amphetamines and benzodiazepines (immunometric assay kits, Instant-View, ASD Inc, Poway, California). Exclusion criteria were: Diagnostic and Statistical Manual for Mental Disorders-Fourth Edition (DSM-IV; American Psychiatric Association, 2000) Axis I disorder, relevant medical or neurological disorders, learning disabilities, use of psychoactive medications, previous use of any other recreational drug for more than 5 occasions lifetime except alcohol and nicotine, lifetime criteria for alcohol abuse or dependence and relevant current alcohol consumption. Current alcohol intake was very low in both study groups, showing a mean ± SD of 5.3 ± 4 units a week in users and 3.1 ± 2.6 units a week in control subjects. On average, cannabis users smoked a mean ± SD of 5.9 ± 5.2 cigarettes a day and control subjects, 2.4 ± 5.9 cigarettes a day. Only three participants (two users and one control subject) smoked more than 10 cigarettes per day. All subjects were right-handed.

Control subjects were required to be male, aged between 18 and 30 years, with at least 10 years of education (15 ± 1 years), showing less than 15 lifetime experiences with cannabis (none in the past month) and negative urine drug screen. Exclusion criteria were identical to the cannabis group. Cannabis users and control subjects showed a mean difference of one year in education ($t = 2.2, P = 0.032$).

Participants were required to refrain from smoking and caffeine 6 h, and alcohol and cannabis 12 h before fMRI. The study consisted of two fMRI assessments. The second fMRI session was carried out in all available participants after a period of 28 days of controlled cannabis abstinence.

2.2. Behavioral assessment

Primary assessments were the State–Trait Anxiety Inventory (STAI; Spielberger et al., 1983) and Rey Auditory-Verbal Learning

Test (RAVLT; Geffen et al., 1990). During the administration of RAVLT, participants were read a list of 15 unrelated words and were asked to recall as many words as they could remember. The same list was repeated over five trials, followed by an interference trial with a new 15-word list, a short-delay free recall trial, and a long-delay free recall trial 20 min later. In this study, the following measurements were considered: "verbal span" (number of words recollected on the first trial), "verbal learning" (over trials; sum of words on trials 1 to 5 minus 5 times the words on trial 1), "recall" (number of recalled words after 20 min) and "forgetting rate" (words on trial 5 minus recalled words after 20 min).

2.3. Image acquisition and preprocessing

A 1.5 Tesla Signa Excite system (General Electric, Milwaukee, WI, USA) equipped with an eight-channel phased-array head coil and single-shot echoplanar imaging (EPI) software was used. The functional sequence consisted of gradient recalled acquisition in the steady state (time of repetition [TR], 2000 m; time of echo [TE], 50 ms; pulse angle, 90°) within a field of view of 24 cm, with a 64 × 64-pixel matrix, and with a slice thickness of 4 mm (inter-slice gap, 1.5 mm). Twenty-two interleaved slices were prescribed parallel to the anterior–posterior commissure line covering the whole-brain. A 6-min continuous resting-state scan was acquired for each participant. Participants were instructed to relax, stay awake and lie still without moving, while keeping their eyes closed throughout. This scan generated 180 whole-brain EPI volumes. The first four (additional) images in each run were discarded to allow magnetization to reach equilibrium.

fMRI data were preprocessed and analyzed using the Statistical Parametric Mapping 8 (SPM8) package, Wellcome Department of Imaging Neuroscience (http://www.fil.ion.ucl.ac.uk/spm/), running on Matlab 7.1 (The Mathworksinc. Natick, Mass). Functional images were realigned (motion corrected), resliced into 2 mm isotropic voxels and spatially warped into the standardized (Montreal Neurological Institute, MNI) SPM template space. A Full Width at Half Maximum (FWHM) 8-mm Gaussian kernel was used to smooth the functional images. All image sequences were inspected for potential acquisition and normalization artifacts. No subjects were excluded because of poor quality images. In addition, we compared both study groups as for potential differences in translational motion, rotational motion, mean interscan displacement and total displacement and found no significant differences in any parameter.

2.4. Functional connectivity analysis

Resting-state functional connectivity was assessed using a seed-based approach as detailed in previous studies (Harrison et al., 2009; Pujol et al., 2012). Functional connectivity maps of Default and Insula networks were generated using regions of interest ("seeds") located in the PCC and anterior insula, respectively. The PCC seed was placed at MNI coordinates $x = 6$, $y = -44$, $z = 37$, which corresponds to the limit between dorsal (anterior) and ventral (posterior) subdivisions of PCC (Leech et al., 2011, 2012; Vogt et al., 2006). The insula seed was placed at $x = 36$, $y = 16$, $z = 2$, which corresponds to the anatomical center (orthocenter) of the anterior insula (Naidich et al., 2004). Although functional connectivity mapping was also carried out using left hemisphere seeds, only data obtained using the right hemisphere seeds are reported for the sake of brevity, as the analysis using left hemisphere seeds gave comparable results.

To generate the maps, the signal time course of a selected seed region was used as a regressor to be correlated with the signal time course of every voxel in the brain, and the obtained voxel-wise regression coefficients served to build first-level output (.con) images. For both locations, seeds were defined as 3.5-mm radial spheres (sampling approximately 25 voxels) using MarsBaR region-of-interest toolbox in MNI stereotaxic space (Brett et al., 2002). Signal values for the seeds were calculated as the average signal of the voxels included in the seed at each time point. In addition, we derived estimates of white matter, CSF, and global brain signal fluctuations to be included as confounding ("nuisance") variables in the analyses.

First-level images generated for each participant were then included in second-level (group) random-effects analyses. One-sample t-statistic maps were calculated to obtain Default and Insula network functional connectivity maps for each group, and two-sample *t*-tests were performed to map between-group differences. Voxel-wise analyses in SPM were also performed to map the correlation between resting-state functional connectivity and behavior ratings (anxiety and memory) and cannabis consumption (average joints per year).

2.4.1. Thresholding criteria

Spatial extent thresholds were determined by 1000 Monte Carlo simulations using AlphaSim (Ward, 2000) as implemented in the SPM REST toolbox (Song et al., 2011). For within-group effects, the input parameters to AlphaSim included an individual voxel threshold probability of 0.005, cluster connection radius of 5 mm, 8 mm FWHM smoothness, incorporating a whole-brain mask volume (256,299 voxels). The estimated minimum cluster size extent was 176 voxels in order to satisfy a family-wise error rate correction of $P_{FWE} < 0.05$. For between-group effects and correlation maps, the incorporated mask instead corresponded to the network maps identified in within-group effects (adding voxels from both cannabis user and control maps), corresponding to 50,427 voxels for the Default network and 64,017 voxels for the Insula network. The respective cluster sizes to satisfy an FWE rate correction of $P < 0.05$ were 102 and 106 voxels. Based on these estimates, clusters greater than 176 voxels with $P < 0.005$ were considered significant (corrected $P < 0.05$) to identify functional connectivity networks in one-sample analyses and clusters greater than 106 voxels with $P < 0.005$ to identify between-group differences and correlation findings.

2.4.2. Hippocampus functional connectivity map

Owing to the relevance of the hippocampus in memory, an additional functional connectivity map was generated for this structure to further characterize the relationship between memory and brain spontaneous activity. The seed region of interest was placed at the midpoint of the hippocampus long axis, corresponding to MNI coordinates $x = 26$, $y = -25$, $z = -14$ (Kahn et al., 2008). The data were analyzed similarly to the main networks of interest as described above.

2.4.3. Statistical analysis of behavioral data

Student-*t* test was used to compare demographic and behavioral variables between groups, and ANCOVA was used instead when covariates were included in the comparison.

3. Results

3.1. Behavioral assessment

Anxiety and memory ratings were within normative values in both study groups (Table 1). Nevertheless, group comparison showed subtle differences that were significant for specific measurements. Cannabis users showed higher anxiety scores, reduced verbal memory span and delayed recall, and increased forgetting rate. After controlling for the effect of education, group differences

Table 1
Cannabis use and behavioral tests.

	Cannabis Mean (SD)
Age of use onset	14.9 (1.0)
Duration of use (years)	6.0 (2.5)
Total lifetime use (joints)	5268 (4265)
Average joints per year	899 (560)

Anxiety and memory ratings	Cannabis Mean (SD)	Controls Mean (SD)	*T*	*P*	F^*	P^*
Trait anxiety (STAI total score)	12.6 ± 4.3	9.0 ± 5.6	2.7	0.009		
State anxiety (STAI total score)	12.3 ± 3.9	9.2 ± 4.4	2.8	0.008		
Verbal memory span (1st trial)	6.1 ± 1.6	7.5 ± 1.9	−2.9	0.006	5.4	0.023
Verbal learning (over 5 trials)	20.6 ± 6.7	20.9 ± 6.0	−0.2	0.848	0.0	0.984
Recall (20-min delayed)	11.2 ± 2.7	13.1 ± 1.8	−3.2	0.002	7.4	0.009
Forgetting rate (5th trial − recall)	1.9 ± 1.5	1.1 ± 1.5	2.1	0.042	3.5	0.069

STAI, State–Trait Anxiety Inventory. Memory assessed with Rey Auditory-Verbal Learning Test (number of words). F^* and P^*, after controlling for years of education.

in memory ratings remained significant for verbal memory span and delayed recall.

