Tesis presentada por
Fco. Javier de Diego Adeliño
para obtener el grado de
Doctor en Psiquiatría y Psicología Clínica

La **depresión mayor** es una enfermedad con elevado riesgo de recurrencia y una respuesta al tratamiento no siempre satisfactoria, que asocia grandes tasas de discapacidad, deterioro psicosocial, morbimortalidad y elevados costes sanitarios. Algunas evidencias señalan que los cambios cerebrales que tienen lugar durante los propios episodios depresivos podrían dejar trazas residuales que aumentaran progresivamente el riesgo de refractariedad y la vulnerabilidad a sufrir nuevas recaídas. En las últimas décadas, el desarrollo e implantación de diversas técnicas de neuroimagen aplicadas a investigación han permitido desgranar al menos parte de las anomalías neuroanatómicas y funcionales subyacentes en depresión, aunque las alteraciones relacionadas con el curso evolutivo han sido menos exploradas. El **objetivo** del presente trabajo de tesis ha sido el de ampliar el conocimiento en torno al sustrato neural relacionado con los procesos de recurrencia y cronicidad, es decir aquellas anomalías identificables mediante técnicas de neuroimagen que se asociarían a la recaída o al fracaso del tratamiento, incluso aquellas que podrían acumularse a lo largo del curso de la enfermedad impidiendo la recuperación clínica. En particular, las dos técnicas en las que se centran los estudios presentados son la **Espectroscopia** y el **Tensor de Difusión por Resonancia Magnética Nuclear (RMN)**.

La tesis se estructura en tres artículos publicados en revistas internacionales indexadas en las bases de datos científicas más populares. Los dos primeros evalúan las alteraciones de la neuroquímica celular asociadas a la refractariedad y a la historia de recurrencias en la **Corteza Prefrontal ventromedial (CPFvm)** e **Hipocampo**, dos áreas claves en la fisiopatología de los trastornos afectivos y sensibles a los efectos potencialmente perniciosos del estrés crónico. El tercer trabajo evalúa las alteraciones en la microestructura de sustancia blanca en los circuitos cortico-corticales y cortico-subcorticales, incluyendo la **conectividad fronto-límbica**.

Los estudios, basados en muestras representativas de un amplio espectro de gravedad de la enfermedad, constatan alteraciones de los niveles de glutamato-glutamina y otros metabolitos en CPFvm e Hipocampo y anormalidades generalizadas de la microestructura de sustancia blanca en los pacientes depresivos claramente sobrerrepresentadas entre aquellos con peor respuesta clínica, antecedentes de más episodios previos o mayor duración de enfermedad. Estos hallazgos son altamente sugestivos del potencial neurotóxico de los estados depresivos y justifican la puesta en marcha de estudios longitudinales que confirmen la posible progresión de estos marcadores en subgrupos de pacientes de peor pronóstico a lo largo del curso de la enfermedad.

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TESIS DOCTORAL | Marcadores de Recurrencia y Resistencia al Tratamiento en Depresión: Estudio de Espectroscopia y Tensor de Difusión por RMN | Fco. Javier de Diego Adeliño

MARCADORES DE RECURRENCIA Y RESISTENCIA AL TRATAMIENTO EN DEPRESIÓN:

Estudio de Espectroscopia y Tensor de Difusión por RMN

Fco. Javier de Diego Adeliño

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Departament de Psiquiatria i Medicina Legal
Universitat Autònoma de Barcelona (UAB)

Barcelona, 2014

Universitat Autònoma de Barcelona

Programa de Doctorado en
Psiquiatría y Psicología Clínica
Departamento de Psiquiatría y Medicina Legal

MARCADORES DE RECURRENCIA Y RESISTENCIA AL TRATAMIENTO EN DEPRESIÓN:

ESTUDIO DE ESPECTROSCOPIA Y TENSOR DE DIFUSIÓN POR RESONANCIA MAGNÉTICA NUCLEAR

Tesis presentada por

Fco. Javier de Diego Adeliño

para optar al grado de Doctor en Psiquiatría y Psicología Clínica

DIRECTORES:

Dr. Enric Álvarez Martínez (Doctor en Psiquiatria i Profesor Titular de la UAB)
Dra. Maria J. Portella i Moll (Doctora en Neurociencias)

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Universitat Autònoma de Barcelona (UAB)

Barcelona, 2014

El **Dr. Enric Álvarez Martínez**,

Doctor en Psiquiatría y Profesor titular de la UAB

y la **Dra. Maria J. Portella i Moll**,

Doctora en Neurociencias

Declaran y confirman que han supervisado la Tesis Doctoral titulada:

MARCADORES DE RECURRENCIA Y RESISTENCIA AL TRATAMIENTO EN DEPRESIÓN:

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presentada por Fco. Javier de Diego Adeliño para optar al grado de

Doctor en Psiquiatría y Psicología Clínica

Firmas,

Dr. Enric Álvarez Martínez **Dra. Maria J. Portella i Moll**

Barcelona, 2014

Para Yani, Andrés y Nicolás

Para mis padres

“Tristeza não tem fim, felicidade sim”

En *A Felicidade*, de **Antonio Carlos Jobim** y **Vinicious de Moraes.**

“Y aunque parezca mentira, tu corazón va a sanar,

va a sanar y va a volver a quebrarse mientras le toque pulsar”

En *Sanar*, de **Jorge Drexler.**

Pensando en aquellos pacientes a quienes estas canciones quizás inspiran otras sensaciones vividas, muy distintas de las cosas de la rutina y de los amores.

Ojalá la investigación siga cambiando las cosas.

“La **TESIS** NO Se **cREA** ni SE **DESTRUYE**... solo **se POSPONE**”

Anónimo.

Pensando en l@s que estáis en ello.

¡Mucho ánimo!

AGRADECIMIENTOS

Llegados a este punto, con los artículos publicados, la introducción escrita, meditadas las conclusiones y a la espera de ultimar los consabidos trámites burocráticos, no le queda a uno sino dar las gracias. Dicen que dar las gracias no cuesta nada, pero aún cuesta menos cuando se dan tras acabar una tesis que es el fruto de tanto tiempo y del esfuerzo de tantos, a muchos de los cuáles os tengo no sólo como compañeros de trabajo sino como amigos.

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Gracias a mis padres y a mi familia por darme la posibilidad de llegar hasta aquí. A todos los amigos que seguís conmigo y sois mi válvula de escape. Por supuesto, gracias a ti, Yani, por tu infinita paciencia y fidelidad, porque -aunque no tengas mucha idea de neurotransmisores, metabolitos o de anisotropías fraccionales (tampoco te creas que yo tengo tanta…)- esta tesis la hemos escrito, cuando menos, a medias. Gracias Andrés porque has nacido y crecido con esta tesis y lleva mucho de ti. Nicolás, a ti te tocará enseñarme a pedir un FIS…

Lo que verdaderamente cuesta no es agradecer sino hacerlo sin dejarte a nadie. Si habéis formado parte de esta tesis o compartís conmigo la alegría de haberla concluido, espero poder daros las gracias en persona.

ÍNDICE

Listado de ABREVIATURAS

Listado de Abreviaturas

ACC = Corteza cingulada anterior

BDNF = Factor neurotrófico derivado del cerebro

Cho = Colina y compuestos derivados

CPF = Corteza prefrontal

CPFvm = Corteza prefrontal ventromedial

CREB = *cyclic-AMP-response-element-binding protein*

CRH = hormona liberadora de corticotropina

DSM-IV = *Diagnostic and Statistical Manual of Mental Disorders*, 4ª versión

DTI = Tensor de difusión por Resonancia Magnética Nuclear

FA = Anisotropía fraccional

GABA = Ácido Gamma-Aminobutírico

Gln = Glutamina

Glu = Glutamato

Glx = Glu/Gln o Glutamato + Glutamina

HAMD = Escala para la evaluación de la depresión de Hamilton

MRS = Espectroscopia por Resonancia Magnética Nuclear

NAA = N-Acetil-Aspartato

NMDA = N-metil-D-Aspartato

PET = Tomografía por emisión de positrones

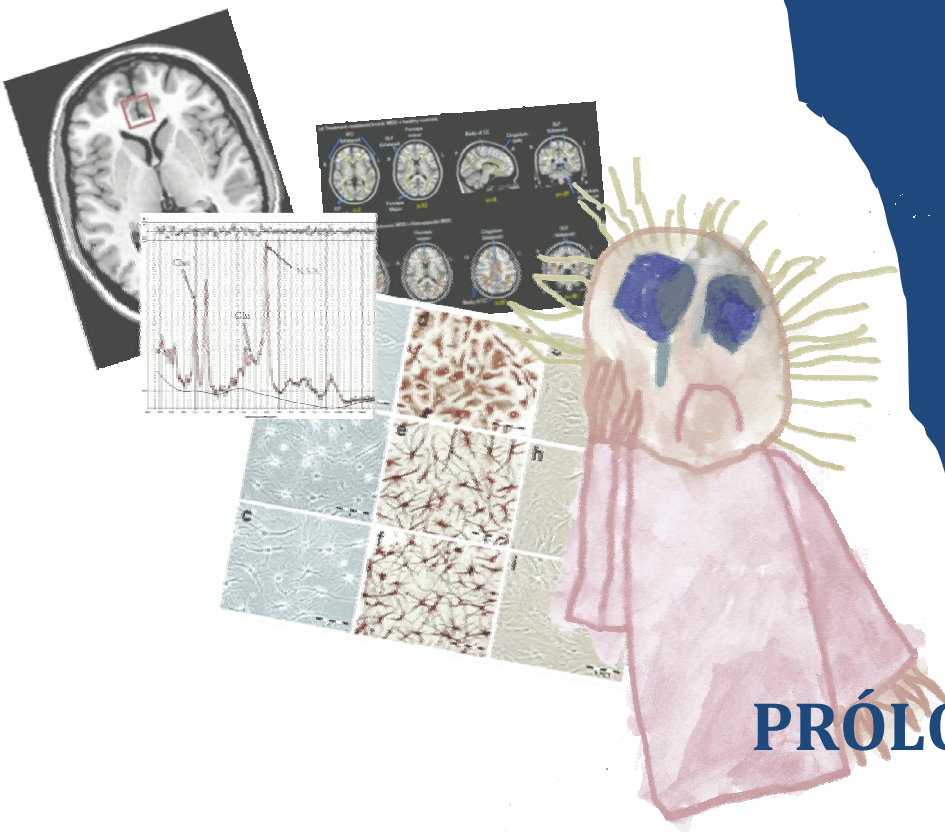
RMN = Resonancia Magnética Nuclear

ROI = Región de interés

TBSS = *Tracted-based spatial statistics*

TEC = Terapia electro-convulsiva

VBM = *Voxel-based morphometry*



PRÓLOGO

PRÓLOGO

Este trabajo de tesis se presenta para obtener el grado de Doctor en Psiquiatría y Psicología Clínica por la Universitat Autònoma de Barcelona. Es el resultado del trabajo realizado entre los años 2009 y 2013 como adjunto del Servicio de Psiquiatría del Hospital de la Santa Creu i Sant Pau, investigador predoctoral del Institut d'Investigació Biomèdica Sant Pau e investigador adscrito al Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM.

Esta tesis ha sido parcialmente financiada por el proyecto PI 10/00372 del Fondo de Investigaciones Sanitarias del Instituto de Salud Carlos III (ISCIII) y mediante un contrato de formación en investigación Río Hortega del ISCIII, con ayuda del Institut d'Investigació Biomèdica Sant Pau y el Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM).

Se ha elaborado de acuerdo a los requisitos establecidos por la Universitat Autònoma de Barcelona para la presentación de **tesis por compendio de publicaciones** y está formada por tres artículos publicados en revistas internacionales de reconocido prestigio, indexadas en las principales bases de datos científicas. Además, se adjunta como anexo otro artículo realizado con anterioridad que sirvió como estímulo y punto de partida para el enfoque del problema y posterior diseño y desarrollo del presente trabajo de tesis.

Los resultados de estos estudios han sido también difundidos en diversos congresos nacionales e internacionales, en forma de pósteres y/o comunicaciones orales.

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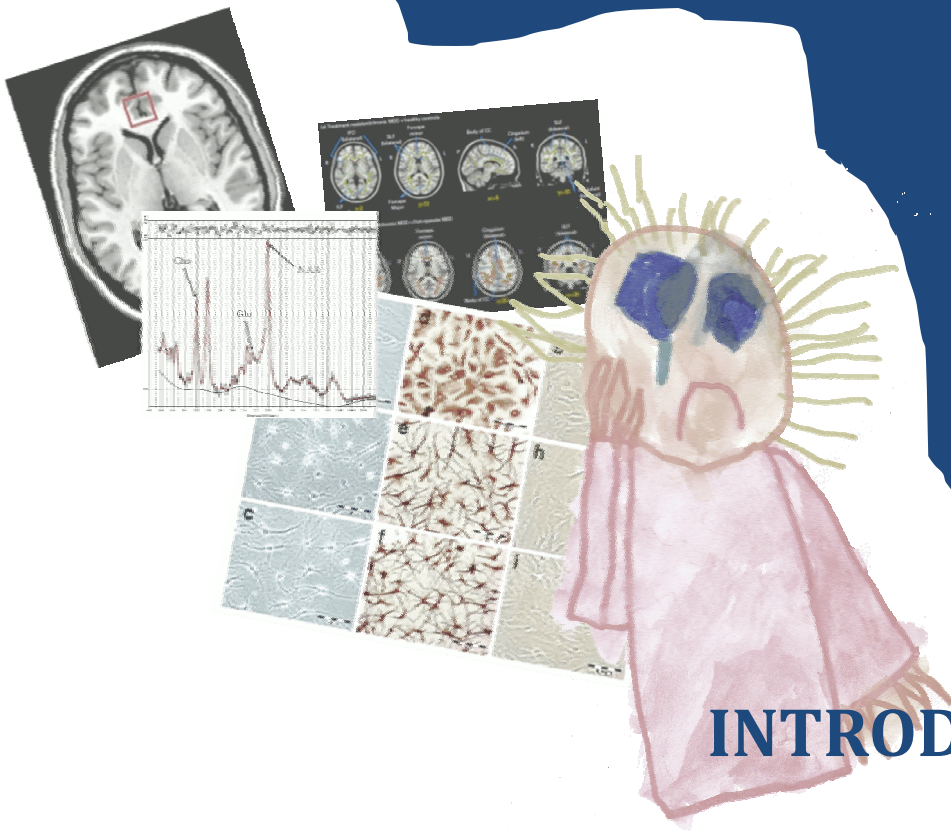

INTRODUCCIÓN

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La **depresión mayor** es uno de los trastornos mentales más frecuentes que, sólo en España, afecta a más de 6 millones de personas (Alonso et al., 2004b). Según el *European Study of the Epidemiology of Mental Disorders,* realizado en nuestro medio, su prevalencia a lo largo de la vida es del 8.9 % para hombres y del 16.5 % para mujeres, y su incidencia parece estar aumentando en las últimas décadas (Alonso et al., 2004b; Haro et al., 2006). La depresión es un trastorno heterogéneo con un elevado riesgo de recaída/recurrencia y una respuesta al tratamiento no siempre satisfactoria que, además de suponer grandes cotas de sufrimiento personal para aquel que la sufre, conlleva un importante índice de morbilidad e incapacidad (Murray & Lopez, 1997) y una reducción de la expectativa de vida estimada entre 7 y 10 años para mujeres y hombres, respectivamente (Chang et al., 2011). No en vano, el riesgo de muerte prematura, especialmente por causas cardiovasculares, casi podría doblar o incluso triplicar el de los individuos libres de depresión (Wulsin et al., 1999) y el riesgo de muerte por suicidio es hasta 18 veces superior al esperado en la población general (Angst et al., 2002).

La recurrencia y refractariedad al tratamiento siguen representando dos de los mayores retos que psiquiatras y psicoterapeutas deben afrontar en su práctica clínica diaria. Los grandes estudios epidemiológicos de seguimiento longitudinal nos indican que, en el mejor de los casos, menos de la mitad de los pacientes con depresión se recuperarán por completo y no sufrirán nuevos episodios y alrededor de un 15% presentará un curso crónico, sin obtener respuesta a múltiples ensayos terapéuticos (Eaton et al., 2008; Mueller et al., 1999; Vuorilehto et al., 2009). Una respuesta insuficiente al tratamiento ha sido extensamente asociada a mayor riesgo de recurrencia, mayores tasas de discapacidad, deterioro psicosocial, comorbilidad y suicidabilidad, junto a un importante incremento de los costes sanitarios (Fekadu et al., 2009; Trivedi et al., 2008). El impacto económico en la sociedad de la depresión, y en especial el de la depresión crónica, va mucho más allá de los costes directos vinculados al uso de servicios

sanitarios, puesto que la mayor parte del gasto es indirecto y deriva de la pérdida de productividad de la persona que la sufre, del absentismo laboral y de la mortalidad prematura asociada (Alonso et al., 2004a; Greenberg et al., 2003; Salvador-Carulla et al., 2011).

La hipótesis de Kindling

Existe un amplio consenso al considerar la depresión como una enfermedad heterogénea y multifactorial, cuyo desarrollo es el fruto de una compleja interacción entre factores psicosociales y biológicos. Sin embargo, múltiples estudios han señalado que los estresores psicosociales jugarían un papel más determinante en la precipitación del primer episodio que en los subsiguientes (Ezquiaga et al., 1987; Perris, 1984). Partiendo de esta observación y de todo un conjunto de investigaciones clínicas y pre-clínicas, Robert M. Post y su equipo formularon una influyente teoría conocida hoy como "*Kindling hypothesis*" (Post et al., 1984) que podría traducirse como hipótesis de *ignición* o de *encendido*. Este autor sugiere que los cambios cerebrales producidos durante los propios episodios podrían dejar trazas residuales que aumentaran progresivamente el riesgo de refractariedad al tratamiento y la vulnerabilidad a sufrir nuevos episodios sin mediación de estresores (Post, 1992). Quizás puede sorprender que ya en 1921, Emil Kraepelin (Kraepelin, 1921), en estrecha relación con todo ello, escribiera estas brillantes impresiones sobre las potenciales causas de la por entonces llamada enfermedad maníaco-depresiva, unas impresiones que el propio Post retoma en su artículo de revisión (Post, 1992):

> "Los episodios empiezan a menudo tras la enfermedad o muerte de familiares cercanos […]. Debemos considerar todos los daños [psíquicos] alegados como posibles disparadores de los episodios pero […] la causa real de la enfermedad debe encontrarse en *cambios internos permanentes* […]. A pesar de eliminar la causa precipitante, el episodio sigue su curso independiente. Pero, finalmente, la aparición de episodios completamente similares en ocasiones completamente distintas o sin causa externa alguna muestra que incluso allí donde hubo una influencia externa, ésta no debe asumirse como un supuesto necesario para la aparición de un episodio."

La "*Kindling hypothesis*" ha sido revisada en diversas ocasiones por su propio autor (Post, 2007; Post et al., 2012), apoyada, a su vez, por los hallazgos de otros investigadores desde diversos ángulos de la neurobiología (Moylan et al., 2013). Las reflexiones de Post, en definitiva, subrayan el potencial neurotóxico del curso de la propia enfermedad y nos llevan a concluir la extrema importancia que podría tener una rápida y adecuada intervención terapéutica en las primeras manifestaciones de la enfermedad de cara a prevenir los eventuales cambios cerebrales y sus perniciosas consecuencias clínicas. Efectivamente, al igual que en los trastornos psicóticos, diversas evidencias nos indican que el intervalo de tiempo que transcurre desde el debut de la enfermedad hasta que se inicia el tratamiento puede resultar crucial para determinar la eficacia del antidepresivo, modulando quizás, tal y como aventuraba Post, los potenciales cambios deletéreos que se producen a nivel cerebral a lo largo de la enfermedad. En esta línea, en 2010 publicamos un primer trabajo (de Diego-Adelino et al., 2010) en el que se observaba como la demora del inicio del tratamiento antidepresivo para un determinado episodio se asociaba con menores tasas de respuesta/remisión entre los pacientes que estaban experimentando su primer cuadro depresivo, con un efecto menos relevante para los pacientes que ya habían sufrido otros episodios en el pasado. Este artículo ha sido incluido aquí como anexo, pues sirvió como estímulo y punto de partida de cara al planteamiento principal del resto de trabajos de investigación que conforman la tesis. El impacto negativo de la prolongación del estado depresivo sin recibir tratamiento sobre las probabilidades de respuesta ha sido posteriormente refrendado por otros estudios (Bukh et al., 2013; Okuda et al., 2010), incluyendo un reciente meta-análisis (Ghio et al., 2014). Además, unos años antes, Yvette Sheline y sus colaboradores (Sheline et al., 2003), publicaron un sugerente artículo mostrando como largos periodos de depresión sin tratar se asociaban a mayores reducciones del volumen del hipocampo. Todos estos hallazgos, en definitiva, sugieren que el debut de este trastorno resulta un periodo crítico en el que podrían tener lugar procesos con potencial neurotóxico capaces de determinar el

pronóstico de la enfermedad, por lo que la investigación de las anomalías cerebrales desde las etapas más tempranas hasta las formas recurrentes y crónicas resulta prioritaria.

En las últimas décadas, el desarrollo e implantación de diversas técnicas de neuroimagen aplicadas a la investigación han permitido desgranar al menos parte de las anomalías neuroanatómicas y funcionales subyacentes en la depresión. Cada vez parece más claro, además, que la depresión no puede concebirse como el resultado de una mera alteración de neurotransmisores o neuropéptidos, ni tampoco de la simple alteración en una única área cerebral. La evidencia acumulada en los últimos años en torno a la fisiopatología de la depresión apunta a la presencia de una disfunción compleja entre redes neuronales en las que intervendría fundamentalmente la corteza prefrontal medial y otras áreas corticales y del sistema límbico estrechamente relacionadas, como la corteza orbitofrontal medial y lateral, la amígdala, el hipocampo o zonas ventromediales de los ganglios basales (Drevets et al., 2008a; Mayberg, 2003). Al hilo de las mencionadas observaciones de Post, el interés del presente trabajo de tesis ha sido el de ampliar el conocimiento en torno al sustrato neural relacionado con los procesos de recurrencia y cronicidad, es decir aquellas anomalías identificables mediante técnicas de neuroimagen que se asociarían a la recaída o al fracaso del tratamiento, incluso aquellas que podrían acumularse a lo largo del curso de la enfermedad impidiendo la recuperación clínica. En particular, las dos técnicas en las que se centrarán los estudios presentados serán la Espectroscopia y el Tensor de Difusión por Resonancia Magnética Nuclear.

Las dos dianas de interés: Hipocampo y Corteza Prefrontal Medial

a) Un poco de contexto: neurotransmisores y cortisol

La **teoría monoaminérgica**, que postula una deficiencia en los sistemas cerebrales de neurotransmisión serotoninérgica, noradrenérgica y –en menor grado- dopaminérgica en la

depresión, estableció unas sólidas bases en la conceptualización neurobiológica de la enfermedad y supuso un gran avance para el desarrollo de los actuales antidepresivos (para revisión, véase Heninger et al., 1996; Ruhe et al., 2007). Sin embargo, la implicación de las monoaminas en la fisiopatología de esta enfermedad parece no ser suficiente para explicar por completo los procesos que precipitan o mantienen el estado depresivo. Conviene recordar que más de un 40% de pacientes no alcanzan una remisión completa después de recibir un tratamiento antidepresivo convencional con Inhibidores de la Recaptación de la Serotonina (ISRS) (Fekadu et al., 2009). Tal y como sugieren Krishnan y Nestler en sus revisiones (Krishnan & Nestler, 2010; Nestler et al., 2002), tampoco hay ninguna razón para considerar que el mecanismo de acción de un tratamiento sea necesariamente el mecanismo opuesto de la fisiopatología de la enfermedad. Probablemente, los fármacos monoaminérgicos funcionan porque dan lugar a cambios similares en una ruta final común que, pasadas unas semanas tras el inicio del tratamiento, podrían ser los responsables definitivos de la respuesta: modulación de segundos mensajeros y señales de transducción celular en las neuronas post-sinápticas, cambios en el estado de factores de transcripción (p.e. la fosforilación del CREB, del inglés *cyclic-AMP-response-element-binding protein*) que interfieren en la expresión genética de proteínas clave en el neurotrofismo y plasticidad (p.e. el Factor neurotrófico derivado del cerebro o BDNF) en regiones cerebrales específicas, regulación al alza o a la baja de receptores postsinápticos, cambios en las concentraciones de neuropétidos (p.e. CRH u hormona liberadora de corticotropina), estimulación del crecimiento dendrítico e incremento en la interconexión neuronal, así como cambios morfológicos y electrofiosiológicos (Reid & Stewart, 2001).

Una creciente línea de investigación se ha dirigido a explorar el papel que pueden estar jugando otros sistemas de neurotransmisión -como el de **glutamato y otros aminoácidos neurotransmisores**- en el desarrollo y evolución de los trastornos afectivos (Kugaya & Sanacora, 2005). Esta interesante línea parte de hallazgos tan sorprendentes como el de la

desaparición de la ideación suicida o incluso la remisión completa del cuadro depresivo en apenas unas horas tras la administración de una única dosis subanestésica de ketamina (un antagonista de los receptores ionotrópicos glutamatérgicos de N-metil-D-aspartato o NMDA) por vía endovenosa, aunque sus efectos no lleguen a sostenerse más allá de unos días, dos semanas a lo sumo (Berman et al., 2000; Price et al., 2009; Zarate et al., 2006). Algunas evidencias apuntan a que la modulación glutamatérgica ejercida por la ketamina daría lugar a algunos de los cambios descritos previamente con los fármacos monoaminérgicos, como p.e. la producción de BDNF en hipocampo o en la corteza prefrontal entre otros, pero de una forma extraordinariamente veloz (para profundizar, véase Niciu et al., 2014).

La **hipótesis neuroendocrina** de la depresión, por su parte, defiende la presencia de una desregulación de la respuesta hipotálamo-hipofisario-adrenal de cortisol al estrés y es también una de las más contrastadas (para revisión, véase Frodl & O'Keane, 2013). El test de dexametasona/CRH (modificación del test de dexametasona ideado inicialmente) no da lugar a una respuesta supresora de la liberación de cortisol hasta en un 80% de los pacientes depresivos (Heuser et al., 1994), dando cuenta de una alteración existente en las vías de señalización del receptor de glucocorticoides entre estos pacientes (Ising et al., 2005; Mokhtari et al., 2013). El test tiene también cierta capacidad predictiva sobre la ulterior respuesta al tratamiento, lo que, en conjunto, lo convierte en un test con capacidad de apoyo al diagnóstico y pronóstico (Ising et al., 2005; Mokhtari et al., 2013) aunque sorprendentemente ha caído en desuso en la práctica diaria. Los sujetos sanos con antecedentes familiares de depresión también muestran una respuesta alterada en este test, con valores intermedios entre los de los individuos sin antecedentes familiares y los de pacientes deprimidos (Holsboer et al., 1995). Además, se han descrito desde elevaciones de los niveles plasmáticos de cortisol o de CRH en líquido cefalorraquídeo en depresiones graves (Burke et al., 2005), hasta reducciones de la expresión de RNA mensajero del receptor de glucocorticoides GR-alfa en sangre periférica (Matsubara et al., 2006) o incrementos de la

expresión de RNA mensajero de CRH y de la propia hormona en hipocampo y otras regiones límbicas claves en la fisiopatología de la depresión (Merali et al., 2004). Lo que resulta aún más interesante es que el exceso de niveles de glucocorticoides, por su importante papel modulador en los procesos de neuroplasticidad y neurogénesis, se ha sugerido como el mecanismo causante más probable de las reducciones del tamaño hipocampal y de diversas alteraciones neuropatológicas observadas en depresión (Campbell & Macqueen, 2004; MacQueen & Frodl, 2011).

b) Alteraciones morfológicas y neuropatológicas del Hipocampo

El hipocampo se considera una región crucial dentro del sistema límbico y desempeña un rol determinante en la regulación emocional y en la fisiopatología del trastorno depresivo mayor (Drevets et al., 2008a). Como se insinuaba previamente, es también una estructura altamente sensible al estrés, ya que una desregulación de la secreción de glucocorticoides inducida por situaciones de estrés –con el consecuente incremento de la actividad de los neurotransmisores aminoácidos excitatorios– podría dar lugar a procesos de remodelación reversibles o incluso a daño celular irreversible en el hipocampo de pacientes con depresión mayor (Campbell & Macqueen, 2004; McEwen & Magarinos, 2001). Un conjunto de evidencia considerable sugiere que el hipocampo puede estar seriamente dañado en pacientes con depresión. Dos meta-análisis (Campbell et al., 2004; Videbech & Ravnkilde, 2004) concluyeron que el hipocampo era bilateralmente de menor tamaño entre pacientes con depresión respecto a controles sanos emparejados. Otros estudios además han asociado dichas reducciones de volumen con una mayor gravedad de la depresión, una edad de debut más precoz, un mayor número de episodios previos, o con la ausencia de respuesta al tratamiento (MacQueen & Frodl, 2011; McKinnon et al., 2009).

