Departamento de Psiquiatría y Psicobiología Clínica
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Anomalías de la estructura y función del hipocampo en la esquizofrenia en relación a los déficits de memoria declarativa

Tesis presentada por

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La **Dr. Carme Junqué i Plaja**, profesora del Departamento de Psiquiatría y Psicobiología Clínica de la Universidad de Barcelona, declara haber supervisado esta tesis doctoral, intitulada "*Anomalías de la estructura y función del hipocampo en la esquizofrenia en relación a los déficits de memoria declarativa"* presentada por Giuseppina Rametti. Así como declara que esta tesis cumple con los requisitos necesarios para ser defendida al fin de obtener el grado de Doctor.

Firma,

Dr. Carme Junqué i Plaja
Departamento de Psiquiatría y Psicobiología Clínica
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Dedico questa tesi a Carlos,
la persona che trova ogni
giorno la forza e l'amore per
sopportarmi.

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Glosario de abreviaturas

ADC	Apparent Diffusion Coefficient
BOLD	blood oxygen level dependent
CI	Cociente de inteligencia
DSM	Diagnostic and Statistical Manual of Mental Disorders
DTI	Diffusion Tensor Imaging
EPI	echoplanar imaging
FA	Fractional Anisotropy
FOV	field of view
FSPGR	fast spoiled gradient
FWE	family wise error
FWHM	full width at half maximum
GM	gray matter
MT	memoria de trabajo
PET	positron emission tomography
RM	resonancia magnética
RMf	resonancia magnética funcional
ROI	Region of Interest
SCID	Structured Clinical Interview for DSM
TE	tiempo de eco
TR	tiempo de repetición
WAIS	Wechsler Adult Intelligence Scale
WM	White matter

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INTRODUCCIÓN TEÓRICA

1 La Esquizofrenia

1.1 La Esquizofrenia: Definición y criterios diagnósticos

Con el término esquizofrenia se describe un grupo de enfermedades mentales que son diferentes en naturaleza y que abarcan un amplio campo de trastornos cognitivos, afectivos y conductuales (Andreasen, 2000).

El termino esquizofrenia deriva del griego σχίζω (*schizo*, escisión) y φρενός (*phrenos*, mente).

Se diferencian fundamentalmente 2 tipos de síntomas:

- los síntomas *positivos*: en los que se incluyen delirios, alucinaciones y trastornos del pensamiento
- los síntomas *negativos*: que se caracterizan por la disminución o desaparición de algunas capacidades normales del sujeto. Los más clásicos son pobreza del lenguaje y de otras funciones comunicativas, ausencia emocional, falta de placer (anhedonia) y de motivación.

Además de estos síntomas clásicos, actualmente los déficits neurocognitivos se consideran como parte de la enfermedad. Los trastornos cognitivos más frecuentes abarcan las funciones de memoria, atención, resolución de problemas, funciones ejecutivas y cognición social (Cirillo y Seidman, 2003; Barch et al., 2005; Fioravanti et al., 2005).

El Manual Diagnóstico y Estadístico (DSM) de la Asociación Psiquiátrica norteamericana proporciona actualmente el sistema más utilizado para diagnosticar y clasificar la esquizofrenia. Según el DSM-IV, para el diagnostico de esquizofrenia, se necesitan los siguientes criterios:

- Síntomas peculiares (positivos o negativos) durante un periodo de un mes
- Pérdida de funciones durante más de seis meses
- Disfunción social o laboral
- No ser debido a trastornos del humor ni a causas orgánicas
- En el caso de un trastorno autista previo, el diagnóstico adicional de esquizofrenia solo es pertinente si hay alucinaciones prominentes.

La correcta identificación del diagnóstico tiene fuerte implicaciones en la investigación, dado que repercute en su validez y replicabilidad.

La esquizofrenia se caracteriza por tener una sintomatología muy heterogénea en la que se diferencian varios subtipos. Los criterios para el diagnostico de los diferentes subtipos de esquizofrenia son los siguientes:

- <u>Criterios para el diagnóstico de Tipo paranoide:</u>
    - Preocupación por una o más ideas delirantes o alucinaciones auditivas frecuentes.
    - No hay lenguaje desorganizado, ni comportamiento catatónico o desorganizado, ni afectividad aplanada o inapropiada.

- <u>Criterios para el diagnóstico de Tipo desorganizado:</u>
    - Lenguaje desorganizado
    - Comportamiento desorganizado
    - Afectividad aplanada o inapropiada
    - No se cumplen los criterios para el tipo catatónico.

- Criterios para el diagnóstico de Tipo catatónico:
    - Inmovilidad motora manifestada por catalepsia (incluida la flexibilidad cérea) o estupor
    - actividad motora excesiva (que aparentemente carece de propósito y no está influida por estímulos externos)
    - negativismo extremo (resistencia aparentemente inmotivada a todas las órdenes o mantenimiento de una postura rígida en contra de los intentos de ser movido) o mutismo
    - peculiaridades del movimiento voluntario manifestadas por la adopción de posturas extrañas (adopción voluntaria de posturas raras o inapropiadas), movimientos estereotipados, manierismos marcados o muecas llamativas
    - ecolalia o ecopraxia

- Criterios para el diagnóstico de Tipo indiferenciado:
    - Un tipo de esquizofrenia en que están presentes los síntomas del Criterio A, pero que no cumple los criterios para el tipo paranoide, desorganizado o catatónico.

- Criterios para el diagnóstico de Tipo residual:
    - Ausencia de ideas delirantes, alucinaciones, lenguaje desorganizado y comportamiento catatónico o gravemente desorganizado.
    - Hay manifestaciones continuas de la alteración, como lo indica la presencia de síntomas negativos o de dos o más síntomas de los

enumerados en el Criterio A para la esquizofrenia, presentes de una forma atenuada (p. Ej., creencias raras, experiencias perceptivas no habituales).

1.2 Hipótesis etiológicas

Aunque la investigación ha tenido un gran impacto en el conocimiento de los síntomas asociados con la esquizofrenia, la fisiopatología y las causas de esta enfermedad siguen siendo no del todo conocidas.

La literatura atribuye la causa de este trastorno a un complejo de factores genético – biológico – psicológicos. Sin embargo, históricamente han dominado dos explicaciones diferentes sobre el origen del trastorno: la hipótesis neurodegenerativa y la hipótesis del neurodesarrollo.

1.2.1 Hipótesis neurodegenerativa

La hipótesis de la neurodegeneración propone, en la esquizofrenia, la existencia de un proceso neurodegenerativo tardío debido a la perdida progresiva de las funciones neuronales. La alteración estaría causada por la poda sináptica (*synaptic pruning*) que se realiza durante la adolescencia (Feinberg, 1982), ocasionada probablemente por una disminución repentina en la disponibilidad de las sustancias químicas (factores tróficos o neurotrofinas) necesarias para la supervivencia de las neuronas y de su infraestructura sináptica asociada (es decir dendritas y terminales axonales). Feinberg especuló que esta poda sináptica podría ser provocada por los cambios hormonales característicos de la pubertad. La edad en la que usualmente empieza a manifestarse la enfermedad (finales de la adolescencia) podría indicar el inicio de un proceso degenerativo. Algún fenómeno patogénico podría influir en la progresión sintomática de la enfermedad. Entre éstos la hipótesis

excito-tóxica propone que las neuronas degenerarían como resultado de una excesiva neurotransmisión glutamatérgica (Deutsch et al., 2001)

Sin embargo, en contra la hipótesis neurodegenerativa, muchos estudios post-mortem (Roberts y Harrison, 2000) no encuentran gliosis en los cerebros de los esquizofrénicos, interpretando esta ausencia como excluyente de un proceso de neurodegeneración típico en la esquizofrenia (Bertolino, 1998).

Arnold (1999), en una exhaustiva revisión sobre el estado neuropsicológico de los pacientes esquizofrénicos, concluye que no existe suficiente evidencia de una pérdida cognitiva progresiva que vaya a favor de un proceso neurodegenerativo.

1.2.2 Hipótesis del neurodesarrollo

El modelo del neurodesarrollo fue propuesto por Weinberger (1987), ganando importancia en los últimos 20 años. Este modelo propone que existen alteraciones cerebrales en una fase muy precoz del desarrollo cerebral del feto (Weinberger, 1987). Específicamente, sugiere que la lesión cerebral ocurre durante el segundo trimestre del embarazo, cuando la diferenciación y el crecimiento del sistema nervioso central (SNC) fetal están teniendo lugar. El daño al SNC puede ocurrir a consecuencia de la exposición maternal a virus y toxinas, una nutrición pobre, hipoxia de nacimiento, hemorragia o un error de la expresión genética *in útero*. Las neuronas enfermas se vuelven incapaces de migrar a las áreas correctas del cerebro y no son capaces de formar las conexiones apropiadas.

Se trataría de una lesión que permanece “silente” hasta que el proceso de desarrollo conecta las estructuras cerebrales afectadas por la lesión (Marenco y Weinberger, 2000). La teoría del neurodesarrollo postula por lo tanto la

existencia de individuos vulnerables o de alto riesgo para padecer esta enfermedad, cuya alteración podría ser secundaria tanto a factores genéticos como ambientales, así como a una combinación de ambos.

Los estudios que apoyan esta hipótesis han demostrado que las complicaciones durante el embarazo y el parto, así como enfermedades víricas, sobretodo durante el segundo trimestre del embarazo, aumentan entre dos y tres veces el riesgo de padecer esquizofrenia (Adams et al., 1993; Barr et al., 1990; Izumoto et al., 1999; Limosin et al., 2003).

1.3 Neuropatología de la esquizofrenia

Las principales diferencias patológicas encontradas en los cerebros de esquizofrénicos incluyen: incremento del tamaño ventricular, volumen y peso cerebral reducidos, número reducido de neuronas en el lóbulo temporal medial así como en otras áreas corticales; todo ello en ausencia de gliosis y de cambios neurodegenerativos (Harrison 1999).