3.2. Functional connectivity maps

The PCC seed consistently identified the elements of the Default network in both groups. Functional connectivity maps included the PCC/precuneus, angular gyri, medial (and lateral) frontal cortex, anterior cingulate cortex and lateral temporal cortex. Compared with control subjects, however, cannabis users showed increased functional connectivity in the ventral part of the PCC and decreased functional connectivity in the dorsal PCC/precuneus junction (Fig. 1 and Table S1).

The insula seed identified a network that included bilateral insula and opercula (extending to the lateral prefrontal cortex and supramarginal gyri), basal ganglia, anterior cingulate cortex and ventral brain structures involving the brainstem and right amygdala in both groups. Cannabis users showed increased functional connectivity relative to controls in the anterior portion of the left insula and supramarginal gyri bilaterally, and reduced functional connectivity in the anterior cingulate cortex and superior brainstem (Fig. 1, Table S1).

Results from mapping the regions showing negative correlations with the seeds were also of interest. The PCC "anticorrelation" map included the Insula network, dorsal sensorimotor cortex, visual areas and cerebellum in both groups. This map additionally included the amygdalae in cannabis users. Compared with control subjects, cannabis users showed stronger anticorrelation specifically with areas of the Insula network (Fig. 2, Table S2). Reciprocally, the insula anticorrelation map included the Default network and part of neighboring networks. In cannabis users, the insula seed showed a stronger anticorrelation specifically with primary Default network areas (ventral PCC, frontal medial cortex and right angular gyrus).

Cannabis users, therefore, showed a pattern of increases and decreases in functional connectivity within the Default and Insula networks and enhanced anticorrelation between both. In a further analysis, we investigated to which extent the observed functional connectivity alterations (extracted at peak group differences) were able to account for group mean differences in behavioral ratings

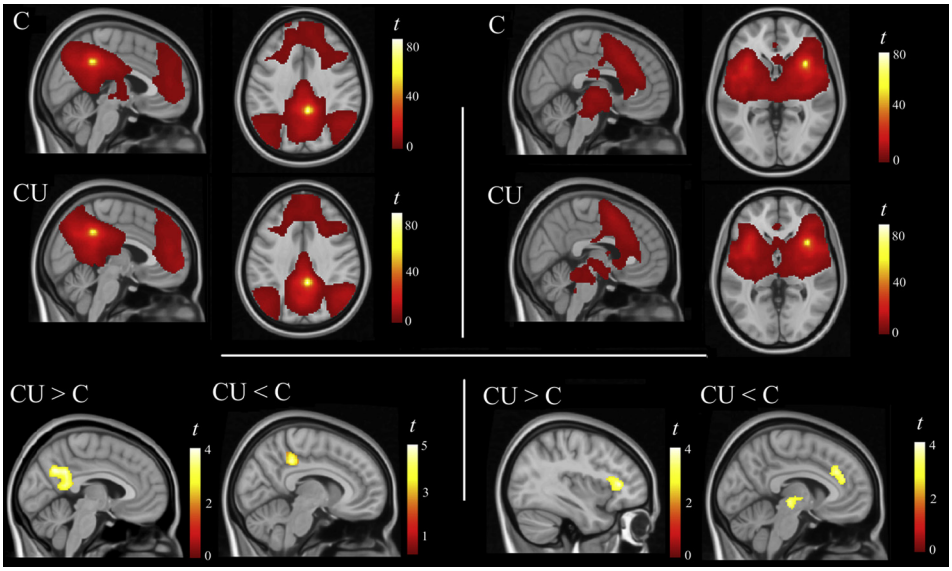


Fig. 1. Default network and Insula network functional connectivity maps, and significant group differences. CU, cannabis users; C, control subjects. The right hemisphere corresponds to the right side of axial views. Bottom right CU > Cview corresponds to MNI $x = -36$.

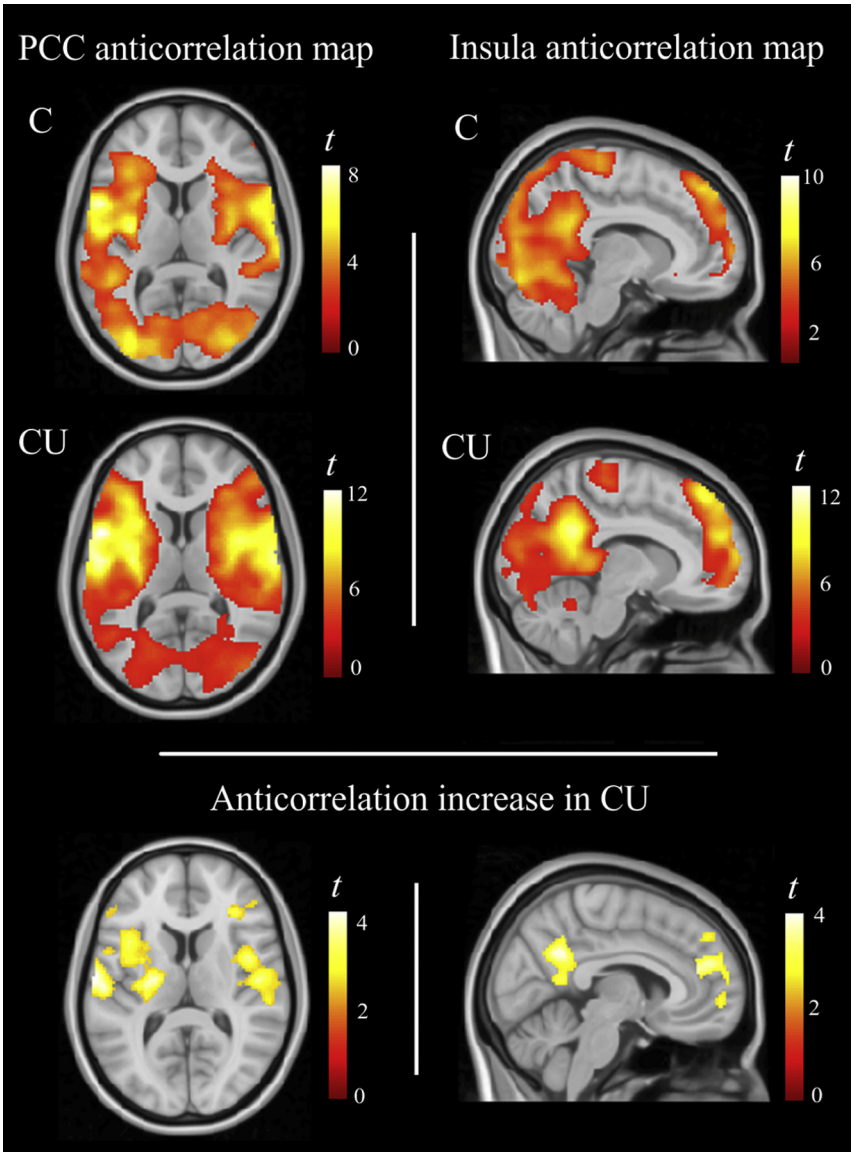


Fig. 2. Functional connectivity maps (negative correlations). PCC, posterior cingulate cortex, and significant group differences. CU, cannabis users; C, control subjects. The right hemisphere corresponds to the right side of axial views.

(i.e., comparing means using ANCOVA with functional connectivity measurements as covariates). Group differences in state anxiety showed a tendency to increase after controlling for ventral PCC functional connectivity ($F = 7.6$ and $P = 0.008$ before and $F = 11.6$ and $P = 0.001$ after removing the effect). This effect was more obvious when controlling for PCC-amygdala anticorrelation (state anxiety group differences; $F = 7.6$ and $P = 0.008$ before and $F = 14.6$ and $P = 0.0003$ after removing the effect). Conversely, we observed that group differences in verbal recall ($F = 7.4$ and $P = 0.009$) were no longer significant when the analysis was controlled for ventral PCC functional connectivity ($F = 3.5$ and $P = 0.066$) and PCC-insula anticorrelation ($F = 2.3$ and $P = 0.133$). Overall, this analysis indicates that the effect of functional connectivity changes was in the direction of reducing anxiety scores and interfering with memory.

3.3. Correlation of functional connectivity with anxiety ratings

A relationship between anxiety ratings and functional connectivity in SPM maps was found involving the right insula (Fig. 3, Table S3, and Supplementary Fig. 1). Specifically, cannabis users showed stronger (than controls) negative correlation between insula functional connectivity and state anxiety (i.e., greater connectivity, less anxiety).

3.4. Correlation of functional connectivity with memory ratings

Memory ratings correlated with functional connectivity measurements in the Default network overlapping with areas showing group differences (i.e., in ventral PCC as a subthreshold but highly specific finding, and dorsal PCC/precuneus junction) (Fig. 3, Table S3, and Supplementary Fig. 1). Specifically, cannabis users showed stronger (than controls) negative correlation between ventral PCC functional connectivity and verbal recall (i.e., greater connectivity, worse recall), and stronger (than controls) positive correlation between dorsal PCC/precuneus functional connectivity and verbal recall (i.e., less connectivity, worse recall). The correlation pattern was marginally affected by controlling for years of education (e.g., dorsal PCC/precuneus showed $F = 13.5$ and $P = 0.0003$ before and $F = 12.9$ and $P = 0.0004$ after controlling for years of education).