Por el contrario, se sabe mucho menos acerca de los cambios anatomopatológicos que subyacen junto al paso del tiempo y la carga de enfermedad en los pacientes con depresión. Hay pocos estudios con muestras post-mortem que hayan probado la existencia de pérdida celular en el hipocampo de pacientes depresivos (Lucassen et al., 2001), pero los trabajos son más consistentes al señalar anomalías sutiles tanto en las neuronas como en la glía de esta estructura, como reducciones del tamaño del soma neuronal o del neuropilo (i.e. la densa maraña de terminales axónicos, dendritas y procesos de las células gliales) (Hercher et al., 2009). En esta misma línea, los modelos animales de depresión en roedores han demostrado que el estrés crónico da lugar a atrofia dendrítica y a reducciones en el recuento o proliferación de células gliales en hipocampo; daños, en definitiva, algunos de los cuáles pueden revertirse o evitarse con ciertos antidepresivos serotoninérgicos, con tratamientos bloqueadores de la síntesis de cortisol o con fármacos moduladores del sistema glutamatérgico (McEwen & Magarinos, 2001). Como antes se apuntaba, la interacción entre alteraciones de la transmisión glutamatérgica y de la secreción cortisol sería la responsable de los cambios en el remodelado dendrítico y población celular que se producirían en los animales de experimentación bajo condiciones de estrés (McEwen & Magarinos, 2001), y tal vez el mismo fenómeno podría explicar los hallazgos morfológicos y neuropatológicos en pacientes depresivos (Campbell & Macqueen, 2004).

c) Alteraciones morfológicas y neuropatológicas de la Corteza Prefrontal Medial

En estrecha relación con el hipocampo, la **corteza prefrontal (CPF),** de la que se postula la mediación de los aspectos más cognitivos y conductuales de la depresión, y en particular la **corteza prefrontal ventromedial (CPFvm)**, incluyendo el cíngulo anterior (ACC), ha sido otro destacado foco de interés en el estudio de la fisiopatología de la depresión (Drevets, 2000; Drevets et al., 2008b; Koenigs & Grafman, 2009). Esta área, bastamente conectada con

regiones parahipocampales y en consiguiente con el hipocampo, es una diana principal de glucocorticoides, pudiendo resultar también potencialmente expuesta a los efectos neurotóxicos de su elevación crónica y sostenida a lo largo del curso de la enfermedad (Gold et al., 2002; Hercher et al., 2009). Múltiples estudios de neuroimagen estructural han revelado una reducción bilateral del volumen en el ACC rostral de pacientes con depresión (Drevets et al., 2008a). Dos meta-análisis de reciente publicación centrados en estudios *Voxel-based Morphometry* (VBM) (Bora et al., 2012; Lai, 2013), señalan esta reducción como el hallazgo volumétrico más consistente en depresión, con una relación inversamente proporcional entre el volumen de esta región y la duración total de la enfermedad. Los estudios de metabolismo con Tomografía por Emisión de Positrones (PET), por su parte, señalan una hiperactivación de la región subgenual del ACC en pacientes deprimidos que se revertiría con la respuesta al tratamiento, independientemente de la estrategia antidepresiva empleada (Drevets et al., 2008a; Hamani et al., 2011).

Existen sólidas evidencias de que esta región cerebral está afectada por procesos neuropatológicos, como así lo indican las reducciones de recuentos y densidad de células gliales observadas en estudios post mortem de la CPF de pacientes depresivos (Rajkowska & Miguel-Hidalgo, 2007). Este hecho tiene mayor interés si se tiene en cuenta que la neuroglia ejerce una misión fundamental en el mantenimiento de la función glutamatérgica y que tales anomalías, por tanto, podrían asociarse con una disposición anómala de neurotransmisores aminoácidos y de otros metabolitos cerebrales (Harrison, 2002). No en vano, estudios con ratas de experimentación han mostrado que la lesión experimental de las células gliales de la CPF induce comportamientos depresivos (Banasr & Duman, 2008), mientras que la terapia electroconvulsiva (TEC) da lugar a una proliferación glial en esta zona que tal vez puede mediar en su potente efecto antidepresivo (Jansson et al., 2009; Ongur et al., 2007). Todas estas premisas subrayan aún más la probable contribución del glutamato y otros sistemas de

neurotrasmisores aminoácidos en la fisiopatología y tratamiento de los trastornos afectivos (Kugaya & Sanacora, 2005).

Como veremos a continuación, algunas de estas alteraciones neuroquímicas y deficiencias en la integridad celular e incluso la posible disminución de población celular (glial y neuronal) en hipocampo y CPF pueden ser estudiadas *in vivo* de forma indirecta mediante la Espectroscopia por Resonancia Magnética Nuclear.

Espectroscopia por Resonancia Magnética Nuclear (MRS)

La **Espectroscopia por Resonancia Magnética Nuclear (MRS)** es una técnica de neuroimagen no invasiva que detecta las señales de radiofrecuencia absorbidas y posteriormente emitidas (i.e. resonancia) por los núcleos atómicos localizados dentro de las moléculas que componen los tejidos humanos, tras haber sido sometidos al potente campo magnético del dispositivo. La frecuencia exacta de resonancia dependerá de las propiedades del núcleo atómico seleccionado (el más empleado es el *protio* o ^{1}H, el isótopo de hidrógeno compuesto por un solo protón) y del entorno que rodea a este núcleo. Del mismo modo que la Resonancia Magnética Nuclear (RMN) convencional, basándose en este principio, obtiene información anatómica a partir de la señal de ^{1}H, la MRS puede obtener información química sobre diversos metabolitos que contienen al ^{1}H. La diferente composición química de los metabolitos cerebrales se refleja en distintas frecuencias de resonancia, por lo que la MRS, sirviéndose de esto, es capaz de ofrecer una representación gráfica del espectro metabólico en la región cerebral a estudio, con diversos picos diferenciales (Figura 1. **Esquema representativo del espectro de la MRS (post-procesado mediante LCModel)**). A pesar de ello, todas las frecuencias de resonancia aparecen en un intervalo muy estrecho lo que da lugar a cierto solapamiento entre las señales de los distintos compuestos e incluso el mismo compuesto puede originar más de una resonancia, lo que dificulta técnicamente la caracterización de los

metabolitos. Aun así, los avances metodológicos en la adquisición y procesado de las imágenes, y la mayor disponibilidad de equipos de RMN de alto campo, han subsanado parte de las limitaciones iniciales ampliando la relación de señal-ruido y ofreciendo una mejor definición de cada uno de los picos de resonancia (Provencher, 2001). La amplitud de estos picos de señal es proporcional al número de núcleos resonados y permite obtener una estimación cuantitativa de la concentración de algunos de los metabolitos cerebrales, entre los que se incluyen los neurotransmisores aminoácidos. Es importante remarcar ante todo que las técnicas convencionales de MRS más que un dibujo detallado de la cinética de los metabolitos, ofrecen tan sólo una mera instantánea del reservorio metabólico cerebral pero, precisamente por esta razón, pueden brindar una valiosa información *in vivo* sobre la integridad de las células o estructuras que los contienen. Los detalles técnicos y metodológicos de la MRS desbordan ampliamente los objetivos de esta tesis y pueden ser revisados en trabajos específicos sobre el tema (Alger, 2010; Dager et al., 2008).

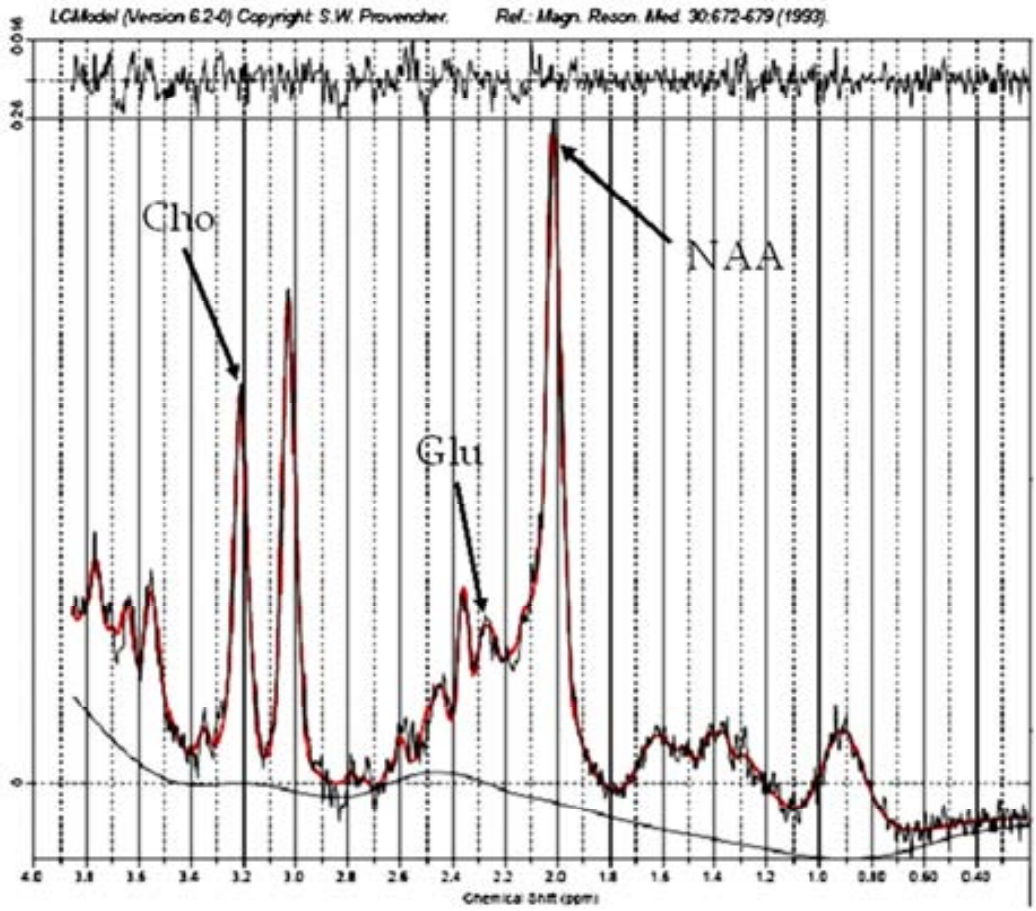


Figura 1. Esquema representativo del espectro de la MRS (post-procesado mediante LCModel)

Los metabolitos detectables por MRS que más comúnmente han sido implicados en el Trastorno Depresivo son **Glutamato/Glutamina** (Glx o Glu/Gln respectivamente), **Colina y sus compuestos derivados** (Cho) y **N-Acetil Aspartato** (NAA) (Yildiz-Yesiloglu & Ankerst, 2006). Cabe mencionar que algunos trabajos iniciales reportaban conjuntamente alteraciones de la señal Glutamato/Glutamina/Ácido-Gamma-Aminobutírico (Glu/Gln/GABA) puesto que estos metabolitos resuenan en zonas muy cercanas del espectro de frecuencias y no se disponía aún de la metodología adecuada para diferenciarlos. A modo de guía podemos decir que los metabolitos relacionados con el glutamato (especialmente Glu y Gln) representan principalmente al reservorio intracelular contenido en las neuronas piramidales glutamatérgicas y en la glía, particularmente en los astrocitos. Por tanto, las reducciones de intensidad en esta señal podrían indicar deficiencias en estas líneas celulares (Yuksel & Ongur, 2010). La señal de resonancia de Cho está predominantemente compuesta por los productos de hidrolisis de la fosfatidilcolina (Klein et al., 1993) por lo que se ha concebido como una medida indirecta del recambio de membrana y del recambio celular (Boulanger et al., 2000). Y NAA, presente principalmente en neuronas, se ha sugerido tradicionalmente como un marcador de la viabilidad y funcionalidad neuronal (Moffett et al., 2007; Wijtenburg et al., 2012).

a) Alteraciones de MRS en la Corteza Prefrontal Medial

A pesar de cierta controversia inicial, los estudios más actuales coinciden en señalar a Glu/Gln/GABA como los metabolitos más comúnmente alterados en áreas prefrontales de pacientes con depresión. Diversos trabajos previos reportaron niveles bajos de Glu/Gln/GABA en el lóbulo frontal de pacientes depresivos comparados con sujetos sanos, ya sean adultos o niños/adolescentes (Ajilore et al., 2007; Auer et al., 2000; Hasler et al., 2007; Michael et al., 2003a; Mirza et al., 2004; Pfleiderer et al., 2003; Rosenberg et al., 2005; Walter et al., 2009). El Glu es el principal neurotransmisor excitatorio que contribuye a un amplio abanico de

funciones cerebrales en condiciones normales, incluyendo la regulación de factores neurotróficos y de la neuroplasticidad, aunque los niveles anormalmente elevados de este metabolito han sido implicados en procesos patológicos como la isquemia cerebral, la lesión cerebral traumática o enfermedades neurodegenerativas crónicas, entre otros (Lipton & Rosenberg, 1994). Basándose en que las células gliales, y los astrocitos en particular, son la principal fuente de suministro energético para las neuronas y representan una pieza esencial en el mantenimiento de la síntesis y del reservorio neuronal de Glu (Magistretti et al., 1999; Rajkowska & Miguel-Hidalgo, 2007), Sanacora y su equipo (Sanacora et al., 2003) propusieron un provocativo modelo en el que se sugería que el deterioro prefrontal de la función glial observado en las muestras post-mortem de enfermos depresivos, daría lugar a una disminución de la recaptación sináptica de Glu con la resultante elevación de sus niveles extracelulares. La disfunción glial y el exceso de Glu extracelular, podría incrementar y acelerar aún más el propio daño glial y neuronal, debido a sus efectos neurotóxicos (Rothstein et al., 1993). A simple vista, todos estos supuestos pueden llegar a parecer contradictorios con las reducciones de los niveles de Glu/Gln/GABA observadas mediante MRS en la CPF de los pacientes con depresión. Sin embargo, debe tenerse en cuenta que este espectro está predominantemente compuesto por el reservorio intracelular presente en neuronas y especialmente en glía (la relación intracelular-extracelular asciende a 5000:1) (Lehmann et al., 1983). Por lo tanto, como anteriormente señalábamos, la disminución de esta señal de resonancia, lejos de reflejar las posibles alteraciones en las concentraciones extracelulares o en la neurotransmisión glutamatérgica per se, podría representar en realidad el deterioro y reducción de población neuroglial presente en los pacientes depresivos. Teniendo en cuenta que se han desarrollado técnicas más sofisticadas de MRS que permiten abordar con mayor definición el pico de solapamiento que conforman Glu/Gln/GABA, determinaciones más específicas de la señal Glu/Gln (Glx) reflejarían con mayor fiabilidad, si cabe, el estado de la glía en los pacientes con depresión.

Por otro lado, aunque la señal de Cho parece estar claramente aumentada en los ganglios basales de los pacientes con depresión, la presencia de anormalidades en el pico de Cho en la CPF es algo más controvertida, con estudios que reportan ascensos (Farchione et al., 2002; Kumar et al., 2002; Steingard et al., 2000), otros que señalan disminuciones (Caetano et al., 2005; Gruber et al., 2003) y otros tantos sin diferencias significativas con individuos sanos (Kaymak et al., 2009; Nery et al., 2009; Yildiz-Yesiloglu & Ankerst, 2006).

El NAA, considerado -como decíamos- un marcador de integridad neuronal, ha sido también investigado en regiones prefrontales de pacientes con depresión con resultados no concluyentes hasta la fecha (Yildiz-Yesiloglu et al., 2006; Ajilore et al., 2007; Walter et al., 2009; Nery et al., 2009; Kaymak et al., 2009; Gonul et al., 2006). Aunque la patología neuronal en depresión parece ser menos prominente que la de la glia (Rajkowska & Miguel-Hidalgo, 2007), ambas líneas celulares están estrechamente relacionadas, ya que, estas últimas son fundamentales para el correcto desarrollo y funcionamiento de las neuronas (Magistretti et al., 1999). Por tanto, el hallazgo de alteraciones en la señal de NAA en pacientes depresivos si no llegara a reflejar una alteración primaria de la integridad neuronal podría, cuando menos, dar cuenta de los daños colaterales en las estructuras neuronales causados por defectos en la glía.

Hasta la fecha, la mayor parte de estudios de MRS en depresión se habían centrado en determinar las diferencias entre pacientes y sujetos controles sanos, describiendo en ocasiones la potencial influencia de la gravedad de los síntomas o los efectos a corto plazo de estrategias terapéuticas (sirvan como ejemplo Walter et al., 2009; Block et al., 2009). No obstante, la relación entre los picos de señal de los metabolitos de la CPFvm y otras variables clínicas relevantes para el curso de la enfermedad (como la duración de la enfermedad, la edad de debut o el número de episodios previos), con contadas excepciones, no habían recibido hasta la fecha la atención que verdaderamente merecían (Nery et al., 2009; Milne et al., 2009; Hasler et al., 2007).

b) Alteraciones de MRS en Hipocampo

A pesar del creciente cuerpo de literatura acumulado en la última década en torno a las alteraciones de metabolitos en depresión, sólo unas pocas investigaciones se han centrado en el hipocampo, arrojando a veces conclusiones poco consistentes. Dos trabajos reportaron niveles disminuidos de Glx en la cabeza del hipocampo izquierdo, uno entre pacientes graves con depresión resistente al tratamiento (Michael et al., 2003b) y otro entre pacientes con un primer episodio depresivo sin tratar (Block et al., 2009), aunque un estudio subsiguiente de Milne y cols. (Milne et al., 2009) no pudo replicar estos hallazgos. Con respecto a las concentraciones de Cho, se han descrito aumentos de la señal en el hipocampo de pacientes con depresión resistente al tratamiento (Mervaala et al., 2000) y con historia de depresión recurrente (Milne et al., 2009), aunque otros autores mostraban niveles normales o incluso disminuidos de Cho en pacientes deprimidos en el momento de la prueba de imagen, con un subsecuente incremento después de obtener una respuesta terapéutica adecuada a TEC (Ende et al., 2000) o al tratamiento farmacológico (Block et al., 2009). Los estudios que se han referido a los niveles hipocampales de NAA, por su parte, no han logrado constatar deficiencias significativas en los pacientes con depresión (Yildiz-Yesiloglu & Ankerst, 2006), a pesar de las alteraciones de neuroplasticidad (Campbell et al., 2004) y posiblemente de neurogénesis que tendrían lugar en esta estructura (Campbell et al., 2004; MacQueen & Frodl, 2011), y que se han sugerido en incontables ocasiones como parte fundamental del sustrato fisiopatológico del trastorno depresivo (Campbell & Macqueen, 2004; MacQueen & Frodl, 2011; Pittenger & Duman, 2008). Aun así, algunos trabajos sí han revelado un incremento de los niveles de NAA asociado a la respuesta al tratamiento (Block et al., 2009; Michael et al., 2003b).

Algunas de las inconsistencias entre los diferentes estudios pueden atribuirse a cuestiones metodológicas (Yildiz-Yesiloglu & Ankerst, 2006) (como p.e. a la falta de consideración de diferencias estructurales en la región estudiada; Milne et al., 2009), mientras

que otras pueden estar relacionadas con diferencias clínicas entre las muestras incluidas en cada estudio. De hecho, hasta la fecha no estaba bien establecida la influencia que variables clínicas relevantes para el curso de la enfermedad (como la duración, el número de episodios previos o la edad de debut) pudieran estar ejerciendo en las concentraciones de los metabolitos.

Alteraciones microestructurales en las redes fronto-límbicas

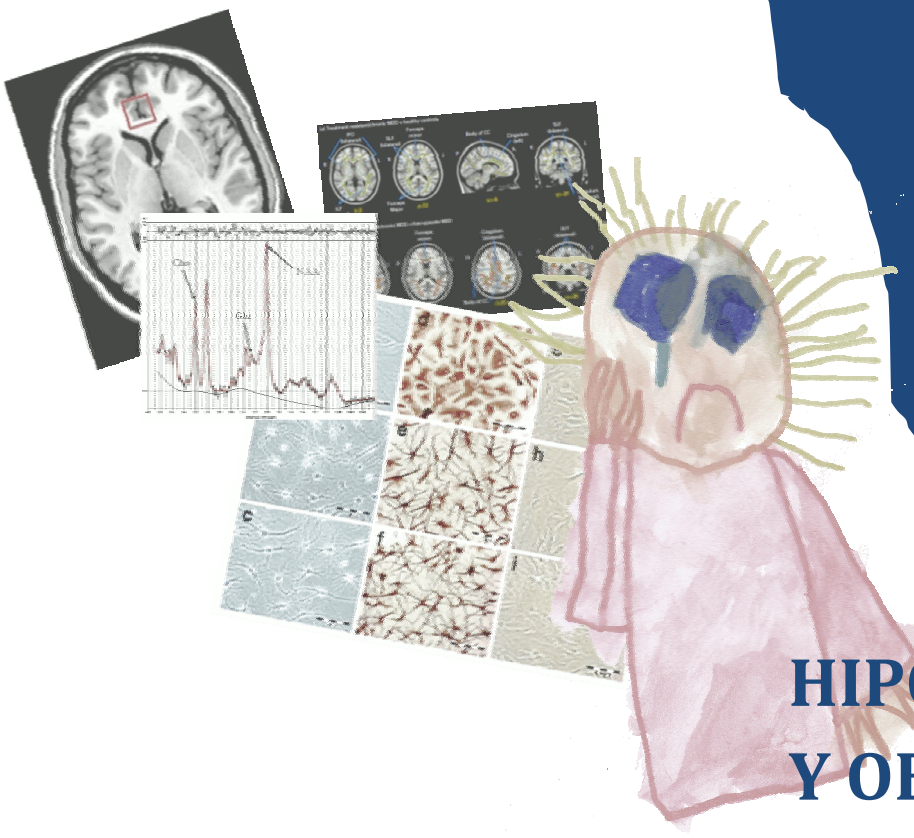
Además de las alteraciones morfológicas, neuroquímicas y neuropatológicas a las que hemos hecho referencia hasta el momento, los estudios de neuroimagen funcional han subrayado la existencia de redes neuronales entre las estructuras fronto-límbicas en las que se distribuirían diversas alteraciones críticas para las manifestación de los estados depresivos y la remisión de la enfermedad (Mayberg, 2003), con un papel destacado de la corteza prefrontal ventromedial en dicha circuitería neural (Drevets et al., 2008a; Johansen-Berg et al., 2008). Los tractos de sustancia blanca son componentes claves de estas redes y las anormalidades en su microestructura pueden ser potencialmente estudiadas in vivo mediante el Tensor de Difusión de Imagen (DTI), una técnica de resonancia magnética que se ha popularizado ampliamente en los últimos años.

Brevemente, la difusión de las moléculas de agua libres presentes en la sustancia blanca del cerebro no es la misma en todas las direcciones, por lo que se la llama anisotrópica, y se ve restringida fundamentalmente por la orientación de las fibras del tracto. Las anomalías microestructurales de la organización intra-axonal, el diámetro y la densidad de las fibras, el grado de mielinización o la coherencia de los tractos, alterarán la señal de difusión anisotrópica, suponiendo una buena estimación indirecta de la integridad de la sustancia blanca. La anisotropía fraccional (FA) es una de las medidas que proporciona el DTI y la más

comúnmente usada para la evaluación de la microestructura de sustancia blanca (para una revisión más extensa sobre la técnica, véase Smith et al., 2006).

Los trabajos previos con DTI habían descrito alteraciones de los valores de FA en regiones fronto-temporales de pacientes con depresión mayor, principalmente en ancianos aunque también en población joven y adultos de edad media (Murphy & Frodl, 2011; Sexton et al., 2009). Sin embargo, la gran mayoría se centraban en identificar diferencias entre un determinado grupo de pacientes con depresión y controles sanos, y la comparación entre diversos estadios o el estudio de la influencia de variables relacionadas con la carga de enfermedad pasada había recibido una atención modesta. En 2012, fueron publicados los resultados de un estudio (Cole et al., 2012) en el que se reportaban alteraciones generalizadas de la microestructura de sustancia blanca en cerebros de pacientes con larga historia de depresión recurrente. En este caso, las alteraciones guardaban además una relación proporcional con la gravedad sintomática. Sólo unos pocos artículos, sin embargo, habían evaluado a pacientes de mediana edad con depresión mayor crónica o resistente al tratamiento antidepresivo (Guo et al., 2012; Hoogenboom et al., 2012; Zhou et al., 2011). Dos de ellos eran estudios prospectivos, aunque metodológicamente distintos, en los que se analizaban las diferencias basales de FA entre pacientes a los que posteriormente se evaluaba la respuesta al tratamiento. Zhou y cols. (Zhou et al., 2011), mediante un análisis basado en vóxel de todo el cerebro, mostraban valores basales bajos de la FA en la región temporal medial de pacientes cuya depresión se hacía resistente al tratamiento. Hoogenboom y cols. (Hoogenboom et al., 2012), mediante un análisis por región de interés (ROI) y una evaluación retrospectiva de la evolución de la enfermedad a través de la revisión de las historias clínicas, reportaban una disminución basal de los valores de FA en el fórnix medial de los pacientes que no alcanzaban la remisión. Guo y cols. (Guo et al., 2012), por su parte, realizaron un estudio transversal con DTI en pacientes con depresión resistente al tratamiento usando el análisis Tract-based spatial statistics (TBSS), un nuevo enfoque metodológico que incrementa la

sensibilidad y la interpretabilidad de los resultados en comparación con los enfoques basados en vóxel tradicionales (Smith et al., 2006). Estos pacientes mostraban valores de FA inferiores en el brazo anterior de la cápsula interna derecha, el cuerpo del Corpus Callosum y en ambas cápsulas externas en comparación con controles sanos, pero la ausencia de un grupo comparador de pacientes no resistentes al tratamiento no permitía profundizar en la interpretación de los hallazgos.



HIPÓTESIS Y OBJETIVOS

HIPÓTESIS Y OBJETIVOS

Los objetivos de los trabajos que conforman esta tesis tienen como denominador común determinar si la presencia de cronicidad/resistencia al tratamiento, así como de variables que estiman una mayor carga de enfermedad pasada (mayor duración de la enfermedad, historia de recurrencias o debut precoz), se asocian con mayores alteraciones en la neuroquímica estructural y en la microestructura de sustancia blanca de la CPFvm y el hipocampo, intentando superar al menos parte de las limitaciones de anteriores estudios.

En particular, nos planteamos testar las siguientes **HIPÓTESIS:**

Hipótesis 1: Los pacientes con depresión crónica/resistente al tratamiento y una mayor carga de enfermedad pasada presentan mayores alteraciones de las concentraciones de Glx, Cho y NAA en la CPFvm que los pacientes con un primer episodio depresivo y que los controles sanos.

Hipótesis 2: Los pacientes con depresión crónica/resistente al tratamiento y una mayor carga de enfermedad pasada presentan mayores alteraciones de las concentraciones de Glx, Cho y NAA en hipocampo que los pacientes con un primer episodio depresivo y que los controles sanos.

Hipótesis 3: Los pacientes con depresión crónica/resistente al tratamiento, mayor gravedad sintomática y una mayor carga de enfermedad pasada presentan valores disminuidos de la FA de forma generalizada y, en particular, en tractos del circuito fronto-límbico, con respecto a pacientes con un primer episodio depresivo y controles sanos.