Sin embargo, aunque los cerebros de pacientes con esquizofrenia presenten ventrículos de tamaño incrementado, la mayor parte de estudios no ha podido identificar a nivel neuropatológico cambios neurodegenerativos y, en particular, de gliosis. Los estudios cuantitativos (Benes, Davidson et al. 1986; Falkai and Bogerts 1986; Pakkenberg 1990) han demostrado una falta de evidencias del proceso gliótico en estructuras como el lóbulo temporal o las regiones periventriculares, sugiriendo que el origen de la enfermedad es debido a un trastorno del neurodesarrollo. Probablemente, durante los primeros meses de vida fetal se produciría una lesión cerebral antes que la maduración glial se pudiese llevar a cabo.

Los estudios post-mortem indican que la esquizofrenia está asociada a modificaciones en el número, la densidad, el tamaño y la distribución neuronal; sin embargo la ubicación, la naturaleza y el significado de los cambios críticos son aún desconocidos (Shapiro, 1993).

Los estudios histológicos en áreas como el lóbulo temporal medial describen anomalías en la posición y distribución laminar cortical del hipocampo y parahipocampo en pacientes esquizofrénicos. Estos resultados se interpretan como debidos a una migración neuronal defectuosa durante el desarrollo (Jakob y Beckmann 1986; Kovelman y Scheibel 1986; Arnold, Hyman et al. 1991). Así, estos estudios sugieren que determinadas neuronas de los pacientes esquizofrénicos no emigran normalmente hacia las capas exteriores de la corteza, sino que se quedan en las capas corticales más profundas. Este desplazamiento dificulta el establecimiento óptimo de conexiones neuronales, que a su vez causa un proceso más excesivo de poda y un empaquetamiento más denso de neuronas. El conjunto de anomalías mencionadas tendrían evidentes implicaciones en el correcto desarrollo cognitivo.

2 Estudios de Neuroimagen en la Esquizofrenia

Los estudios de neuroimagen, tanto estructural como funcional, han reanimado la idea de que la esquizofrenia es una enfermedad cerebral, de obvia base orgánica.

2.1 Resonancia magnética estructural

Los estudios volumétricos han encontrado que este trastorno está asociado con anomalías en casi todas las estructuras corticales y sub-corticales del cerebro. Las más consistentes son: incremento de los ventrículos laterales y el tercer ventrículo, decrementos en el lóbulo temporal (el complejo amígdala/hipocampo y el giro temporal superior) y el lóbulo frontal, incrementos y decrementos en los ganglios basales, y atrofia en el tálamo y el cerebelo. También se han observado reducciones del volumen intracraneal total y del cuerpo calloso (para revisión, ver Shenton et al., 2001).

2.1.1 Signos de atrofia cerebral difusa

La dilatación de los ventrículos (índice de la atrofia del parénquima cerebral) ha sido investigada inicialmente mediante la relación entre las dimensiones ventriculares y las cerebrales (VBR- ventricular-brain ratio) (Andreasen et al., 1982).

Las anomalías más frecuentemente encontradas en el líquido cefalorraquídeo de los pacientes esquizofrénicos se refieren al volumen de los ventrículos laterales (Sanfilipo et al., 2000) y, en parte, al tercer ventrículo (Degreef et al., 1992), mientras que el cuarto ventrículo parece estar inalterado (McCarley et al., 1999). La dilatación ventricular podría indicar que otras partes adyacentes

del cerebro no se han desarrollado debidamente, lo que permite que los ventrículos aumenten su tamaño. A este propósito, la proximidad del tercer ventrículo con el tálamo ha sugerido que un incremento de fluido podría estar asociado en la esquizofrenia con una reducción del volumen talámico (Shenton et al., 2001).

2.1.2 Hallazgos corticales

2.1.2.1 Lóbulo temporal

Gyrus temporal superior

Una de las regiones temporales más comúnmente implicada en la esquizofrenia es el gyrus temporal superior (Hirayasu et al., 1998; McCarley et al., 1999), que comprende el córtex auditivo y el planum temporal (GTS posterior). La literatura ha descrito frecuentemente una pérdida de volumen del GTS mas pronunciada en el lado izquierdo (Barta et al., 1990; Shenton et al., 1992), área relacionada con el procesamiento lingüístico y auditivo (Galaburda et al., 1978), a menudo afectados en la esquizofrenia. La reducción del volumen del GTS izquierdo ha sido relacionada con alucinaciones auditivas (Levitan et al., 1999), así como con trastornos del pensamiento y del lenguaje (Vita et a., 1995).

Planum temporal

El planum temporal es la estructura cerebral más asimétrica del cerebro humano, siendo más grande en el hemisferio izquierdo en la mayor parte de los individuos (Geschiwind & Levitsky, 1968). Los estudios en esquizofrenia del planum temporal demuestran su reducción o una inversión en su asimetría izquierda (Barta et al., 1997; McCarley et al., 1999; Shapleske et al., 1999). Así

mismo, esta región, normalmente involucrada en la comprensión y producción lingüística, ha sido correlacionada en la esquizofrenia con los trastornos del pensamiento (Petty et al., 1995).

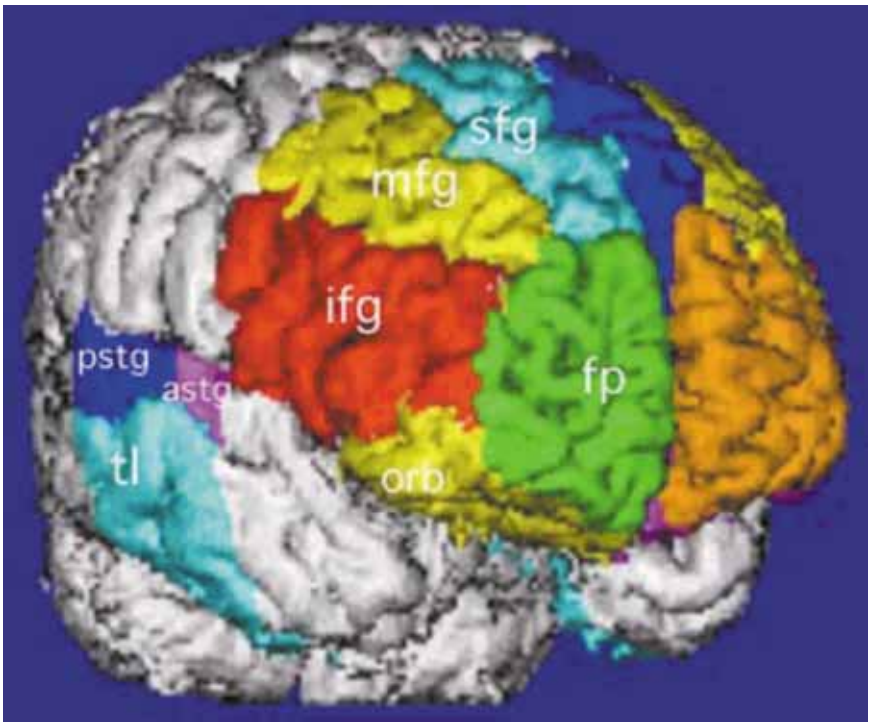

Figura 1. Reconstrucción tridimensional de las regiones temporales y prefrontales (tomada de Kasai et al., 2002)

Sfg = gyrus frontal superior

mfg = gyrus frontal medial

ifg =gyrus frontal inferior

orb = gyrus orbitofrontal

fp = polo frontal

astg = gyrus temporal anterior

pstg = gyrus temporal superior

tl = lobo temporal

2.1.2.2 Lóbulo frontal

El lóbulo frontal es una de las regiones corticales más implicada en la esquizofrenia. Muchos estudios encuentran que los individuos afectados de esquizofrenia se caracterizan por síntomas como desinhibición, auto-conciencia

inadecuada, conducta inapropiada, cambios de personalidad y un enlentecimiento psicomotor, lo que caracteriza también los trastornos frontales (Liddle y Morris, 1991).

Los estudios de meta-análisis encuentran una disminución en el volumen del lóbulo frontal en prácticamente la mitad de los trabajos (McCarley et al., 1999); aunque hay que tener presente que a menudo los cambios en esta región son demasiado sutiles para ser detectados (Shenton et al., 2001).

Se han evidenciado además en esta región cerebral diferencias de género relativas a una mayor reducción de volumen en la corteza dorso-medial en pacientes varones y en la corteza orbito-frontal en el caso de las mujeres (Gur et a., 2000).

Weinberger (1992) hipotetiza una conexión anormal del circuito fronto-límbico que podría explicar la correlación entre el volumen de la corteza prefrontal y el lóbulo temporal (Breier et al., 1992). Otra importante asociación evidenciada se produce entre las anormalidades frontales y los síntomas negativos (Wilbe et al., 1995).

2.1.3 Hallazgos sub-corticales

2.1.3.1 El cuerpo calloso

El cuerpo calloso es la mayor comisura ínter-hemisférica, conectando la actividad y permitiendo el paso de la información entre los 2 hemisferios cerebrales. Es una de las comisuras neuronales que mas tardíamente madura, continuando su desarrollo durante la joven edad adulta (Pujol et al., 1993). A menudo se ha discutido sobre una transferencia alterada de la información

entre los dos hemisferios en los esquizofrénicos o sobre una errónea sincronización de su actividad (Crow, 1997). Estudios meta-analíticos de MRI describen una reducción significativa de toda el área del cuerpo calloso en esquizofrenia (Woodruff et al., 1995; McCarley et al., 1999). Se ha hallado también una asociación entre síntomas negativos y un decremento del área callosa (Woodruff et al., 1997).