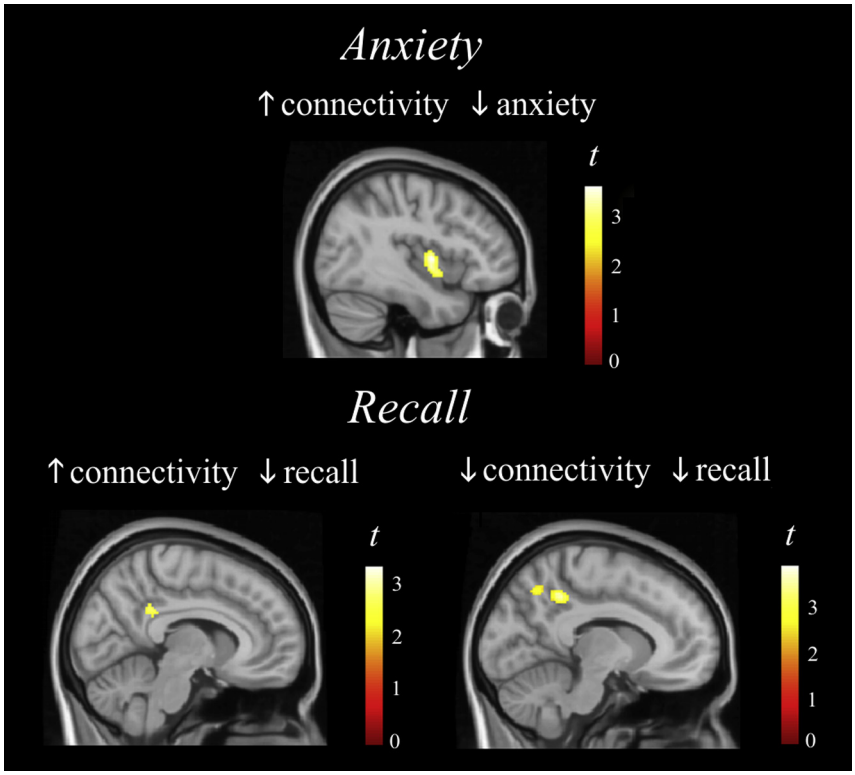


Fig. 3. Correlation between functional connectivity and behavior ratings in the Insula (top) and Default network maps (bottom). Significant interactions between group and correlation pattern are reported. Cannabis users showed a stronger (than controls) negative association between insula functional connectivity and state anxiety, and stronger negative (ventral PCC) and positive (dorsal PCC/precuneus) associations between functional connectivity and verbal recall. Top view corresponds to MNI $x = 38$.

3.5. Correlation with the amount of cannabis used

Average joints per year in cannabis users showed a positive correlation with the strength of functional connectivity in the PCC (subthreshold) and insula (Fig. 4, Table S4).

3.6. Hippocampus seed analysis

The hippocampus seed identified a typical hippocampal connectivity map including medial temporal lobe structures extending to the retrosplenial region and upper brainstem (Fig. 5, Table S5). Compared with control subjects, cannabis users showed an area of reduced functional connectivity in the right hippocampus, and stronger positive correlation between left parahippocampus functional connectivity and verbal recall (i.e., less connectivity, worse recall).

We investigated the extent to which hippocampus alterations were able to account for memory impairment in cannabis users. We observed no reduction in group differences for verbal recall before ($F = 7.4$ and $P = 0.009$) and after ($F = 8.4$ and $P = 0.006$) controlling for hippocampal functional connectivity (at peak group differences).

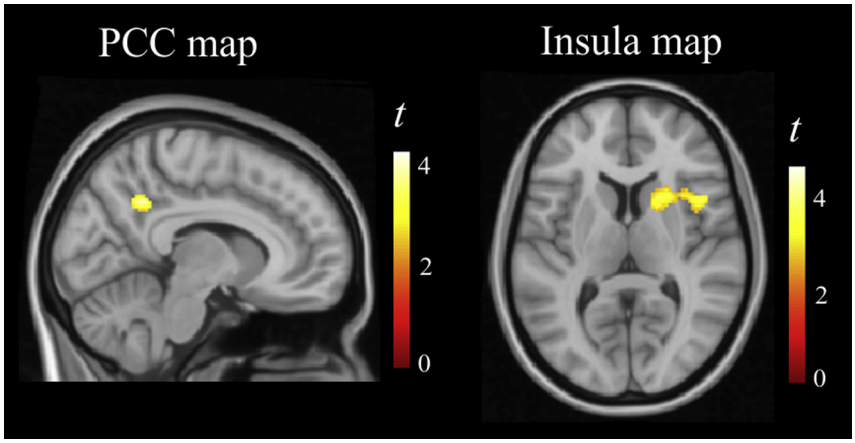


Fig. 4. Correlations between functional connectivity measurements and amount of cannabis used. In cannabis users, strength of functional connectivity correlated positively with average joints per year in primary regions of the Default and Insula networks (subthreshold cluster extent in the case of PCC). The right hemisphere corresponds to the right side of the axial view.

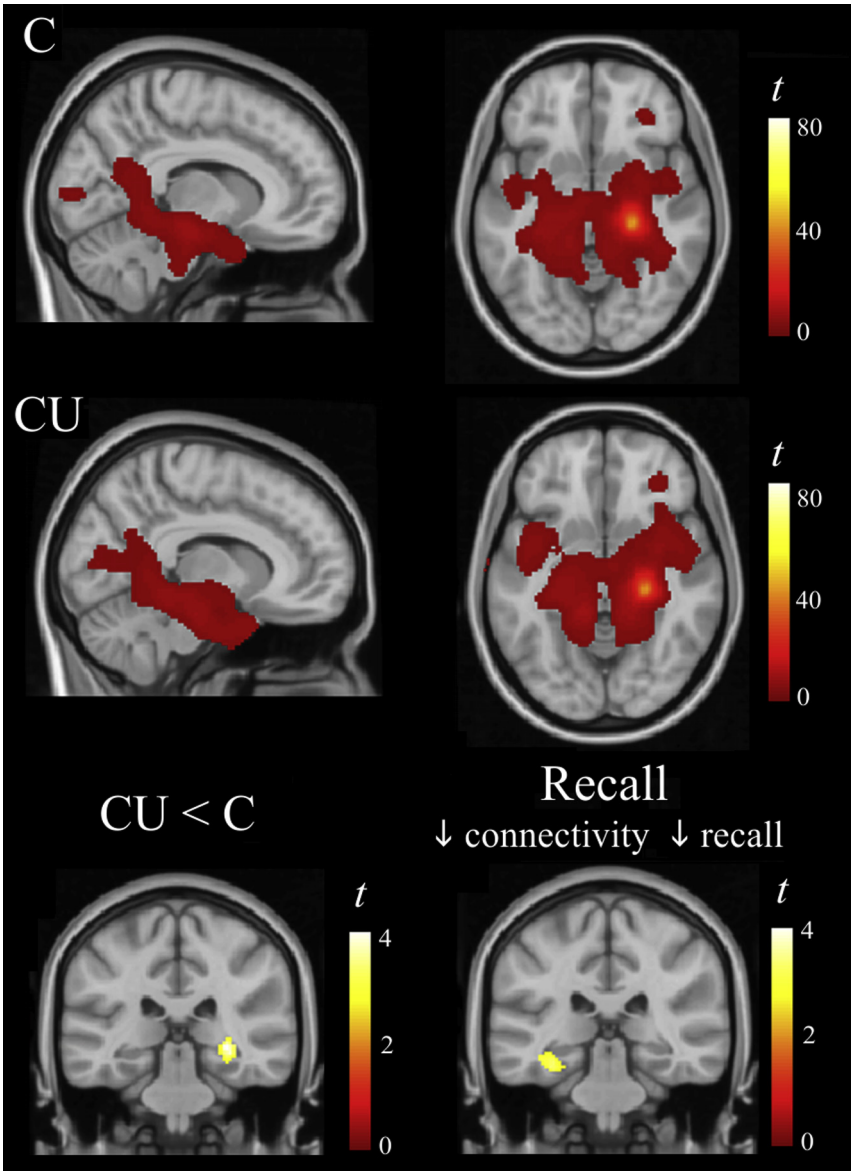


Fig. 5. Hippocampus functional connectivity maps, significant group differences (bottom left) and correlation between functional connectivity and the memory performance (bottom right). Significant interaction between group and correlation pattern is reported for verbal recall. CU, cannabis users; C, control subjects. The right hemisphere corresponds to the right side of the axial and coronal views.

3.7. Long-term cannabis use effect on functional connectivity

After one month of abstinence, there was a general tendency for the magnitude of observed functional alterations to be reduced. Nevertheless, between-group differences persisted for connectivity increases and decreases in the Default network and connectivity increases in the Insula network (Fig. 6).

4. Discussion

Chronic cannabis use was associated with functional connectivity alterations in brain networks relevant to self-awareness. Compared with control subjects, cannabis users showed a specific combination of increases and decreases in functional connectivity within the Default and Insula networks and selective enhancement of fMRI signal anticorrelation between both. These alterations were associated with behavioral measurements in a direction suggesting both anxiety score reduction and interference with memory performance. The functional changes were related to the amount of cannabis used and partially persisted after one month of abstinence.