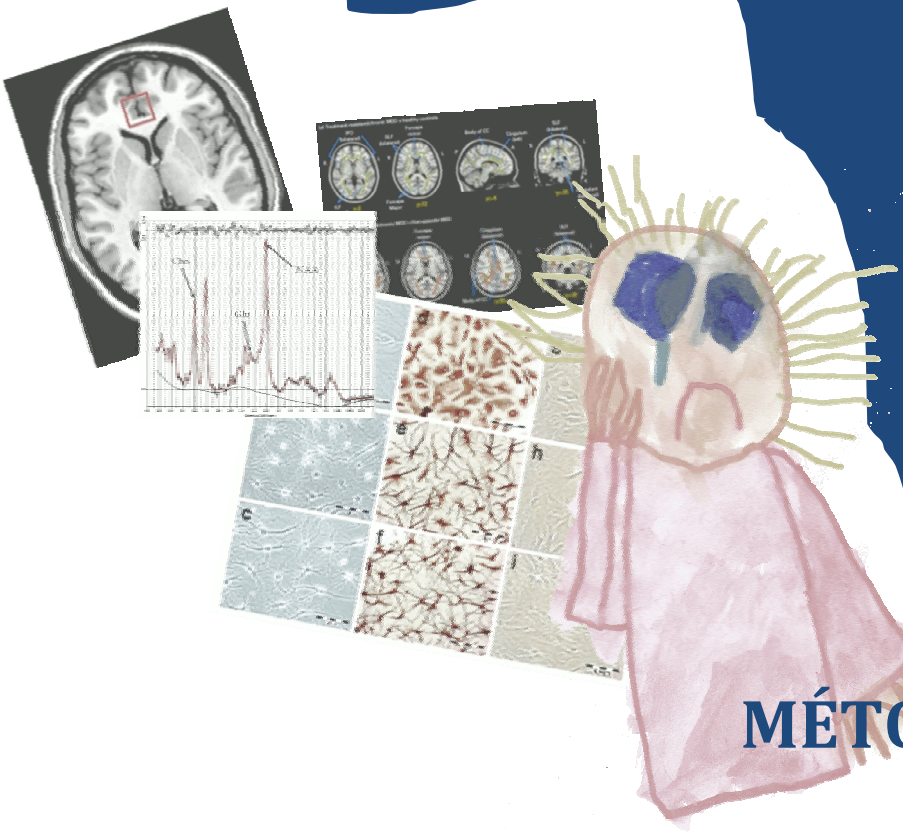
OBJETIVOS ESPECÍFICOS

Principales

1. Determinar las diferencias en las concentraciones de Glx, Cho y NAA en la CPFvm entre controles sanos y pacientes con depresión en diversos estadios de la enfermedad (i.e. depresión crónica/resistente al tratamiento, depresión recurrente en fase de remisión y primer episodio depresivo) – **Trabajo 1**
2. Determinar las diferencias en las concentraciones de Glx, Cho y NAA en hipocampo derecho e izquierdo entre controles sanos y pacientes con depresión en diversos estadios de la enfermedad – **Trabajo 2**
3. Determinar las diferencias en la microestructura de sustancia blanca de todo el cerebro entre controles sanos y pacientes con depresión en diversos estadios de la enfermedad – –**Trabajo 3** (estudio mediante *TBSS* de todo el cerebro)
4. Determinar las diferencias en la microestructura de sustancia blanca de la CPFvm entre controles sanos y pacientes con depresión en diversos estadios de la enfermedad – –**Trabajo 3** (estudio mediante *TBSS* con ROI en CPFvm)

Secundarios

1. Analizar la relación entre las concentraciones de Glx, Cho y NAA en CPFvm, la gravedad sintomática y las variables relacionadas con la carga de enfermedad pasada (i.e. mayor duración de la enfermedad, mayor número de episodios y debut precoz).
2. Analizar la relación entre las concentraciones de Glx, Cho y NAA en hipocampo, la gravedad sintomática y las variables relacionadas con la carga de enfermedad pasada.
3. Analizar la relación entre los valores de la FA en cerebro, la gravedad sintomática y las variables relacionadas con la carga de enfermedad pasada.



MÉTODOS

MÉTODOS

Esta tesis está formada por tres artículos publicados en revistas internacionales indexadas en las principales bases de datos bibliográficas científicas. Aunque las características de las muestras, el diseño, los parámetros de adquisición y post-procesamiento de las imágenes y los procedimientos estadísticos están exhaustivamente descritos en cada uno de los artículos originales, en este apartado se exponen los aspectos más relevantes.

Se trata de tres trabajos observacionales de diseño caso-control cuyas muestras reunían las mismas características. Por un lado fueron reclutados **pacientes** ambulatorios adultos **con diagnóstico de Trastorno Depresivo Mayor** (según criterios DSM-IV) que realizaban seguimiento en las Consultas Externas del Servicio de Psiquiatría del *Hospital de la Santa Creu i Sant Pau* de Barcelona. Estos pacientes se dividían en tres grupos:

- Uno compuesto por pacientes con **Trastorno Depresivo Crónico/resistente al tratamiento**, cuyo episodio en curso tenía una duración de al menos 2 años sin respuesta a múltiples estrategias antidepresivas, un índice de resistencia de *Thase-Rush* ≥3 (i.e. fracaso con al menos tres ensayos terapéuticos, incluyendo antidepresivos tricíclicos, a dosis y tiempos suficientes; Thase & Rush, 1997) y una puntuación superior a 14 en la *Hamilton Depression Rating Scale* (HAM-D).
- Otro compuesto por pacientes con **Trastorno Depresivo Mayor Recurrente en remisión**, con al menos 3 episodios depresivos previos y que cumplieran criterios de remisión clínica (puntuaciones en la HAM-D ≤7) de forma sostenida (al menos 6 meses de recuperación desde el último episodio).

- Y otro compuesto por pacientes que experimentaban su **Primer Episodio Depresivo Mayor,** con un cuadro depresivo activo, recientemente diagnosticado y tratado en el momento de la evaluación.

Todos ellos debían ser diestros y de una edad similar en el momento de la exploración radiológica. Los dos primeros grupos eran representativos de pacientes con una elevada carga de enfermedad pasada aunque diferenciados por el estado sintomático actual y la respuesta al tratamiento. El tercer grupo era representativo de aquellos pacientes con una baja carga de enfermedad, i.e. sin episodio previos, con una menor duración total de la enfermedad y un debut, en general, más tardío.

Por último, se reclutó un cuarto **grupo control** de individuos sanos, diestros, sin historia personal previa de ningún diagnóstico psiquiátrico, tampoco de enfermedades neurológicas o somáticas relevantes y sin antecedentes psiquiátricos conocidos en ningún familiar de primer grado. En todos los casos, los individuos con historia de traumatismo cerebral, enfermedad neurológica, abuso o dependencia de alcohol u otras drogas, excepto tabaco, fueron excluidos.

Todos los individuos eran evaluados por miembros del *staff* con experiencia clínica mediante entrevistas semiestructuradas y revisión de sus cursos clínicos, incidiendo en particular en la historia de la enfermedad, comorbilidades, edad de debut y número de episodios previos. El estado sintomático además era evaluado mediante la HAM-D. Una vez confirmados los criterios de inclusión/exclusión, el paciente era sometido a una exploración radiológica mediante un protocolo de RMN específicamente diseñado, cuyos detalles se recogen en los respectivos artículos.

En los tres trabajos, tras examinar las posibles diferencias en variables sociodemográficas o clínicas, la hipótesis principal era testada mediante un análisis multivariante tipo ANOVA con contraste unilateral, tomando al grupo como el factor inter-sujetos y las concentraciones de metabolitos o la FA, en cada caso, como variables dependientes. El estudio DTI no sólo

comparaba los valores de la FA media entre los cuatro grupos sino también las diferencias regionales de la microestructura de sustancia blanca mediante la metodología *TBSS*. Para cada uno de los estudios, se consideró la inclusión de factores adicionales o covariables potencialmente confusoras en los análisis: el género en el primer trabajo (puesto que existían diferencias significativas entre grupos), el volumen total del hipocampo y la composición relativa de sustancia gris/blanca en el volumen de interés en el segundo trabajo (con intención de superar las limitaciones de anteriores estudios publicados) y una estimación cuantitativa de la carga de medicación en el tercer trabajo (mediante un método previamente descrito por otros autores (Almeida et al., 2009; Sackeim, 2001) con el fin de controlar la posible influencia del tratamiento farmacológico sobre las variables a estudio). En los trabajos de MRS, aquellos metabolitos que mostraban diferencias en los análisis multivariantes eran incluidos en análisis de correlación con el fin de explorar la influencia de las variables clínicas relacionadas con la carga de enfermedad. En el caso del estudio DTI, se incluyeron las variables clínicas como potenciales predictores independientes de los cambios de FA (variable dependiente) mediante análisis de regresión múltiple.

RESULTADOS

TRABAJO 1:

Portella, M. J., de Diego-Adelino, J., Gomez-Anson, B., Morgan-Ferrando, R., Vives, Y., Puigdemont, D., et al. (2011). Ventromedial prefrontal spectroscopic abnormalities over the course of depression: a comparison among first episode, remitted recurrent and chronic patients. J Psychiatr Res, 45(4), 427-434.

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Ventromedial prefrontal spectroscopic abnormalities over the course of depression: A comparison among first episode, remitted recurrent and chronic patients

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ABSTRACT

Structural and neuropathological alterations in the ventromedial prefrontal cortex (vmPFC) described in depression (MDD) might become even more pronounced over the course of illness. Measurement of brain metabolites by means of Magnetic Resonance spectroscopy (MRS) can indirectly deliver information about glial and neuronal integrity or potential cellular loss. The aim of this study was to investigate whether Glutamate (Glu), Choline (Cho) and total N-acetylaspartate (total-NAA) levels in the vmPFC differed among MDD patients in distinct stages of illness and healthy controls. We hypothesized that high-past illness-burden would represent more metabolite abnormalities independently of mood state. A 3-Tesla MR facility was used to measure these metabolites in vmPFC of 45 depressive patients (10 first-episode-MDD, 16 remitted-recurrent-MDD and 19 chronic-MDD) and 15 healthy controls. Multivariate and correlation analyses were carried out to explore the influence of duration of illness, age at onset and mood-state. Levels of Glu were significantly decreased in remitted-recurrent and chronic patients compared with both first-episode and controls (up to 28% mean reduction; $p < 0.001$, Cohen's $d = 2.88$) and were negatively correlated with illness duration ($r = -0.56$; $p < 0.001$). Cho levels showed an opposite pattern: highest values were detected in chronic patients, correlating positively with duration of illness ($r = 0.32$; $p = 0.03$). Total-NAA levels were significantly lowered in remitted-recurrent and chronic patients, which were associated with an earlier age at onset ($r = 0.50$; $p = 0.001$). Our data suggest that abnormalities in Glu, Cho and total-NAA levels are consistently related to the course of MDD, supporting the hypothesis that cellular changes would take place in vmPFC over time.

1. Introduction

Major depression (MDD) is a heterogeneous disorder with a high risk of relapse/recurrence, an inconsistent response to treatment, and no established mechanism. Given these characteristics, the nature of neural abnormality that precipitates or maintains MDD has yet to be described. Even more, the aberrant brain changes that could take place along the course of the illness are still little known. Numerous studies have sought to identify the key brain areas implied in the pathogenesis of depressive symptoms. The involvement of prefrontal cortex has been an important focus, particularly the ventromedial prefrontal cortex (vmPFC), including anterior cingulate cortex (ACC) (Drevets, 2000; Drevets et al., 2008a; Koenigs and Grafman, 2009). This area, densely connected with parahippocampus and subsequently with hippocampus, is a major target for glucocorticoids, being potentially exposed to the neurotoxic effects of a chronic sustained elevation over the illness course (Gold et al., 2002; Hercher et al., 2009). Despite the many studies reporting volumetric and functional neuroimaging abnormalities in this region in patients with MDD versus healthy comparison subjects (Drevets et al., 2008a; Mayberg et al., 1999; Liotti et al., 2002; Greicius et al., 2007), not so many have addressed the presence of deficiencies in prefrontal

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cellular neurochemistry in MDD. There is evidence that this brain region is affected by neuropathological processes that could be associated with altered availability of metabolites (Harrison, 2002) including amino acid neurotransmitters. Given the importance of glia in maintaining proper function of the glutamatergic system, the observation of abnormal reductions in prefrontal glial cell counts and density in postmortem studies of MDD (Rajkowska and Miguel-Hidalgo, 2007) is likely to have a strong link with the emerging line of research that highlights the contribution of glutamate and other amino acid neurotransmitters systems in the pathophysiology and treatment of mood disorders (Kugaya and Sanacora, 2005).

Magnetic resonance spectroscopy (MRS) provides a useful approach for quantization of in vivo amino acid neurotransmitters and other metabolites in brain (see Yildiz-Yesiloglu and Ankerst, 2006 for a review). Conventional MRS applications give only a snapshot of metabolite pool size rather than of pool kinetics, but they can indirectly provide in vivo helpful information about cellular integrity and potential cellular loss. Even though conflicting results have been reported, recent studies converge to suggest Glutamate/Glutamine/G-AminoButyric-Acid (Glu/Gln/GABA, respectively) as the most common metabolites to show abnormalities in prefrontal areas of MDD patients. Several previous works have reported lower Glu/Gln/GABA values in the frontal lobes of adult and pediatric patients with MDD compared to healthy controls (Auer et al., 2000; Michael et al., 2003a; Pfleiderer et al., 2003; Mirza et al., 2004; Rosenberg et al., 2005; Hasler et al., 2007; Ajilore et al., 2007; Walter et al., 2009). Glutamate serves as an important excitatory neurotransmitter that contributes to a wide array of normal brain functions, including regulation of neurotrophic factors and neuronal plasticity although excess levels of this metabolite have been involved in pathological conditions such as cerebral ischemia, traumatic brain injury or chronic neurodegenerative states among others (Lipton and Rosenberg, 1994). Based on the fact that glial cells, and particularly astrocytes, supply the primary source of energy to neurons and also furnish the major pathway for neuronal glutamate synthesis (Magistretti et al., 1999; Rajkowska and Miguel-Hidalgo, 2007), a provocative model proposed by Sanacora et al. (2003) suggests that the impaired glial cell function in amygdala and prefrontal cortex structures, observed in postmortem mood disorder samples, may result in decreased synaptic glutamate uptake with a resultant elevation of extracellular glutamate levels. The glial disruption and elevated extracellular glutamate may further accelerate neuronal and glial damage by its neurotoxic effect (Rothstein et al., 1993). These assumptions could seem contradictory with reductions of Glu/Gln/GABA levels found in prefrontal cortex of MDD patients by MRS studies. It should be mentioned that this MRS spectrum is overpoweringly composed by the intracellular pool contained in neurons and especially in glia. Therefore, decreased signal of these metabolites, instead of revealing potential abnormalities in extracellular concentrations or in glutamatergic neurotransmission *per se*, might account for the described anomalies of glial cells in prefrontal areas of depressed patients. Taking into account that further MRS techniques have been developed to overcome the Glu/Gln/GABA peak overlapping, specific Glu concentration at 3T field strength will better mirror the status of glia.

By contrast, though Choline-containing compounds (Cho) seem to be clearly increased in the basal ganglia of patients with MDD, the presence of abnormalities in the Cho peak in prefrontal areas is somewhat more controversial (Yildiz-Yesiloglu and Ankerst, 2006; Nery et al., 2009; Kaymak et al., 2009; Milne et al., 2009). Since Cho is hugely present in oligodendrocytes (Urenjak et al., 1993), observed increases of Cho levels in MDD could also reflect abnormalities in glial function and/or myelinization (Yildiz-Yesiloglu and Ankerst, 2006). Moreover, Cho is over-released from membrane stores normally under pathological conditions and could be therefore conceived as a marker of cellular membrane turnover and active neurodegeneration (Malhi et al., 2002).

N-acetylaspartate (NAA), that is considered to be a marker of neuronal density or integrity, has also been investigated in MDD patients in prefrontal regions yielding inconclusive findings to date (Yildiz-Yesiloglu and Ankerst, 2006; Gonul et al., 2006; Ajilore et al., 2007; Walter et al., 2009; Nery et al., 2009; Kaymak et al., 2009). Although pathology of neurons in depression seems to be less prominent than that of glial cells (Rajkowska and Miguel-Hidalgo, 2007), both cellular lines are closely related, since the latter is known to be crucial for the efficient development and functioning of neurons (Magistretti et al., 1999). Therefore, one could expect to find alterations in prefrontal NAA peak in MDD, which would reflect a primary neuronal damage or, at least, the downstream effects on neuronal structures caused by defects in glia.

So far, most spectroscopic studies in MDD have focused on the differences between patients and healthy controls, and on the potential influence of illness severity, acute effects of therapeutic strategies or treatment resistance (see for example Walter et al., 2009; Block et al., 2009; Price et al., 2009). Nevertheless, the relationship between metabolic changes in vmPFC and other relevant clinical variables, including length of depressive disorder, age at onset or illness subtype, has not received nearly the attention it deserves (Nery et al., 2009; Milne et al., 2009; Hasler et al., 2005).

The aim of this study was to investigate whether Glu levels in the vmPFC differed between MDD patients in distinct stages of the illness and healthy controls. In addition, we also investigated differences of Cho and total NAA concentrations among these groups of patients. We hypothesized that high-past illness burden would imply more metabolite abnormalities independently of mood state.

2. Materials and methods

2.1. Subjects

Sixty right-handed patients with MDD (DSM-IV-TR) aged between 20 and 60 years old entered the study to undergo a specifically designed MR protocol. Patients were split into three different groups. One group of depressed participants was comprised of individuals who were presenting for a first depressive episode ($n = 20$, first-episode). The second group of patients included individuals who had experienced three or more previous episodes of MDD and were currently euthymic –for at least six months– ($n = 20$, remitted-recurrent). The third group was comprised of patients who had a chronic depressive disorder, with a duration of last episode of more than 2 years with no response to antidepressant strategies ($n = 20$, chronic). Likewise, twenty right-handed healthy controls matched by age were also recruited. Exclusion criteria for healthy participants were: lifetime psychiatric diagnoses, first degree relatives with psychiatric diagnoses, and clinically significant physical or neurological illness. Semi-structured interviews were carried out for all participants to collect demographics and clinical information, including comorbid Axis I conditions according to DSM-IV-TR criteria. Subjects with a history of head injury, neurological illness, alcohol or substance abuse were excluded from the study. Current depressive symptoms were assessed using the Hamilton Depression Rating Scale (HDRS) by experienced raters of the clinical staff. The study was approved by the Research Ethics Board of *Hospital de la Santa Creu i Sant Pau* and was carried out in accordance with the Declaration of Helsinki. All subjects gave informed consent after a full explanation of the study protocol.

2.2. MRS scanning procedure

MR studies were obtained using a 3T Philips Achieva facility (software version 2.1.3.2), and the SENSE 8-channel head-coil, using a dedicated acquisition protocol. This included a 3D-MPRAGE whole brain sequence (Turbo Field Echo, TR = 6.7, TE = 3.1, Voxel size = 1 × 1 × 1.2), on which ^{1}H MRS (SVS-PRESS; TR = 2000; TE = 38; NSA = 128; VOI = 2 × 2 × 2 cm, AutoWS-Prescan) was obtained from the ventromedial region (Fig. 1A), which included the rostral cingulate gyrus, and Broadmann's areas 10, 24 and 32 (Talairach and Tournoux, 1988).

^{1}H MRS raw data were exported, and then post-processed using the LCModel (Provencher, 2001). This is an external reference method that provides concentrations (mmol/l) of the single metabolite peaks. For the purposes of the current study, we analyzed Glu, Glx (Glutamate + Glutamine), total NAA (N-acetylaspartate + N-acetylaspartylglutamate), Cho (Glycerophosphocholine and Phosphocholine compounds) and Cr (Creatine). Apart from standard manufacturer's quality assurance (QA), weekly acquisitions of a home-built NAA-phantom were also performed. All spectra were fit with LCModel (SD% based on the Cramer-Rao lower bound), evaluated by an experienced observer (BGA), and only good or acceptable quality spectra (having standard deviations below 30% for quantifications of the main metabolites) were accepted.

2.3. Data analyses

Statistical analyses were performed in SPSS version 17. Demographics and clinical characteristics of groups were compared by one-way analysis of variance. MRS measures were analyzed with multivariate analysis using Wilk's Lambda, and post hoc comparisons (Bonferroni test). Subsequently, metabolites were included in bivariate correlation analyses in order to explore the influence of duration of illness, age at onset and mood state. The results would then be tested by means of a multivariate ANCOVA to confirm validity of associations.

3. Results

After visual assessment of the spectra, 70% of cases resulted good/acceptable for analyses. Included spectra had SD% less than 22% for Glu and Glx, 16% for total NAA, 6% for Cr and 12% for Cho. An exploratory analysis showed that the assumption of homoscedasticity among study groups was satisfied for all metabolites, except for total NAA (Levene's test was not significant for Glu, Glx, Cho and Cr; and for total NAA, $F = 4.09$, $p = 0.01$). Sample sizes were as follows: first-episode patients: $n = 10$; remitted-recurrent patients: $n = 16$; chronic patients: $n = 19$; healthy controls: $n = 15$. Demographics and clinical data are summarized in Table 1. The four groups were comparable for age. The number of females was significantly higher in the remitted-recurrent and chronic groups, when compared to first-episode patients, and healthy controls. For this reason, gender was included as an additional factor in multivariate analyses. Antidepressant treatment was not distributed similarly across the patients groups. As it can be observed in the table, chronic patients received more second-line antidepressants, antipsychotics and combined regimens compared with remitted-recurrent and first-episode patients. Importantly, no relevant differences regarding treatment were found between remitted-recurrent and first-episode patients. Before performing MANOVA analyses, an ANOVA was carried out so as to determine whether to utilize absolute concentrations or ratios with Cr. Given that Cr showed significant differences among the four groups ($F = 8.28$; $p < 0.001$), absolute metabolite levels were then used.

3.1. Metabolite differences between groups

Metabolites concentrations in vmPFC of patients and healthy controls are displayed in Table 2. Two-way MANOVA showed a strong effect of group ($F = 2.90$; $p < 0.001$), but no effect for neither gender ($F = 1.97$; $p > 0.05$), or group × gender ($F = 0.79$; $p > 0.05$). Between-subjects effects appeared for Glutamate ($F = 21.70$; $p < 0.001$), total NAA ($F = 4.82$; $p = 0.005$) and Cho ($F = 3.96$;

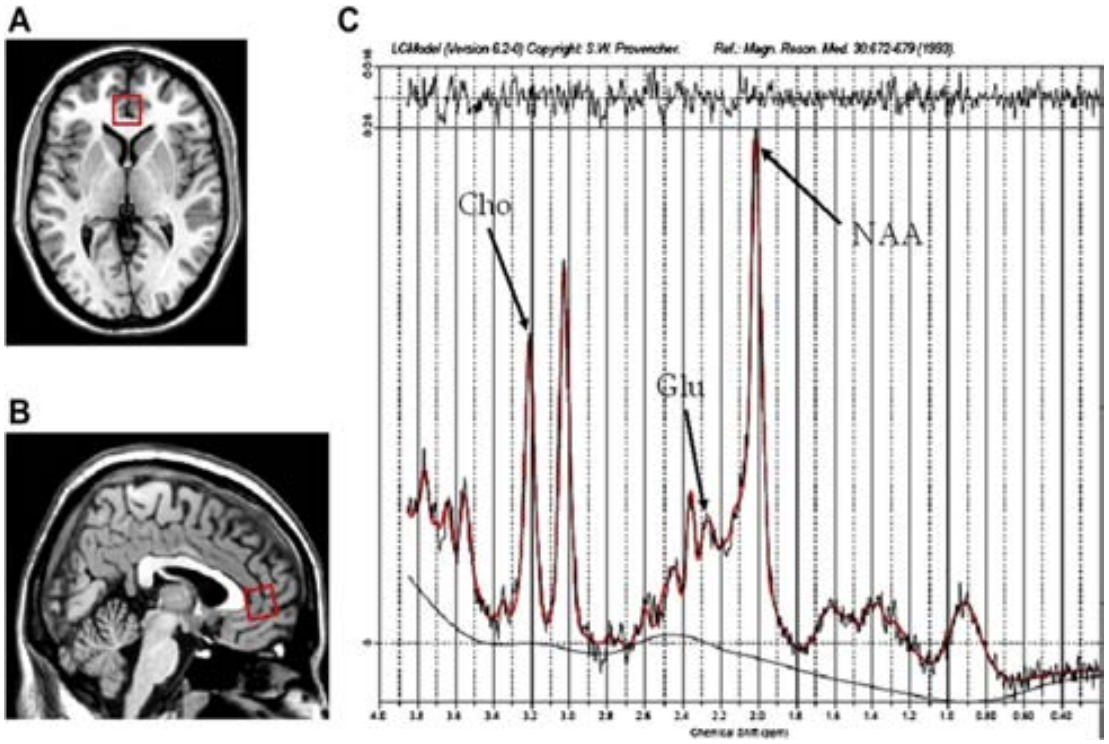

Fig. 1. Axial (A) and sagital (B) images showing a typical ventromedial prefrontal Volume of Interest (VOI); (C) Representative, post-processed (LCModel), ^{1}H MR spectrum: PRESS acquisition at 3T, with TR = 2000 ms and TE = 38 ms.

Table 1
Summary of demographics and clinical variables.

	Healthy Controls ($n = 15$)	First Episode ($n = 10$)	Remitted-Recurrent Patients ($n = 16$)	Chronic Patients ($n = 19$)
Gender (% females)[a]	66%	50%	94%	79%
Age	40.47 ± 11.6	44.50 ± 8.7	45.44 ± 13.9	50.95 ± 7.3
Age at onset[b]	NA	43.50 ± 8.2	25.25 ± 8.1	27.84 ± 9.1
Duration of illness (years)	NA	1.2 ± 1.8	21.1 ± 9.8	22.8 ± 10.1
Number of previous episodes[b]	NA	NA	6.19 ± 5.0	6.56 ± 6.8
HDRS at recruitment	2.46 ± 1.7	13 ± 9.7	4.44 ± 3.6	21.39 ± 4.5
Treatment				
• Antidepressant				
SSRI or SSNRI	NA	100%	75%	83.3%
TCA or MAOI[c]	NA	10%	12.5%	44.4%
Others[d]	NA	0%	18.8%	61.1%
Combination[d]	NA	10%	18.8%	83.3%
No antidepressant	NA	0%	12.5%	0%
• Stabilizer	NA	0%	18.8%	22.2%
• Antipsychotic[c]	NA	10%	12.5%	44.4%
• Benzodiazepine	NA	30%	31.3%	50%

Values represent percentages or mean scores (± standard deviations).
Abbreviations: *HDRS* = Hamilton Depression Rating Scale; *SSRI* = Selective Serotonine Reuptake Inhibitors; *SSNRI* = Selective Serotonine and Noradrenaline Reuptake Inhibitors; *TCA* = Tricyclic Antidepressant; *MAOI* = Monoamine Oxidase Inhibitors; *Others* = Noradrenaline Reuptake Inhibitors, Noradrenaline and Dopamine Reuptake Inhibitors, Tetracyclic antidepressants, Mirtazapine, Metilfenidate or Trazodone; *Combination* designs concomitant use of antidepressants with different mechanisms of action (e.g. SSRI with reboxetine). *Stabilizer* includes anticonvulsivants and mostly lithium. *Antipsychotic* comprehends mainly atypical antipsychotics associated with antidepressants.
[a] Significant differences among the 4 groups.
[b] Significant differences between first-episode and the two other patients groups.
[c] Significant differences between chronic and remitted-recurrent group.
[d] Significant differences between chronic and the two other patients groups.

$p = 0.01$). Bonferroni post hoc analyses showed statistically significant lower values of Glu (Fig. 2, left side) and total NAA (Fig. 2, middle) in remitted-recurrent and chronic patients, compared to first-episode patients ($p < 0.01$), and to healthy controls ($p < 0.05$). Interestingly, the concentrations of Cho showed the opposite pattern (Fig. 2, right side): the highest values were observed in chronic patients and the lowest in first-episode patients ($p < 0.04$).

3.2. Influence of duration of illness, age at onset and mood-state

Correlation analyses showed that Glu was negatively associated to illness duration ($r = -0.56$, $p < 0.001$; see Fig. 3, left side), the higher levels of this metabolite being present in patients having a shorter duration. By contrast, longer illness duration was correlated to higher levels of Cho ($r = 0.32$, $p = 0.03$; Fig. 3, middle). A significant, negative correlation was also observed between illness duration and total NAA, but, as we will see below, this association was dispelled in MANCOVA analysis. In the opposite direction, an earlier age at onset was related to lower levels of total NAA (r=0.50, $p = 0.001$; Fig. 3, right side). There was no significant relation between metabolite levels and HDRS scores in our sample ($p > 0.2$).

To avoid potential confusing effects and to confirm the robustness of our results, a MANCOVA was performed including those metabolites that showed differences among groups as dependent variables, clinical variables as covariates (duration of illness, age at onset and HDRS scores), and gender as a factor. The results showed that, indeed, duration of illness had a significant main effect on metabolite concentrations ($F = 3.94$, df = 3,36, $p = 0.016$). Between subjects effects were observed on Glu ($p = 0.02$) and on Cho ($p = 0.009$), but not on total NAA, vanishing its apparent relation with duration of illness. Although age at onset did not reach significance in main multivariate comparison ($p = 0.08$), a between-subjects effect was now observed on total NAA ($p = 0.03$). The rest of the variables did not show any effect on metabolites.