2.1.3.2 Complejo amígdala-hipocampo

Las anomalías más frecuentemente encontradas en el lóbulo temporal se refieren a la parte medial: hipocampo, parahipocampo, amígdala y corteza entorinal (Nelson et al., 1998; Weiss et al., 2005).

La mayoría de los estudios de neuroimagen combinan el hipocampo y la amígdala como si fueran una estructura conjunta a causa de las dificultades en separarlos, demostrando una reducción del complejo amígdala-hipocampo en sustancia gris (Shenton et al., 2001), con una clara predominancia izquierda.

Estudios meta-analíticos de volumetría han demostrado que el hipocampo es acerca de un 6 % más pequeño en la esquizofrenia, a nivel bilateral (Nelson et al. 1998; Wright et al., 2000). Hirayasu et al. (1998) investigaron, mediante ROIs manuales, las diferencias volumétricas en primeros episodios psicóticos, identificando reducciones significativas en el gyrus temporal superior y en el complejo amígdala-hipocampo, ambos izquierdos.

2.1.3.3 Ganglios Basales

La literatura refiere fundamentalmente un incremento del volumen de los ganglios basales (Shenton et al., 2001) que parece tener relación con la medicación antipsicótica. Gur et al., (1998) describen como pacientes expuestos a un tratamiento neuroléptico típico durante un tiempo largo exhiben

un aumento en el volumen de los ganglios basales y del tálamo respecto a pacientes nunca tratados y a los controles sanos. Además Corson et al., (1999) refieren, por un lado, una correlación positiva entre el volumen de los ganglios basales y la exposición acumulativa a neurolépticos típicos y, por otro, una correlación negativa entre el volumen de los ganglios basales y la exposición a neurolépticos atípicos.

2.1.3.4 Tálamo

Medir los núcleos del tálamo resulta a menudo difícil dado que esta región presenta áreas en las que la sustancia gris y la blanca no tienen límites bien definidos. Además los cambios podría estar restringidos a núcleos talámicos específicos y no a la estructura entera (Clinton et al., 2004). Los resultados relativos a su medida volumétrica son inconsistentes refiriendo tanto una reducción (Gur et al., 1998; Gilbert et al., 2001; Salgado-Pineda et al., 2003) como ningún cambio en su volumen (Bridle et al., 2002; Deicken et al., 2002).

Portas et al., (1998) encuentran una correlación positiva entre el volumen talámico reducido y la sustancia blanca de la corteza prefrontal. Así mismo, ha sido descrita una asociación entre el reducido volumen del tálamo en pacientes de inicio precoz y el rendimiento pobre en aprendizaje, abstracción y flexibilidad cognitiva (Jeste et al., 1998). Finalmente, Gur et al., (1998) refieren una asociación entre un aumento del volumen talámico y la gravedad de los síntomas positivos.

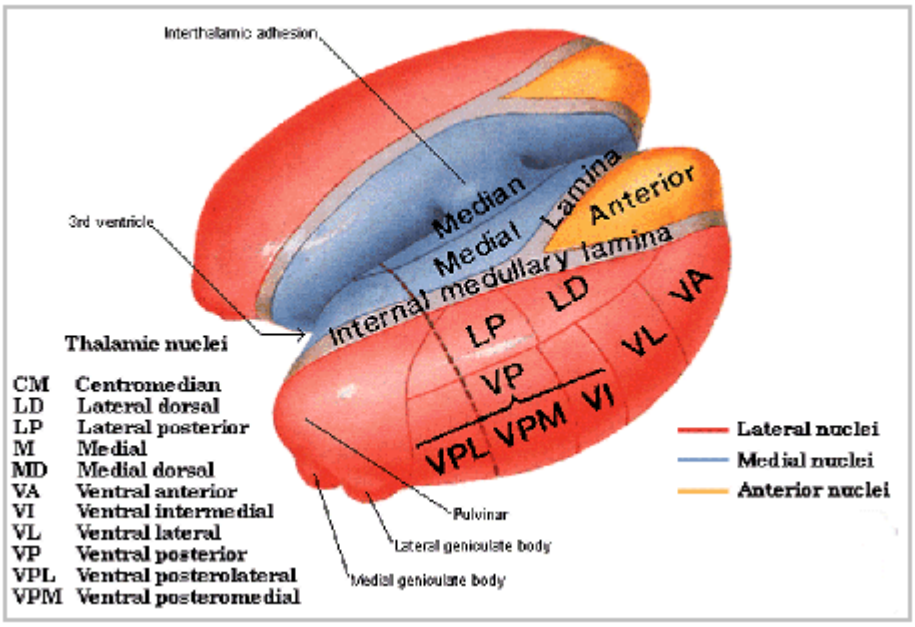

Figura 2. *Núcleos del Tálamo* (tomada de www.alpha.furman.edu)

2.2 Resonancia magnética funcional

El desarrollo de las técnicas de neuroimagen funcional (PET, SPECT, fMRI) ha representado una considerable contribución al estudio y comprensión de los correlatos neuronales implicados en los procesos cognitivos.

Una de las técnicas de neuroimagen funcional más utilizada, la fMRI, se basa en la respuesta BOLD (Blood oxygenation level dependent), dependiente del nivel de oxigenación en la sangre. Esta técnica se fundamenta en el hecho de que un aumento de la actividad neuronal durante la realización de una tarea cognitiva se acompaña de un aumento en el flujo sanguíneo local de las áreas implicadas en la o las operaciones cognitivas necesarias. Esto lleva a un aumento de la oxí-hemoglobina oxigenada que, a su vez, comporta un aumento de la señal de resonancia magnética. Este riego sanguíneo aumentado provee

así las neuronas metabólicamente activas del oxígeno necesario para desarrollar una tarea particular (Menon et al., 1992).

En la esquizofrenia, las principales funciones cerebrales estudiadas son relativas fundamentalmente al lóbulo frontal (funciones ejecutivas y atencionales) y al lóbulo temporal (funciones de memoria).

2.2.1 Alteraciones funcionales del lóbulo frontal

El uso del término "hipo-frontalidad" suele describir una reducción de la respuesta cerebral frontal durante tareas cognitivas frecuentemente asociadas con la respuesta pre-frontal. Se trata esencialmente de tareas ejecutivas y de atención, tales como el Wisconsin Card Sorting Task, la Torre de Londres, fluidez verbal, y el Continuous Performance Test (CPT).

La mayor parte de los estudios han sugerido un menor flujo cerebral en regiones pre-fontales durante la realización de tareas de funciones ejecutivas (Davidson et al., 2003). Salgado-Pineda et al. (2004) encuentran, durante la aplicación de un test de atención sostenida (CPT), una hipoactivación, particularmente en el hemisferio derecho, en la corteza DLPF, en regiones temporales y parietales inferiores, así como a nivel sub-cortical en el tálamo. Sin embargo, se ha sugerido que esta “hipo-frontalidad” puede ser el resultado de una incapacidad en activar las regiones frontales durante los procesos cognitivos, más que una disminución de la actividad frontal en sí (Parellada et al., 1998).

Otro proceso cognitivo estudiado respecto a esta región cerebral es la memoria de trabajo (MT): la habilidad de mantener y manipular ítems durante cortos

periodos de tiempo. Esta función, estudiada mediante la tarea N-back, ha demostrado estar también relacionada en la esquizofrenia fundamentalmente con una reducción de la actividad de la corteza dorsolateral prefrontal (Barch et al., 2005).

2.2.2 Alteraciones funcionales del lóbulo temporal

La función más frecuentemente estudiada respecto a los lóbulos temporales, especialmente en la región medial, es la memoria.

Numerosos estudios han examinado la activación cerebral durante el desempeño de tareas de memoria declarativa, encontrando esencialmente una hipo-actividad de la corteza temporal (Ganguli et al., 1997; Ragland et al., 1998, 2001; Crespo-Facorro et al., 2001).

Del mismo modo, varios estudios funcionales han proporcionado una evidencia relativamente coherente de una activación anormal del hipocampo en la esquizofrenia, tanto en la codificación (Jessen et al. 2003) como en el proceso de recupero de la información (Heckers et al. 1998; Jessen et al. 2003; Weiss et al. 2003) (Estos estudios se detallarán más detenidamente en el capitulo 3).

Los estudios funcionales en esta región se han centrado además en investigar la posible implicación del lóbulo temporal en la sintomatología positiva, sobre todo las alucinaciones auditivas, típicas de la patología esquizofrénica (Shergill et al., 2000; Font et al., 2003).

3 El Hipocampo en la esquizofrenia

El hipocampo forma parte del sistema límbico y sus funciones cognitivas primarias se refieren a la memoria y a la organización espacial (Andersen et al., 2007). Es una estructura central en la investigación de la esquizofrenia. Las evidencias de su alteración provienen de estudios en vivo (neuropsicológicos, estructurales y de neuroimagen funcional) así como de los estudios post-mortem (histológicos, neuroquímicos y genéticos).

3.1 Neuropatología (estudios post-mortem)

Los estudios post-mortem evidencian una dilatación del asta temporal del ventrículo izquierdo debida a reducciones en el volumen de estructuras temporo-limbicas más próximas, la amígdala y el gyrus parahippocampal, así como el propio hipocampo (Bogerts et al. 1985; Heckers et al. 1990; Jeste y Lohr, 1989).

La literatura sobre la densidad de las neuronas del hipocampo no es siempre consistente, refiriendo una disminución (Jeste y Lohr 1989; Benes et al. 1998), un aumento (Zaide1 et al. 1997) o ningún cambio (Heckers et al. 1991; Arnold et al. 1995). Igualmente, el tamaño medio de las neuronas piramidales del hipocampo ha sido descrito como reducido (Arnold et al., 1995; Zaidel et al. 1997) o invariado (Benes et al., 1998). Los cambios morfológicos podrían ser explicados por una alteración sináptica, dendrítica o en la organización axonal (Harrison, 2004). En síntesis, los resultados referentes a la citoarquitectura del hipocampo sugieren básicamente una reducción en el tamaño neuronal, más que una pérdida de neuronas (Harrison, 2004).