Increased functional connectivity within the Default network involved the ventral (posterior) portion of the PCC. This region has a key role in the context of Default network function as its connectivity pattern (Leech et al., 2012) and prominent participation in different testing conditions suggest. In an early study, we found activation in this PCC area when normally developing adolescents responded to moral dilemmas and passively viewed their outcomes (Pujol et al., 2008). In a subsequent adult study, the ventral/posterior region was again the PCC portion more strongly connected during spontaneous rest, more activated during moral dilemma and more deactivated during a Stroop task (Harrison et al., 2008). Cannabis use, therefore, appears to be associated with increased connectivity in an area highly representative of the PCC (and Default network) function. By contrast, functional connectivity reduction was identified in an area that overlaps with a cognitive control network (see below).

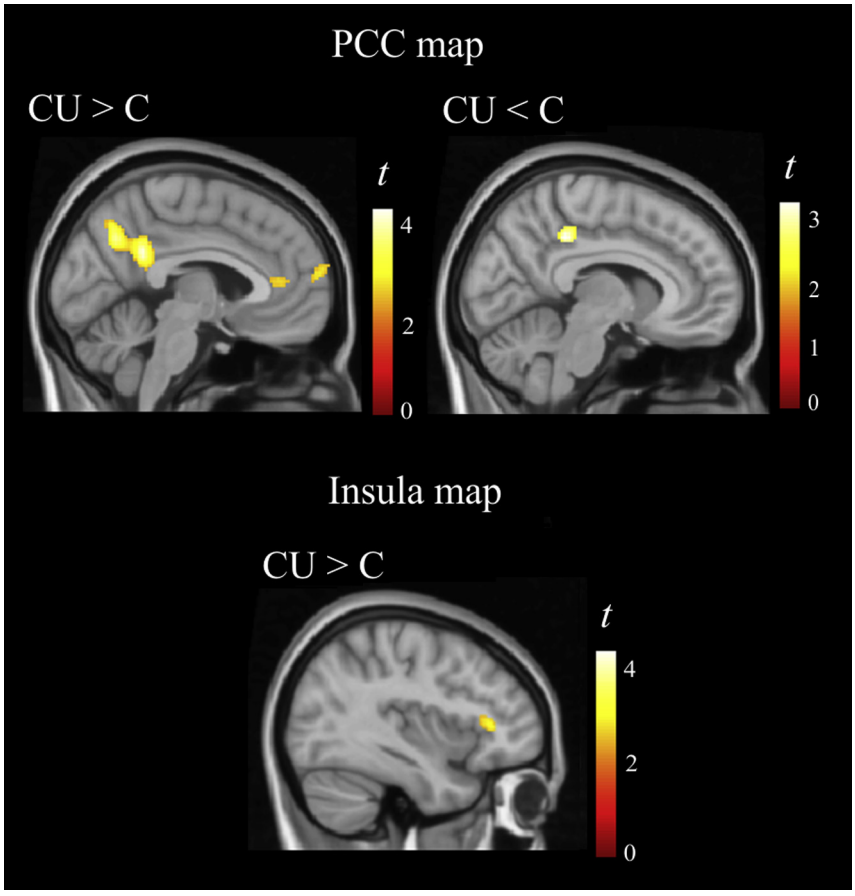


Fig. 6. Persistent effects on functional connectivity after one month of cannabis abstinence. Group differences in the Default (top) and Insula (bottom) networks are reported. CU, cannabis users; C, control subjects. Bottom right view corresponds to MNI $x = -36$.

The Default network, as a functional unit, is active in situations involving self-referential mental activity, as in moral dilemma solving, self-judgments, conceiving the viewpoint of others, autobiographical memory recall and prospective thinking (Buckner and Carrol, 2007; Greicius et al., 2003; Gusnard et al., 2001; Northoff et al., 2006). In these situations indeed, it is supposed that a representation of oneself is projected into active mental processes to generate the subjective perspective (see full argumentation in Buckner and Carroll, 2007). To converge with traditional conceptions of Default network function (Buckner et al., 2008; Mesulam, 1990; Small et al., 2003), it was argued that a representation of self can be generated upon awareness of the body in space (Shulman et al., 1997). Brain lesions involving the PCC and right angular gyrus typically interfere with awareness of both the body (somatoagnosia) and the extrapersonal space (neglect) (Mesulam, 1981, 1990).

Increased functional connectivity within the Insula network involved the anterior insula cortex. This brain area is a highly convergent node participating in a variety of functions covering a full range from emotion to cognition (Caseras et al., 2010, 2011; Cauda et al., 2012; Craig, 2009; Singer et al., 2009). At the level of basic brain operations, however, it is proposed that the anterior insula primarily underlies interoceptive or visceral awareness (Caseras et al., 2011; Critchley et al., 2004). This paralimbic cortex is highly coupled with the anterior cingulate cortex and amygdala, which together are likely critical for integrating interoceptive information into emotion (Craig, 2002; Critchley et al., 2004; Naqvi and Bechara, 2009). In a previous study, for example, we found that anxiety provocation and interoceptive (heartbeat) awareness activated similar portions of the anterior insula and anterior cingulate cortices (Caseras et al., 2011). In cannabis users, we have found increased functional connectivity within the anterior insula, but decreased connectivity to the anterior cingulate cortex (and the thalamus/midbrain junction). Interestingly, both anterior cingulate cortex and thalamus/midbrain are areas of the Insula network overlapping with the Default network (Fig. 1). The anatomy of the findings therefore suggests a markedly specific effect of chronic cannabis use on network tuning (and coupling) involving increased functional connectivity in core areas and reduced connectivity in areas bordering with other networks.

The increase in connectivity within the anterior insula seems to be associated with anxiety score reduction. This finding may give further support to a model of addiction which proposes that the ability of addictive drugs to enhance visceral sensations via insula activation is likely to modify an individual's affect state (and contribute to promote addiction), as these sensations themselves may be pleasurable and rewarding (Naqvi and Bechara, 2009). It is also important, however, that a relevant part of the Insula network overlaps with the classical dopamine rewarding system at the level of the medial frontal cortex, hippocampus and amygdale (Morales and Pickel, 2012; Swanson, 1982), and that cannabis-related alterations within the dopamine system have also been described (Iversen, 2003; Nestor et al., 2010; van Hell et al., 2010). Therefore, it appears that cannabis use could influence motivated behavior by a direct action on the classical rewarding system and indirectly via insula modulation.

The effect of psychoactive drugs on the sense of well-being is frequently associated with memory disturbances (Robbins et al., 2008; Solowij and Battisti, 2008). In our study, increased functional connectivity in the ventral PCC and reduced functional connectivity in the dorsal PCC/precuneus junction were both associated with impaired verbal recall. The dorsal and ventral parts of the PCC are histologically and functionally distinct (Leech et al., 2011; Vogt et al., 2006). The dorsal PCC is a Default network area, but is also highly connected to a dorsal cognitive control network relevant to working memory (Leech et al., 2011, 2012). In the memory task used in our study, verbal recall scores notably depend on verbal span, which is a typical form of working memory (65% of verbal recall variance was explained by the combination of verbal span and forgetting rate in our data). Dorsal PCC alterations could indeed affect verbal recall by interfering with working memory processes. It has previously been observed that reduced PCC connectivity with the other Default network areas at rest predicts poorer performance during working memory tasks (Hampson et al., 2006).

The ventral PCC may fulfill a functional role more conventionally associated with the Default network. This PCC area is activated during memory operations requiring an internal focus of attention, such as autobiographical memory retrieval (Svoboda et al., 2006). In our study, increased ventral PCC functional connectivity at rest was associated with impaired verbal recall. This is a paradoxical finding, as lower (as opposed to higher) ventral PCC connectivity has been reported to predict poorer memory performance in older individuals (Wang et al., 2010). Studies using nicotine may shed light on how increased PCC connectivity could also interfere with verbal recall. In contrast with the effects of cannabis on memory, nicotine may improve accuracy for word recall (Heishman et al., 2010). When tested using fMRI, nicotine was associated with reduced PCC activity during rest (Newhouse et al., 2011; Tanabe et al., 2011). One hypothesis is that nicotine may enhance cognitive performance by suppressing Default network activity (Tanabe et al., 2011). By analogy, cannabis use could impair verbal memory due to defective suppression of Default network activity. Relevantly, memory success is critically related to the ability to switch from PCC deactivation during encoding to PCC activation during retrieval (Daselaar et al., 2009; Kim et al., 2010). Moreover, the retrosplenial PCC is thought to be responsible for the ability to switch from first-person to observer perspectives during recall (Vann et al., 2009). Therefore, one hypothesis to be further tested is whether cannabis use interferes with memory performance by reducing PCC flexibility.