4. Discussion

To our knowledge, these exploratory results constitute the first comparison of prefrontal brain metabolite concentrations among different stages of MDD. The observation in our sample of higher values of Glu at the beginning of the disorder and its progressive decrement extends previous MRS studies, which had reported reductions in Glu levels in the ACC and prefrontal brain cortex when comparing depressive patients to healthy controls. The decrease was of a relevant magnitude, reaching up to 25% in chronic vs. first-episode patients. An opposite pattern was observed for Cho: first-episode patients, together with healthy controls, showed the lowest levels of Cho, having remitted-recurrent and chronic patients the highest values. Interestingly, the tendency of progressive changes of Glu and Cho metabolites was clearly related to illness duration regardless the state of mood at scanning time. Controls and patients with a first depressive episode in our sample

Table 2
Absolute metabolite concentrations in the ventromedial prefrontal region.

Metabolites	Healthy Controls ($n = 15$)	First Episode Patients ($n = 10$)	Remitted-Recurrent Patients ($n = 16$)	Chronic Patients ($n = 19$)
Glutamate (Glu)	9.33 ± 0.84	8.99 ± 0.64	7.18 ± 1.24	6.72 ± 0.97
Glutamate + Glutamine (Glx)	11.54 ± 1.34	11.67 ± 1.62	10.28 ± 2.09	9.91 ± 1.59
N-acetylaspartate + N-acetylaspartylglutamate (total NAA)	6.94 ± 0.39	7.20 ± 0.72	6.22 ± 1.06	6.25 ± 0.45
Choline-containing compounds (Cho)	1.56 ± 0.25	1.56 ± 0.32	1.76 ± 0.29	1.84 ± 0.20
Creatine (Cr)	6.05 ± 0.41	5.81 ± 0.62	5.44 ± 0.45	5.31 ± 0.42

Values represent mean scores and standard deviations of metabolite concentrations expressed in mmol/l.

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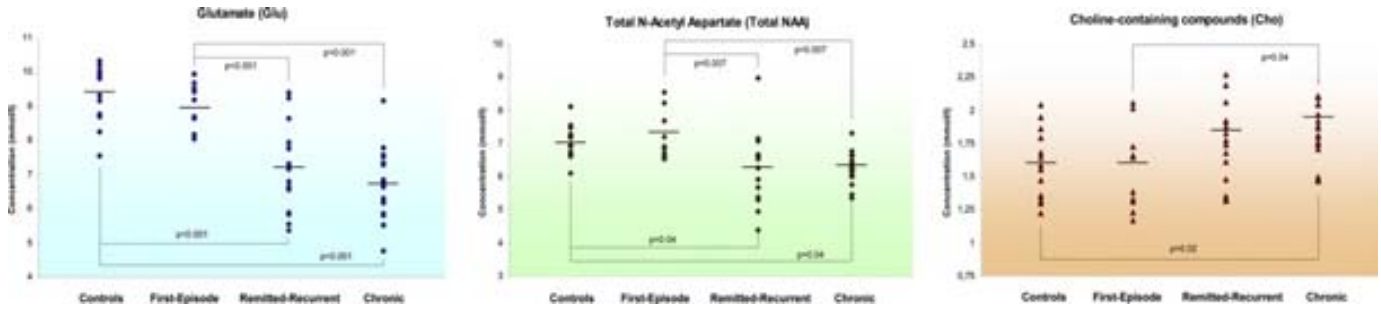

Fig. 2. Absolute metabolites concentrations (Glu, total NAA and Cho) in vmPFC of healthy controls, patients with first-episode, remitted-recurrent and chronic depression. Group means are displayed as horizontal bars. Remitted-recurrent patients were 23% lower than healthy controls ($p < 0.001$, Cohen's $d = 2.03$) and 20% lower than first-episode ($p < 0.001$, Cohen's $d = 1.83$) in Glu concentrations. A similar pattern is observed for total NAA concentrations, remitted-recurrent being 10.4% lower than controls ($p = 0.04$, Cohen's $d = 0.97$) and 13.6% lower than first-episode ($p = 0.007$, Cohen's $d = 1.08$). Chronic patients were 28% lower than healthy controls ($p < 0.001$, Cohen's $d = 2.88$), and 25.3% lower than first-episode ($p < 0.001$, Cohen's $d = 2.76$) in Glu concentrations; moreover, they were 10% lower in total NAA values than healthy controls ($p = 0.04$, Cohen's $d = 1.65$) and 13.2% lower than first-episode ($p = 0.007$, Cohen's $d = 1.65$). Regarding Cho levels, chronic patients were 18% higher than controls and first-episode ($p = 0.02$, Cohen's $d = -1.08$ and $p = 0.04$, Cohen's $d = -1.07$, respectively). p values derived by Bonferroni's post hoc tests.

showed higher total NAA levels compared to remitted-recurrent and chronic patients. Association analyses showed, however, that levels of such metabolite were not actually related to duration of illness but to age at onset (that was earlier for our long-standing depressive patients), so the disorder would begin before in those patients with lower vmPFC total NAA levels.

Previous MRS studies in depression have already reported concentrations of Glutamate/Glutamine to be abnormally reduced in the ACC (Auer et al., 2000; Pfleiderer et al., 2003; Mirza et al., 2004; Rosenberg et al., 2005; Walter et al., 2009), dorsomedial, dorsolateral and ventromedial prefrontal cortex (Michael et al., 2003a; Hasler et al., 2007) and subcortical regions including amygdala (Michael et al., 2003b; Ajilore et al., 2007). It is noteworthy that by means of LCModel post-processing single Glu peaks were obtained for the present study. Although hypothetically it would reflect the combined intracellular and extracellular pool, Glu signal is dominated overwhelmingly by the intracellular pool contained in neurons and glia (ratio of intracellular–extracellular up to 5000:1; Lehmann et al., 1983). Hence, it is unlikely that the abnormalities in Glu observed in the current study mirror changes in the extracellular compartment but they may account for the abnormal reduced density of astrocytes and pyramidal glutamatergic neurons found in postmortem brains in MDD (Rajkowska and Miguel-Hidalgo, 2007). Indeed, contribution of amino acid systems to the pathophysiology of depression has raised great expectation in the last decade, heightened by promising results of NMDA glutamatergic receptor antagonists (Berman et al., 2000; Brennan et al., 2010). In this regard, the model proposed by Sanacora et al. (2003) described how impaired glial function could lead to decreases in Glu pools in depressed patients. The regions displaying reductions in glial cell counts include the dorsal anterolateral prefrontal cortex, ACC, orbitofrontal cortex, and amygdala (Cotter et al., 2001; Rajkowska et al., 1999; Ongür et al., 1998; Bowley et al., 2002; Cotter et al., 2002; Uranova et al., 2004). Our results implicate that these histopathologic changes in vmPFC would occur along the progression of the disease.

On the other hand, Cho increase found in our study in long-standing MDD subjects as compared to first-episode MDD and controls could be interpreted at least in two different ways. Since oligodendrocytes are particularly rich in this metabolite (Urenjak et al., 1993), vmPFC abnormalities in myelinization and/or glial function along the course of the illness may underlie the augmented Cho concentrations observed herein. Alternatively, Cho signal is predominantly composed of by-products of phosphotidylcholine hydrolysis (Klein et al., 1993), therefore increased Cho levels may also reflect neuronal membranes breakdown and/or alterations in cellular signal transduction systems in the prefrontal cortex caused by past illness burden. Similarly, a previous study has recently reported Cho to be significantly increased in the hippocampus of patients with extensive past illness (Milne et al., 2009). Considered part of the limbic system, the vmPFC mediates many behaviours influenced by stress, since it is also a major target for glucocorticoids. This area displays reciprocal connections to the parahippocampal gyrus, and thus to the hippocampus, which plays an important role in the response to stress, being presumably exposed to its potential neurotoxic effects in chronic stress conditions. Considering that chronic stress could act as a trait-dependent risk factor in MDD, one might predict that structures strongly

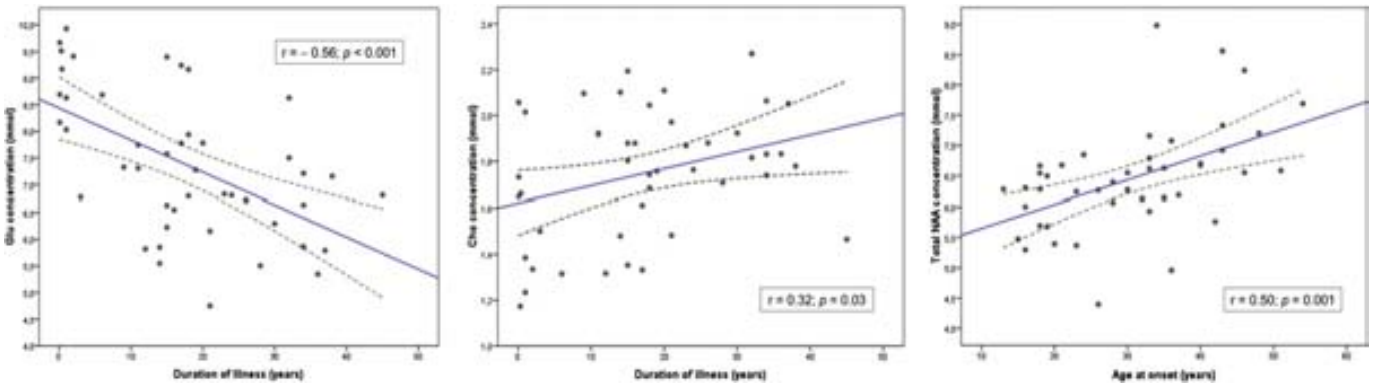

Fig. 3. Scatter plots showing correlation of duration of illness with Glu and Cho concentrations in subjects with major depression ($n = 45$) and correlation of age at onset with total NAA in these patients ($n = 45$). Bold and dotted lines represent the interpolation lines and their 95% confidence intervals, respectively.

connected to the hippocampus, such as the vmPFC, are more likely to be affected at the cellular level. In this regard, metabolic alterations observed in our study could feasibly reflect such affectation. Therefore, abnormal glial reductions observed in postmortem MDD patients might be related to remodeling processes in the prefrontal areas either as a cause or consequence of illness burden, which could be determined in vivo by means of Glu and Cho spectra.

Our finding of decreased levels of total NAA in vmPFC of patients with chronic and remitted-recurrent depression associated with an earlier onset leads us to believe that it would play a modulating role in the establishment of the illness, probably acting as a vulnerability neurobiological trait, since the decrease was independent of symptomatic state. It should be noted that our first-episode sample was on average middle-aged at the debut of the illness, which could explain significant differences with the rest of depressive patients, who had had an earlier beginning of the disorder. NAA has been postulated as a marker of neuronal density or integrity (Urenjak et al., 1993), being a key factor in cell signaling between neurons and glial cells (Baslow, 2000). For this reason, diminished total NAA levels might represent a reduction of neuronal population within this area or, at least, neuronal dysfunction. The former hypothesis would seem to be in agreement with previous neuroimaging studies that have reported structural changes and volumetric gray matter reductions in medial prefrontal regions, specially in subgenual ACC (Coryell et al., 2005), but neuropathological postmortem studies have revealed that these abnormalities would be more associated with reductions in glia than in neurons (Drevets et al., 2008b). More feasibly, low total NAA levels could involve more complex damages in prefrontal neuronal cells, as those reported by Cotter et al. (2001), who described neuronal size reductions without significant changes in neuronal density in the ACC of MDD patients. Such anomalies, might, in turn, augment the vulnerability to suffer from depression.

There are several methodological issues of the present study which merit comment. First of all, the MRS data were acquired using a 3-T MR facility, which provides a higher signal-to-noise ratio and a better separation among metabolite peaks. For this reason, by means of the LCModel, analysis of the specific glutamatergic resonances was possible. Secondly, the location of VOI is centered in a key area implicated in affective disorders, which includes the ACC and portions of the adjacent prefrontal gyri. The selected voxel size permitted an acceptable spatial resolution; however, it precluded the ability to distinguish metabolic differences across these specific anatomical subregions or grey/white matter segments. In this regard, it would have been clarifying to weigh up the magnitude of metabolic changes by means of volumetric differences or tissue segmentation, as previously highlighted by Milne et al. (2009) and Brennan et al. (2010). Nevertheless, our VOI was not located on a unique brain structure, but on a wider region, not allowing determination of precise volumes. Thirdly, individual absolute metabolite concentrations were obtained. Early spectroscopic studies have used total Cr as an internal standard. However, this is an acceptable approach only if the absolute concentration of Cr is stable, and it is still quite controversial whether this can be assumed in both unipolar and bipolar depression, at least in the prefrontal cortex (Gruber et al., 2003; Frey et al., 2007). In fact, in our study notable differences in Cr among groups were also observed, showing diminished Cr levels in depressed samples. Cr plays an important role in brain energy metabolism and its levels can be modulated by conditions of energy production and demand (Frey et al., 2007) thus, one might be tempted to understand these findings as a proof of abnormalities in prefrontal cellular energy production. Nonetheless, we preferred to be cautious with such interpretation since no a priori hypothesis was made and this topic would have deserved further careful analyses. More importantly, the results bring into question again the advisability of using Cr as an internal standard or reference ratio in mood disorders spectroscopic research. Fourthly, although there were gender differences among groups, after controlling for its effect, the results were not affected. Fifthly, age at onset was later in first-episode patients. Since it is well established that there are brain metabolite changes associated with normal aging (Kaiser et al., 2005), all participants were required to be similarly aged at the time of scanning in order to avoid the potential confounding effect. This, in turn, yielded to ineluctable age differences at the debut of illness. In our opinion this is the best option to explore the effect of illness burden on metabolite changes through a cross-sectional design. It is well known that this kind of design provides limited information, so longitudinal studies, that track spectroscopic changes in MDD patients over disease onset and through follow-up, would be more adequate. Sixthly, all patients were on medication which could imply a strong limitation, since previous works had already reported metabolite changes caused by antidepressant treatments (Block et al., 2009; Price et al., 2009). We aimed at recruiting a representative sample of non-elderly adults with moderate-severe MDD in different stages of illness. One might argue that the metabolite changes observed in our study could be explained in terms of how and for how long these patients had been treated rather than duration of illness itself. However, this possibility seems unlikely because the direction of metabolite changes reported herein is in agreement with previous studies, even with those focusing on drug-free patients (Mirza et al., 2004; Rosenberg et al., 2005; Hasler et al., 2007). Additionally, whereas chronic patients appear to receive more often combined treatments (comprising more than one antidepressant and/or atypical antypsychotics), remitted-recurrent and first-episode patients did not differ in their prescription regimens. Therefore, the potential confounding effects of medication would then be equally represented in short and long-standing depression groups and, in any case, would have underestimated the differences, especially between healthy controls and first-episode depressive patients.

5. Conclusions

In summary, we found decreased Glu and total NAA concentrations and increased Cho levels in the vmPFC of long-standing MDD patients. Abnormalities in Glu and Cho resonances were more pronounced in patients with chronic or remitted-recurrent depression and consistently related to illness duration, suggesting that the course of the illness may be an important factor to explain such metabolite abnormalities, rather than mood state. These results would provide support for the hypothesis that there are cellular changes, mainly in glia, taking place within the ventromedial prefrontal region over the course of MDD. It remains to be elucidated whether these deficiencies in cellular neurochemistry are primary or secondary to disease progress, but, anyway, they could still have significant pathogenic implications. More importantly, the present findings would open up new diagnostic approaches and therapeutic goals, stressing the consequences of being ill for longer periods and, thus, intending more adequate and specific antidepressant treatments (perhaps with novel mechanisms of action as glutamatergic modulating agents) from the early stages of illness in order to avoid the potential neurotoxic changes presumably associated with further relapses and chronicity.

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Contributors

MJP, VP, EA and BGA designed the study and wrote the protocol. MJP and JDA managed the literature searches and analyses. BGA, RMF and YV performed and analyzed the NMR spectroscopy. JDA, DP, RP, EA and VP performed the recruitment and psychopathological assessment of the patients. MJP and JDA undertook the statistical analysis, and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

Dr. Víctor Pérez declares having received educational honoraria from: Sanofi-Aventis, Lundbeck, Pfizer, AstraZenecaand Eli Lilly, and research funding from Boehringer-Ingelheim for this work. Dr. Enric Alvarez has received consulting and educational honoraria from several pharmaceutical companies including Eli Lilly, Sanofi-Aventis, Lundbeck and Pfizer, and he has participated as main local investigator in clinical trials from Eli Lilly, Bristol-Myers and Sanofi-Aventis and also as national coordinator of clinical trials from Servier and Lundbeck. The rest of authors declare no financial interests or potential conflicts of interest related directly or indirectly to this work.

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Research Paper

Hippocampal abnormalities of glutamate/glutamine, *N*-acetylaspartate and choline in patients with depression are related to past illness burden

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Background: Smaller hippocampal volumes in major depressive disorder (MDD) have been linked with earlier onset, previous recurrences and treatment refractoriness. The aim of our study was to investigate metabolite abnormalities in the hippocampus associated with past depressive illness burden. **Methods:** Glutamate/glutamine (Glx), *N*-acetylaspartate (NAA) and choline (Cho), potential markers of glial/neuronal integrity and membrane turnover, respectively, were measured in adults with depression and healthy controls using a 3 T magnetic resonance spectroscopy scanner. Voxels were placed in the head of the right and left hippocampus. We controlled for systematic differences resulting from volume-of-interest (VOI) tissue composition and total hippocampal volume. **Results:** Our final sample comprised a total of 16 healthy controls and 52 adult patients with depression in different stages of the illness (20 treatment-resistant/chronic, 18 remitted-recurrent and 14 first-episode), comparable for age and sex distribution. Patients with treatment-resistant/chronic and remitted-recurrent depression had significantly lower levels of Glx and NAA than controls, especially in the right hippocampal region ($p \leq 0.025$). Diminished levels of Glx were correlated with longer illness duration (left VOI r = –0.34, $p = 0.01$). By contrast, Cho levels were significantly higher in patients with treatment-resistant/chronic depression than those with first-episode depression or controls in the right and left hippocampus (up to 19% higher; all $p \leq 0.025$) and were consistently related to longer illness duration (right VOI r = 0.30, $p = 0.028$; left VOI r = 0.38, $p = 0.004$) and more previous episodes (right VOI r = 0.46, $p = 0.001$; left VOI r = 0.44, $p = 0.001$). **Limitations:** The cross-sectional design and the inclusion of treated patients are the main limitations of the study. **Conclusion:** Our results support that metabolite alterations within the hippocampus are more pronounced in patients with a clinical evolution characterized by recurrences and/or chronicity and add further evidence to the potential deleterious effects of stress and depression on this region.

Introduction

The hippocampus is considered a crucial brain region within the limbic system, playing a determinant role in emotion regulation and in major depressive disorder (MDD).[1] It is also a highly stress-sensitive structure, since a dysregulation of glucocorticoid secretion in stress-induced situations — and the accompanying increased activity of excitatory amino acid neurotransmitters — could result in either potentially reversible remodelling or irreversible cell damage in the hippocampus of

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patients with MDD.[2] There is considerable evidence suggesting that the hippocampus could be seriously affected in patients with depression. Two meta-analyses[3,4] concluded that the hippocampus was bilaterally smaller in people with MDD than matched controls. Numerous studies have linked such volume reductions to greater severity of depression, younger age at onset of illness, greater number of previous episodes or nonresponsiveness to treatment.[5]

In contrast, little is known about the neuropathological hippocampal changes that underly the past burden of illness in patients with MDD. Few postmortem works have proven cellular loss in the hippocampus of depressed patients,[6] but reports are consistent about more subtle abnormalities in neurons and glia, such as those showing reductions in neuronal soma size and in neuropil (i.e., the dense tangle of axon terminals, dendrites and glial cell processes).[7] These deficiencies in the cellular integrity and potential cellular loss in the hippocampus should be indirectly detectable by proton magnetic resonance spectroscopy (MRS), as it is a noninvasive neuroimaging technique that allows in vivo quantification of diverse metabolites in localized brain regions. Glutamate/glutamine (Glx or Glu/Gln, respectively), choline-containing compounds (Cho) or *N*-acetylaspartate (NAA) are some of the metabolites measurable by MRS that are most commonly implicated in MDD.[8] Glutamate-related metabolites mainly represent the intracellular pool contained in pyramidal glutamatergic neurons and glia, particularly in astrocytes. Therefore, decreases on that signal may account for defects in these cellular lines.[9] Resonance of Cho can be conceived as an indirect measure of membrane turnover.[10] Primarily present in neurons, NAA is suggested to be a marker of neuronal viability and functionality.[11]

In last decade, a large and compelling body of literature on biochemical changes in MDD has appeared, but only a few works have focused on the hippocampus, sometimes leading to inconsistent conclusions. Two studies reported diminished levels of Glx within the head of the left hippocampus in severely ill patients with treatment-resistant depression[12] and untreated patients with first-episode depression,[13] although a subsequent study by Milne and colleagues[14] was unable to confirm such a finding. With regard to Cho concentrations, patients with treatment-resistant depression[15] or with previous recurrences[14] have been shown to have a greater spectroscopic signal in the hippocampus, although other authors have reported low or normal Cho levels in patients with a current depressive episode, with subsequent increases after successful response to electroconvulsive therapy[16] or pharmacological treatment.[13] Previous studies of hippocampal levels of NAA have failed to observe deficiencies in depressed patients[8] despite alterations in neuroplasticity and/or neurogenesis having been repeatedly suggested to underlie MDD.[3,17] Nevertheless, some works have revealed increases in NAA levels associated with treatment response.[12,13]

Some of the inconsistencies among the studies can be attributed to methodological issues[8] (e.g., the lack of consideration for structural differences on the region studied[14]) whereas others can be related to clinical differences of the samples included. In fact, the influence of relevant illness course variables (e.g., duration of illness, number of previous episodes, age at onset of illness) on metabolite changes has not been well established until now. Our group recently reported abnormalities in Glu, NAA and Cho that were consistently related to the course of illness within the ventromedial prefrontal cortex (vmPFC) of patients with MDD, supporting the idea that greater past illness burden (measured by longer illness duration, earlier onset of illness, recurrences and treatment resistance) entails more defects in cellular neurochemistry.[18]

The present study investigated whether alterations of Glx, NAA and Cho levels in the left and right hippocampus differed between patients with MDD in distinct stages of illness and healthy controls, as previously observed in the vmPFC. We hypothesized that a substantial past illness burden would imply more metabolite abnormalities in the medial temporal region, independent of mood state.

Methods

Participants

A group of right-handed adult patients with MDD (DSM-IV-TR criteria) underwent a specifically designed magnetic resonance protocol. We recruited them from the outpatient service of the Department of Psychiatry, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, as part of a bigger project whose main purpose was to establish in vivo neuroimaging markers of clinical illness burden. Most of the patients included in the present study were different from those included in previous work by our group[18] (only 11 patients were used in both studies), as the quality of spectra varied considerably from vmPFC to medial temporal volume-of-interest (VOI) locations. Patients were split into 3 different groups. One comprised depressed participants with greater past illness burden (≥ 3 previous episodes of MDD) who were euthymic (score < 8 on the Hamilton Rating Scale for Depression [HAM-D][19]) in at least the 6 months preceding the study (remitted-recurrent group). The second group comprised patients who had a chronic depressive disorder and whose last episode had a duration of more than 2 years, with no response to several antidepressants (treatment-resistant/chronic group). The third group comprised patients with a newly diagnosed first episode of depression who were currently depressed and being treated at the time of scanning. The first-episode group was representative of depressed patients with low illness burden (i.e., no previous episodes, shorter illness duration and later age at onset of illness), and participants were of similar age to those in the rest of the groups. Likewise, we recruited right-handed healthy controls with comparable age and sex distribution to that in the patient groups from among restaurant staff, technical support workers and graduate students at Hospital de la Santa Creu i Sant Pau. Controls received monetary compensation for their participation. Exclusion criteria for controls were lifetime psychiatric diagnoses, first-degree relatives with psychiatric diagnoses and clinically important physical or neurologic illness. We conducted semistructured interviews for all participants to collect demographic and clinical information, including comorbid Axis I conditions according to

DSM-IV-TR criteria. Experienced clinical staff assessed current depressive symptoms using the HAM-D. Participants with a history of head injury, neurologic illness, alcohol or substance abuse were excluded from the study. The study was approved by the Research Ethics Board of Hospital de la Santa Creu i Sant Pau and was carried out in accordance with the Declaration of Helsinki. All participants provided informed consent after a full explanation of the study protocol.

MRS scanning procedure

We obtained MRS images using a 3 T Philips Achieva scanner (software version 2.1.3.2) and a SENSE 8-channel head coil with a dedicated acquisition protocol. This included a 3-dimensional magnetization-prepared rapid-acquisition gradient echo (3D-MPRAGE) whole-brain sequence (turbo field echo, repetition time [TR] 6.7 ms, echo time [TE] 3.1 ms, voxel size 1 × 1 × 1.2), on which ^{1}H-MRS (single-voxel spectroscopy with point resolved excitation spin-echo sequence; TR 2000 ms, TE 38 ms, numbers of signals averaging 128, VOI 2 × 2 × 2 cm, AutoWS-Prescan) images were obtained from the left and right medial temporal regions (Fig. 1, left side). First, borders of hippocampal voxels were aligned to the head of the hippocampus bilaterally as the anterior edge on the sagittal view. Then, the medial edge was set to be lateral to the medial border of the right and left hippocampus using the coronal series. Therefore, VOIs included the head of the hippocampus and surrounding white and grey matter, as well as cerebrospinal fluid (CSF).

The ^{1}H-MRS raw data were exported and then post-processed using LCModel.[20] This is an external reference method that provides concentrations (in millimoles) of the single metabolite peaks. For the purposes of the present study, we included the following metabolites: Glx (Glu/Gln peak, mainly dominated by Glu[12]), NAA (*N*-acetylaspartate + *N*-acetylaspartate-glutamate) and Cho (glycerophosphocholine and phosphocholine compounds). Apart from standard manufacturer's quality assurance, weekly acquisitions of a home-built NAA phantom were also performed. All spectra were fit with LCModel (percent standard deviation [SD%] based on the Cramér–Rao lower bound), evaluated by an experienced observer (B.G.A.), and only good quality spectra (SD < 20% for quantifications of the main metabolites) were accepted (see Fig. 1, right side, for a representative ^{1}H-MRS). We used absolute metabolite levels instead of ratios with creatine to avoid a bias through systematic drifts in the magnitude of the creatine resonance, as suggested by previous studies.[18,21–24]

VOI tissue segmentation and total hippocampal volume

To control for the effect of potential structural differences in the region studied, we assessed tissue composition within the VOIs and total hippocampal volume as follows. The amount of each tissue — grey matter, white matter and CSF — was quantified for each VOI, so the scans were first separated in the 3 different tissues using SPM8 software (Wellcome Trust Centre for Neuroimaging, www.fil.ion.ucl.ac.uk/spm) running under MATLAB 7.8.0 (MathWorks). We then manually reoriented the scans according to Montreal Neurological Institute space, aligning the anterior and posterior commissure in the same axis. When the images were segmented we used the "native space" option, which produces a tissue class image (c*) in the same anatomic space of the original nonsegmented image. After that, the VOI of 2 cm^{3} was resituated in the original images using ITK-SNAP software version 2.0.0.[25] Finally, the mask of each participant was multiplied by his or her own image for the 3 required tissues using MATLAB, and then we quantified the volume of the resulting multiplied images. Following this procedure, we obtained the amount of grey matter, white matter and CSF of the left and

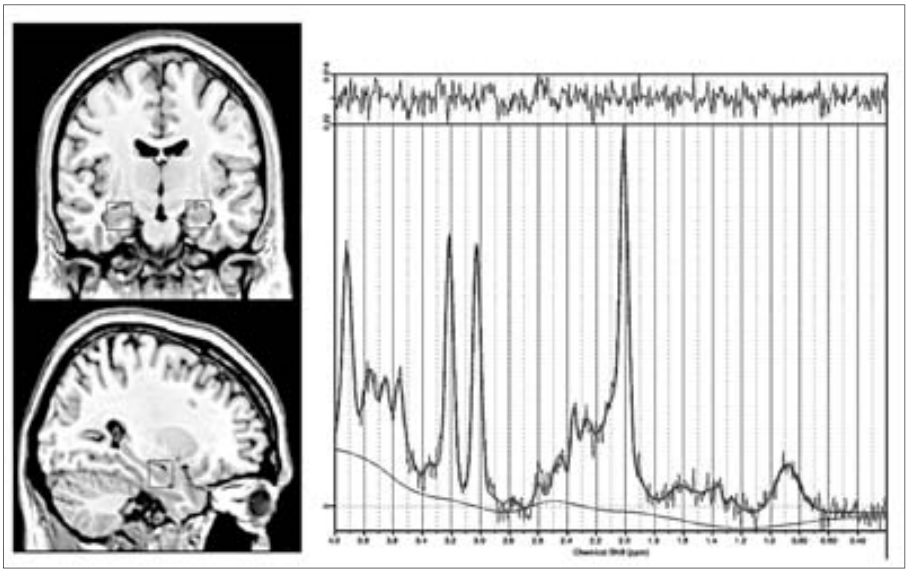

Fig. 1: Magnetic resonance images showing (**left**) the placement of the volumes of interest in a coronal and sagittal section of the medial temporal region and (**right**) a representative ^{1}HMRS postprocessed with LCModel: PRESS acquisition at 3 T, with a repetition time of 2000 ms and echo time of 38 ms.

right VOI. Measurement of hippocampal volumes was conducted automatically for all the participants using FreeSurfer image analysis software (http://surfer.nmr.mgh.harvard .edu/). Further details of the procedure used can be found elsewhere.[26]

Statistical analysis

We performed statistical analyses using SPSS software version 18. We compared the demographic and clinical characteristics of the groups using 1-way analysis of variance or non-parametric tests, as appropriate. Magnetic resonance spectrocopy measures were analyzed using multivariate analysis of covariance (MANCOVA; 1 per side), with group as the between-subjects factor, metabolite values as the dependent variables and total hippocampal volumes and VOI tissue differences as covariates. We considered these results to be significant at $p < 0.05$. Subsequently, metabolites exhibiting significant differences in the MANCOVA were included in bivariate correlation analyses to explore the influence of clinical variables. Given the exploratory purposes of the correlation analyses and the risk of type I errors associated with multiple comparisons, all patients with MDD were examined together, and we considered results to be significant at $p < 0.01$.