3.2 Neuroimagen del hipocampo

3.2.1 Neuroimagen estructural

La mayor parte de los estudios de neuroimagen estructural han encontrado evidencias de una reducción del volumen del hipocampo en la esquizofrenia. Nelson et al. (1998), en un estudio meta-analítico, encuentran una reducción volumétrica bilateral del 4% del hipocampo en los pacientes esquizofrénicos comparados con los controles sanos; por tanto se trataría de un cambio evidente aunque sutil. En pacientes de primer episodio los resultados son parecidos (Hirayasu et al., 1998; Velakoulis et al., 1999), lo cual contradice la hipótesis neurodegenerativa, indicando que la atrofia del hipocampo no sería secundaria a la evolución de la enfermedad ni tampoco a su tratamiento.

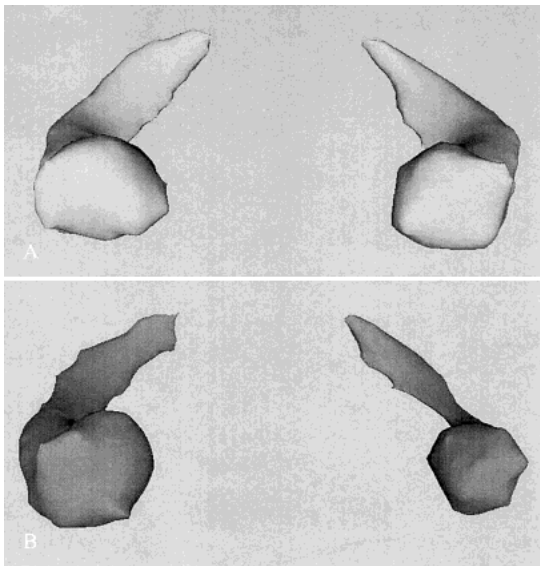

Figura 3. Morfometría del hipocampo en la esquizofrenia en sujetos normales (A) y en pacientes esquizofrénicos (B). (Tomado de Heckers et al., 2001)

Csernansky et al. (2002) refieren anomalías estructurales en la forma del hipocampo, sobre todo en su cabeza, y proponen una alteración en la conectividad con la corteza prefrontal.

A pesar de estas evidencias, aún no está claro qué región del hipocampo (anterior/posterior o derecha/izquierda) contribuye más a la reducción volumétrica. Respecto a la lateralidad, Shenton et al., (2001) hallan una predominancia izquierda en la atrofia del hipocampo. Respecto a la división de la estructura en subregiones anterior y posterior, ha sido se ha encontrado que la reducción afecta primariamente a la parte anterior del hipocampo (Pegues et al., 2003; Szeszko et al. 2003), aunque también existen estudios demostrando lo contrario (Yamasue et al., 2004)

Algunas investigaciones plantean que las diferencias en volumen podrían ser debidas a anomalías presentes en sustancia blanca (Colter et al., 1987, Heckers et al., 1991), que causarían una desconexión de las fibras aferentes y eferentes del hipocampo.

Otra cuestión de alto interés es la relación que puede tener la reducción del hipocampo con los trastornos neuropsicológicos que están asociados a la enfermedad. En esta línea, se han encontrado correlaciones significativas entre el volumen del hipocampo anterior y las funciones ejecutivas (abstracción, fluencia verbal y motora). Estos datos sugieren una vez más un deterioro en el sistema responsable de la funcionalidad temporo-frontal (Bilder et al., 1995).

3.2.2 Neuroimagen funcional

Los estudios que han relacionado la esquizofrenia con anomalías funcionales del lóbulo temporal han realizado 3 tipos diferentes de inferencias (Heckers et al., 2001). Así, la esquizofrenia estaría asociada con:

1. una actividad anormal del lóbulo temporal medial en reposo

2. un incremento de la actividad del lóbulo temporal medial durante la vivencia de alucinaciones auditivas
3. un reclutamiento deficitario del hipocampo durante la realización de tareas de memoria.

Los primeros trabajos en esquizofrenia refirieron una actividad metabólica regional cerebral de la glucosa inferior en esta región (Buchsbaum et al., 1992; Tamminga et al., 1992). Gur et al., (1995) encontraron una disminución en el metabolismo temporal izquierdo, predominantemente en pacientes con síntomas negativos o con alucinaciones graves. En la misma línea, investigando el flujo sanguíneo cerebral en pacientes durante alucinaciones auditivas (Silbersweig et al., 1995; Dierks et al., 1999) se ha observado una evidente implicación de la formación hipocampal.

Respecto a la actividad funcional durante la realización de tareas de memoria, Heckers et al., (1998), en un estudio de PET, encuentran una hipoactivación del hipocampo derecho durante una tarea verbal de memoria episódica. Jessen et al., (2003) observan en pacientes esquizofrénicos una activación inferior en el hipocampo izquierdo durante una tarea de codificación verbal y una hipoactivación bilateral durante una tarea de reconocimiento verbal. En pacientes de primer episodio, se ha encontrado también una hipoactivación hipocámpica durante una tarea de codificación semántica (Achim et al., 2007).

Sin embargo, la relación entre la actividad cerebral del hipocampo y el rendimiento en memoria aún no ha sido del todo confirmada, y se han propuesto hipótesis explicativas que implican las regiones frontales (Mozley et al., 1996; Blackwood et al., 1999).

3.3 Neuropsicología

El hipocampo ha sido tradicionalmente concebido como la principal estructura responsable de la consolidación de la información almacenada desde el corto al largo plazo, permitiendo así la adquisición y retención de nueva información (Squire & Zola-Morgan, 1991). La literatura sugiere la existencia de déficits neuropsicológicos relacionados con funciones temporo-hipocámpicas tales como aprendizaje y memoria (Goldberg et al., 1994; Rubin et al., 1995).

De interés particular para el conocimiento de la esquizofrenia es el rol jugado por el hipocampo en los procesos de memoria, como la codificación y la recuperación de la información, puesto que existe un consenso general acerca de un deterioro mnésico global en la esquizofrenia (Aleman et al., 1999). En particular, el deterioro ha sido detectado en un subsistema de la memoria declarativa, la memoria episódica (Cirillo and Seidman, 2003).

Se ha asociado la alteración del rendimiento en tarea de memoria verbal y espacial con la reducción del volumen del hipocampo izquierdo (Goldberg et al., 1994) y del hipocampo bilateral (Gur et al., 2000; Sanfilipo et al., 2002) Además, se ha encontrado una relación inversa entre el volumen del hipocampo izquierdo y la memoria verbal demorada (Toulopoulou et al., 2004).

Contrariamente a estos resultados, Delisi et al., (1991) no hallaron ninguna asociación entre el volumen del hipocampo y la memoria verbal y espacial tanto en pacientes crónicos como en los de primer episodio.

Asimismo, el volumen del hipocampo correlaciona con funciones normalmente atribuidas a la integridad del lóbulo frontal: funciones ejecutivas y motoras (Szeszko et al., 2002; Bilder et al., 1995), así como con el cociente intelectual (Toulopoulou et al., 2004). Obviamente, la correlación no implica causalidad y

podría ser que una mayor reducción del hipocampo simplemente este indicando mayor afectación del paciente y esta mayor gravedad sea la responsable de las alteraciones cognitivas asociadas a la enfermedad.

El amplio interés hacia el conocimiento tanto anatómico como funcional del hipocampo se explica porque, hipotéticamente, podría contribuir a las anomalías clínicas, psicofisiológicas y cognitivas encontradas en la esquizofrenia (Weinberger and Lipska, 1995).

4 El trastorno de memoria en la esquizofrenia

4.1 Aspectos generales

La memoria es una de las funciones neurocognitivas más investigadas en la esquizofrenia. El sistema de memoria se divide normalmente en memoria a corto plazo y memoria a largo plazo. La memoria a corto plazo, a su vez, puede dividirse en inmediata y de trabajo, mientras que la memoria a largo plazo incluye la memoria declarativa (explicita) y la no-declarativa (implícita) (Lezak et al., 2004)

La especificidad del tipo de memoria deteriorado en la esquizofrenia no está aún bien delimitada, obviamente como en otras patologías neurológicas y psiquiátricas algunos aspectos de la memoria están más afectados que otros (Aleman et al., 1999). En esta línea, se ha demostrado que los individuos con esquizofrenia presentan un mayor deterioro en el aprendizaje de nueva información (Holthausen et al., 2003); dificultad que ha sido atribuida a una codificación insuficiente. Varios estudios neurocognitivos han concluido que el fracaso en los procesos de codificación sería central en el deterioro de memoria en la esquizofrenia (Gold et al., 2004), y esta función podría estar más deteriorada que otros procesos como la evocación y el reconocimiento (Aleman et al., 1999; Holthausen et al., 2003).

Memoria declarativa

La memoria declarativa se define como el recuerdo consciente de hechos, ideas, historias o acontecimientos que han sido aprendidos previamente o que el sujeto ha experimentado conscientemente (Cirillo y Seidman, 2003).