Memory impairment as a collateral effect of sedative drugs may result from direct action on the hippocampal system (Robbins et al., 2008). Cannabis receptors are notably present in the hippocampus and hippocampus alterations have been proposed to account for memory impairment in cannabis users (Iversen, 2003; Yücel et al., 2008). We found a reduction in functional connectivity within the hippocampus. In our analysis, however, group memory differences were better explained by changes in Default network connectivity than by hippocampal alterations. Nevertheless, the Default network and the hippocampus systems are closely related (Vann et al., 2009). The ventral PCC, for example, is reciprocally connected with the hippocampus and parahippocampal region and with the anterior thalamus, closing the classical Papez circuit relevant to episodic memory (Vann et al., 2009). Overall, the scenario suggests that cannabis abuse has potential to critically interfere with the integration of self-referential processes into the storage of personal experiences.

This study was limited in that cannabis users and control subjects had a mean education level difference of one year. Although participants in both groups were selected to have a minimum of 10 years education, the difference could potentially affect memory performance. To partially circumvent this limitation, we specifically covaried for years of education in our analyses. Secondly, while the study design has allowed us to establish significant associations between chronic cannabis use and brain functional changes, is not appropriate for making direct statements regarding the causal role of cannabis. Nonetheless, the observed correlations between amount of cannabis use and functional connectivity suggest such relationships may exist. The current findings may also express a relatively long-lasting effect on brain functional connectivity, as the pattern of alterations persisted after one month of abstinence. On the other hand, these alterations had a tendency to be less pronounced in the follow-up assessment, which begs the question of their potential reversibility.

In conclusion, we have identified specific patterns of altered functional connectivity associated with chronic cannabis use that appear to involve the tuning and coupling of brain networks relevant to self-awareness. The Default and Insula networks, in turn, show anatomical overlap and strong functional connection with brain networks devoted to cognitive control, storage of personal experiences and motivated behavior. The results suggest potential mechanisms for recreational drugs to interfere with higher-order network interactions generating conscious experience.

Authors' contribution

RMS, RTF and JP were responsible for the study design. AF, AB, MF and MT conducted the clinical characterization and selection of participants. LBH, MLS, CSM, JD and JP designed the fMRI protocols, performed the neuroimaging, and analyzed fMRI results. JP wrote the initial manuscript draft. JAC, SB, BJH, and SN provided critical revision of the manuscript for important intellectual content. All the authors have supervised and approved the final version of the manuscript.

Role of the funding source

Funding organizations had no role in the following: design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

This study was supported in part by Ministerio de Sanidad y Consumo, PNSD PI101/2006, PI041731/2011 and SOC/3386/2004 and Fondo de Investigación Sanitaria, ISCIII-FEDER, RTA:RD06/0001/1009. The Agency of University and Research Funding Management of the Catalonia Government participated in the context of Research Groups SGR 2009/718, 1435 and 1450. Ms. Blanco-Hinojo is supported by the PFIS grant FI10/00387 from the Carlos III Health Institute. Dr. López-Solà is supported by the FPU grant AP2005-0408 from the Ministry of Education of Spain. Dr. J.A. Crippa receives a CNPq (Brazil) productivity award (1B). Dr. Soriano-Mas is funded by a Miguel Servet contract from the Carlos III Health Institute (CP10/00604). Dr. Harrison is supported by a National Health and Medical Research Council of Australia (NHMRC) Clinical Career Development Award (I.D. 628509).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jpsychires.2013.12.008.

References

American Psychiatric Association. Diagnostic and statistical manual of mental disorders. text revision. 4th ed. Washington, DC: American Psychiatric Press; 2000.

Augustine JR. Circuitry and functional aspects of the insular lobe in primates including humans. Brain Res Rev 1996;22:229–44.

Batalla A, Bhattacharyya S, Yücel M, Fusar-Poli P, Crippa JA, Nogué S, et al. Structural and functional imaging studies in chronic cannabis users: a systematic review of adolescent and adult findings. PLoS One 2013;8:e55821.

Bishop S, Duncan J, Brett M, Lawrence AD. Prefrontal cortical function and anxiety: controlling attention to threat-related stimuli. Nat Neurosci 2004;7:184–8.

Bluhm RL, Clark CR, McFarlane AC, Moores KA, Shaw ME, Lanius RA. Default network connectivity during a working memory task. Hum Brain Mapp 2011;32:1029–35.

Brett M, Anton JL, Valabregue R, Poline JB. Region of interest analysis using an SPM toolbox [abstract]. In: Presented at: the 8th international conference on functional mapping of the human brain June 2–6, 2002. Sendai, Japan. Available on CD-ROM in Neuroimage 16(2).

Buckner RL, Carroll DC. Self-projection and the brain. Trends Cogn Sci 2007;11:49–57.

Buckner JD, Bonn-Miller MO, Zvolensky MJ, Schmidt NB. Marijuana use motives and social anxiety among marijuana-using young adults. Addict Behav 2007;32:2238–52.

Buckner RL, Andrews-Hanna JR, Schacter DL. The brain's default network: anatomy, function, and relevance to disease. Ann N Y Acad Sci 2008;1124:1–38.

Caseras X, Mataix-Cols D, Trasovares MV, López-Solà M, Ortiz H, Pujol J, et al. Dynamics of brain responses to phobic-related stimulation in specific phobia subtypes. Eur J Neurosci 2010;32:1414–22.

Caseras X, Murphy K, Mataix-Cols D, López-Solà M, Soriano-Mas C, Ortiz H, et al. Anatomical and functional overlap within the insula and anterior cingulate cortex during interoception and phobic symptom provocation. Hum Brain Mapp 2011. http://dx.doi.org/10.1002/hbm.21503.

Cauda F, Torta DM, Sacco K, Geda E, D'Agata F, Costa T, et al. Shared "core" areas between the pain and other task-related networks. PLoS One 2012;7(8):e41929.

Craig AD. How do you feel? Interoception: the sense of the physiological condition of the body. Nat Rev Neurosci 2002;3:655–66.

Craig AD. How do you feel – now? The anterior insula and human awareness. Nat Rev Neurosci 2009;10:59–70.

Crippa JA, Zuardi AW, Martín-Santos R, Bhattacharyya S, Atakan Z, McGuire P, et al. Cannabis and anxiety: a critical review of the evidence. Hum Psychopharmacol 2009;24:515–23.

Critchley HD, Wiens S, Rotshtein P, Ohman A, Dolan RJ. Neural systems supporting interoceptive awareness. Nat Neurosci 2004;7:189–95.

Daselaar SM, Prince SE, Dennis NA, Hayes SM, Kim H, Cabeza R. Posterior midline and ventral parietal activity is associated with retrieval success and encoding failure. Front Hum Neurosci 2009;3:13.

Eickhoff SB, Amunts K, Mohlberg H, Zilles K. The human parietal operculum. II. Stereotaxic maps and correlation with functional imaging results. Cereb Cortex 2006;16:268–79.

Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc Natl Acad Sci U S A 2005;102:9673–8.

Geffen G, Moar KJ, O'Hanlon AP, Clark CR, Geffen LB. Performance measures of 16- to 86-year old males and females on the auditory verbal learning test. Clin Neuropsychol 1990;4:45–63.

Greicius MD, Krasnow B, Reiss AL, Menon V. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. Proc Natl Acad Sci U S A 2003;100:253–8.

Gusnard DA, Akbudak E, Shulman GL, Raichle ME. Medial prefrontal cortex and self-referential mental activity: relation to a default mode of brain function. Proc Natl Acad Sci U S A 2001;98:4259–64.

Hall W, Solowij N. Adverse effects of cannabis. Lancet 1998;352:1611–6.

Hampson M, Driesen NR, Skudlarski P, Gore JC, Constable RT. Brain connectivity related to working memory performance. J Neurosci 2006;51:13338–43.

Harrison BJ, Pujol J, López-Solà M, Hernández-Ribas R, Deus J, Ortiz H, et al. Consistency and functional specialization in the default mode brain network. Proc Natl Acad Sci U S A 2008;105:9781–6.

Harrison BJ, Soriano-Mas C, Pujol J, Ortiz H, López-Solà M, Hernández-Ribas R, et al. Altered corticostriatal functional connectivity in obsessive-compulsive disorder. Arch Gen Psychiatry 2009;66:1189–200.

Harrison BJ, Pujol J, Contreras-Rodríguez O, Soriano-Mas C, López-Solà M, Deus J, et al. Task-induced deactivation from rest extends beyond the default mode brain network. PLoS One 2011;6(7):e22964.

Heishman SJ, Kleykamp BA, Singleton EG. Meta-analysis of the acute effects of nicotine and smoking on human performance. Psychopharmacology (Berl) 2010;210:453–69.

Iversen L. Cannabis and the brain. Brain 2003;126:1252–70.

Kahn I, Andrews-Hanna JR, Vincent JL, Snyder AZ, Buckner RL. Distinct cortical anatomy linked to subregions of the medial temporal lobe revealed by intrinsic functional connectivity. J Neurophysiol 2008;100:129–39.

Kim H, Daselaar SM, Cabeza R. Overlapping brain activity between episodic memory encoding and retrieval: roles of the task-positive and task-negative networks. Neuroimage 2010;49:1045–54.

Large M, Sharma S, Compton MT, Slade T, Nielssen O. Cannabis use and earlier onset of psychosis: a systematic meta-analysis. Arch Gen Psychiatry 2011;68:555–61.