Results

We recruited 60 adult patients with MDD (20 in the remitted-recurrent group, 20 in the treatment-resistant/chronic group and 20 in the first-episode group) and 20 controls for participation in this study. After visual assessment of the spectra, 87.5% of the participants recruited had results that were adequate for analysis. The average SD% of included spectra was of 12% for Glx, 9% for NAA and 4.2% for Cho. An exploratory analysis showed that the assumption of homoscedasticity among study groups was satisfied for all metabolites ($p > 0.05$). On the basis of this assessment, our final sample included 52 patients with MDD (14 in the first-episode group, 18 in the remitted-recurrent group and 20 in the treatment-resistant/chronic group) and 16 healthy controls.

Demographic and clinical data are summarized in Table 1. After selection of good spectra, the 4 groups did not significantly differ in age or in sex. Antidepressant treatment was not distributed similarly across the patient groups. Treatment-resistant/chronic patients received more second-line antidepressants, antipsychotics, mood stabilizers and combined regimens than the other patients.

Table 2 displays absolute hippocampal volumes across the participant groups. There were significant group effects for the left ($F = 4.5$, $p = 0.006$) and the right hippocampus ($F = 6.1$, $p = 0.001$), with smaller volumes in the remitted-recurrent group (right $p = 0.039$) and the treatment-resistant/chronic group (right $p < 0.001$; left $p = 0.004$) than in the first-episode group. Table 2 also includes the percentage of white matter, grey matter and CSF segmentations of the selected VOIs. Both white and grey matter segments differed among the

Table 1: Participant demographic and clinical characteristics

Characteristic	Group; mean (SD)*			
	Control, $n = 16$	First-episode depression, $n = 14$	Remitted-recurrent depression, $n = 18$	Treatment-resistant/chronic depression, $n = 20$
Sex, % female	61.1	62.5	88.9	80
Age, yr	43.4 (9.1)	42.5 (8.7)	47.3 (9)	49.6 (7.6)
Years of education	14.2 (3.1)	13.8 (3.3)	13.4 (3.1)	13.2 (3.5)
Age at onset, yr†‡	NA	41.8 (8.7)	26.3 (9.4)	27.9 (8.5)
Duration of illness, mo†‡	NA	6.8 (4.3)	245.8 (125.9)	280.3 (142.4)
No. of previous episodes†‡	NA	1.0 (0)	5.1 (4.7)	6.0 (6.2)
HAM-D at scanning†§¶**	2.8 (1.4)	12.6 (7.4)	2.6 (1.5)	20.9 (4.6)
Treatment, %				
Treatment				
Antidepressant				
SSRI or SSNRI†	NA	100	72.2	85.0
TCA or MAOI‡	NA	6.3	16.7	40.0
Others‡¶	NA	6.3	22.2	55.0
Combination‡¶	NA	12.5	27.8	80.0
No antidepressant	NA	0	11.1	3.7
Stabilizer‡	NA	0	16.7	30.0
Antipsychotic‡¶	NA	6.3	11.1	40.0
Benzodiazepine	NA	50.0	33.0	50.0

Antipsychotic = mainly atypical antipsychotics associated with antidepressants; Combination = designs concomitant use of antidepressants with different mechanisms of action (e.g., SSRI with reboxetine); HAM-D = Hamilton Depression Rating Scale;[19] MAOI = monoamine oxidase inhibitors; NA = not applicable; Others = noradrenaline reuptake inhibitors, noradrenaline and dopamine reuptake inhibitors, tetracyclic antidepressants, mirtazapine, metilfenidate or trazodone; SD = standard deviation; SSNRI = selective serotonin and noradrenaline reuptake inhibitors; SSRI = selective serotonin reuptake inhibitors; Stabilizer = includes anticonvulsants and mostly lithium; TCA = tricyclic antidepressant.
*Unless otherwise indicated.
†Significant differences between first-episode and remitted-recurrent depression.
‡Significant differences between first-episode and treatment-resistant/chronic depression.
§Significant differences between first-episode depression and control.
¶Significant differences between treatment-resistant/chronic and remitted-recurrent depression.
**Significant differences between treatment-resistant/chronic depression and control.

4 groups (right VOI: $F = 4.55$, $p = 0.006$ and $F = 3.13$, $p = 0.032$, respectively; left VOI: $F = 5.45$, $p = 0.002$ and $F = 4.96$, $p = 0.004$, respectively). This difference indicates that patients with remitted-recurrent depression had a significantly higher proportion of grey matter and significantly lower proportion of white matter than healthy controls and patients with treatment-resistant/chronic depression. We found no differences in CSF among the 4 groups. Therefore, total hippocam-pal volumes and segmentation proportions of grey and white matter were included as covariates in the subsequent multivariate analyses.

Metabolite differences among groups

Metabolite concentrations in the medial temporal region of patients and controls are displayed in Figure 2. The 1-way MANCOVA for right VOI showed an effect of group ($F = 3.9$, $p < 0.001$), but no effect for either white matter ($F = 0.7$, $p = 0.58$), grey matter ($F = 1.3$, $p = 0.28$) or hippocampal volume ($F = 1.2$, $p = 0.31$). Between-subjects effects appeared for Glx ($F = 2.7$, $p = 0.05$), NAA ($F = 6.6$, $p = 0.001$) and Cho ($F = 6.8$, $p < 0.001$). Bonferroni post hoc analyses showed significantly lower values of Glx and NAA in patients with remitted-recurrent and treatment-resistant/chronic depression than in controls ($p \leq 0.025$). Concentrations of Cho showed the opposite pattern ($p < 0.001$), where the highest values were observed in treatment-resistant/chronic depression and the lowest in first-episode depression and healthy controls (all $p \leq 0.034$).

The 1-way MANCOVA for the left VOI also indicated a group effect ($F = 2.6$, $p = 0.007$), regardless of tissue composition ($F = 1.2$, $p = 0.33$ for white matter and $F = 1.7$, $p = 0.17$ for grey matter) or hippocampal volume ($F = 1.1$, $p = 0.36$). In this case, between-subject effects were significant for Glx ($F = 2.7$, $p = 0.049$) and Cho ($F = 4.9$, $p = 0.004$) and for NAA at a trend level ($F = 2.5$, $p = 0.07$). Cho levels were significantly higher in patients with treatment-resistant/chronic depression than in those with first-episode depression or in controls ($p = 0.006$ and $p = 0.025$, respectively), and Glx concentrations were significantly lower in patients with treatment-resistant/chronic depression than in controls ($p = 0.024$). Other post hoc analyses did not reach significance.

Influence of clinical variables

As shown in Figure 3, higher Cho concentrations were related to longer illness duration (right VOI r = 0.30, $p = 0.028$; left VOI r = 0.38, $p = 0.004$) and to more previous episodes (right VOI r = 0.46, $p = 0.001$; left VOI r = 0.44, $p = 0.001$). On the other hand, diminished levels of Glx were correlated with longer illness duration (left VOI r = –0.34, $p = 0.008$). To ensure that outliers were not responsible for the whole effect, we performed nonparametric Spearman rho correlations with the same variables, and the results were confirmed for Cho but not for Glx (data not shown). By contrast, concentrations of NAA did not correlate with any of the clinical variables ($p > 0.10$), nor was there a significant association between metabolite levels and HAM-D scores in our sample ($p > 0.20$).

Discussion

Our data indicated that patients with MDD whose course of illness was characterized by multiple relapses or a chronic evolution presented lower levels of Glx and NAA than healthy controls, especially in the right medial temporal region. When patients with greater illness burden were compared with patients with a more benign course of MDD (i.e., first episode, later onset and shorter duration), differences emerged in both hemispheres for Cho concentrations, whereas patients with treatment-resistant/chronic illness displayed the highest levels. Changes of Glx and Cho peaks correlated in the opposite direction with a longer duration of illness, and higher Cho concentrations were also consistently associated with more previous depressive episodes.

Selected VOIs were situated in a medial temporal region

Table 2: Bilateral hippocampal volumes and proportions of grey matter, white matter and cerebrospinal fluid of left and right medial temporal voxels of interest

Measure	Control, $n = 16$	First-episode depression, $n = 14$	Remitted-recurrent depression, $n = 18$	Treatment-resistant/chronic depression, $n = 20$
Hippocampal volume, mean (SD) mm^3				
Left	4055.1 (419.7)	4280.3 (482.4)	3924.2 (494.5)	3767.3 (460.5)*
Right	4157.9 (323.1)	4458.7 (427.8)	4045.9 (568.4)†	3858.3 (355.5)*
Tissue segmentation, %				
Left VOI				
Grey matter	70.2	73.2	74.8‡§	70.4
White matter	24.8	22.4	19.8‡§	25.1
Cerebrospinal fluid	5.1	4.8	5.4	4.5
Right VOI				
Grey matter	69.3	70.2	73.4	70.2
White matter	25.9	24.6	21.2‡§	25.0
Cerebrospinal fluid	4.9	5.2	5.4	4.8

SD = standard deviation; VOI = volumes of interest.
*Significant differences between first-episode and treatment-resistant/chronic depression (left $p = 0.004$; right $p < 0.001$).
†Significant differences between first-episode and remitted-recurrent depression ($p = 0.039$).
‡Significant differences between remitted-recurrent depression and controls (left VOI: grey matter $p = 0.018$, white matter $p = 0.014$; right VOI: white matter $p = 0.009$).
§Significant differences between remitted-recurrent and treatment-resistant/chronic depression (left VOI: grey matter $p = 0.011$, white matter $p = 0.003$; right VOI: white matter $p = 0.033$).

that mainly included the head of the hippocampus. Converging evidence from clinical and preclinical studies underlines the central role of this highly stress-sensitive brain structure on MDD. Volumetric hippocampal reductions have been replicated in depressed patients, and they are preferentially observed in patients with an earlier onset of illness, recurrent evolution or treatment refractoriness,[5] as confirmed by our present results.

On the other hand, scant literature on hippocampus neuropathological analyses of patients with MDD reveals increases in the mean densities of neurons and glia contrasting with reductions in neuronal soma size[27] and diminished levels of neurotrophic and astrocyte viability markers, which have been putatively related to dysfunctional adult hippocampal neurogenesis.[7] An earlier study observed neuronal loss in CA1 and CA4 regions, but the phenomenon, although convincing, was only moderate.[6] However, reductions in neuropil seem to be prominent[27] and were the most probable explanation for the cumulative hippocampal shrinkage.[28] Whatever the deficiencies in the cellular integrity and neuropil, these would be likely to underlie the abnormalities in spectroscopic resonances reported herein. It has to be stressed that metabolic alterations found in our patients were evident even when we controlled for differences in total hippocampal volumes and VOI tissue composition.

Milne and colleagues[14] did not find defects in Glx concentration in a sample of patients with recurrent MDD compared with healthy controls. In contrast, Michael and colleagues[12] reported lower Glx levels in the hippocampus/amygdala of severely ill patients with treatment-resistant depression compared with healthy controls; the levels recovered after successful electroconvulsive therapy. Diminished levels of Glx were also described in a sample mainly composed of untreated patients with a first depressive episode.[13]

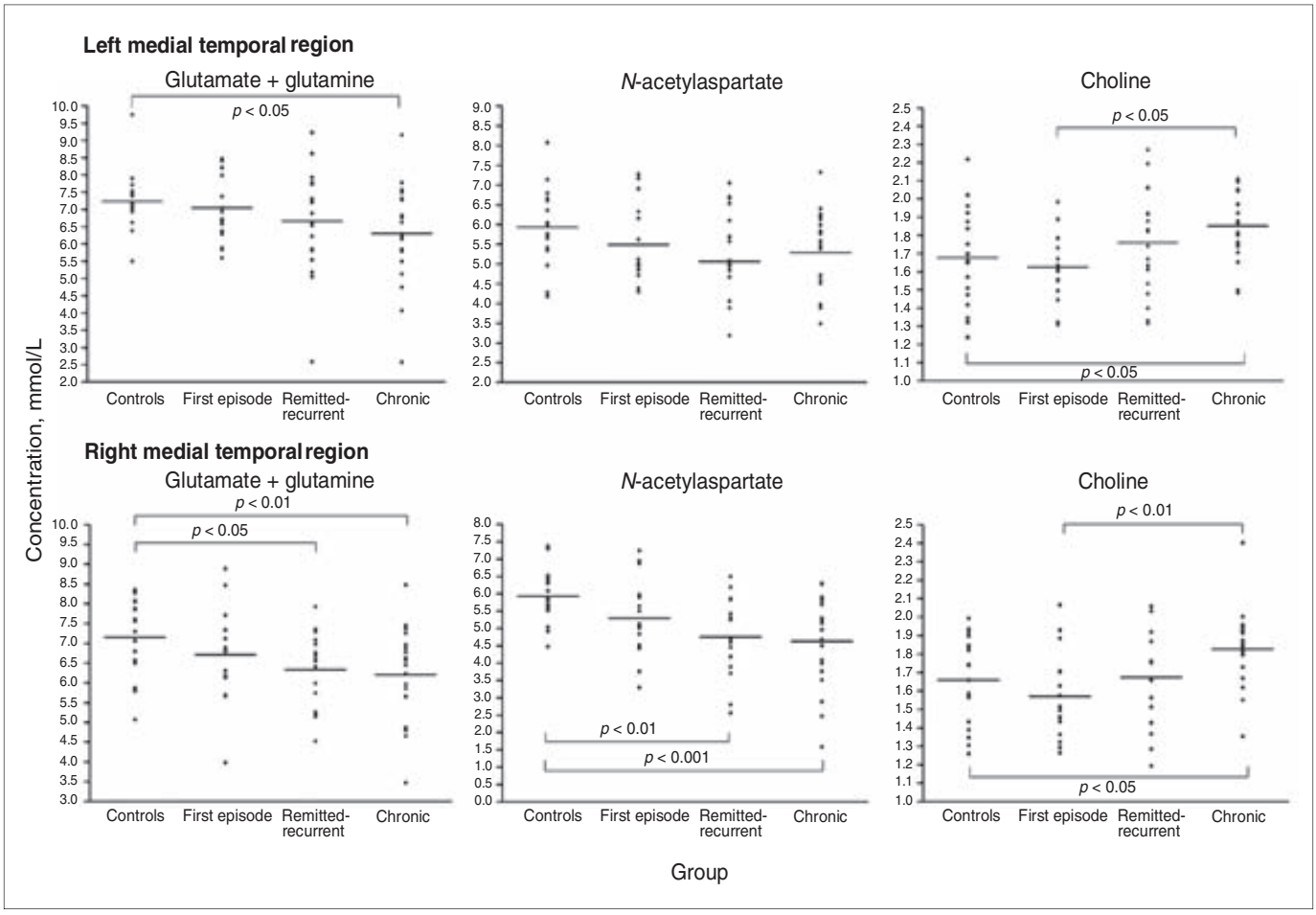

Fig. 2: Absolute metabolite concentrations (glutamate/glutamine [Glx], *N*-acetylaspartate [NAA] and choline [Cho]) in left and right medial temporal regions of healthy controls and patients with first-episode, remitted-recurrent or treatment-resistant/chronic depression. Group means are displayed as horizontal lines. The *p* values are derived from Bonferroni post hoc tests. (**Top**) The Glx levels in patients with treatment-resistant/chronic depression were 14% lower than those in controls ($p = 0.024$, Cohen $d = 0.81$). The Cho concentrations in patients with treatment-resistant/chronic depression were 19% higher than those in patients with a first episode ($p = 0.006$, Cohen $d = 1.89$) and 12% higher than those in controls ($p = 0.025$, Cohen $d = 0.80$). Differences of NAA did not reach statistical significance. (**Bottom**) The Glx levels in patients with treatment-resistant/chronic depression were 15% lower than those in controls ($p = 0.008$, Cohen $d = 1.04$), and their NAA levels were 21% lower than those in controls ($p < 0.001$, Cohen $d = 1.11$). The Glx levels in patients with remitted-recurrent depression were 14% lower than those in controls ($p = 0.025$, Cohen $d = 1.05$), and their NAA levels were 17% lower than those in controls ($p = 0.002$, Cohen $d = 1.04$). The Cho concentrations in patients with treatment-resistant/chronic depression were 13% higher than those in patients with a first episode ($p = 0.002$, Cohen $d = 0.9$) and 6% higher than those in controls ($p = 0.034$, Cohen $d = 0.4$).

It has been assumed that Glx signal reflects the status of glia and pyramidal glutamatergic neurons.[9] In the present study, the pattern of reduced Glx concentrations seen in the patients with treatment-resistant/chronic and remitted-recurrent depression was not apparent in patients presenting for a first episode, suggesting that long-lasting depressive history may have an important influence on the glial integrity and glutamatergic metabolism of the hippocampus. The presence of diminished Glx levels in patients with remitted-recurrent illness in our sample extends the previously reported findings, emphasizing the possibility that these abnormalities can remain within the hippocampus after resolution of a depressive episode.

A quite similar pattern of lower concentrations in chronic and recurrent forms of MDD was observed for NAA in our sample. *N*-acetylaspartate has been described as a sensitive marker of neuronal functionality/viability,[10] and defects on that signal could reflect damage or loss of neurons, reductions of interneuronal neuropil, neuronal or axonal metabolic dysfunction or some combination of these processes.[29] Reductions of NAA have been observed in patients with bipolar[30] and pediatric depression,[31] but there is no consistent agreement for altered NAA levels in the hippocampus in previous MRS studies of adult unipolar depression.[8] The lack of control for VOI tissue composition in some of these studies might represent a serious limitation, which we attempted to

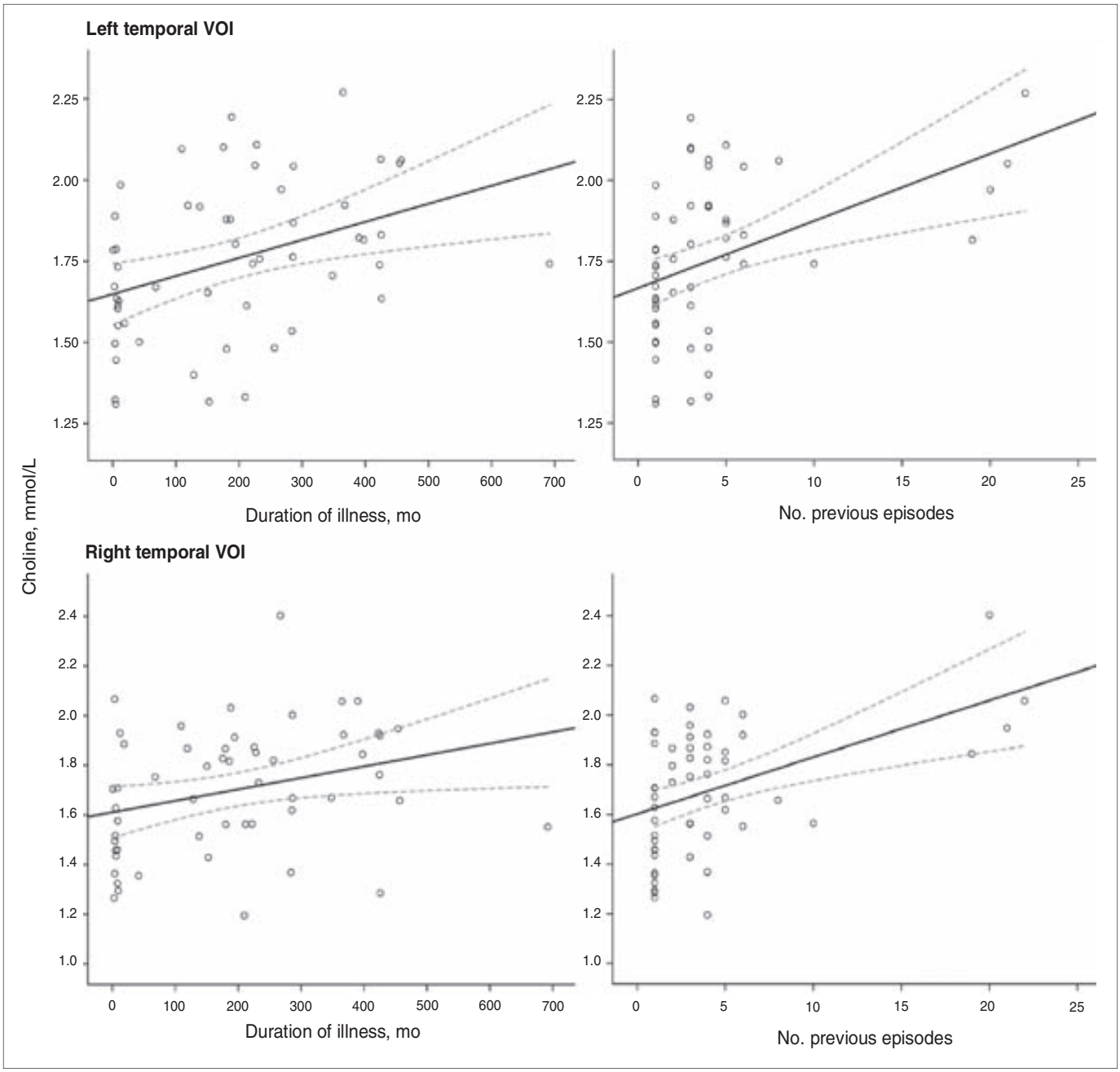

Fig. 3: Scatter plots showing correlation of choline levels within the left and right medial temporal regions of depressed patients (*n* = 52) with duration of illness and number of previous episodes. Bold and dotted lines represent the interpolation lines and their 95% confidence intervals, respectively. VOI = volumes of interest.

correct herein. At least 2 independent groups reported increases of NAA concentrations after successful response to either electroconvulsive or pharmacological therapy.[12,13] These findings suggest that antidepressant treatments could elicit neurotrophic effects, with a positive role in restoring neuronal integrity being therefore relevant for clinical recovery. Strikingly, 2 preclinical reports revealed decreased NAA concentrations associated with reductions of hippocampal volume and neurogenesis in chronically stressed animals, changes that were prevented with antidepressant treatment.[32,33] A recent postmortem study in which quantification of metabolites was performed directly on brain tissue reported NAA decrements in different subcortical structures, including the hippocampus and amygdala, in patients with long-term, severe MDD.[29] Although in the present study NAA did not correlate with any of the explored clinical variables, reduction of its levels in the right hippocampus was conspicuous, reaching up to 17% and 21% among patients with remitted-recurrent and treatment-resistant/chronic depression, respectively. These findings support the notion that hippocampal neuronal damage could be present in depression when the burden of illness is prominent, even in asymptomatic states.

The increased Cho signal seen bilaterally in patients with treatment-resistant/chronic depression compared with healthy controls and patients with first-episode depression represents one of the most intriguing findings. The Cho resonance mainly reflects changes in the concentrations of phosphocholine and glycerophosphocholine, a precursor and a degradation product, respectively, of membrane phospholipids. For this reason, it is considered a potential biomarker for the status of membrane metabolism.[11] Our results are in agreement with those of previous studies that found increased Cho/creatine ratios in a sample of patients with treatment-resistant MDD[15] and higher absolute Cho concentrations in patients with extensive past illness (but not in those with a first episode) than age-matched healthy controls.[14] We also describe a robust association between higher Cho levels and longer duration of illness and more recurrences. This association provides preliminary evidence that increases in Cho concentrations might reveal an augmented membrane turnover related to past illness burden.

In contrast with the treatment-resistant/chronic group, our sample of patients with a first depressive episode displayed the lowest concentrations of Cho. Block and colleagues[13] also reported marginally diminished baseline Cho peaks in untreated patients with a first episode of depression. Interestingly, they reported that low baseline Cho levels and a subsequent increase after 8 weeks of treatment were associated with good response. An earlier study[16] had already described lower Cho concentrations that increased after a successful course of electroconvulsive therapy in patients with current, severe depression. Therefore, low Cho levels represent a marker of good response to treatment, but increments of such levels seem to be necessary for recovery from depression. However, in light of our results, excessive levels of Cho would mark resistance to treatment. From a neurobiological perspective, increases of Cho would reflect changes in membrane turnover, precipitated by either antidepressant treatment or endogenous mechanisms, as expression of the brain's efforts to restore abnormal neural functioning. These changes, however, might end up being insufficient and even pathologically maintained in chronic stages of depression. In fact, it would be possible that increases in Cho peak occur at the expense of membrane precursors (as phosphocholine) during initial stages of treatment and at the expense of degradation products (as glycerophosphocholine) in patients with chronic depression that is unresponsive to treatment, although current MRS techniques do not allow this distinction to be made.

Abnormalities of Glx, NAA and Cho signal described herein within the hippocampus are in accordance with those reported in the vmPFC in a previous study of our group composed of patients in different stages of MDD.[18] Alterations in vmPFC cellular neurochemistry were also consistently related to illness-course variables. The well-established structural and functional connection between the hippocampus, parahippocampal gyrus and prefrontal areas — all strongly linked with the physiopathology of mood disorders[1] — may explain the similarity of metabolite alterations observed in our 2 studies. In fact, these areas, particularly the hippocampus, are known to play an important role in the response to stress, being extremely sensitive to its potential neurotoxic effects under chronic stress conditions. Glial and neuronal pathology and remodelling processes — as reflected by reductions of Glx and NAA and by increases in Cho, respectively — in both the vmPFC and medial temporal region of patients with MDD would then be considered a phenomenon associated with the burden of the disease.

Although other studies have reflected metabolite changes associated with treatment response or symptom severity, we did not obtain similar results. Our sample included a variety of patients, some of them entirely comparable in terms of age at onset of illness and illness duration but extremely different in terms of symptomatic state (treatment-resistant/chronic v. remitted-recurrent), who finally displayed notable similarities on metabolite abnormalities. This is a preliminary cross-sectional study designed to explore the role of past illness burden on brain metabolites, but we do not rule out the possibility of an association between metabolite resonances and symptoms from alternative approaches (e.g., analysis of specific groups of patients, longitudinal designs).

Limitations

There are some methodological limitations of this study that need to be mentioned. First, the hippocampus is a complex anatomic region that often entails spectral quality–related problems. However, MRS data were acquired using a 3 T magnetic resonance scanner, which provides a higher signal-to-noise ratio and better separation among metabolite peaks than commonly used 1.5 or 2 T scanners. Second, the present study was spatially limited to the anterior region of the hippocampus, and it remains to be clarified whether our results could be extrapolated to the whole structure. Nonetheless, we report data for both hemispheres in contrast to many previous works that have centred exclusively on the left temporal

region.[12–15] Third, a systematic bias in voxel placement/selection could have given rise to different tissue composition of the VOIs among the groups and accounted for differences in the measured spectra. To overcome this potential limitation, we controlled for VOI tissue segmentation in the main analyses. Fourth, the sample comprised a fairly large number of individuals, including healthy controls and a representative, well-characterized group of outpatients in different stages of illness, but the cross-sectional nature of the study should lead us to be cautious in interpreting the findings. Longitudinal studies are warranted to provide definitive evidence of the role of illness burden on brain metabolites in patients with MDD. Finally, the lack of a drug washout period represents an additional limitation. Given that the hypothesis to be tested required the inclusion of severely ill outpatients, there were inherent ethical challenges (e.g., patients' conditions could worsen without medication). Moreover a washout period that was not long enough might have yielded metabolic changes related more to psychotropic withdrawal than with the illness, per se. To address these challenges, we decided to maintain medication in all patients and recruited an age- and sex-matched group of patients treated for a first episode as well as a non-treated healthy control group. The presence of treatment did not entail substantial metabolic differences between these latter groups. But, as might have been expected, patients with treatment-resistant/chronic illness more often received combined treatments (more than 1 antidepressant and/or atypical antipsychotics). For their part, patients with remitted-recurrent or first-episode depression received an almost equivalent antidepressant regimen, although the former group had a longer history of treatment. Nevertheless, the direction of the metabolic changes observed in our sample fit in with previous studies based on medication-free patients.[12,13] Altogether, these comments suggest that the abnormalities described in the present study cannot be merely attributable to the treatment itself.