En una revisión sobre el déficit de la memoria declarativa en la esquizofrenia, Weiss y Heckers (2001) resumen las evidencias más importantes:

1. Aunque la esquizofrenia ha sido asociada con un amplio espectro de déficits cognitivos, el de memoria parece particularmente pronunciado.
2. La memoria declarativa está especialmente afectada, mientras que la no-declarativa aparece relativamente intacta.
3. Los estudios de memoria declarativa se han enfocado más en la memoria episódica (memoria de eventos personales) que en la semántica (memoria de hechos), aunque las dos parecen estar deterioradas.
4. El recuerdo libre estaría más deteriorado que el reconocimiento. Esta alteración podría estar debida a una organización semántica pobre durante la codificación o a una estrategia insuficiente en la evocación de la información.
5. Los déficits de memoria no serían debidos únicamente a concentración pobre, falta de motivación, síntomas positivos distractores o al efecto de la medicación.
6. La disfunción de la memoria verbal es el mejor predictor del funcionamiento social y ocupacional deteriorado de estos pacientes

El rendimiento deficitario en la memoria de los esquizofrénicos ha sido a menudo atribuido a limitaciones en la capacidad atencional (Neuchterlein y Dawson, 1984) así como a anomalías en los procesos de control ejecutivo (Goldberg et al., 1989). Aunque un meta-análisis de 70 estudios (Aleman et al., 1999) concluye que la hipótesis de que estos deterioros sean secundarios a

una disfunción atencional no parece muy plausible. En un estudio más reciente se cuestiona si otros déficits cognitivos puedan causar el deterioro en memoria. El papel de la velocidad de procesamiento y las funciones ejecutivas en el rendimiento en memoria fueron examinados mediante un análisis de regresión múltiple por cada participante. En este trabajo se pone en evidencia cómo procesos cognitivos como la atención y la memoria de trabajo ejercen una influencia modesta en la memoria a largo plazo (Holthausen et al., 2003).

Los paradigmas usados para medir la efectividad del sistema de memoria han sido clásicamente dos: la evocación de la información (recuerdo) y el reconocimiento. El recuerdo se refiere al proceso de búsqueda activo de la información previamente codificada, mientras que el reconocimiento representa la identificación de ítems previamente aprendidos (Cirillo y Seidman, 2003).

Los pacientes esquizofrénicos suelen exhibir un recuerdo deficitario en tareas de memoria respecto a controles sanos, siendo mayor la diferencia cuando se comparan recuerdo y reconocimiento (Goldberg et al., 1989). En la esquizofrenia el recuerdo se considera una habilidad limitada, mientras que el reconocimiento parece ser más variable refiriéndose tanto un rendimiento inferior (Gold et al., 1992; Goldberg et al., 1989) como comparable a controles normales (Beatty et al., 1993).

Memoria de trabajo

La memoria de trabajo se define como un sistema cognitivo multicomponente que sirve para mantener “on-line” una cantidad limitada de información durante un espacio de tiempo corto y manipularla simultáneamente para que esté

disponible durante un posible procesamiento cognitivo adicional (Baddeley et al., 1992). Este tipo de memoria se compone de dos almacenes a corto plazo: el visuo-espacial y el fonológico. Los 2 sistemas están conectados a un ejecutor central, encargado de la coordinación y del almacenamiento de la información.

La memoria de trabajo es una de las funciones más comprometidas en la esquizofrenia, siendo tanto la verbal como la no verbal disfuncionales (Conklin et al 2000; Okada 2002).

En la esquizofrenia, el deterioro en MT ha sido utilizado principalmente para caracterizar y cuantificar la disfunción en las áreas corticales prefrontales (Nestor et al., 2002), empleando tanto técnicas neuropsicológicas como de neuroimagen (Jansma et al., 2004).

Investigando el modelo de memoria de Baddeley en individuos con esquizofrenia, Salamé et al., (1998) han identificado un deterioro en todos los componentes del sistema; no sólo en funciones atribuidas al ejecutivo central sino también en el llamado "bucle fonológico".

El grupo de Weinberger ha trabajado durante años sobre la asociación entre el gene COMT y las funciones prefrontales, demostrando repetidamente la activación anormal de la CPFDL durante tareas de memoria de trabajo (para revisión, ver Weinberger et al 2001).

Goldman-Rakic (1999) incluso llega a sugerir que los síntomas de la esquizofrenia podrían surgir como resultado de un deterioro en los procesos de la memoria de trabajo.

4.2 Bases neuroanatómicas del trastorno de memoria en la esquizofrenia

La literatura sugiere que las regiones prefrontales y temporales podrían jugar un rol importante en los trastornos de memoria.

Los primeros estudios que correlacionan funciones cognitivas con medidas volumétricas refieren una relación entre memoria a largo plazo y volumen del lóbulo frontal, en particular de la corteza prefrontal dorsolateral (CPFDL) (Seidman et al., 1994). Análogamente, el volumen de la CPFDL se ha relacionado con la memoria verbal, y más específicamente, con la capacidad de utilizar el contexto y la información organizada para la recuperación de la información (Maher et al., 1995). El recuerdo inmediato de material verbal y visual ha sido asociado con un volumen reducido de la corteza prefrontal (Baaré et al., 1999). Así mismo se ha observado una relación entre la memoria de trabajo y el volumen de sustancia blanca y gris frontal (Nestor et al., 2002).

En el lóbulo temporal, el hipocampo es la estructura más implicada en el funcionamiento de la memoria. La disminución del volumen del mismo se ha asociado a un peor rendimiento en memoria verbal y espacial (Gur et al., 2000; Sanfilipo et al., 2002). Se ha encontrado además una relación inversa entre el volumen del hipocampo izquierdo y la memoria demorada (Toulopoulou et al., 2004). Entre los resultados negativos, Delisi et al., (1991) hallaron una falta de asociación entre el volumen del hipocampo y la memoria verbal y visual en pacientes de primer episodio y crónicos. Adicionalmente, Torres et al. (1997) no encontraron diferencias en el volumen del hipocampo entre pacientes diferenciados por habilidad alta y baja en la memoria demorada.

El gyrus parahipocampal, del mismo modo, es una parte crucial del sistema de memoria del lóbulo temporal medial. Nestor et al., (1993) encontraron una

asociación entre el volumen del gyrus superior temporal izquierdo y el rendimiento en una tarea de memoria verbal asociativa.

En resumen, el lóbulo temporal medial, y en especial el hipocampo, juegan un rol importante en el déficit de memoria presente en esta patología, al que podría contribuir también de forma consistente la corteza prefrontal. La literatura científica sugiere una disfunción de la red fronto-temporal como explicativa del deterioro en memoria presente en la esquizofrenia (Weinberger et al., 1992).

4.3 Bases neurofuncionales del trastorno de memoria en la esquizofrenia

Las técnicas de neuroimagen funcional han permitido aumentar considerablemente el conocimiento sobre el funcionamiento de la memoria y su relación con la actividad regional cerebral mediante la identificación de aquellas estructuras involucradas en el momento de la ejecución de una tarea. En la esquizofrenia las regiones más implicadas en las funciones de memoria son la corteza prefrontal dorsolateral, los lóbulos temporales laterales y el tálamo dorso-medial (Weiss y Heckers, 2001).

Mozley et al., (1996) encuentran una correlación entre el rendimiento en memoria y el aumento en la actividad de la corteza prefrontal inferior izquierda, mientras que Blackwood et al., (1999) describen una asociación con una actividad reducida de la misma región. Otros trabajos asocian el déficit en la evocación verbal con un decremento bilateral en la activación de la CPFDL y en el cortex cingulado anterior (Ganguli et al., 1997; Fletcher et al., 1998). En un estudio de PET, se observó una hipoactivación del córtex prefrontal a nivel bilateral relacionada con un peor rendimiento en recuerdo (Hazlett et al., 2000).

Aunque la mayor parte de estudios encuentren una actividad cerebral reducida de la región frontal, estos resultados no son siempre consistentes, ya que también se ha descrito una hiperactividad de la región (Ragland et al., 1998; Carpenter et al., 1988).

En relación con la ejecución de tareas de memoria de trabajo en pacientes esquizofrénicos, varios estudios describen una activación anormal en la CPF, mientras que la actividad de la CPFVL parece estar inalterada (Barch et al. 2002, Menon et al. 2001).

Respecto a la actividad del lóbulo temporal, muchos estudios demuestran una hipoactividad de la corteza temporal (Ganguli et al., 1997; Ragland et al., 1998; Hazlett et al., 2000) y del hipocampo (Heckers et al., 1998) durante tareas de memoria. Las evidencias referidas son relativas a tareas de recuerdo libre (Ganguli et al., 1997; Hazlett et al., 2000), recuerdo mediante pistas (Heckers et al., 1998) y reconocimiento (Ragland et al., 1998). La implicación del hipocampo en el funcionamiento mnésico está detallada en el capítulo anterior dedicado a esta estructura.

Finalmente, existen pocos estudios que han identificado una actividad disminuida del tálamo referida a la realización de tareas de memoria verbal en pacientes esquizofrénicos (Ganguli et al., 1997; Crespo-Facorro et al., 1999; Heckers et al., 2000).

HIPÓTESIS Y OBJETIVOS

Objetivos

El objetivo general de esta tesis es investigar las anomalías estructurales y funcionales del hipocampo en pacientes con esquizofrenia y que presentan déficit cognitivos de tipo mnésico.

Para responder a este objetivo general se analizan mediante técnicas de Resonancia Magnética estructural y funcional la sustancia gris del hipocampo, su conectividad con las estructuras adyacentes y su actividad funcional durante el desempeño de dos diferentes tareas de memoria declarativa, respectivamente verbal y visual.

Hipótesis

Las hipótesis que nos planteamos con la presente tesis son las siguientes:

Hipótesis I

Los pacientes con esquizofrenia presentarán una atrofia de la sustancia gris del hipocampo.

Hipótesis II

La atrofia de la densidad del hipocampo en los pacientes esquizofrénicos correlacionará con el deterioro en el rendimiento de la memoria verbal declarativa.

Hipótesis III

Los pacientes con esquizofrenia presentarán un déficit en la conectividad de las fibras que relacionan el lóbulo temporal, en particular el hipocampo, con las regiones frontales.

Hipótesis IV

La anomalía presente en la sustancia blanca de esta región correlacionará con el déficit de memoria de los pacientes esquizofrénicos.