Leech R, Kamourieh S, Beckmann CF, Sharp DJ. Fractionating the default mode network: distinct contributions of the ventral and dorsal posterior cingulate cortex to cognitive control. J Neurosci 2011;31:3217–24.

Leech R, Braga R, Sharp DJ. Echoes of the brain within the posterior cingulate cortex. J Neurosci 2012;32:215–22.

Mesulam MM. A cortical network for directed attention and unilateral neglect. Ann Neurol 1981;10:309–25.

Mesulam MM. Large-scale neurocognitive networks and distributed processing for attention, language, and memory. Ann Neurol 1990;28:597–613.

Morales M, Pickel VM. Insights to drug addiction derived from ultrastructural views of the mesocorticolimbic system. Ann N Y Acad Sci 2012;1248:71–88.

Naidich TP, Kang E, Fatterpekar GM, Delman BN, Gultekin SH, Wolfe D, et al. The insula: anatomic study and MR imaging display at 1.5 T. Am J Neuroradiol 2004;25:222–32.

Naqvi NH, Bechara A. The hidden island of addiction: the insula. Trends Neurosci 2009;32:56–67.

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

Newhouse PA, Potter AS, Dumas JA, Thiel CM. Functional brain imaging of nicotinic effects on higher cognitive processes. Biochem Pharmacol 2011;82:943–51.

Northoff G, Heinzel A, de Greck M, Bermpohl F, Dobrowolny H, Panksepp J. Self-referential processing in our brain – a meta-analysis of imaging studies on the self. Neuroimage 2006;31:440–57.

Ochsner KN, Gross JJ. The cognitive control of emotion. Trends Cogn Sci 2005;9:242–9.

Ogborne AC, Smart RG, Weber T, Birchmore-Timney C. Who is using cannabis as a medicine and why: an exploratory study. J Psychoact Drugs 2000;32:435–43.

Pujol J, Reixach J, Harrison BJ, Timoneda-Gallart C, Vilanova JC, Pérez-Alvarez F. Posterior cingulate activation during moral dilemma in adolescents. Hum Brain Mapp 2008;29:910–21.

Pujol J, Batalla I, Contreras-Rodríguez O, Harrison BJ, Pera V, Hernández-Ribas R, et al. Breakdown in the brain network subserving moral judgment in criminal psychopathy. Soc Cogn Affect Neurosci 2012;7:917–23.

Reilly D, Didcott P, Swift W, Hall W. Long-term cannabis use: characteristics of users in an Australian rural area. Addiction 1998;93:837–46.

Robbins TW, Ersche KD, Everitt BJ. Drug addiction and the memory systems of the brain. Ann N Y Acad Sci 2008;1141:1–21.

Ruben J, Schwiemann J, Deuchert M, Meyer R, Krause T, Curio G, et al. Somatotopic organization of human secondary somatosensory cortex. Cereb Cortex. 2001;11:463–73.

Shulman GL, Fiez JA, Corbetta M, Buckner RL, Miezin FM, Raichle ME, et al. Common blood flow changes across visual tasks: II. Decreases in cerebral cortex. J Cogn Neurosci 1997;9(5):648–63.

Singer T, Critchley HD, Preuschoff K. A common role of insula in feelings, empathy and uncertainty. Trends Cogn Sci 2009;3:334–40.

Small DM, Gitelman DR, Gregory MD, Nobre AC, Parrish TB, Mesulam MM. The posterior cingulate and medial prefrontal cortex mediate the anticipatory allocation of spatial attention. Neuroimage 2003;18:633–41.

Solowij N, Battisti R. The chronic effects of cannabis on memory in humans: a review. Curr Drug Abuse Rev 2008;1:81–98.

Song XW, Dong ZY, Long XY, Li SF, Zuo XN, Zhu CZ, et al. REST: a toolkit for resting-state functional magnetic resonance imaging data processing. PLoS One 2011;6(9):e25031.

Spielberger C, Gorsuch R, Lushene R, Vagg P. Manual for the state–trait anxiety inventory (form Y). Palo Alto, CA: Consulting Psychologists Press; 1983.

Svoboda E, McKinnon MC, Levine B. The functional neuroanatomy of autobiographical memory: a meta-analysis. Neuropsychologia 2006;44:2189–208.

Swanson LW. The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res Bull 1982;9:321–53.

Sylvester CM, Corbetta M, Raichle ME, Rodebaugh TL, Schlaggar BL, Sheline YI, et al. Functional network dysfunction in anxiety and anxiety disorders. Trends Neurosci 2012;35:527–35.

Tanabe J, Nyberg E, Martin LF, Martin J, Cordes D, Kronberg E, et al. Nicotine effects on default mode network during resting state. Psychopharmacology (Berl) 2011;216:287–95.

Torrens M, Serrano D, Astals M, Pérez-Domínguez G, Martín-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;161:1231–7.

van Hell HH, Vink M, Ossewaarde L, Jager G, Kahn RS, Ramsey NF. Chronic effects of cannabis use on the human reward system: an fMRI study. Eur Neuropsychopharmacol 2010;20:153–63.

Vann SD, Aggleton JP, Maguire EA. What does the retrosplenial cortex do? Nat Rev Neurosci 2009;10:792–802.

Vogt BA, Vogt L, Laureys S. Cytology and functionally correlated circuits of human posterior cingulate areas. Neuroimage 2006;29:452–66.
Wang L, Laviolette P, O'Keefe K, Putcha D, Bakkour A, Van Dijk KR, et al. Intrinsic connectivity between the hippocampus and posteromedial cortex predicts memory performance in cognitively intact older individuals. Neuroimage 2010;51:910–7.
Ward BD. Simultaneous inference for FMRI data. Available at: http://stuff.mit.edu/afs/sipb.mit.edu/project/seven/doc/AFNI/AlphaSim.ps; 2000 [accessed 11.06.12].
Yücel 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;65:694–701.

Universitat de Barcelona

Comissió de Doctorat,
Facultat de Medicina

C/ Casanova 143
080360 Barcelona

Tel. +34 934 035 250
Fax +34 934 035 254
secretariamedicina@ub.edu
www.ub.edu/medicina

VALORACIÓ DEFINITIVA TESI DOCTORAL

ALBERT BATALLA CASES

Data de lectura: 23 de juliol de 2014

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

Segons el resultat de l'escrutini dels vots secrets emesos pels membres del tribunal de la tesi, la valoració definitiva és:

EXCEL·LENT "CUM LAUDE"

El President del Comissió de Doctorat

Josep M. Grau Junyent

Barcelona, 23 de juliol de 2014

EUROPEAN PSYCHIATRIC ASSOCIATION
22nd EUROPEAN
CONGRESS OF PSYCHIATRY
Munich, Germany 1-4 March 2014

Oral Presentation Certificate

This is to certify that the abstract entitled

CATECHOL O-METHYLTRANSFERASE VAL158MET

GENOTYPE AND NEURAL MECHANISMS RELATED TO

RESPONSE INHIBITION IN CHRONIC CANNABIS USERS

Was presented by

A. Batalla, A.B. Fagundo, L. Blanco-Hinojo, C. Soriano-Mas,

R. Navinés, M. Farré, M. Udina, R. De la Torre, S. Bhattacharyya,

J.A. Crippa, J. Pujol, M. Torrens, R. Martín-Santos (*Spain*)

22nd European Congress of Psychiatry
1-4 March 2014
Munich, Germany

Danuta Wasserman
EPA President

Peter Falkai
Local Organising Committee Chair

Hans-Jürgen Möller
Local Organising Committee Honorary Chair

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*

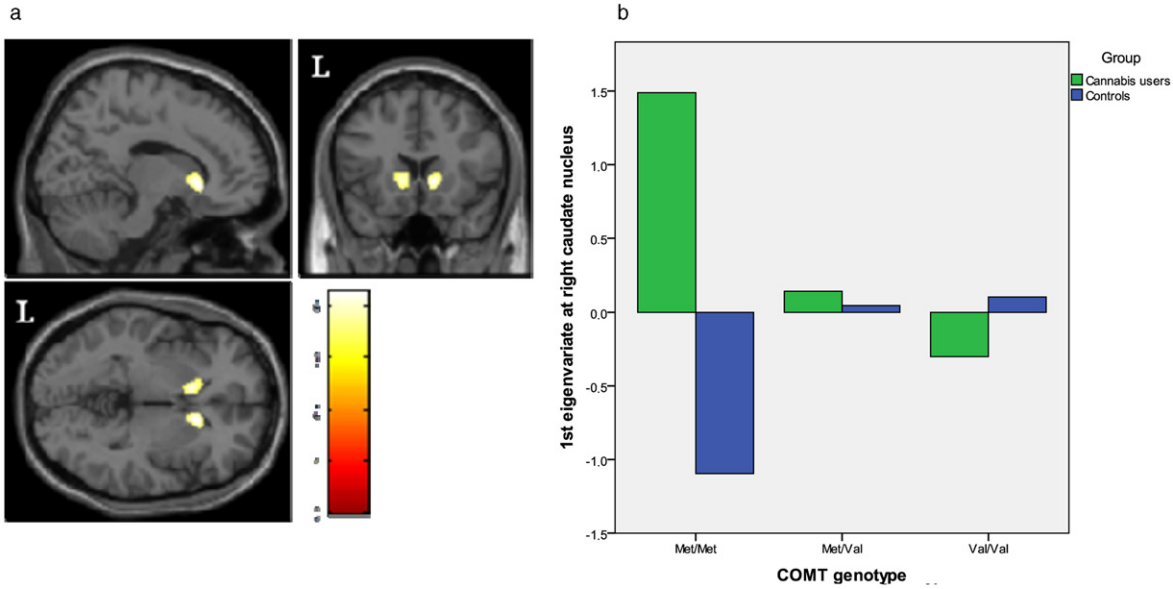


Figure 1 Regions of interaction between catechol-O-methyltransferase (COMT) genotype and brain morphology superimposed on selected slices of a normalized brain (ROI analysis). (a) In the right and left ventral caudate nucleus, while gray matter volume was negatively correlated with the number of *Val* 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