Conclusion

Our data support the notion that metabolite alterations within the hippocampus are more pronounced in patients with MDD whose clinical evolution is characterized by recurrences and/or chronicity. This adds further evidence to the link between hippocampal formation and the potential neurotoxic effects of stress and depression, suggesting that abnormalities in Glx, NAA and Cho spectra are closely related to the past burden of illness. Defects of glial/neuronal integrity and membrane turnover could underlie the metabolic changes reported herein, although further research is needed to establish the specific cellular processes involved in MDD over the course of the illness.

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Contributors: J. de Diego-Adeliño, M.J. Portella, B. Gómez-Ansón and V. Pérez designed the study and acquired and analyzed the data. O. López-Moruelo, M. Serra-Blasco, Y. Vives, D. Puigdemont, R. Pérez-Egea and E. Alvarez also acquired the data. J. de Diego-Adeliño and M.J. Portella wrote the article, which all authors reviewed and approved for publication.

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ORIGINAL ARTICLE

Microstructural white-matter abnormalities associated with treatment resistance, severity and duration of illness in major depression

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Background. Although white-matter abnormalities have been reported in middle-aged patients with major depressive disorder (MDD), few data are available on treatment-resistant MDD and the influence of relevant variables related to clinical burden of illness is far from being well established.

Method. The present study examined white-matter microstructure in a sample of 52 patients with MDD in different stages (treatment-resistant/chronic MDD, n=18; remitted-recurrent MDD, n=15; first-episode MDD, n=19) and 17 healthy controls, using diffusion tensor imaging with a tract-based spatial statistics approach. Groups were comparable in age and gender distribution, and results were corrected for familywise error (FWE) rate.

Results. Widespread significant reductions of fractional anisotropy (FA) – including the cingulum, corpus callosum, superior and inferior longitudinal fascicule – were evident in treatment-resistant/chronic MDD compared with first-episode MDD and controls (p<0.05, FWE-corrected). Decreased FA was observed within the ventromedial prefrontal region in treatment-resistant/chronic MDD even when compared with the remitted-recurrent MDD group (p<0.05, FWE-corrected). Longer duration of illness (β=–0.49, p=0.04) and higher depression severity (at a trend level: β=–0.26, p=0.06) predicted lower FA in linear multiple regression analysis at the whole-brain level. The number of previous episodes and severity of symptoms were significant predictors when focused on the ventromedial prefrontal area (β=−0.28, p=0.04; and β=−0.29, p=0.03, respectively). Medication effects were controlled for in the analyses and results remained unaltered.

Conclusions. Our findings support the notion that disruptions of white-matter microstructure, particularly in fronto-limbic networks, are associated with resistance to treatment and higher current and past burden of depression.

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Key words: Diffusion tensor imaging, fractional anisotropy, major depression.

Introduction

Recurrence and treatment refractoriness in major depressive disorder (MDD) represent two of the major challenges that mental health professionals have to face in their daily clinical practice. In the best of cases, less than a half of depressed patients will completely recover and will have no further episodes and about 15% of cases will present a chronic unremitting course, failing to respond to multiple treatment trials (Mueller *et al.* 1999; Eaton *et al.* 2008; Vuorilehto *et al.* 2009). Incomplete remission has been extensively associated with higher risk of relapse and higher rates of disability, psychosocial impairment, comorbidity, suicidality and medical costs (Trivedi *et al.* 2008; Fekadu *et al.* 2009).

Some brain abnormalities might predispose to relapse or to treatment failure in MDD whereas others might also accumulate over time and hinder full recovery. This neural substrate is far from being well established. Structural and functional neuroimaging studies have identified alterations located in fronto-limbic networks that seem to be critical for illness remission (Mayberg, 2003) and the ventromedial prefrontal cortex plays a crucial role in the neural circuitry of

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MDD (Johansen-Berg *et al.* 2008). White-matter tracts are key components of these networks and abnormalities in their microstructure can potentially be studied *in vivo* by means of diffusion tensor imaging (DTI), a magnetic resonance imaging (MRI) technique that is becoming increasingly popular. Fractional anisotropy (FA) quantifies how strongly directional the fibre tract organization is (Smith *et al.* 2006) and it is the most commonly used measure in the assessment of white-matter microstructure.

Prior DTI reports have described altered fronto-temporal FA values mostly in geriatric, but also in young and middle-aged population with MDD (for detailed reviews, see Sexton *et al.* 2009; Murphy & Frodl, 2011). However, most of them have focused on identifying differences between a single group of depressed patients *versus* healthy controls, and the study of the influence of relevant variables related to clinical burden of illness has received modest attention. Moreover, only a few have evaluated middle-aged patients with chronic or treatment-resistant depression (Zhou *et al.* 2011; Guo *et al.* 2012; Hoogenboom *et al.* 2012). Two prospective reports, with a completely different study design, examined baseline FA differences between depressed patients in which response to treatment was subsequently evaluated. Zhou *et al.* (2011), using a voxel-based morphometry approach, showed low baseline FA values within the hippocampal region among patients who developed a treatment-resistant depression. Hoogenboom *et al.* (2012), using a region of interest (ROI) analysis based on legacy data, revealed low baseline FA values in the medial fornix among MDD patients who failed to achieve remission. Guo *et al.* (2012), for their part, performed a cross-sectional DTI study in patients with treatment-resistant depression using tract-based spatial statistics (TBSS) methodology, a recent approach that increases the sensitivity and the interpretability of the results compared with conventional voxel-based approaches (Smith *et al.* 2006). These patients showed lower FA values than healthy controls in the right anterior limb of the internal capsule, the body of the corpus callosum and the bilateral external capsule, but the absence of a group of non-treatment-resistant patients precluded further interpretation of their findings.

In this study, we aimed to investigate whole-brain white-matter microstructure using TBSS in a sample of patients with treatment-resistant/chronic MDD as compared with patients with remitted-recurrent MDD, patients with a first-episode MDD and healthy control subjects. Given the prominent role of the ventromedial prefrontal cortex in treatment refractoriness (Johansen-Berg *et al.* 2008), an additional regional analysis centred on this area was carried out. We also examined the relationship between clinical variables and white-matter abnormalities, hypothesizing that FA values would be diminished in those patients with treatment-resistant/chronic depression and higher current and past illness burden (i.e. greater severity of symptoms, longer duration of illness, earlier age at onset and/or more previous episodes).

Method

Participants and assessments

A total of 52 right-handed adult patients with MDD [Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria] and 17 right-handed healthy controls were recruited to undergo a MRI protocol specifically designed for the present study. All patients were recruited from the out-patient clinic of the Department of Psychiatry of the University Hospital de la Santa Creu i Sant Pau. Since it is well known that age-related structural changes can occur in the brain, all participating individuals were similarly aged. Depressed participants were split into three different groups. The first group consisted of patients with chronic depressive disorder, whose last episode had a duration of more than 2 years with no response to multiple antidepressant strategies, a Thase–Rush index of treatment resistance $\geqslant 3$ and a score above 14 in the Hamilton Depression Rating Scale (HAMD) ($n=18$, 'treatment-resistant/chronic' group). The second comprised patients who had experienced three or more major depressive episodes and were currently euthymic (score <8 in the HAMD) for at least 6 months before the enrolment ($n=15$, 'remitted-recurrent' group). Euthymia in the 6 previous months was determined during the clinical interview and checked out with clinical records. The third group comprised currently depressed patients who were suffering from their first episode: ($n=19$, 'first-episode' group). The latter was representative of depressed patients with lower clinical burden of illness (i.e. no previous episodes, shorter illness duration and later age at onset). Enrolled healthy individuals received a small monetary compensation for their participation. To satisfy the inclusion criteria, controls had to have no history of psychiatric diagnoses, no first-degree relatives with psychiatric diagnoses and no clinically significant physical or neurological illnesses. The exclusion criteria for all subjects included: mental retardation, history of head injury, neurological illness, clinically significant physical illness, and other psychiatric disorders such as bipolar disorder, schizophrenia, any other psychotic disorder, or alcohol and other substance abuse/dependence. Presence of co-morbid anxiety symptoms did not

constitute an exclusion criterion if MDD was the primary diagnosis.

Semi-structured interviews were carried out for all participants to collect demographics and clinical information according to DSM-IV text revision (DSM-IV-TR) criteria. Current depressive symptoms were assessed using the HAMD. We estimated a composite measure of medication load for each patient according to a previously established method (Sackeim, 2001; Almeida *et al.* 2009), attempting to control for the effects of psychotropic drugs. In brief, doses of each antidepressant, mood stabilizer, antipsychotic and anxiolytic medication were coded as 0=absent, 1=low or 2=high, following the rating system described by Sackeim (2001) and adapted by Almeida *et al.* (2009); a composite measure of medication load for each patient was obtained by summing all individual medication codes for each medication category.

The study was approved by the Research Ethics Committee of the Hospital de la Santa Creu i Sant Pau and was carried out in accordance with the Declaration of Helsinki. Written consent was obtained from each participant. This work is part of a wider project that aims to investigate *in vivo* neuroimaging markers of the clinical burden of depression.

MRI acquisition

All brain MRI data were acquired on a 3 T Philips Achieva MR Scanner (software version 2.1.3.2; Philips Healthcare, The Netherlands) with an eight receive-channel head-coil at the Department of Neuroradiology, Hospital de la Santa Creu i Sant Pau, Barcelona. Acquisition parameters for DTI data were the following: sensitivity-encoded (SENSE) single-shot echo-planar imaging, SENSE factor of 2, repetition time (TR)=8166 ms, echo time (TE)=60 ms, slice thickness=2 mm, field of view (FOV)=224×224×120 mm, reconstruction matrix=128×128 with 60 contiguous axial slices, voxel dimensions=1.75×1.75×2 mm. A diffusion sensitizing gradient was applied along 15 directions (b-value=800 s/mm^2) and one volume without diffusion weighting (b=0 s/mm^2, b0). For each subject, high-resolution three-dimensional magnetization-prepared rapid gradient-echo imaging (3D-MPRAGE) images were also acquired (whole-brain coverage; TR=6.7 ms, TE=3.2 ms, 170 slices, voxel size=0.89×0.89×1.2 mm, reconstruction matrix =288×288×170; FOV=256×256×204 mm, slice thickness=1.2 mm), with a sagittal slice orientation, T1 contrast enhancement, flip angle 8°, grey matter as a reference tissue, acquisition matrix (ACQ matrix M×P)=256×240 and turbo-field echo (TFE) shots=218.

Image processing

DTI data processing was performed at the Port d'Informació Científica (PIC) in Barcelona through the so-called PICNIC (PIC NeuroImaging Center) platform with version 4.1.4 of the Functional MRI of the Brain (FMRIB) Software Library (FSL) (Smith *et al.* 2004). An experienced neuroradiologist (B.G.-A.) and a brain neuroimaging researcher (P.P.) conducted visual inspection of all DTI image data to ensure quality. Unweighted b0 images were extracted and used for head motion correction by an affine registration to the same subjects' weighted images, using Eddy Current Correction of the FMRIB Diffusion Toolbox (FDT). A brain extraction tool (Smith *et al.* 2002) with a fractional intensity threshold of f=0.3 was used to create a binary brain mask for further analyses. FA maps were generated by fitting a diffusion tensor for each voxel using FDT DTIFIT. Voxel-wise statistical analysis of the FA data was performed with the TBSS package (Smith *et al.* 2006) implemented in FSL. First, a non-linear registration was used in order to align all participants' FA images into a standard space (FMRIB58-FA) through the FMRIB's Non-Linear Image Registration Tool (FNIRT). The mean FA image was created and thinned (0.2 threshold value) to obtain the mean FA skeleton that represents the centres of all tracts common to the group. Each individual's FA map was projected onto the threshold mean FA skeleton and these data were entered into voxel-wise statistics. In addition, a ROI was defined using the medial frontal cortex mask provided by FSL within the TBSS-generated FA skeleton overlaid on the mean FA map. To determine approximately which white-matter tracts showed significant differences, two different atlases were used: an MRI atlas of human white matter (Oishi *et al.* 2011) and the JHU White-Matter Tractography Atlas included in the FSL program. Additionally, high-resolution 3D-MPRAGE images were segmented into grey matter, white matter and cerebrospinal fluid using the standard segmentation model in SPM8 (http://www.fil.ion.ucl.ac.uk/spm).

Statistical analysis

Demographic, clinical and brain-tissue segmentation analyses were performed with the statistical package SPSS version 18 (IBM, USA) using analyses of variance (ANOVAs) and the χ^2 test for quantitative and categorical variables, respectively. The level of statistical significance was set at $p<0.05$. One-way ANOVA was performed to detect differences among groups in mean FA values of whole-brain and ventromedial prefrontal ROIs. FA data were also analysed using voxel-wise statistics by the FSL RANDOMISE procedure

Table 1. *Demographics, clinical variables and brain volumetric data*

	Healthy controls (n=17)	First-episode MDD (n=19)	Remitted-recurrent MDD (n=15)	Treatment-resistant/chronic MDD (n=18)	F or χ^2	p
Mean age, years (S.D.)	43.4 (11.4)	44.2 (6.9)	47 (9.4)	48.5 (7.3)	1.3	0.3
Gender, n					6.6	0.1
Male	5	8	1	3		
Female	12	11	14	15		
Mean duration of education, years (S.D.)	14.1 (2.9)	14.5 (2.4)	13.4 (2.9)	13.4 (3.4)	0.7	0.6
Mean HAMD (S.D.)	1.9 (1.7)*†	14.6 (7.5)	2.1 (0.9)*†	20.8 (4.9)	64.8	<0.001
Mean age at onset, years (S.D.)	N.A.	43.6 (6.8)	26.4 (10.0)*	26.1 (8.4)*	25.8	<0.001
Mean duration of illness, months (S.D.)	N.A.	4.9 (2.88)	238.1 (133.2)*	272.7 (125.8)*	36.9	<0.001
Mean no. of previous episodes (S.D.)	N.A.	1 (0)	5.1 (3.6)*	7.1 (7.2)*	10.7	<0.001
Treatment,% SSRI or SSNRI	N.A.	100	73.3	83.3	5.4	0.07
TCA or MAOI	N.A.	5.3	13.3	33.3	5.3	0.07
Others[a]	N.A.	5.9	20†	55.6*	12.4	0.002
Combination[b]	N.A.	10.5	20*†	77.8*	20.5	<0.001
No antidepressant	N.A.	0	13.3	0	5.1	0.08
Stabilizer[c]	N.A.	5.3	13.3	33.3	5.3	0.07
Antipsychotic[d]	N.A.	10.5	13.3†	44.4*	7.1	0.03
Benzodiazepine	N.A.	52.6	33.3	50	1.4	0.5
Mean medication load (S.D.)	N.A.	2.7 (1.64)	2.8 (1.70)†	4.9 (1.59)*	11.9	<0.001
Structural volumetric data						
Mean grey matter, cm^3 (S.D.)	482.9 (61.1)	491.8 (51.9)	481.7 (79.8)	458.6 (57.8)	0.9	0.4
Mean white matter, cm^3 (S.D.)	425.1 (65.9)	442.3 (48.9)	437.6 (73.3)	410.6 (49)	1.0	0.4
Mean CSF, cm^3 (S.D.)	211.3 (33.2)	220.1 (33.9)	215.7 (39.3)	215.6 (33.4)	0.2	0.9
Mean TIV, cm^3 (S.D.)	1119.4 (154.4)	1154.1 (127.3)	1134.5 (186.6)	1084.8 (129.7)	0.7	0.6

MDD, Major depressive disorder; S.D., standard deviation; N.A., not applicable; HAMD, Hamilton Depression Rating Scale; SSRI, selective serotonin reuptake inhibitors; SSNRI, selective serotonin and noradrenaline reuptake inhibitors; TCA, tricyclic antidepressant; MAOI, monoamine oxidase inhibitors; CSF, cerebrospinal fluid; TIV, total intracranial volume.

[a] 'Others' includes noradrenaline reuptake inhibitors, noradrenaline and dopamine reuptake inhibitors, tetracyclic antidepressants, Mirtazapine, Metilfenidate or Trazodone.

[b] Combination indicates concomitant use of antidepressants with different mechanisms of action (e.g. SSRI with reboxetine).

[c] Stabilizer includes anticonvulsants and mostly lithium.

[d] Antipsychotic includes mainly atypical antipsychotics associated with antidepressants.

* Value was significantly different from that of the first-episode patients (p<0.05).

† Value was significantly different from that of the treatment-resistant/chronic patients (p<0.05).

included in FSL 4.1.4 (Nichols & Holmes, 2002), with 5000 permutations. One-way ANOVA was used to detect group differences in FA across the skeletonized brain maps. Correction for multiple comparisons was carried out with a familywise error (FWE) rate at a p<0.05 threshold, after threshold-free cluster enhancement (TFCE). Likewise, the same process was applied for the ROI analysis by projecting each subject's aligned FA data onto the ventromedial prefrontal region mask at a p<0.05 threshold significance (FWE) after TFCE. Finally, backwards stepwise multiple regression analyses were computed across the three groups of patients to identify clinical variables that were independently associated with white-matter microstructure.

Results

Demographic and clinical data

Demographics, clinical variables and brain volumetric data are summarized in Table 1. Healthy controls and patients were comparable in age, gender and years of education. According to the design of the study, there were expected significant differences among distinct MDD groups relative to relevant clinical variables such as severity at recruitment (see HAMD scores), duration of illness, number of episodes or age at illness onset. The remitted-recurrent and treatment-resistant/chronic groups were comparable in terms of illness duration and age at debut but totally opposed in terms of symptomatic state. Patients with a

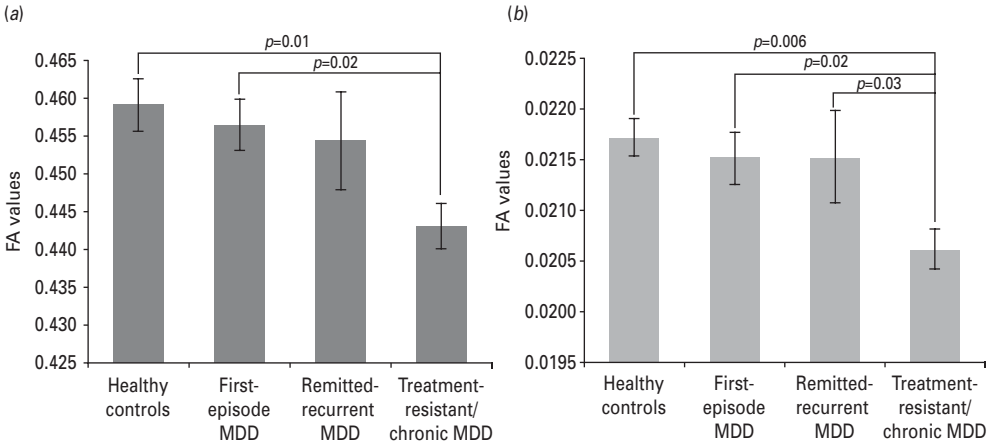

Fig. 1. Whole-brain (*a*) and ventromedial prefrontal region of interest (*b*) values of fractional anisotropy (FA) for healthy control and major depressive disorder (MDD) groups. Values are means, with standard deviations represented by vertical bars.

first episode, for their part, showed intermediate HAMD scores, lesser duration of illness and older age at onset than the other patients, consistent with a low clinical burden profile. Information regarding current medication is also presented in Table 1. Treatment- resistant/chronic patients received more second-line antidepressants and combined regimens with stabilizers, antipsychotics or other antidepressants, resulting in a higher medication load. No significant differences at this level were found between first-episode and remitted-recurrent groups, although medication exposure in the latter was obviously longer than in first-episode patients. On the other hand, there were no significant differences among groups in any of the whole-brain or prefrontal ROI volumetric measurements (data not shown for the latter).

Differences in whole-brain FA

Whole-brain mean FA values of patients and controls are displayed in Fig. 1*a*. The one-way ANOVA showed a significant main effect of group ($F_{3,65}$=3.14, p=0.03). This effect was maintained when medication load was included as a covariate ($F_{3,72}$=3.64, p=0.016). *Post-hoc* comparisons showed that treatment-resistant/chronic patients exhibited a significant decrease of mean FA values compared with healthy controls (p=0.01) and with first-episode patients (p=0.02). Voxel-wise whole-brain analyses revealed a generalized significant reduction in FA in treatment-resistant/chronic patients compared with healthy controls (p<0.05, FWE-corrected; cluster size: 30176 mm^2), mostly affecting the following white-matter tracts: bilateral inferior fronto-occipital fasciculus, bilateral inferior longitudinal fasciculus (ILF), bilateral superior longitudinal fasciculus (SLF), forceps major and forceps minor, body of corpus callosum and bilateral cingulum (see Fig. 2*a*). A significant decrease in FA was also observed in treatment-resistant chronic patients compared with first-episode patients (p<0.05, FWE-corrected; cluster size: 10458 mm^2), affecting the body of the corpus callosum, bilateral SLF, forceps minor, forceps major, bilateral cingulum, and bilateral ILF (see Fig. 2*b*).

Differences in regional FA (ventromedial prefrontal cortex)

Mean FA values within medial prefrontal ROI are presented in Fig. 1*b*. The one-way ANOVA showed a significant group effect ($F_{3,65}$=3.28, p=0.03), which was maintained when medication load was controlled for ($F_{3,64}$=2.97, p=0.04). *Post-hoc* tests showed that treatment-resistant/chronic MDD patients had significantly lower FA values than healthy controls (p=0.006), first-episode MDD (p=0.02) and also remitted-recurrent MDD patients (p=0.03). As can be observed in Fig. 3, voxel-wise analyses confirmed a significant decrease of FA in treatment-resistant chronic patients compared with healthy controls and with the other patients (p<0.05, FWE-corrected; cluster size: 164 mm^2). Subregions of the uncinate fasciculus and corpus callosum were affected within this area.

Influence of clinical variables on FA

HAMD scores, duration of illness, age at onset, number of previous episodes and medication load were entered in the stepwise multiple regression models. Duration of illness was the unique predictor of whole-brain mean FA, with a significant negative linear relationship (β=–0.49, p=0.04). HAMD scores showed a marginal significant effect (β=–0.26, p=0.06). The regression equation accounted for 15% of variance (R^2=0.15, F=2.83, p=0.05). Within ventromedial

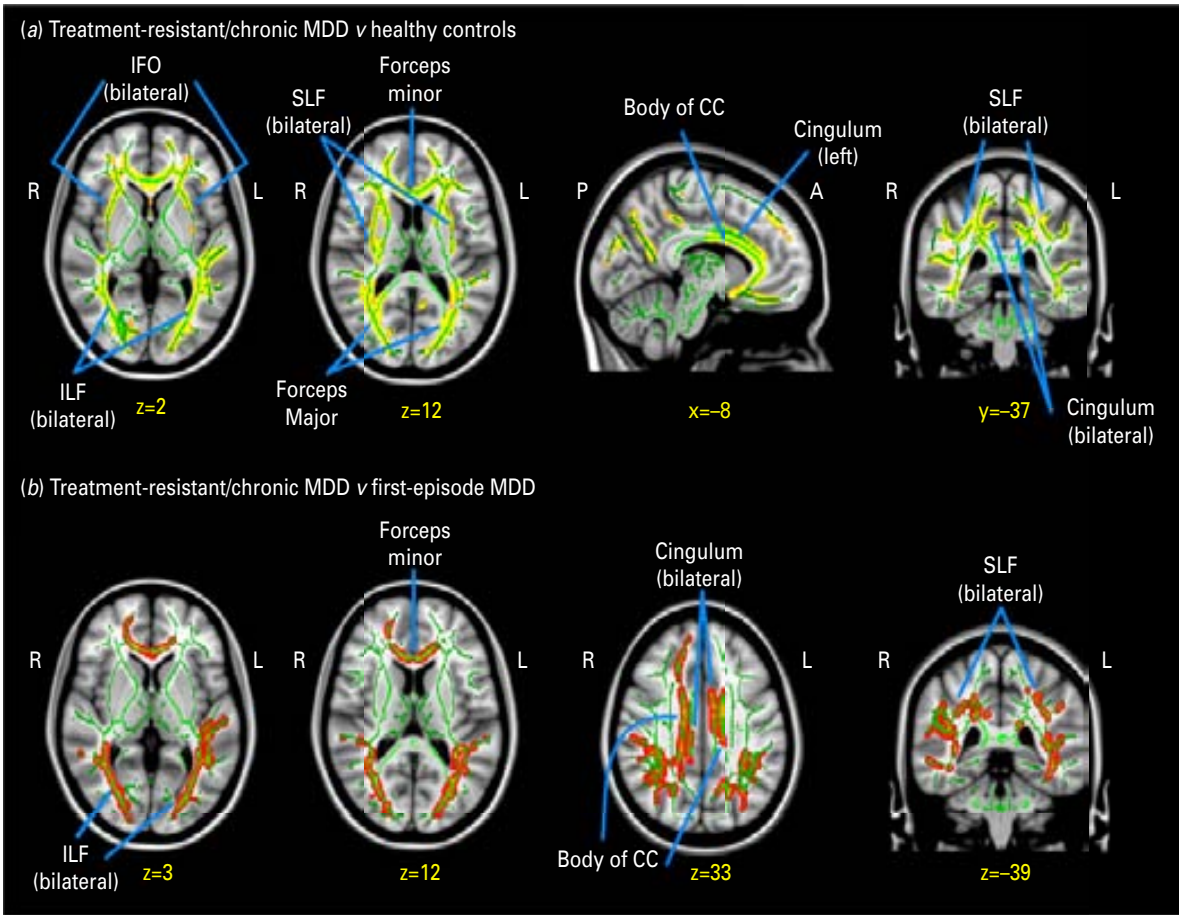

Fig. 2. Diffusion tensor imaging voxel-wise analysis of whole brains. Rows show selected axial, sagittal and coronal slices in which tracts with a significant decrease of fractional anisotropy (FA) is observed for healthy controls *versus* treatment-resistant/chronic major depressive disorder (MDD) patients (*a*) and for first-episode MDD patients *versus* treatment-resistant/chronic MDD patients (*b*). The background image is the Montreal Neurological Institute (MNI152) standard template 1×1×1 mm brain template (MNI coordinates). Green voxels represent the FA white-matter skeleton. Red voxels represent regions in which FA was significantly decreased. IFO, Inferior frontal-occipital; R, right; L, left; ILF, inferior longitudinal fasciculus; CC, corpus callosum; SLF, superior longitudinal fasciculus.

prefrontal ROI, HAMD scores (β=–0.29, p=0.03) and number of previous episodes (β=–0.28, p=0.04) were significant predictors of FA values, showing again a negative linear relationship; the regression model explained 12% of variance (R^2=0.12, F=3.17, p=0.05). No other clinical variable contributed significantly to the prediction of FA values in any of the regression models.

Discussion

We have observed widespread alterations in white-matter microstructure of patients with treatment-resistant/chronic MDD when compared with patients with a first-episode MDD and with healthy controls. Moreover, longer duration of illness and higher severity (this latter at a trend level) were associated with greater white-matter disruptions. Focusing on the ventromedial prefrontal region, the treatment-resistant/chronic group showed multiple affected fibres with significant FA reductions even when compared with patients with remitted-recurrent MDD. In this region, more previous episodes and higher severity of symptoms predicted white-matter abnormalities. Interestingly, findings remained unchanged after controlling for medication status. To our knowledge, this is the first DTI study that has investigated white-matter microstructure in different stages of non-geriatric MDD using TBSS methodology. Our results extend previous findings showing that microstructural white-matter anomalies may be present in middle-aged subjects with MDD but these are particularly heightened in patients with a chronic course and higher current and past burden of illness.