Hipótesis V

Los pacientes con esquizofrenia presentarán un decremento de activación del hipocampo durante la realización de tareas de memoria de tipo verbal y visual y presentarán un rendimiento cognitivo análogo a los controles.

Hipótesis VI

La activación cerebral tendrá un efecto de lateralización cerebral dependiente de la naturaleza del estímulo (verbal o visual).

MATERIALES Y MÉTODOS

Esta tesis doctoral consta de un compendio de tres estudios, dos de los cuales ya han sido publicados y el tercero está en fase de revisión:

- Rametti, G., Segarra, N., Junqué, C., Bargalló, N., Caldú, X., Ibarretxe, N., Bernardo, M. (2007). Left posterior hippocampal density reduction using VBM and stereological MRI procedures in schizophrenia. *Schizophrenia research*, 96(1-3), 62-71.

- Rametti, G., Junqué, C., Falcón, C., Bargalló, N., Catalán, R., Penadés, R., Garzón, B., Bernardo, M. A voxel-based diffusion tensor imaging study of temporal white matter in patients with schizophrenia (En revisión en la revista *Psychiatry Research: Neuroimaging*).

- Rametti, G., Junqué, C., Vendrell, P., Catalán, R., Penadés, R., Bargalló, N., Bernardo, M. Hippocampal underactivation in an fMRI study of word and face memory recognition in schizophrenia. (En revisión en la revista *European Archives of Psychiatry and Clinical Neuroscience*)

PAPER I

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Left posterior hippocampal density reduction using VBM and stereological MRI procedures in schizophrenia

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Abstract

Structural deficits in the hippocampus have been implicated in the pathophysiology of schizophrenia. However the role played by structural impairments in the hippocampus in the memory deficits of schizophrenic patients remains unclear. Magnetic resonance imaging was used in this study to investigate left, right, anterior and posterior hippocampal volume and density in 28 schizophrenic patients and 33 normal controls. Voxel-based morphometry analysis showed that schizophrenics had significantly lower density in the right and posterior hippocampus than controls. MRI stereological analysis revealed significant differences in left posterior hippocampus than controls. MRI stereological analysis revealed significant differences in anterior and posterior on both sides, with the left posterior region predominating. Schizophrenics showed significant impairments in verbal learning and long term retention ($P<0.001$). The correlation analyses between hippocampal density and memory variables yielded a significant correlation between forgetting and density of the anterior hippocampus. These findings support the hypothesis of a regional atrophy within the hippocampus in schizophrenic patients.

Keywords: Hippocampus; Memory; MRI; Stereology; Neuropsychology; Schizophrenia

1. Introduction

In recent years interest in the study of structural cerebral changes in schizophrenia has grown rapidly due to the enormous progress made by neuroimaging techniques, particularly magnetic resonance. Several investigators have focused their attention on changes in the hippocampus, because this portion of the brain can partially explain the memory impairment associated with schizophrenia (for review see Wright et al., 2000).

Volumetric studies carried out with magnetic resonance imaging generally report a global reduction in the hippocampus (Bogerts et al., 1985; Buchanan et al., 1993; Nelson et al., 1998; McCarley et al., 1999; Weiss et al., 2005) and differences in shape compared to controls (Velakoulis et al., 2001; Csernansky et al., 1998; Wang et al., 2001; Styner et al., 2004). However, several other studies found no volume reductions in patients (Delisi et al., 1991; Laakso

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et al., 2001; Marsh et al., 1999; Niemann et al., 2000; Rajarethinam et al., 2001; Sanfilipo et al., 2002).

A meta-analysis of lateralization carried out in 1998 showed a bilateral volume reduction of 4%, but no significant differences were found between the right and the left hippocampus (for a review see Nelson et al., 1998). However, the more recent review of 49 MRI studies by Shenton et al. (2001) reported a clear left lateralization of the amygdala–hippocampal complex.

A neuropathological study suggests that most structural differences in the hippocampus in neuronal size and density are found in the posterior region (Benes et al., 1991). However the anterior–posterior regional contrast has not been thoroughly investigated by means of MRI procedures. Pegues et al. (2003) observed bilateral anterior hippocampal reduction in chronic patients. Similar results were obtained in antipsychotic-naive patients with a first-episode (Szeszko et al., 2003; Narr et al., 2004). In contrast, other authors found reductions in the posterior part (Narr et al., 2001; Becker et al., 1996; Yamasue et al., 2004).

Few studies have investigated the correlation between hippocampal reductions and memory impairment, and the results are contradictory (for review, see Antonova et al., 2004). Impaired memory has been associated with left (Goldberg et al., 1994) and bilateral hippocampus reduction (Gur et al., 2000; Sanfilipo et al., 2002). However, Torres et al. (1997) did not observe hippocampal differences between schizophrenic patients with or without memory deficits. Delisi et al. (1991) found that the Associative Learning and Logical Memory subtests of the Wechsler Memory Scale did not correlate with the hippocampus/amygdale size, but did correlate with bilateral parahippocampus size.

The contradictory results on volumetric data in schizophrenia may be due to the different procedures used to quantify the volume of the hippocampus. In the present study we investigated right and left asymmetries and anterior–posterior gradients using two different methods of MRI analysis in the same sample. The second objective was to correlate hippocampal reductions with memory impairment.

2. Methods

2.1. Subjects and clinical assessments

The patient group comprised 28 subjects (mean age± standard deviation (SD)=27.7±4.4) who met DSM-IV criteria for schizophrenia (American Psychiatric Association, 1994). The patients were recruited from the Psychiatry Service of the Hospital Clinic in Barcelona. Consensus on patients' diagnoses was established by two expert psychiatrists after independent assessments. The structured interview SCID I (Structured Clinical Interview for DSM-IV-TR) was also used. The clinical characteristics of patients were quantified using the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorman, 1962) and the scales for assessment of positive and negative symptoms assessment (Andreasen, 1983, 1984). All patients were receiving stable doses of antipsychotic medication. The mean of chlorpromazine equivalents was 270.59 mg/day (±255.04). No patients were taken anticholinergic drugs. Clinical characteristics of patients and treatment are detailed in Table 1.

The control group consisted of 33 healthy subjects (mean age±SD=28.09±5.1 years) matched for age, gender, handedness and parental socio-economic status with the patient group. The normal control version of SCID was administered to healthy subjects in order to exclude DSM-IV disorder. None of the controls had any history of mental, neurological or physical illness or recent substance abuse. Table 1 shows the demographic and clinical characteristics of the subjects.

All subjects were administered a short battery of neuropsychological tests covering the cognitive functions that are mainly impaired in schizophrenic patients: memory and frontal lobe functions. Global cognitive performance was estimated using the Vocabulary subtest of the Wechsler Adult Intelligence Scale (WAIS-III).

Table 1
Demographics and clinical characteristics of the sample

	Schizophrenics		Controls	
Number of subjects	28		33	
Gender (Male/Female)	21/8		21/12	
Handedness (Right/Left)	25/3		29/4	
	Mean	SD	Mean	SD
Age (years)	27.7	4.4	28.09	5.1
Illness duration (years)	5.5	2.9		
BPRS	33.6	7.5		
Positive symptom severity	12.4	5.07		
Negative symptom severity	17.2	5.6		
Total symptom severity	59.5	15.4		

Antipsychotic treatments (dose range)	*n*
Quetiapine (800–1066/day)	3
Aripiprazole (400–600/day)	2
Risperidone (50–300/day)	11
Risperidone C (150–300/day)	3
Olanzapine (200–250/day)	2
Clozapine (114–171/day)	4
Amisulpiride (225/day)	1
Olanzapine/Risper. C (200+300/day)	1

BPRS: Brief Psychiatric Rating Scale.

For memory assessment we used Rey's auditory verbal learning test (RAVLT) since it is among the most sensitive verbal memory test because of its relative freedom from associative context (Lezak et al., 2004). In the RAVLT procedure, during five consecutive presentations, we recorded the number of words learned from a list of fifteen words, and the number of words recalled after a 20-minute delay. The percentage of words recalled after delay with respect to the last trial was taken as a measure of forgetting. To assess frontal lobe dysfunctions of the patients we used: TMT (Trail Making Test), COWAT (Controlled Oral Word Examination Test) and CPT (Continuous Performance Test).

The study was approved by the ethics committee of the Hospital Clínic of Barcelona. All participants were informed about the procedure and their written informed consent was obtained.

2.2. MRI acquisition and preprocessing

MRI data were obtained on a GE Signa 1.5 T scanner (NV/Cv1 8.4 General Electric, Milwaukee, WI). High resolution whole brain T1-weighted fast spoiled gradient-recalled three dimensional sequence (time of repetition/time of echo=12.1/5.2 ms, time of inversion=300, FOV=24×24 cm, 256×256 matrix, flip angle 20°) was acquired in an axial plane yielding contiguous slices of thickness 1.5 mm. MR images were analysed using the voxel-based morphometry approach by SPM2 software (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, University College London, UK, http://www.fil.ion.ucl.ac.uk/spm), running in Matlab 6.5 (MathWorks, Natick, Massachusetts, USA).

For the image preparation, a single investigator performed the prior manual steps (line determination of the anterior and posterior commissures and image reorienting) for all images. We followed the methodology of Good et al. (2001). First, we created a prior anatomical study-specific template obtained from all 61 subjects (patients and controls). The normalized structural data from the 61 subjects were smoothed with an 8 mm full-width at half-maximum (FWHM) isotropic Gaussian kernel, and a mean image was created with the previous smoothed files. The spatially normalized images were automatically segmented into separate images representing probability maps for gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF). The tissue classification method is described in detail in Ashburner and Friston (1997). A separate GM template was created by averaging all the 61 smoothed normalized GM images. All the original T1 images were then segmented into GM, WM and CSF files. The new extracted GM images were normalized to the specific GM template created previously. The segmented and normalized GM images were smoothed with an isotropic Gaussian kernel 8 mm in FWHM. These final images to be compared between groups can be taken to represent regional differences in density of GM. For more information about VBM procedures and interpretation see Mechelli et al. (2005).