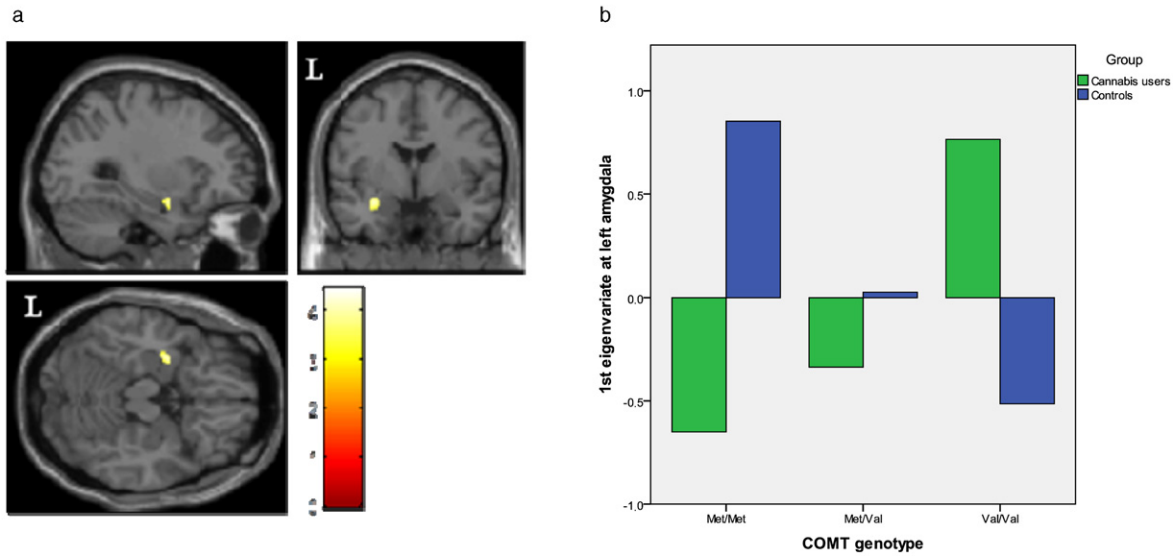


Figure 2 Regions of interaction between catechol-O-methyltransferase (COMT) genotype and brain morphology superimposed on selected slices of a normalized brain (ROI analyses). (a) In the amygdala of the left hemisphere, gray matter volumes were correlated with the number of *Val* alleles in chronic cannabis 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

DETALLE DE JUSTIFICANTES DE GASTOS

RELACIÓN DE JUSTIFICANTES DE GASTOS PRESENTADOS EN LA DELEGACIÓN DEL GOBIERNO PARA EL PLAN NACIONAL SOBRE DROGAS						
Nº EXPEDIENTE: 2011/050	AÑO DE LA CONVOCATORIA: 2011		JUSTIFICACIÓN FINAL DE ANUALIDAD 3ª			
FECHA INICIAL DEL PERIODO DE JUSTIFICACIÓN DE GASTOS: 01/06/2014			FECHA FINAL DEL PERIODO DE JUSTIFICACIÓN DE GASTOS: 07/11/2014			
ORGANISMO: Fundació Clínic per a la Recerca Biomèdica (FCRB)						
TÍTULO DEL PROYECTO: Repercusión del inicio precoz del consumo continuado (crónico) de substancias de abuso sobre la red atencional: estudio de conectividad funcional cerebral y modulación genética.						
INVESTIGADOR PRINCIPAL: Dra. Rocío Martín-Santos						
CONCEPTO DE GASTO AUTORIZADO	Nº ORDEN DEL JUSTIFICANTE (1)	TIPO DE JUSTIFICANTE DE GASTO (2)	Nº FACTURA O JUSTIFICANTE	FECHA DE LA FACTURA O JUSTIFICANTE	IMPORTE DEL JUSTIFICANTE	CANTIDAD IMPUTADA AL PROYECTO
GASTOS DE PERSONAL						
Personal contratado						
BATALLA CASES, ALBERT	1	Nómina/TC2	HR FU00136	30/06/2014	1.191,64	1.191,64
BATALLA CASES, ALBERT	2	Nómina/TC2	HR FU00125	31/07/2014	1.191,64	1.191,64
BATALLA CASES, ALBERT	3	Nómina/TC2	HR FU00116	31/08/2014	1.191,64	1.191,64
BATALLA CASES, ALBERT	4	Nómina/TC2	HR FU00114	30/09/2014	1.191,64	1.191,64
BATALLA CASES, ALBERT	5	Nómina/TC2	HR FU00114	31/10/2014	1.191,64	1.191,64
BATALLA CASES, ALBERT	6	Nómina/TC2	HR FU00002	07/11/2014	753,27	753,27
					TOTAL GASTOS DE PERSONAL:	6.711,47
GASTOS DE FUNCIONAMIENTO						
					TOTAL GASTOS DE FUNCIONAMIENTO:	0,00
					TOTAL GASTOS DIRECTOS:	6.711,47
					GASTOS INDIRECTOS (3):	1.006,72
TOTAL COSTE DE LA ACTIVIDAD SUBVENCIONADA: GASTOS DIRECTOS + INDIRECTOS:						7.718,19
COSTE TOTAL DEL PROYECTO:						52.959,77
TOTAL FINANCIACIÓN RECIBIDA PARA EL DESARROLLO DEL PROYECTO (*):						54.209,00

CONCEPTO DE GASTO AUTORIZADO	Nº ORDEN DEL JUSTIFICANTE (1)	TIPO DE JUSTIFICANTE DE GASTO (2)	Nº FACTURA O JUSTIFICANTE	FECHA DE LA FACTURA O JUSTIFICANTE	IMPORTE DEL JUSTIFICANTE	CANTIDAD IMPUTADA AL PROYECTO
FECHA, NOMBRE, FIRMA Y DEL REPRESENTANTE LEGAL DE LA ENTIDAD: 5/01/2015 Elias Campo Güerri			SELLO DE LA ENTIDAD: FUNDACIÓ CLÍNIC BARCELONA Rosselló, 149-153 - 08036 BARCELONA	FECHA, NOMBRE Y FIRMA DEL INVESTIGADORA PRINCIPAL: 5/01/2015 Rocío Martín-Santos		

(1) Serie numerada. Por favor, no olvide numerar los justificantes según esta relación.

(2) Nómina, recibo, contrato, nombramiento becario, factura, certificado, hoja de liquidación de viajes, etc. En el concepto de Gastos de viaje es obligatorio acompañar hoja de liquidación que agrupe los justificantes de gastos del mismo y debidamente motivada o relacionada con el proyecto.

(3) Los gastos de gestión o costes indirectos se justificarán con una certificación del representante legal de la entidad en la que conste la cantidad ingresada en contabilidad por este concepto. Igualmente, se presentará un certificado con la imputación de IVA que se haya cargado a la subvención, en función del IVA soportado por la entidad.

(*) En caso de haber obtenido otra financiación para el desarrollo del proyecto, se presentará un certificado firmado por el representante legal de la entidad en el que se informe de los gastos soportados con otra financiación, según lo establecido en el Artículo 30, 4 de la Ley 38/2003, de 17 de noviembre, General de Subvenciones: "Cuando las actividades hayan sido financiadas, además de con la subvención, **con fondos propios u otras subvenciones o recursos**, deberá acreditarse en la justificación el importe, procedencia y aplicación de tales fondos a las actividades subvencionadas".

Agencia Tributaria
www.agenciatributaria.es

Delegación Especial de CATALUÑA

Unidad de Gestión de Grandes Empresas de CATALUÑA

PZ DR.LETAMENDI, 13-22
08007 BARCELONA (BARCELONA)
Tel. 932911100
Fax. 932911698

Nº de Remesa: 00050120009

Nº Comunicación: 1566846100810

FUNDACIO PRIVADA CLINIC PER A LA RECERCA BIOMEDICA
C ROSSELLO 149
08036 BARCELONA
BARCELONA

CERTIFICADO

Nº REFERENCIA: 20150143160

Presentada solicitud de certificado acreditativo de encontrarse al corriente de sus obligaciones tributarias a efectos de obtener una subvención otorgada por las Administraciones Públicas, de acuerdo con lo establecido por la Ley 38/2003, de 17 de noviembre, General de Subvenciones, o financiada con cargo a fondos de la Unión Europea, de acuerdo con la normativa comunitaria aplicable y con las normas nacionales de desarrollo o transposición de aquella, o un beneficio en la cotización a la Seguridad Social, de conformidad con su normativa reguladora, por:

N.I.F.: **G59319681** RAZÓN SOCIAL: **FUNDACIO PRIVADA CLINIC PER A LA RECERCA**
DOMICILIO FISCAL: **C ROSSELLO NUM 149 08036 BARCELONA**

La Agencia Estatal de Administración Tributaria,

CERTIFICA: Que conforme a los datos que obran en esta Unidad, el solicitante arriba referenciado se encuentra al corriente de sus obligaciones tributarias de conformidad con lo dispuesto en el artículo 74 del Reglamento general de las actuaciones y los procedimientos de gestión e inspección tributaria y de desarrollo de las normas comunes de los procedimientos de aplicación de los tributos, aprobado por el Real Decreto 1065/2007, de 27 de julio.