The reported abnormal FA values in patients with treatment-resistant/chronic depression involved multiple white-matter tracts. Among them, the SLF, cingulum, corpus callosum (including body, forceps

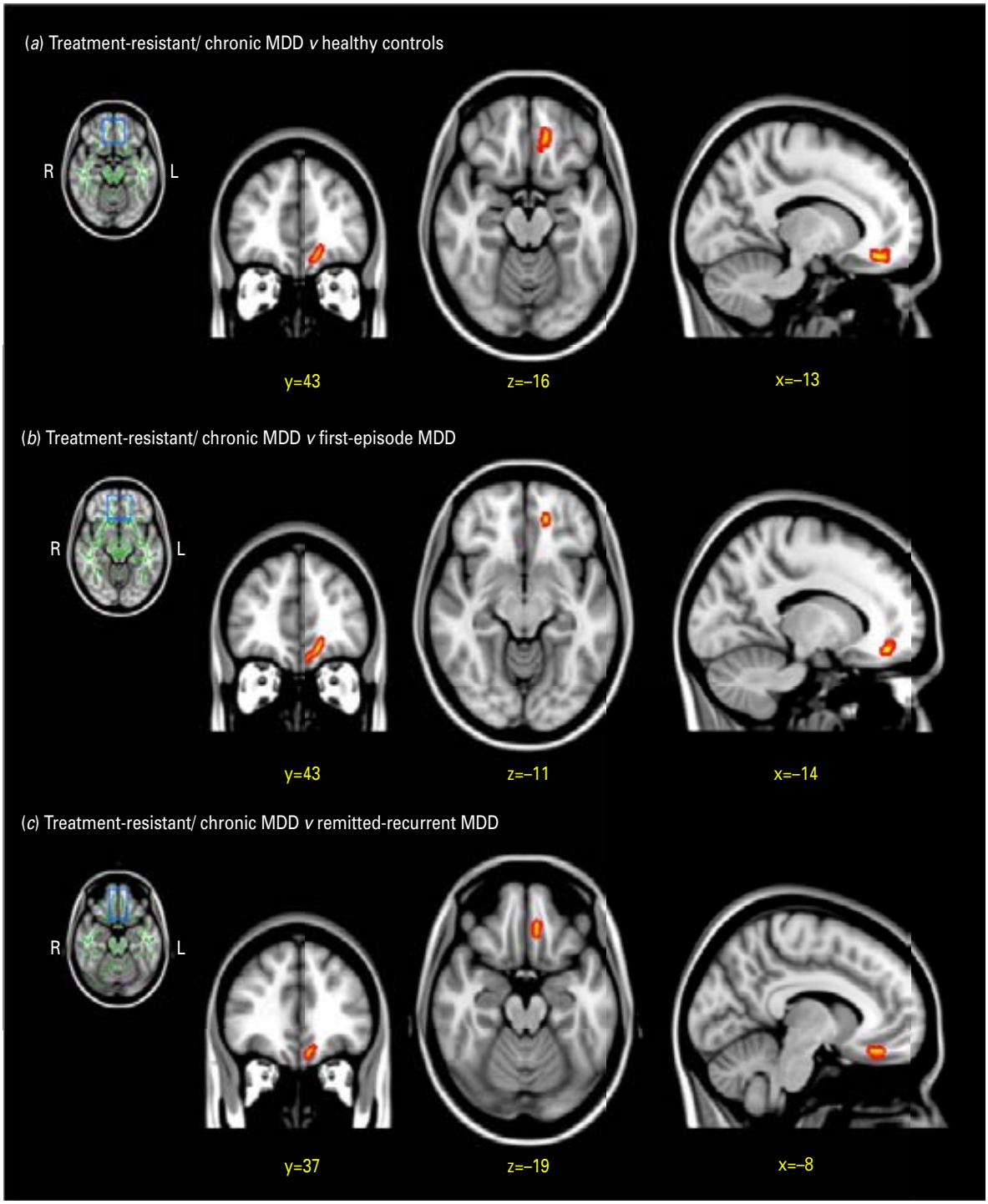

Fig. 3. Diffusion tensor imaging voxel-wise analysis of the ventromedial prefrontal cortex (MFC). Blue squares indicate the place of the MFC mask provided by FSL (Smith *et al.* 2004) (left side). Red blobs correspond to significant reduced fractional anisotropy values ($p<0.05$, family-wise error-corrected) in treatment-resistant/chronic major depressive disorder (MDD) patients compared with: (*a*) healthy controls (cluster size: 164 mm^2), (*b*) first-episode MDD patients (cluster size: 116 mm^2) and (*c*) remitted-recurrent MDD patients (cluster size: 74 mm^2). Images represent coronal, axial and sagittal views, respectively. R, Right; L, left.

major and forceps minor) and ILF deserve special attention given that significant differences within these tracts were found in comparison with both healthy controls and patients with a first-episode MDD. The SLF is a big bundle of association fibres that connects the dorsolateral prefrontal cortex and other frontal regions with the temporal, parietal and occipital lobes (Schmahmann & Pandya, 2006). The cingulum bundle lies within the cingulate gyrus and is an important association pathway linking prefrontal

and parahippocampal cortices (Schmahmann & Pandya, 2006). Therefore, both of these tracts constitute key components of the fronto-temporal and fronto-limbic connections whose dysfunction is thought to underlie many of the emotional, cognitive and behavioural deficits associated with depression (Mayberg *et al.* 1999). On the other hand, diverse alterations in the shape and size of the corpus callosum – the largest interhemispheric bundle of the human brain – have been previously (although not consistently) described in depression (Walterfang *et al.* 2009; Sun *et al.* 2009; MacMaster *et al.* 2013). The ILF is an association fibre tract that connects the occipital and temporal lobes, including the hippocampus and amygdala (Schmahmann & Pandya, 2006), and could be related to emotion and visual processing information deficits of depression (Leyman *et al.* 2007; Desseilles *et al.* 2009). Significant white-matter FA reductions observed herein along all these tracts agree well with preliminary DTI studies based on middle-aged patients with treatment-resistant depression (Zhou *et al.* 2011; Hoogenboom *et al.* 2012; Guo *et al.* 2012). Cole *et al.* (2012) have also reported extensive white-matter alterations heightened with increasing severity of symptoms in patients with long-lasting recurrent MDD. Our findings strengthen the view that a generalized altered neurocircuitry is present in MDD, especially in the most chronic and severe treatment-resistant forms of depression.

Longer duration of illness was predictive of greater white-matter microstructural abnormalities. Abe *et al.* (2010) observed a negative correlation between total days depressed and FA values in the right anterior cingulate and left frontal cortex. However, other DTI studies have failed to detect a relationship with duration of illness (Ma *et al.* 2007; Zou *et al.* 2008; Zhu *et al.* 2011; Guo *et al.* 2012). This discrepancy could be due in part to differences in sample characteristics, such as low number of subjects or limited subsets of patients (including only those with either low or high past burden of illness). Previous evidence linking lifetime duration of illness with structural brain changes in depression is predominantly based on grey-matter volume data, in particular of the hippocampus and fronto-limbic regions (Frodl *et al.* 2008; McKinnon *et al.* 2009; Bora *et al.* 2012). Although the cross-sectional study design demands a cautious interpretation, our findings expand these observations to white-matter microstructure, warning of further potential risks associated with long-term depression.

Defects observed within the ventromedial prefrontal cortex were provocative, since this brain region is known to play a crucial role on refractoriness to antidepressant treatment. Patients with treatment-resistant/chronic MDD showed abnormalities in subregions of the uncinate fasciculus and corpus callosum compared with the rest of the subjects, including patients with a remitted-recurrent MDD. These two groups shared a similar past clinical background except in the response to antidepressants. Consistently, current severity of symptoms as measured by the HAMD predicted lower FA values within the ventromedial region. This observation complements a previous study in which another altered measure of the uncinate fasciculus microstructure was associated with greater severity in patients with MDD (Zhang *et al.* 2012). The uncinate fasciculus connects the medial prefrontal cortex, including the subgenual region, with temporo-limbic structures crucial for mood disorders, such as the amygdala or hippocampus (Schmahmann & Pandya, 2006). Modulation of neural activity in this distributed fronto-limbic network has been suggested to underlie the promising therapeutic effects of deep brain stimulation in the subgenual area of patients with severe forms of chronic treatment-resistant depression (Johansen-Berg *et al.* 2008). Our findings bring up the question whether deep brain stimulation achieves its effects by resolving or bypassing the white-matter anomalies observed in this subset of patients. Number of previous episodes was also associated with FA reductions within the ventromedial prefrontal area, which, taken as a whole, suggests that white-matter microstructure of this area may be related to recurrence and persistence of depression.

Changes in FA could represent multiple anatomopathological processes, such as changes in axonal density or axonal diameter, abnormal myelination or altered coherence of the fibre tracts (Sexton *et al.* 2009). The few post-mortem studies that have focused on middle-aged patients support an increased prevalence of deep white-matter lesions in unipolar depression (for a review, see Tham *et al.* 2011). In particular, they have reported decreases in oligodendrocyte density (Uranova *et al.* 2004) and in deep white-matter myelin staining intensity within prefrontal areas (Regenold *et al.* 2007). Additional indirect evidence of white-matter damage in MDD come from nuclear magnetic spectroscopic studies that have described abnormal decreased *N*-acetylaspartate and increased choline-containing compound levels in the prefrontal cortex and hippocampus, particularly in patients with treatment-resistant/chronic depression and high past illness burden (Portella *et al.* 2011; de Diego-Adeliño *et al.* 2013). These alterations in *N*-acetylaspartate and choline signals presumably reflect neuronal/axonal integrity and aberrant cell membrane turnover, respectively (Wijtenburg *et al.* 2013).

Contrary to some prior studies (Ma *et al.* 2007; Zhu *et al.* 2011), we did not detect any significant FA difference between patients with a first-episode MDD and healthy controls. These patients had started medication shortly before the scanning; hence, the likelihood that restorative effects of antidepressant treatment could have already emerged seems to be low. On the other hand, current analyses were specifically designed to test differences between patients with a low and high illness burden. We performed an ANOVA with four groups (rather than simply two) and applied a significance threshold based on FWE correction, the most rigorous method for avoiding type I errors and probably the one that unequivocally reflects true population-level differences (Lieberman & Cunningham, 2009). Therefore, we cannot rule out the existence of white-matter anomalies in the first stages of MDD, but, in light of our results, these could be more subtle and perhaps limited to those patients who will subsequently develop a treatment-resistant disorder. In this regard, Zhou *et al.* (2011) reported an association between low baseline FA values in the hippocampus and poor subsequent antidepressant response in a sample of non-geriatric patients.

Limitations

Several methodological issues of the present study deserve a comment. We employed an established protocol for the Philips 3 T scanner with a SENSE factor of 2, which provides a good balance between scan time and signal-to-noise ratio. Nevertheless, according to the current state of the art (e.g. Jones *et al.* 2013), there are now better acquisition parameters to ensure improved image quality for DTI research. For example, current protocols recommend a minimum of 20 gradient directions, b-values of at least 1000 s/mm^2 or higher numbers of b=0 volumes. The 'crossing fibre problem' is an acknowledged concern in DTI research (Jbabdi *et al.* 2010). Some of the observed differences in FA among groups may actually correspond to differences in the number of crossing fibres. With regard to the analyses of data, we applied a TBSS approach using FWE correction and TFCE, which confers a meaningful advantage in ensuring accuracy and robustness of the findings. The sample was large and included a representative, well-characterized group of MDD outpatients, covering a broad spectrum of clinical illness burden, although our conclusions should be considered in the context of a cross-sectional study design. All patients were medicated at the time of scanning, which could represent a major limitation of the study. The inclusion of severely chronic ill patients prevented us from establishing a drug wash-out period because of obvious ethical concerns. The effects of treatment on white-matter microstructure have not been well established yet; several studies have not reported a clear influence (McIntosh *et al.* 2008; Wang *et al.* 2008; Sussmann *et al.* 2009) but some evidence suggests that medication might even attenuate FA abnormalities in certain patients (Yoo *et al.* 2007; Versace *et al.* 2008). Even though, we included an established index of medication load (Sackeim, 2001; Almeida *et al.* 2009) in the analyses to control for the potential confounding effects, and the results remained unaltered.

Conclusions

In summary, the present study aimed to investigate microstructural white-matter differences between patients with MDD in distinct stages of the illness and healthy controls, applying TBSS on DTI data. Our findings showed a generalized decrease of FA in patients with the poorest clinical response and the highest current and past illness burden. Longer duration of illness predicted lower FA at the whole-brain level whereas more previous episodes and greater severity of the symptoms were the negative predictors when focused on the ventromedial prefrontal area. These observations, in sum, support the notion that a disruption in cortical–subcortical networks, particularly the fronto-limbic connection, is associated with persistence of symptoms and treatment refractoriness. Whether abnormalities in white-matter microstructure are actually cumulative over time because of prolonged exposure to depression should be confirmed with longitudinal studies.

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Declaration of Interest

V.P. declares having received educational honoraria from: Servier, Lundbeck, Bristol-Myers, Pfizer, AstraZeneca and Eli Lilly, and he has participated as main local investigator in clinical trials for AstraZeneca, Eli Lilly and Bristol-Myers. E.Á. has received consulting and educational honoraria from several pharmaceutical companies including Servier, Eli Lilly, Lundbeck and Pfizer, and he has participated as main local investigator in clinical trials for Eli Lilly, Bristol-Myers and also as national coordinator of clinical trials for Servier and Lundbeck.

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DISCUSIÓN

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Aunque la investigación de los metabolitos relacionados con glutamato y otras alteraciones de la neuroquímica celular mediante MRS había despertado un gran interés en los últimos años en el campo de los trastornos afectivos, la mayor parte de los estudios apenas habían prestado atención a la influencia del curso evolutivo, incluyendo la historia de recurrencias o la presencia de refractariedad al tratamiento. El primer trabajo de esta tesis representa la primera comparación publicada de las concentraciones de metabolitos en CPF entre pacientes con depresión en diversos estadios de la enfermedad. De acuerdo a nuestras hipótesis iniciales, en él se demostraba que los pacientes con depresión y una mayor carga de enfermedad pasada, ya fueran pacientes con depresión crónica y resistente al tratamiento o pacientes con larga historia de recurrencias depresivas en fase de remisión, presentaban menores concentraciones de Glx y NAA en la CPFvm con respecto a controles sanos y a pacientes con un primer episodio depresivo. Cabe destacar que, en el caso de los niveles de Glx, la diferencia era de una notable magnitud, puesto que entre los pacientes con depresión crónica resistente al tratamiento las concentraciones eran hasta un 25 y 28% menores que las de los pacientes que presentaban su primer episodio y las de los controles sanos, respectivamente. El patrón con respecto a las concentraciones de Cho era exactamente el opuesto, con los niveles más elevados entre los pacientes con depresión crónica resistente al tratamiento y los niveles más bajos entre los sujetos sanos y los pacientes con su primer episodio depresivo. De forma consistente, las variaciones en la señal de Glx y Cho correlacionaban significativamente (cada una en direcciones opuestas) con los años de duración de la enfermedad, sin relación aparente con el estado sintomático en el momento de la exploración. Los niveles de NAA también se relacionaban con la duración de la enfermedad, aunque en ese caso análisis estadísticos adicionales señalaban una relación más robusta con la

edad de debut de la enfermedad, de modo que la depresión aparecía antes entre aquellos con menores concentraciones de NAA en la CPFvm.

En estrecha congruencia con estos hallazgos, los resultados del segundo trabajo demostraban alteraciones de los mismos metabolitos en hipocampo también asociadas a la carga clínica de enfermedad. Los pacientes con depresión de larga evolución cuya enfermedad se caracterizaba por un curso crónico resistente al tratamiento o bien por múltiples recurrencias previas aun estando en remisión, presentaban niveles disminuidos de Glx y NAA en hipocampo con respecto a controles sanos, especialmente en hemisferio derecho. Por el contrario, los pacientes con depresión y un curso evolutivo más benigno (i.e. sin episodios previos, con edad debut más tardía y una menor duración total de enfermedad) no mostraban diferencias significativas con los sujetos sanos, mientras que las concentraciones de Cho en ambos hipocampos eran significativamente menores en esos pacientes con un primer episodio y en los controles comparados con los pacientes con depresión crónica y resistente al tratamiento. Asimismo, también aquí los valores de Glx y Cho correlacionaban con el tiempo de evolución de la enfermedad. Además, mayores concentraciones de Cho se asociaban a un mayor número de episodios depresivos previos, sin relación alguna entre el estado sintomático y la señal de ninguno de los metabolitos. Los resultados son aún más interesantes si tenemos en cuenta que las alteraciones de metabolitos no se explican por la mera reducción del volumen del hipocampo en las fases más avanzadas de la depresión, como pudo controlarse en los análisis estadísticos.

Tal y como queda revisado con detalle en el apartado de discusión de los respectivos artículos, todos estos hallazgos coinciden, en líneas generales, con los de los estudios de MRS y depresión publicados con anterioridad, y de forma especialmente sólida en lo que respecta a las alteraciones de Glx. No obstante, quizás por abarcar un espectro más amplio de enfermos con depresión, nuestras observaciones se extienden a otros metabolitos implicados de forma

menos constante, con tamaños del efecto relevantes en todos los casos. Por otra parte, los resultados subrayan la prominencia de las anormalidades metabólicas entre las formas más severas y de más larga evolución de la enfermedad, unas anormalidades que parecen persistir incluso tras la resolución de los síntomas y, tal vez, agravarse con el paso del tiempo. La estrecha conexión estructural y funcional entre el hipocampo y la corteza prefrontal, y su más que contrastada implicación con la fisiopatología de los trastornos afectivos, justificaría la similitud de las alteraciones de metabolitos observadas en ambos trabajos. Estas regiones, y el hipocampo en particular, desempeñan un importante rol en la respuesta al estrés, quedando especialmente expuestas a sus potenciales efectos neurotóxicos bajo condiciones de estrés crónico.

El hallazgo de menores niveles de Glx apoya las hipótesis que postulan anormalidades en el metabolismo glutamatérgico en depresión (Kugaya & Sanacora, 2005) y es coherente con la reducción del número de astrocitos y neuronas glutamatérgicas descrita en ACC y otras regiones de la CPF de enfermos depresivos post-mortem (Rajkowska & Miguel-Hidalgo, 2007) o con las alteraciones de marcadores de viabilidad astrocitaria observadas en hipocampo (Czeh & Lucassen, 2007). Los estudios concluyen que, en depresión, la afectación de la neuroglia sería más prominente que el de las propias neuronas (Rajkowska & Miguel-Hidalgo, 2007), aunque el deterioro glial previsiblemente se traduciría en una alteración del funcionamiento neuronal (Haydon, 2001; Magistretti et al., 1999) y también han podido demostrarse alteraciones estructurales sutiles en las neuronas de ACC e hipocampo, como la reducción del tamaño de sus cuerpos celulares (Cotter et al., 2001; Stockmeier et al., 2004). Además, la contrastada reducción de los volúmenes del hipocampo en depresiones de larga evolución se ha relacionado con disminuciones del neuropilo en esta estructura, incluyendo el decremento de la arborización y espinas dendríticas o el de los procesos gliales (Cobb et al., 2013; Stockmeier et al., 2004). Las disminuciones en la señal de NAA observadas en los trabajos de esta tesis podrían reflejar esas disfunciones y alteraciones de la integridad neuronal. Los

aumentos en la señal de Cho, por su parte, tal y como habíamos mencionado con anterioridad, se han relacionado con el recambio celular y de membrana (Boulanger et al., 2000). Así pues, en conjunto, las alteraciones de las concentraciones de Glx, NAA y Cho presentes entre los pacientes con depresión crónica y recurrente apoyarían la existencia de anormalidades en glía, neuronas y procesos de remodelación en CPFvm e hipocampo, y sugerirían que esos cambios celulares estarían ligados a la carga clínica de la enfermedad y a su curso evolutivo.

Junto a las alteraciones de la neuroquímica celular en territorios fronto-límbicos, los estudios de neuroimagen funcional avalan la hipótesis de una disfunción subyacente en la conectividad entre estas y otras áreas cerebrales en la depresión (Drevets et al., 2008a; Mayberg, 2003). Aunque la mayor parte de los estudios DTI que revelaban alteraciones microestructurales de sustancia blanca en depresión se basaban en muestras de pacientes ancianos, algunos trabajos apuntaban ya a la existencia de alteraciones de la FA en un espectro más amplio de la pacientes depresivos (Murphy & Frodl, 2011; Sexton et al., 2009). El tercer trabajo de esta tesis es la primera publicación que compara la microestructura de sustancia blanca entre diferentes estadios de depresión no geriátrica, usando DTI y metodología TBSS. Los resultados del estudio amplían las observaciones de otros autores y suscriben las hipótesis iniciales al demostrar extensas disminuciones de la FA entre pacientes con depresión crónica resistente al tratamiento en comparación con sujetos controles sanos y pacientes con un primer episodio depresivo. Los años de evolución de la enfermedad y, de forma marginal, la intensidad de los síntomas, se asociaban con mayores disrupciones de sustancia blanca.

Los tractos mayormente afectados eran el fascículo longitudinal superior, el cingulum, el cuerpo calloso (incluyendo regiones del cuerpo, el fórceps major y el fórceps minor) y el fascículo longitudinal inferior. De entre ellos, es particularmente remarcable la afectación del fascículo longitudinal superior y el cingulum, dada la congruencia con la hipótesis de afectación de la conectividad fronto-temporal y fronto-límbica en depresión. El cingulum es una ruta de

asociación fundamental entre las cortezas prefrontales y parahipocampales mientras que el fascículo longitudinal superior es un importante haz de fibras de asociación que conecta la corteza prefrontal dorsolateral con otras regiones frontales y con los lóbulos temporal, parietal y occipital (Schmahmann & Pandya, 2006). La afectación del fascículo superior longitudinal en depresión, además, se ha visto respaldada por un meta-análisis en el que también se relacionaba la disminución de la FA en esta región con la duración de la enfermedad y la gravedad de los síntomas (Murphy & Frodl, 2011). La disfunción de la conexión entre las regiones prefrontales y témporo-límbicas parece el principal sustrato neural de la mayor parte de déficits emocionales, cognitivos y comportamentales asociados a depresión y los daños en la microestructura de sustancia blanca de estas regiones ligan con las anormalidades metabólicas reportadas en las mismas áreas por los otros dos trabajos de esta tesis.

No obstante, la reducción de FA descrita aquí también compromete a otras regiones cerebrales. Esto coincide con los resultados de otro reciente estudio en el que se observaba una afectación extensa de la microestructura de sustancia blanca en pacientes con depresión de larga evolución, asociada asimismo a la gravedad de los síntomas (Cole et al., 2012). Nuestros hallazgos refuerzan la presencia de una disfunción generalizada de la neurocircuitería en depresión, especialmente en las formas más graves y resistentes al tratamiento, y esta disfunción parece claramente ligada al curso evolutivo y a la carga clínica de enfermedad.

Cuando el análisis se focalizó en la región prefrontal ventromedial, los pacientes con depresión crónica resistente mostraban disminuciones significativas de la FA en comparación al resto de grupos, incluso en comparación a los pacientes con depresión recurrente en actual remisión. Entre los tractos afectados aquí, se incluían subregiones del fascículo uncinado (otro tracto esencial en la conexión prefronto-límbica) y del cuerpo calloso (el mayor haz de conexión cerebral interhemisférica) (Schmahmann & Pandya, 2006). En esta zona, el número de episodios previos y la gravedad sintomática eran predictores independientes de las

anormalidades de FA, de modo que cuantas más recurrencias y mayor intensidad de los síntomas, menores eran los niveles de FA. Los hallazgos, en su conjunto, son coherentes con el trascendente rol que parece jugar la CPFvm en la recurrencia, la refractariedad y la persistencia de los síntomas. La modulación de la actividad neural en esta zona se postula como la responsable de los alentadores efectos terapéuticos de la DBS en el área cingulada subgenual entre los pacientes con las formas más graves de depresión crónica (Johansen-Berg et al., 2008). Curiosamente, algunos de los pacientes que sufrían depresión crónica resistente al tratamiento y que fueron reclutados para este estudio, fueron posteriormente tratados mediante DBS con buenos resultados (Puigdemont et al., 2012). Queda por discernir si los efectos terapéuticos de la DBS se alcanzan resolviendo o simplemente eludiendo las alteraciones microestructurales de la sustancia blanca descritas en esta región.

Las variaciones de la FA pueden representar diversos procesos anatomopatológicos, como cambios en la densidad o el diámetro axonal, anormalidades de la mielinización o alteraciones en la coherencia de los tractos fibrosos (Sexton et al., 2009). Los pocos estudios post-mortem focalizados en pacientes adultos de mediana edad avalan la presencia de lesiones de la sustancia blanca profunda en la depresión unipolar (Tham et al., 2011). En concreto, se han descrito disminuciones en la densidad de oligodendrocitos (células gliales encargadas de la mielinización de las fibras en el sistema nervioso central) o reducciones en la intensidad de la tinción de mielina en la sustancia blanca profunda de regiones prefrontales (Regenold et al., 2007; Uranova et al., 2004). Las alteraciones de la FA han sido relacionadas con cambios de la neuroquímica celular en investigaciones previas (Wijtenburg et al., 2012). Las alteraciones microestructurales de la sustancia blanca que acabamos de describir casan coherentemente con las presuntas alteraciones de la integridad neuronal/axonal y del recambio de membrana reflejadas por las anormales concentraciones de NAA y Cho reportadas en los dos primeros trabajos.

En resumen, tanto las variaciones de los niveles de Glx y el resto de metabolitos en CPFvm e hipocampo como las disminuciones generalizadas de la FA mostradas en los pacientes depresivos, apoyan la probable implicación de la neuroglia en la fisiopatología de la depresión, y la sobrerrepresentación de estas alteraciones entre los pacientes con peor respuesta clínica y mayor carga de enfermedad subraya su importancia en los procesos de recurrencia y resistencia al tratamiento. Aunque sin esclarecer por completo en qué grado las alteraciones de los metabolitos y de la microestructura de sustancia blanca son preexistentes o secundarias a la progresión de la enfermedad, estos hallazgos son altamente sugestivos del potencial neurotóxico de los estados depresivos y respaldan, al menos de forma preliminar, algunos de los enunciados de la hipótesis de *kindling* (Post, 1992). Los resultados podrían abrir las puertas a nuevos enfoques diagnósticos e incluso a nuevos objetivos terapéuticos, puesto que hacen hincapié en las consecuencias de permanecer enfermo por largos periodos de tiempo e invitan a explorar pautas de tratamiento antidepresivo más adecuadas y específicas (quizás con nuevos mecanismos de acción como los agentes moduladores glutamatérgicos) desde las primeras etapas de la enfermedad con el fin de evitar los potenciales cambios neurotóxicos deletéreos presumiblemente asociados a las recaídas y la cronicidad.

Fortalezas y limitaciones generales

Aunque los aspectos metodológicos han sido discutidos en profundidad en cada uno de los artículos incluidos en esta tesis, se citarán a continuación las fortalezas y limitaciones más relevantes y comunes a los tres trabajos presentados. Todas las exploraciones radiológicas se realizaron con un equipo de RMN Philips de 3 Teslas de intensidad de campo magnético, el cuál provee una mejor relación de señal-ruido que los dispositivos más convencionales de 1’5 o 2 Teslas y mejora la resolución de los picos metabólicos y de las imágenes del tensor de difusión. Las muestras de pacientes ambulatorios incluidas en los tres trabajos eran

relativamente grandes, cubriendo un espectro más amplio de gravedad y carga clínica de la enfermedad que investigaciones anteriores, lo que permitió testar las hipótesis. Además, por tratarse de pacientes que -en su mayoría- llevaban largo tiempo de seguimiento en nuestras Consultas Externas, los casos estaban muy bien caracterizados, mejorando así la fiabilidad de los diagnósticos y las variables clínicas empleadas. No obstante, a pesar de la consistencia interna de los hallazgos y de las relaciones observadas en torno a la duración de la enfermedad o al número de episodios previos, la interpretación de los resultados está inevitablemente condicionada por las limitaciones propias de los diseños caso-control. Así pues, la presunta existencia de marcadores de rasgo/vulnerabilidad y el posible empeoramiento de los parámetros metabólicos y microestructurales de sustancia blanca a lo largo del tiempo deben ser confirmados mediante estudios longitudinales prospectivos, algunos de los cuáles ya hemos puesto en marcha. Por último, el hecho de que los pacientes estuvieran medicados en el momento de la exploración radiológica representa probablemente la mayor limitación de la investigación. La necesaria inclusión de pacientes graves, especialmente en el caso del grupo de pacientes con depresión crónica, hacía inviable la posibilidad de retirar la medicación por razones éticas. Sin embargo, como queda referido en detalle en los respectivos trabajos de la tesis, la dirección de los resultados coincide, en general, con la de estudios previos, incluso con la de aquellos que se han basado en muestras de pacientes no tratados. Varias investigaciones apoyan la falta de influencia de la medicación antidepresiva en los metabolitos o la FA (Sussmann et al., 2009; Taylor et al., 2010; Wang et al., 2008) y otras han sugerido que el tratamiento podría, si cabe, atenuar las alteraciones de estos parámetros (Michael et al., 2003b; Taylor et al., 2012; Versace et al., 2008; Yoo et al., 2007). En el último de los trabajos de la tesis, además, se calculó una medida estimativa de la carga de tratamiento para cada paciente y fue incluida como covariable en los análisis sin que modificara las conclusiones. Por todo ello, parece improbable que los resultados observados sean meramente atribuibles a la influencia del tratamiento.