2.3. Statistical analysis

2.3.1. VBM group analysis

Voxel-based morphometry (VBM) analysis was carried out on SMP2. VBM compares images of cerebral gray matter obtained from segmented MRI images using statistical parametric mapping to identify and make inferences about regionally specific differences. We analyzed gray matter density, performing a group comparison of hippocampal GM using a one-sided t test with two different contrasts: patients>controls and patients<controls. The resulting t-statistic maps were thresholded at a P-value of <0.05 corrected for multiple comparisons using the FWE approach. We selected this high level of significance to avoid type I errors. We performed two left and right ROI (region of interest) analyses using WFU-Pickatlas toolbox software for the SPM version (Maldjian et al., 2003). Only clusters larger than 20 contiguous voxels were considered in the analysis. For the clusters we also used a corrected level of significance of $P<0.05$.

2.4. Stereological analysis

We carried out a stereological analysis to measure hippocampus volume using ANALYZE 6.0 software. Stereology is a semi-automated method whose accuracy and validity for volume estimation has been reported in several studies. Stereological measurements yield high repeatability and precision (Ronan et al., 2006).

Prior to measurement, MR image volumes were reoriented according to the anterior–posterior commissure. An observer used fixed grid stereology to estimate hippocampus volumes. The hippocampus volume was measured using a 2.5×2.5 mm^2 rigid grid with random starting position and horizontal angle of deviation. The grid was superimposed on every third coronal slice. The interslice increment and grid size chosen yielded an error coefficient between 0.01–0.04. The orthogonal tool of the software allowed three orthogonal views of each grid at the same time, thus enabling us to decide more accurately which cross is contained in the

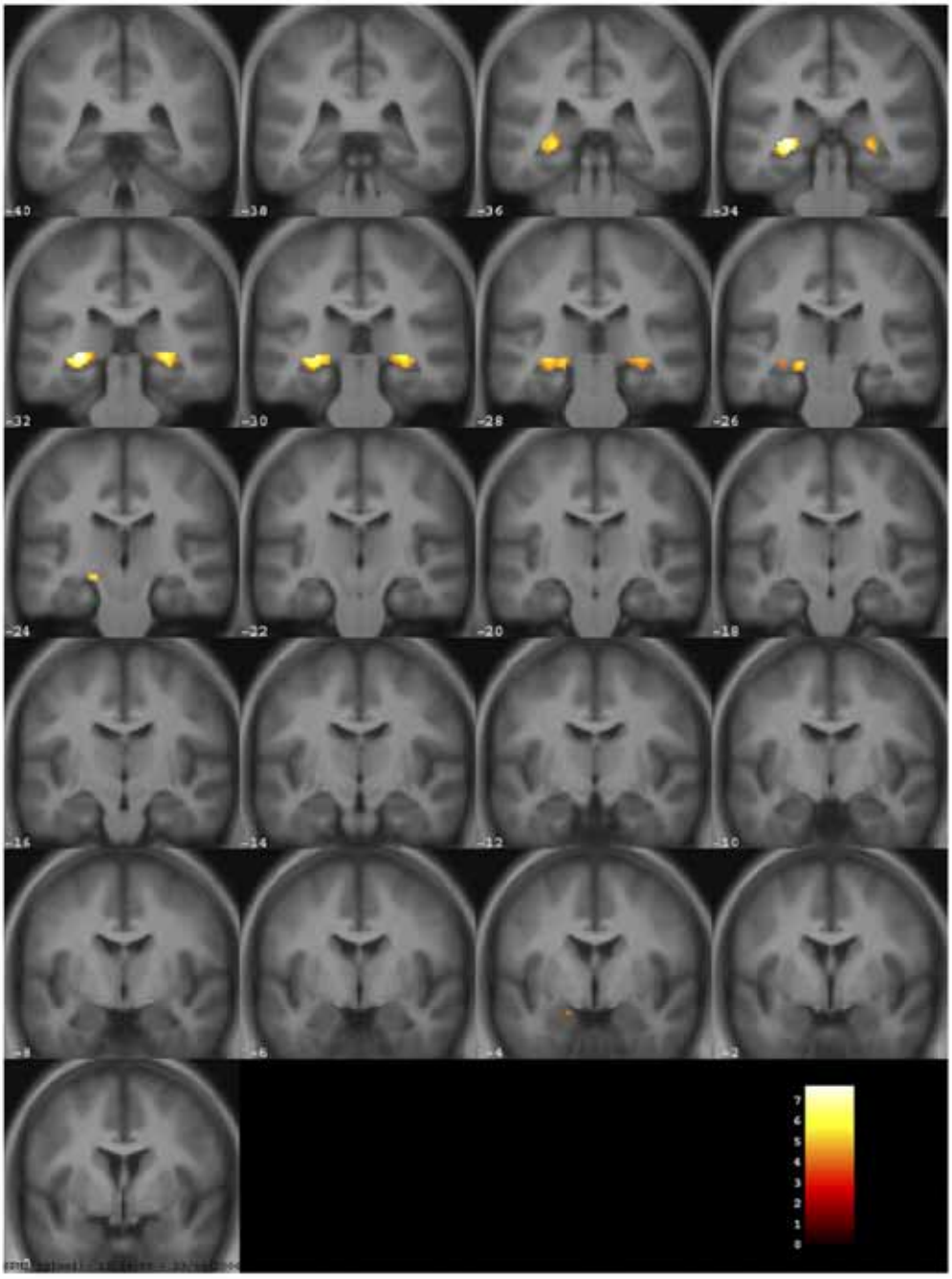

Fig. 1. Coronal MRI slices in which the hippocampus is present. Cut-40 is the most posterior and cut 0 the most anterior. Regions of increased gray matter concentration are superimposed on a normalized single subject image. The colour bar represents the *t* score: Yellow indicates higher statistical significance than orange or red. The statistical parametric maps follow the standard neuroradiological representation, the left side of the images corresponding to the left hemisphere. The number of slices corresponds to the relative position in the *X* axis. There are statistically significant gray matter decreases in schizophrenic patients compared to controls in both hippocampi, but in the left hemisphere the density changes are more extensive and the *P* values higher than those of the right hemisphere. The density changes are predominantly in the posterior cuts.

Table 2
Results of volume of the hippocampus, hippocampal subregions and ICV

	Group				*t*	*P*
	Patients		Controls			
	Mean	SD	Mean	SD		
Left anterior volume (cm^3)	1.05	0.06	1.06	0.05	−1.18	0.24
Left anterior hippocampus/ICV Ratio × 100	0.06	0.007	0.06	0.006	−2.58	0.01
Right anterior volume (cm^3)	1.03	0.08	1.06	0.07	−1.71	0.09
Right anterior hippocampus/ICV Ratio × 100	0.06	0.008	0.06	0.006	−2.91	**0.005**
Left posterior volume (cm^3)	0.96	0.11	1.03	0.07	−2.98	**0.004**
Left posterior hippocampus/ICV Ratio × 100	0.05	0.008	0.06	0.007	−3.82	**<0.001**
Right posterior volume (cm^3)	0.93	0.10	0.96	0.09	−1.39	0.17
Right posterior hippocampus/ICV Ratio × 100	0.05	0.007	0.06	0.008	−2.87	0.006
Hippocampus volume (cm^3)	3.98	0.24	4.13	0.17	−2.91	**0.005**
Hippocampal volume/ICV Ratio × 100	0.24	0.02	0.26	0.02	−3.59	**0.001**
Intracranial volume (cm^3)	1665.73	205.82	1566.16	136.74	2.25	0.028

ICV: intracranial volume.
In bold the *P* values that achieved statistical significance after Bonferroni's correction.

hippocampus structure. Finally, we obtained direct values from 4 hippocampal subregions: right-anterior, right-posterior, left-anterior and left-posterior. The hippocampal boundaries were defined using the same traces as Pantel et al. (2000). For the anterior boundary the alveus was used as a border between amygdala and hippocampus, and the posterior boundary was formed by the CSF of the lateral ventricle.

Following the procedure described by Sullivan et al. (1995), anterior and posterior portions of hippocampus were designated by dividing the total number of slices in half. When the total slice number was an odd number, the larger number of slices was assigned to the anterior division. The size of the grid extension along the hippocampus was between 65 and 86 slices. An inter/intra-rater reliability study was carried out by two raters who calculated hippocampal volumes. For the inter-rater reliability, Intraclass Correlation Coefficients (ICC) was 0.91 for the anterior region and 0.87 for the posterior; for the intra-rater reliability ICC was 0.95 for anterior and 0.96 for the posterior region.

Since the hippocampal and the intracranial volumes (ICV) are related, we performed a ratio (regional brain volume in mm^3/ICV in $mm^3 \times 100$).

Group comparison was performed using *t* test for independent samples from SPSS 12.0. Following the Bonferroni correction for multiple comparisons, the level of significance was established at *P* equal or inferior to 0.005.

2.5. Correlations

We performed "simple regression" (correlation) SPM2 analysis through the gray-matter density maps and the scores of the schizophrenic subjects on the memory tests. The results of this analysis were thresholded at $P<0.001$ (uncorrected). We also restricted the correlation analysis to the hippocampal region using the MRIcro toolbox.

Correlations between volumetric data from stereological analysis were performed by SPSS 12.0. For these analyses, we used the Bonferroni correction for multiple comparisons. The Bonferroni adjustments lowered the alpha for each test to 0.004.