El presente certificado se expide a petición del interesado, tiene carácter POSITIVO y una validez de seis meses contados desde la fecha de su expedición, se expide al efecto exclusivo mencionado y no origina derechos ni expectativas de derechos en favor del solicitante ni de terceros, no pudiendo ser invocado a efectos de interrupción o de paralización de plazos de caducidad o prescripción, ni servirá de medio de notificación de los expedientes a los que pudiera hacer referencia, sin que su contenido pueda afectar al resultado de actuaciones posteriores de comprobación o investigación. Todo ello, de conformidad con lo dispuesto en la normativa citada.

Documento firmado electrónicamente (Real Decreto 1671/2009) por la Agencia Estatal de Administración Tributaria, con fecha 12 de enero de 2015. Autenticidad verificable mediante Código Seguro Verificación SRLQ8FPCYNHSCRDE en www.agenciatributaria.gob.es.

GOBIERNO DE ESPAÑA
MINISTERIO DE EMPLEO Y SEGURIDAD SOCIAL

TESORERÍA GENERAL DE LA SEGURIDAD SOCIAL

CERTIFICADO DE ESTAR AL CORRIENTE EN LAS OBLIGACIONES DE SEGURIDAD SOCIAL

DATOS IDENTIFICATIVOS DE LA EMPRESA

RAZÓN SOCIAL	CÓDIGO CUENTA DE COTIZACIÓN PRINCIPAL
FUNDACIó PRIVADA CLíNIC RECERCA BIOMèDICA	0111 08018424968
CÓDIGO DE IDENTIFICACIÓN	**N.A.F.**
0G59319681	

IDENTIFICADORES ASOCIADOS

	CLV
08161112275, 08172279403, 08151653866.********** ***	7GB ***
Total CLV	1GM

De los antecedentes obrantes en esta Tesorería General se CERTIFICA que:

NO tiene pendiente de ingreso ninguna reclamación por deudas ya vencidas con la Seguridad Social.

Y para que conste, a petición del interesado, se expide la siguiente certificación positiva a los solos efectos de lo establecido en el apartado e) del artículo 13 de la Ley 38/2003, de 17 de noviembre, General de Subvenciones, que no originaría derechos ni expectativas de derechos a favor de los solicitantes o de terceros, ni podría ser invocada a efectos de interrupción o paralización de plazos de caducidad o prescripción, ni servirá de medio de notificación de los expedientes a que pudiera hacer referencia ni afectará a ulteriores actuaciones de comprobación e investigación relativas a la situación a que está referida.

De conformidad con los términos de la autorización número 18036, concedida en fecha 24/08/1999 a FUNDACIO CLINIC PER A LA RECERCA BIOMEDICA cuyo titular es D/Dª FERNANDO AGUADO GARCIA NIF: 039181121T por la Tesorería General de la Seguridad Social, certifico que estos datos han sido transmitidos y validados por la misma e impresos de forma autorizada, surtiendo efectos en relación con el cumplimiento de las obligaciones conforme al artículo uno de la Orden ESS/484/2013 de 26 de marzo (BOE de 28 de marzo).

El Titular de la autorización,

Fdo.:

CODIFICACIONES INFORMÁTICAS

REFERENCIA:	FECHA:	HORA:	HUELLA:	PÁGINA:
RCC11411000001	27-11-2014	09:54:33	GI2BK4LO	1 de 1

Este documento no será válido sin las codificaciones informáticas

ANEXO V

AUTORIZACIÓN PARA QUE LA DELEGACIÓN DEL GOBIERNO PARA EL PLAN NACIONAL SOBRE DROGAS PUEDA RECABAR DATOS A LA AGENCIA ESTATAL DE ADMINISTRACION TRIBUTARIA Y A LA TESORERÍA GENERAL DE LA SEGURIDAD SOCIAL SOBRE EL CUMPLIMIENTO DE LAS OBLIGACIONES CON DICHOS ORGANISMOS PARA PODER SER BENEFICIARIO DE AYUDAS Y SUBVENCIONES PÚBLICAS.

El abajo firmante autoriza a la Delegación del Gobierno para el Plan Nacional sobre Drogas a solicitar de la Agencia Estatal de Administración Tributaria y de la Tesorería General de la Seguridad Social los datos relativos a las obligaciones de la entidad que represento con dichos Organismos, para comprobar el cumplimiento de los requisitos establecidos a los beneficiarios de subvenciones, según regula el artículo 22.4 del Reglamento de la Ley 38/2003, General de Subvenciones.

La presente autorización se otorga exclusivamente a los efectos del reconocimiento, seguimiento y control de la subvención o ayuda que pudiera conceder la Delegación del Gobierno para el Plan Nacional sobre Drogas en la convocatoria que se cita.

ENTIDAD :	CIF
FUNDACIÓ CLÍNIC PER A LA RECERCA BIOMÈDICA (FCRB)	G-59319681
APELLIDOS Y NOMBRE DEL REPRESENTANTE LEGAL	DNI
CAMPO GÜERRI, ELÍAS	40.908.557-K
Convocatoria: 2011 Orden SPI/1728/2011, de 15 de junio	Concesión de ayudas económicas para el desarrollo de proyectos de investigación sobre drogodependencias

Firma y sello de la entidad

En Barcelona a 5 de Enero de 2015

D. Elías Campo Güerri
Director FCRB

ANEXO VI

DECLARACIÓN RESPONSABLE ACREDITATIVA DE QUE LA ENTIDAD SOLICITANTE, NO ESTÁ INCURSA EN LAS PROHIBICIONES PARA OBTENER LA CONDICIÓN DE BENEFICIARIO DE SUBVENCIONES, ESTABLECIDAS EN LOS APARTADOS 2 Y 3 DEL ARTÍCULO 13 DE LA LEY 38/2003, DE 17 DE NOVIEMBRE, GENERAL DE SUBVENCIONES Y ARTÍCULO 25 DEL R.D. 887/2006, DE 21 DE JULIO, POR EL QUE SE APRUEBA EL REGLAMENTO DE LA CITADA LEY

D. Elías Campo Güerri con DNI 40.908.557-K y con domicilio en C/ Rosselló 149-153, 08036 Barcelona en representación de la entidad Fundació Clínic per a la Recerca Biomèdica (FCRB), con NIF, G-59319681 en su calidad de Director General, DECLARA que la entidad que represento:

a).-No ha sido condenada mediante sentencia firme a la pena de pérdida de la posibilidad de obtener subvenciones o ayudas públicas.

b).- No ha solicitado la declaración de concurso, no ha sido declarada insolvente en cualquier procedimiento, no se ha declarado en concurso, no esta sujeta a intervención judicial ni ha sido inhabilitada conforme a la ley concursal.

c).- No ha dado lugar, por causa de la que hubiese sido declarada culpable, a la resolución firme de cualquier contrato celebrado con la Administración.

d).- Los responsables de la entidad no están incursos en ninguno de los supuestos de la ley 5/2006, de 10 de abril, de regulación de conflictos de intereses de los miembros del Gobierno y de los Altos Cargos de la Administración General del Estado, de la ley 53/1984, de 26 de diciembre, de incompatibilidades del Personal al servicio de las Administraciones Públicas, o tratarse de cualquiera de los cargos electivos regulados en la Ley Orgánica 5/1985, de 19 de junio, del Régimen Electoral General, en los términos establecidos en la misma o en la normativa autonómica que regule estas materias.

e).- Se halla al corriente en el cumplimiento de las obligaciones tributarias o frente a la Seguridad Social, impuestas por las disposiciones vigentes, en la forma que se determina reglamentariamente.

f).- No tiene residencia fiscal en un país o territorio calificado reglamentariamente como paraíso fiscal.

g).- No tiene pendiente el pago de obligaciones por reintegro de subvenciones.

h).- No ha sido sancionada mediante resolución firme con la pérdida de la posibilidad de obtener subvenciones según esta Ley General de Subvenciones o la Ley General Tributaria.

i).- No está incursa en las causas de prohibición previstas en los apartados 5 y 6 del artículo 4 de la Ley Orgánica 1/2002, de 22 de marzo, reguladora del Derecho de Asociación.

En Barcelona a 5 de Enero de 2015

Firma del representante legal y sello de la entidad

FUNDACIÓ CLÍNIC BARCELONA
Rosselló, 149-153 - 08036 BARCELONA

Dr. Elías Campo Güerri
Director General FCRB