CONCLUSIONES

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En esta tesis se han presentado y discutido las múltiples alteraciones de metabolitos estructurales y de la microestructura de sustancia blanca presentes en regiones fronto-límbicas de pacientes con depresión crónica o con gran carga clínica de enfermedad pasada. De acuerdo a los resultados obtenidos en los distintos trabajos y a las hipótesis y objetivos planteados al inicio de esta tesis, se enumeran las siguientes conclusiones finales:

Trabajo 1 – *Alteraciones MRS en CPFvm asociadas a la recurrencia y cronicidad*

1. Los pacientes con depresión de larga evolución cuya enfermedad se caracteriza por un curso crónico resistente al tratamiento presentan niveles disminuidos de Glx y NAA y niveles aumentados de Cho en CPFvm con respecto a controles sanos y a pacientes que sufren su primer episodio depresivo.
2. Los pacientes con depresión de larga evolución cuya enfermedad se caracteriza por múltiples recurrencias, aun hallándose en situación de remisión clínica, presentan concentraciones de Glx y NAA en CPFvm igualmente disminuidas con respecto a controles sanos y a pacientes que sufren su primer episodio depresivo.
3. Las respectivas anomalías en los niveles de Glx y Cho en CPFvm correlacionan significativamente con el tiempo de evolución de la enfermedad. La disminución de los valores de NAA, por su parte, se asocia a una edad de debut más temprana.

Trabajo 2 – *Alteraciones MRS en hipocampo asociadas a la recurrencia y cronicidad*

1. Los pacientes con depresión de larga evolución cuya enfermedad se caracteriza por un curso crónico resistente al tratamiento presentan niveles disminuidos de Glx y NAA en hipocampo (especialmente en hemisferio derecho) con respecto a controles sanos. Los niveles de Cho en hipocampo, por el contrario, son significativamente superiores entre los pacientes con depresión crónica y resistente al tratamiento.

2. Los pacientes con depresión de larga evolución cuya enfermedad se caracteriza por múltiples recurrencias, aun hallándose en situación de remisión clínica, presentan concentraciones de Glx y NAA en hipocampo derecho igualmente disminuidas con respecto a controles sanos.
3. Los pacientes con un curso de enfermedad más benigno (es decir, aquellos que sufren su primer episodio depresivo con una edad de debut más tardía y una menor duración de enfermedad) no muestran diferencias con sujetos sanos en los valores de metabolitos estructurales de hipocampo, pero sí presentan concentraciones significativamente menores de Cho bilateralmente con respecto a los pacientes con depresión crónica y resistente al tratamiento.
4. Las respectivas anormalidades de los niveles de Glx, NAA y Cho son independientes de (o no se explican exclusivamente por) el menor volumen total de hipocampo observado entre los pacientes con larga historia de recurrencias o refractariedad.
5. Las alteraciones de los valores de Glx y Cho correlacionan en direcciones opuestas con el tiempo de evolución de la enfermedad y mayores concentraciones de Cho, además, se asocian con un mayor número de episodios depresivos previos.

Trabajos 1 y 2 – *Alteraciones espectroscópicas en CPFvm e hipocampo*

1. Las anomalías en las concentraciones de Glx, Cho y NAA descritas tanto en CPFvm como hipocampo guardan más relación con variables relacionadas con la carga de enfermedad (i.e. cronicidad y refractariedad al tratamiento, historia de múltiples recurrencias, mayor duración de la enfermedad o debut precoz) que con el propio estado sintomático en el momento de la exploración.
2. Estos resultados son congruentes con los hallazgos post-mortem de pacientes con depresión y apoyarían la existencia de cambios celulares en CPF y en hipocampo que estarían asociados al curso evolutivo de la depresión. Las disminuciones de Glx y NAA y los

incrementos de Cho observados en la CPFvm e hipocampo podrían reflejar alteraciones de la glía, alteraciones de la integridad neuronal y procesos de remodelación celular, respectivamente.

Trabajo 3 - *Alteraciones microestructurales de sustancia blanca asociadas a recurrencia y cronicidad*

1. Los pacientes con depresión crónica resistente al tratamiento presentan una alteración generalizada de la microestructura de sustancia blanca con respecto a sujetos controles sanos y pacientes con un primer episodio depresivo, que resulta independiente de la carga de medicación que toman. Los tractos mayormente afectados son el fascículo longitudinal superior e inferior, el cuerpo calloso y el cingulum, implicando, por tanto, a redes cortico-corticales y cortico-subcorticales, entre las que se incluye el circuito fronto-límbico. La duración de la enfermedad, desde su debut, es la variable clínica más claramente asociada con estas anormalidades.
2. Al poner el foco en la CPFvm, los pacientes con depresión crónica resistente al tratamiento presentan disrupciones de la microestructura de sustancia blanca en subregiones del fascículo uncinado y del cuerpo calloso con respecto a controles sanos, a pacientes con un primer episodio, e incluso a pacientes con depresión recurrente de larga evolución en fase de remisión. El número de episodios previos y la gravedad sintomática son aquí los predictores de esas alteraciones. Estas observaciones son consistentes con el papel crucial en la respuesta al tratamiento que se atribuye a la CPFvm y señalan la posible relación de la microestructura de la sustancia blanca en esta zona con la recurrencia y refractariedad de la depresión.

Nuevas líneas de investigación

Los resultados y conclusiones de esta tesis han sugerido la apertura de nuevas líneas de trabajo, algunas de ellas ya en desarrollo:

- Partiendo de un diseño caso-control similar al de los trabajos presentados, resultaría de gran interés la exploración de marcadores asociados a recurrencia, refractariedad y carga de enfermedad pasada:
  a) Mediante otras técnicas y modalidades de neuroimagen (p.e. análisis de grosor y/o volumen mediante VBM o estudios de Default-Mode Network con RMN funcional)
  b) Poniendo el foco en otras dianas específicas que se hayan mostrado relevantes en los trastornos del ánimo (p.e. amígdala, núcleo accumbens o habénula).
- Partiendo de un diseño longitudinal prospectivo centrado en los pacientes con un primer episodio depresivo mayor, resultaría de gran valor:
  a) La exploración de marcadores de neuroimagen al inicio del tratamiento capaces de predecir la respuesta terapéutica y el curso de la enfermedad
  b) El estudio de la posible progresión en las concentraciones de metabolitos estructurales y alteraciones de la microestructura de sustancia blanca de CPFvm, hipocampo u otras regiones a través de estudios seriados con RMN en los pacientes con mala respuesta al tratamiento o historia de recurrencias a lo largo del tiempo.
  c) La evaluación de nuevas dianas terapéuticas (p.e. moduladores glutamatérgicos y de la glía) y de variables clínicas relacionadas con el tratamiento (p.e. duración de la depresión sin tratar) en su capacidad para modular los cambios identificables con la neuroimagen y mejorar el pronóstico de la enfermedad.
  d) La investigación de otros marcadores biológicos (p.e. genéticos o bioquímicos, tales como agentes inflamatorios) relacionados con las alteraciones de la neuroimagen estructural y el curso evolutivo.

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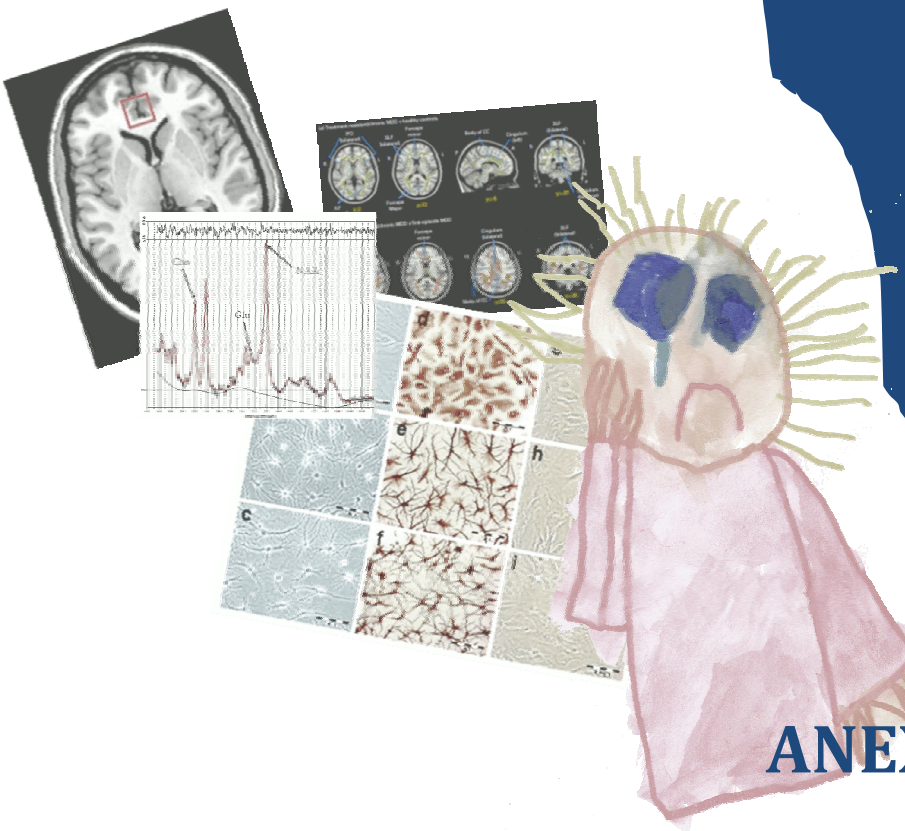

ANEXO

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de Diego-Adelino, J., Portella, M. J., Puigdemont, D., Perez-Egea, R., Alvarez, E., & Perez, V. (2010). A short duration of untreated illness (DUI) improves response outcomes in first-depressive episodes. J Affect Disord, 120(1-3), 221-225.

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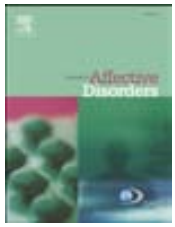

Brief report

A short duration of untreated illness (DUI) improves response outcomes in first-depressive episodes

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ABSTRACT

Background: Few studies have addressed the implication of the duration of untreated illness (DUI) on the clinical outcome of mood disorders. Although not focusing on DUI, previous findings suggest that the longer it takes to start appropriate treatment, the worse will be the evolution of depressive disorder. We sought to determine the effect of the duration of untreated episode (DUE) on 1) rates of response to treatment, 2) time to attain a sustained response and 3) rates of remission of MDD, dealing specially with first-depressive episodes.

Methods: 141 patients with MDD were grouped into long DUE (>8 weeks) and short DUE (≤8). Statistical analyses were performed to determine differences in outcome variables. The same analyses were repeated by splitting the sample between first-episode and recurrent depression.

Results: The percentage of patients who achieved a sustained response was significantly higher in the group with a short DUE [OR = 2.6; 95% CI 1.3–5.1]. Survival analyses showed that patients with a long DUE delayed longer time to attain a sustained response [39 vs. 20 days, $p = 0.012$]. Once the sample was split, these results were even more pronounced in the subsample of first-depressive episode patients.

Limitations: Given that the sample was originally recruited for two clinical trials, the follow-up period of this study is only six weeks long.

Conclusions: Our results indicate that response to antidepressant treatments is faster when the no-treatment interval is reduced. The earliest treatment of first-depressive episodes seems to be crucial since a shorter duration of untreated illness implies better response outcomes.

1. Introduction

In the last two decades, the duration of untreated illness (DUI) has been considered to have implications on the clinical outcome and on the course of different psychiatric disorders, mainly in schizophrenia and psychotic disorders (Barnes et al., 2008; Melle et al., 2008; Marshall et al., 2005; Perkins et al., 2005). However, only a few studies have focused on the possible role of the DUI on the clinical outcome and course of mood disorders (Altamura et al., 2007, 2008; Goldberg and Ernst, 2002). Recently, Altamura et al. (2007, 2008) performed two studies based on different patients' samples with recurrent depression and showed that a DUI longer than 12 months involved poorer outcomes. However, the most robust association found by these authors was an earlier age of onset, which could have acted as a confusing variable. Although not focusing strictly on DUI, previous studies had reported that a longer no-treatment interval in a given depressive episode entailed higher risk of chronicity (Scott et al., 1992; Gormley et al., 1999) while others failed to found such association (Furukawa et al., 2000). Hence, it seems clear that the longer it takes to start appropriate treatment, the worse will be the evolution of the disorder. In any case, little is known about this effect on the early stages of the illness, and in particular, on the first-depressive episode.

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Table 1
Clinical and demographic characteristics of the total sample ($n=141$) and both subgroups: first-depressive episodes ($n=83$) and recurrent depressive episodes ($n=58$).

	Total sample				First-depressive episodes				Recurrent depressive episodes			
	DUE≤8 ($n=75$)	DUE>8 ($n=66$)	Stats (F/χ^2)	p	DUE≤8 ($n=39$)	DUE>8 ($n=44$)	Stats (F/χ^2)	p	DUE≤8 ($n=36$)	DUE>8 ($n=22$)	Stats (F/χ^2)	p
Gender (% females)	74.7	66.7	1.1	0.30	71.8	65.9	0.3	0.56	77.8	68.2	0.7	0.42
Age	40.9 (12.2)	42.9 (11.6)	0.9	0.35	41.3 (11.8)	42.9 (11.5)	0.4	0.52	40.6 (12.8)	42.8 (11.9)	0.4	0.53
Age at onset	37.2 (12.5)	40.6 (12.3)	2.5	0.11	41.2 (11.7)	42.9 (11.7)	0.5	0.49	32.9 (12.1)	36.1 (12.6)	0.9	0.35
Recurrent depression (%)	48	33.3	3.1	0.08	NA				NA			
No. of previous episodes	0.8 (1.1)	0.5 (0.8)	3.4	0.07	NA				1.7 (1.1)	1.5 (0.7)	0.9	0.36
Baseline HDRS score	23.5 (3.5)	23.1 (3.4)	0.5	0.49	23.1 (3.3)	23.4 (3.3)	0.07	0.8	23.8 (3.7)	22.5 (3.6)	1.7	0.19
Final HDRS score	7.2 (4.8)	10.2 (7.1)	7.6	0.007	7.5 (4.9)	10.7 (7.4)	4.8	0.03	6.9 (4.8)	9.4 (6.8)	2.3	0.1
Treatment received (%)												
SSRI + Pindolol	53.3	47.0	0.6	0.45	66.7	50	2.4	0.13	38.9	59.1	0.02	0.88
SSRI + Placebo	46.7	53.0			33.3	50			61.1	40.9		

This is a preliminary study of a project dealing with first-depressive episodes, currently carried out in the Psychiatry Department of Hospital de Santa Creu i Sant Pau, in which first-onset MDD patients are followed-up. Using data of two previous clinical trials we sought to determine the effect of the duration of untreated episode on: 1) rates of response to treatment 2) time to attain a sustained response and 3) rates of remission in a sample of major depressive patients, with special focus on those who presented their first episode.

2. Methods

2.1. Sample

Consecutive eligible patients 18 years or older referred by General Practitioners of the Primary Care Centres or from the Psychiatric Emergency Services (Catalonian Public Health Service) were recruited for two randomised 6-week long clinical trials of SSRIs (fluoxetine and citalopram) plus placebo versus Pindolol, a 5-HT1$_A$ receptor antagonist (Pérez et al., 1997; Portella et al. not published yet). All these patients were screened by trained psychiatrists, researchers of this study, at the Affective Disorders Unit of the Hospital de Sant Pau (Barcelona, Spain). Those same patients were subsequently used for the present study to examine the influence of the duration of the index episode on the time to attain a sustained response and on the response rates.

The inclusion criterion was a diagnosis of unipolar major depression on DSM-IV with moderate to severe symptoms (score ≥ 18 on the Hamilton 17-items Depression rating scale—HDRS). The sample was composed by first-depressive episode and recurrent patients. The enrolled subjects had to be naïve (no previous antidepressant treatment) or antidepressant free for at least six months and with no previous resistance to SSRI treatment. Reasons for exclusion were: concomitant psychiatric pathology (DSM-IV axes I, II clusters A or B); suicide risk score ≥ 3 on the HDRS; participation in other drug trials within the previous month; organic brain disease or history of seizures; delusions or hallucinations (whether or not mood congruent); history of drug abuse (including alcohol) in the past three months; pregnancy or lactation; serious organic illnesses (not chronic well controlled diseases) such as hypo or hyperthyroidism, cardiac arrhythmias, asthma or diabetes mellitus; myocardial infarction in the past 6 months; frequent or severe allergic reactions; concomitant use of other psychotropic drugs (benzodiazepines were allowed), β-blockers or catecholamine-depleting agents; and current structured psychotherapy. A total of 164 patients with major depression diagnosis entered the study.

2.2. Variables of study

Demographic and clinical data were collected, which included among others age, gender, personal and familiar history of psychiatric disorders. Clinical variables relevant to the study describing the depressive disorder were severity of current episode, time of evolution, number of previous episodes, age at onset, and medical history. Depression severity was assessed with the HDRS. Response to treatment was defined as a reduction of at least 50% from the HDRS baseline score and Sustained Response was defined as a maintained reduction of at least 50% from HDRS baseline score until the end of the study or the patient's endpoint. Remission was defined as a HDRS score of 8 or lower.

2.3. Procedure

Given that the fluoxetine trial implied a response to placebo run-in period, all those patients who displayed such a response were excluded for the main analyses of this study (final sample $n=141$). For the purpose of the study, the sample was divided into two groups: patients with a duration of untreated index episode (DUE)>8 weeks ($n=66$) and DUE≤8 ($n=75$). The DUE was defined as the interval between the onset of the index episode and the beginning of antidepressant treatment (determined by the clinical trial). The threshold value of 8 weeks for the DUE was established after the calculation of the median for the whole sample. Parametric and non-parametric tests were used to determine which variables could exhibit differences between these two groups. A survival analysis was carried out to determine whether the groups differed in the number of days to attain a

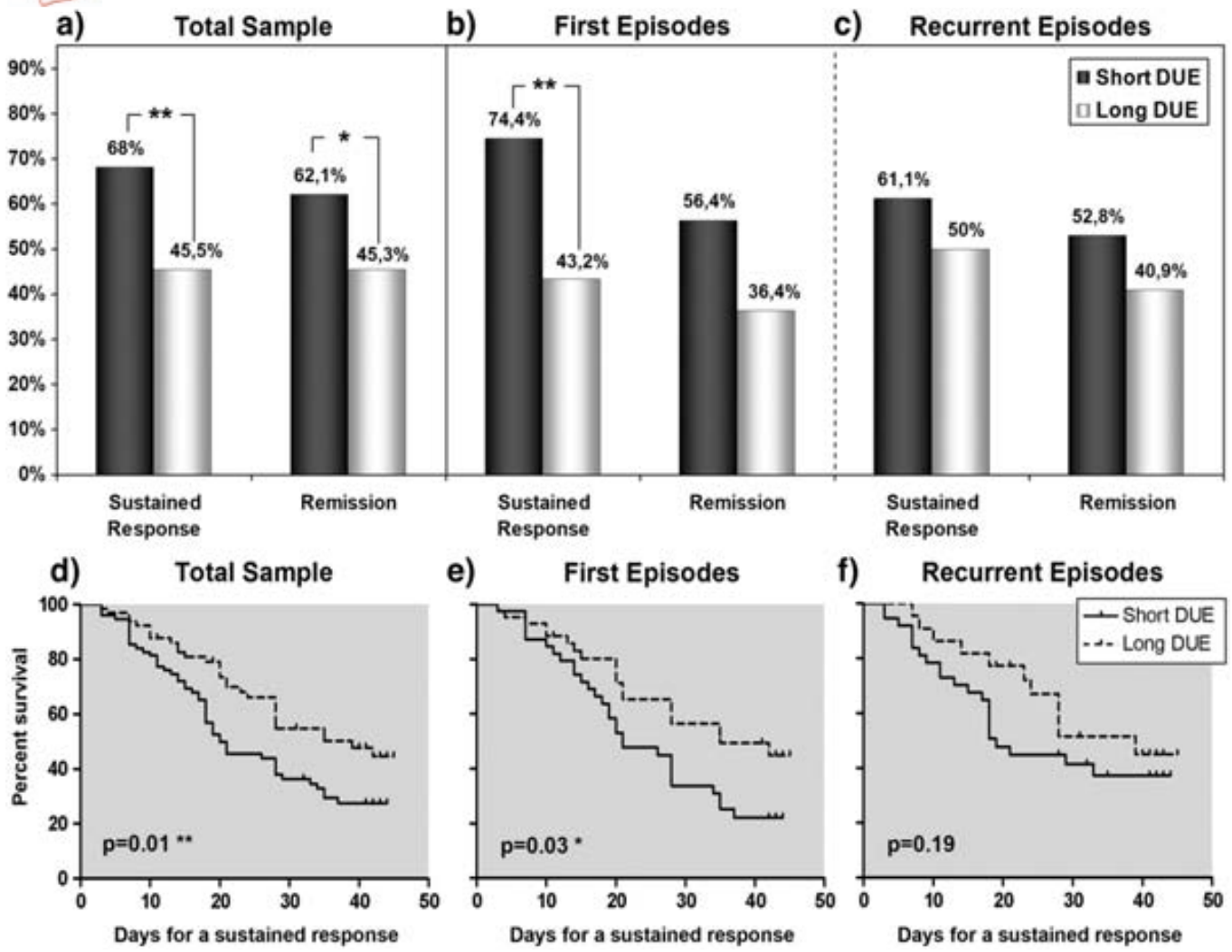

Fig. 1. Upper side represents the different rates of response outcomes considering the duration of untreated episode (DUE) in the whole sample (a), the first episodes (b) and the recurrent episodes (c). Lower side displays survival curves comparing short and long DUE in the whole sample (d), the first episodes (e) and the recurrent episodes (f). * $p \leq 0.05$; ** $p \leq 0.01$.

sustained response. Afterwards, these analyses were repeated separately by segmenting the sample between first-episode and recurrent MDD.

3. Results

The initial sample of 164 was comparable to the final sample ($n = 141$) used in the subsequent analyses (data not shown). An *intention-to-treat* (ITT) analysis approach was established in order to deal with missing data by means of *last observation carried forward* (LOCF) method. The rate of dropouts after the six-week study period was sufficiently low (14.9%) so as not to interfere in the results. The characteristics of the sample are displayed in Table 1. After the division in terms of DUE, both groups did not differ in any demographic characteristic. Patients with a short DUE had been a mean of 6.9 (SD = 1.8) weeks untreated, and those with a long DUE, a mean of 13.8 (SD = 5.2) weeks. There were more patients with recurrent depression among short DUE group, although this did not reach significance. Treatment received was comparable between short and long DUE groups.

Final HDRS scores were significantly different between short and long DUE patients (see Table 1). As it can be observed in Fig. 1a, the percentage of patients who achieved a sustained response was significantly higher in the short DUE group (OR = 2.6; 95% CI 1.3–5.1). Moreover, patients with a shorter DUE exhibited higher rates of remission (OR = 1.9; 95% CI 1.1–3.9).

Based on the Kaplan–Meier Survival Analysis, a significant difference in the number of days to respond to antidepressant treatment was found (median 20 vs. 39 days; see Fig. 1d) where DUE>8 delayed longer time to attain a sustained response. The point estimate and 95% CI was 1.746 (1.136 to 2.778).

3.1. Effects of DUE in first-depressive episodes

Neither demographics nor baseline clinical variables revealed differences between short and long DUE groups (Table 1). Patients with a short DUE had lower final HDRS scores than patients with a long DUE.

As it can be seen in Fig. 1b, patients with a short DUE were significantly more likely to attain a sustained response (OR = 3.8; 95% CI 1.5–9.7). There was also a trend towards higher remission rates in these patients (OR = 2.3; 95% CI 0.9–5.5).

The survival analysis showed that patients with a short DUE spent fewer days to reach a sustained response than patients with longer DUE (21 vs 35 days, respectively; Fig. 1e). The point estimate and 95% CI was 1.833 (1.06 to 3.426).

3.2. Effects of DUE in recurrent depressive episodes

Among patients with a recurrent episode, there were no significant differences in demographic and clinical features between both groups, short and long DUE (Table 1). Regarding final HDRS scores, response outcomes and survival curves, we failed to find any effects related to duration of untreated episode in this subsample (Fig. 1c and f).

4. Discussion

Our results imply that a longer duration of no-treatment interval in a given depressive episode implies lower rates of sustained response, more time to attain treatment response and lower likelihood to remit after 6 weeks of antidepressant medication. These effects, except remission rates, emerge particularly when considering first-depressive episodes alone.

The period prior to treatment has already been determined to be a predictive factor of persistence of depressive symptoms and even chronicity (Scott et al., 1992; Gormley et al., 1999). It is worth noting that patients were followed-up over a different period in these two previous studies: until remission in the first, and up to 12 months in the second. The present report was originally a clinical trial, so the patients were followed for only 6 weeks (trial endpoint). Interestingly, consequences of the delay in starting antidepressant medication in the early stages of the illness can already be observed even within such a short period of treatment. However, the short follow-up is clearly a limitation of this study, since the possibility that with a longer period of follow-up the differences between both groups might vanish cannot be rejected. Given that the design of the clinical trial aimed to test another hypothesis, more investigations should be performed to ascertain whether these differences can be observed in longer periods.

When we segment the sample, the effects of duration of untreated episode appear only in patients with a first episode of depression, as those patients who had a DUE lower than 8 weeks display almost four-fold likelihood to achieve a sustained response. The delay in treatment prescription in the first-depressive episode corresponds with the notion of duration of untreated illness (DUI). Although DUI has been mainly focused as a predictor of outcome in schizophrenia (Barnes et al., 2008; Melle et al., 2008; Marshall et al., 2005; Perkins et al., 2005; Marshall et al., 2005), Altamura et al. (2007, 2008) have already published some works dealing with major depressive disorder. They found that patients with a longer DUI presented a higher number of recurrences and more frequent comorbid axis I disorders (Altamura et al., 2007). However, only longer duration of the illness and earlier age at onset were replicated in the second study (Altamura et al., 2008). This latter variable, which has been repeatedly described as an independent factor for bad prognosis, could account for their findings. Another weakness of these studies is the crude estimation of the DUI and the posterior categorisation on the basis of a 12-month threshold after several years of illness evolution. Given that the length of the episode relied on the accuracy of patients' self-reports, the measurement of the DUE was limited in those studies. In contrast, our sample was interviewed at an early stage of the episode, ensuring a more acceptable estimate of the measure.

It is unlikely that our results are influenced by other factors since none of the relevant variables, including treatment received, showed significant differences (see Table 1). Nevertheless, Pindolol—a 5-HT1$_A$ receptor antagonist able to reduce response latency—might have acted as a confusing factor, as patients receiving SSRI + Pindolol tended to be overridden among those with a short DUE in the first-depressive episode subgroup. In order to rule out such effect, we repeated all the analyses for this subgroup considering only the patients who had received SSRI + Placebo. Our main findings were maintained in this case (data not shown).

Unexpectedly, our results were not confirmed in the subsample of recurrent patients. The recruitment criteria for the clinical trial excluded patients who had history of previous failures to SSRI treatment, chronicity, and comorbidity in DSM-IV axes I and II. The selection of the sample, which could have yielded a special good-prognosis subgroup, may have missed the effects of DUE on response outcomes. In any case, it seems reasonable that an early treatment might be more beneficial in the first stages of the illness rather than afterwards.

5. Summary

Our results indicate that response to antidepressant treatments is boosted when the no-treatment interval is reduced. The earliest treatment of first-depressive episodes seems to be crucial since a shorter DUI implies better response outcomes. Although the real extent to which hold-up in starting treatment in depressive disorder onset has not been proven in this study, DUI ought to be considered as a key factor that might have long-term prognostic implications. Further initiatives should be directed at emphasising the importance of early recognition and treatment of depressive disorder by clinicians.

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Conflict of interest

The authors do not have any affiliation or financial interest in any organization that might imply a conflict of interest.

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