3. Results

Results from the VBM analysis showed reductions in posterior hippocampus in schizophrenics compared to controls. These reductions were robustly significant for both right (*t* statistic = 6.53; corrected $P<0.001$) and left posterior regions (*t* statistic = 7.67; corrected $P<0.001$) (see Fig. 1). The cluster size for the right hemisphere was 86 and the MNI coordinates for the voxel of the maximum significance were 28 −32 −2; the cluster size for the left hemisphere was 180 and the MNI coordinates for the voxel of maximum significance were −28 −32 −4.

Table 3
Rey's memory variables

	Group				*t*	*P*
	Patients		Controls			
	Mean	SD	Mean	SD		
Learning	50.82	9.77	58.91	7.13	−3.72	<0.001
Delayed recall	10.57	2.60	12.97	2.14	−3.94	<0.001
Forgetting	20.82	3.60	86.79	19.52	−17.60	<0.001

Table 4
Neuropsychological results

	Group				t	P
	Patients		Controls			
	Mean	SD	Mean	SD		
WAIS-III — Vocabulary	46.14	6.45	47.61	10.50	−0.64	0.52
TMT-A	33.29	10.25	28.03	7.07	2.35	0.022
TMT-B	83.25	36.20	57.39	16.54	3.67	0.001
COWAT	32.68	10.28	42.55	10.30	−3.73	<0.001
CPT — Omission errors	2.14	4.03	0.97	2.45	1.36	0.17
CPT — Commission errors	9.14	7.35	7.06	6.25	1.17	0.24
CPT — Reaction time (ms)	321.82	4.19	232.03	2.45	−1.37	0.17

Note. WAIS-III: Wechsler Adult Intelligence Scale-Third edition; TMT: Trail Making Test; COWAT: Controlled Oral Word Examination Test; CPT: Continuous Performance Test.

In the MRI stereological analyses, we found strong statistical differences between patients and controls in global hippocampal volume, and also for the ratios controlling for the intracranial cerebral volume. The descriptive data and group comparison for each hippocampal subregions are reported in Table 2. The left posterior hippocampal region reached the strongest statistical significance. Direct values of the left and right anterior and the right posterior sections did not differ between schizophrenics and controls, but on applying the intracranial volume correction several sub-regional differences reached statistical significance. The strongest difference was seen in the left posterior region (see Table 2).

A repeated measure ANOVA with two within-subject factors, right-left and anterior–posterior, and one between-subjects factor, patients-controls, were performed. Three main effects were significant: right-left ($F=16.70$; $P<0.001$), anterior–posterior ($F=40.62$; $P<0.001$) and patients-controls ($F=8.51$; $P<0.005$). Only one significant interaction was found, between the anterior–posterior factor and the right-left factor ($F=8.51$; $P<0.006$).

Patients differed from controls on all the memory measures of the RAVLT: learning, delayed recall and forgetting and also differed from controls in several frontal lobe functions (see Tables 3 and 4). There was a significant correlation between forgetting and hippocampal density assessed by VBM. The cluster size was 45 for the right hippocampus and 40 for the left (see

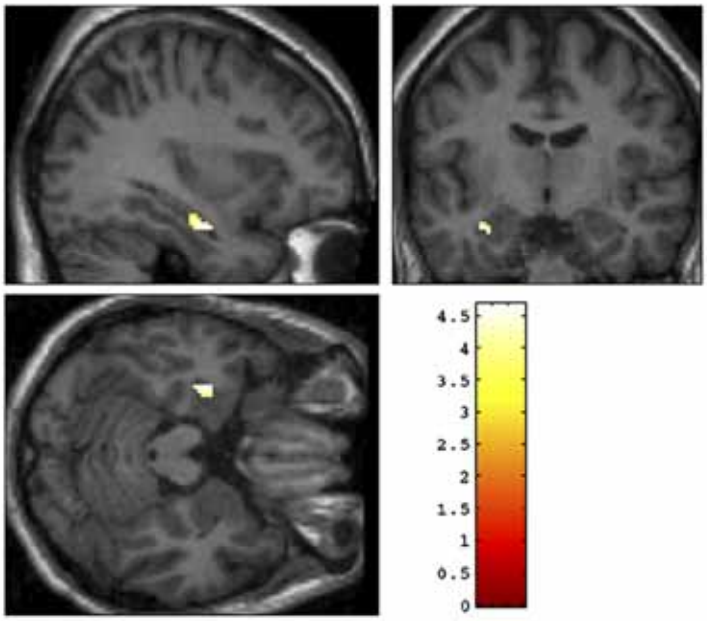

Fig. 2. This figure illustrates the correlations between verbal forgetting and decreases in gray matter in the hippocampus of schizophrenic patients. In yellow the hippocampal regions that achieved statistical significance. The three images correspond to a sagittal, coronal and axial planes. The bars indicate the P values. P values are 0.037 FWE-corrected for the left hemisphere and 0.033 FWE-corrected for the right. The r for the voxel local maxima is 0.54 (MNI coordinates −34 −10 −22).

Fig. 2). Moreover, for the volumetric analysis performed by stereology we observed a trend towards significance between delayed recall and the left anterior region ($r=0.39$; $P=0.038$). There was no significant correlation between hippocampal volume and RAVLT variables in controls. No significant correlations were seen for frontal lobe variables or IQ estimation.

4. Discussion

The aim of this study was to investigate regional hippocampal reductions in schizophrenic patients using two complementary MRI approaches. Voxel-based morphometry showed bilateral density reduction, while volumetry by stereological analysis revealed density reduction mainly in the left posterior region.

As regards lateralization, we found significant differences in density in the right and left hippocampus between patients and controls, but the left hippocampus had a larger cluster and higher statistical significance. Volumetric analyses performed by stereological procedures also showed a clear left predominance. Both direct and corrected values of the left hippocampal volume achieved statistical significance. In contrast, the right hippocampal volume was significant only after intracranial volume correction. This significance is probably due to the greater intracranial volume in patients compared to controls. The left hippocampal volumetric reduction is a consistent finding (Csernansky et al., 1998; Wang et al., 2001; Yamasue et al., 2004), and also coincides with the results of a study of shape differences in the amygdale–hippocampus complex (Shenton et al., 2002). The laterality differences are less evident in neuropathological studies when neuronal size is considered. Zaidel et al. (1997a,b) found that neuronal size was reduced in the left CA1 and CA2 regions, and in the right CA3, while others have demonstrated cellular loss and disorganization in bilateral subiculum, CA3 and CA4 fields (Harrison and Eastwood, 2001).

Since different studies have found neuronal density to be increased (Zaidel et al., 1997a,b) decreased (Jeste and Lohr, 1989), or unchanged (Benes et al., 1991; Arnold et al., 1995), the issue remains controversial. Our MRI findings suggest bilateral density decreases. The measurements of gray matter density in VBM might reflect the underlying cytoarchitecture related to the organization of layers or the density of neurons (Luders et al., 2005) although they should not be confused with cell packing density measured cytoarchitectonically (Mechelli et al., 2005).

The density loss observed in VBM was bilateral but predominantly posterior. The left posterior predominance was clearly seen in the volumetric analysis carried out by stereology. This finding is consistent with other studies which report a reduced volume in the posterior part of the amygdala–hippocampal complex (Bogerts et al., 1993; Becker et al., 1996; Hirayasu et al., 1998; Yamasue et al., 2004). However, in some studies in which the hippocampus was divided into posterior and anterior, the reduction was more pronounced in the anterior part (Pegues et al., 2003; Szeszko et al., 2003). This discrepancy may reflect the use of different techniques to analyse the anterior and posterior sub-regions. Our results agree with a three-dimensional mapping study that found posterior hippocampal volume, length and width reductions (Narr et al., 2001), and another that demonstrated hippocampal reductions in the posterior two-thirds (Velakoulis et al., 2001).

Finally, the first DTI study of connectivity, which separated the four hippocampal sub-regions, reported only a significant bilateral reduction of anisotropy values in the posterior hippocampus (Kalus et al., 2004). In terms of structural connections the posterior hippocampus projects to the dorsolateral region of the prefrontal cortex (Goldman-Rakic et al., 1984). Moreover, there is a decrease in neuronal size, particularly of pyramidal-neurons, in the posterior hippocampus of schizophrenic patients (Benes et al., 1991).

Voxel-based morphometry is a sensitive technique for the detection of hippocampal impairment in schizophrenia. Honea et al. (2005) reported that the left medial temporal region was the region most frequently reported to be decreased in VBM studies (in 69% of studies). Negative results using VBM could be due to the selection of VBM procedures. Sensitivity can be increased by using a small smoothing kernel and a small-volume correction, as well as a study-specific template. Small kernels (4–8 mm) are appropriate for smaller structures such as the hippocampus.

Intracranial volume was greater in patients than in controls. This difference is difficult to explain, because in the classical literature using manual measures of ICV, no differences or reductions were observed in patients. According to the review by Shenton et al. (2001), 39 of 50 MRI studies reported negative findings in ICV. Using the automatic methods provided by VBM, no significant difference was found between patients and controls (for review see Honea et al., 2005). Only one study observed larger ICV in patients (Shapleske et al., 2002).

The neuropsychological study showed that patients have verbal memory impairment according to both learning and forgetting measures. In the correlation analysis we observed only a significant association between forgetting and gray matter in the anterior hippocampus. There was no correlation between hippocampal volume and RAVLT

variables in controls. Similar findings have previously been reported in normal adult subjects. Correlations between memory and hippocampal volumes are often seen in aged or pathological subjects (see Van Petten, 2004 for a review). To our knowledge, this is the first study to correlate regional hippocampal gray matter volume with memory performance in patients using voxel-based morphometry. Previous reports with the classical volumetric approach have reported an association between left hippocampus and verbal memory (Goldberg et al., 1994; Gur et al., 2000; Sanfilipo et al., 2002), but these studies did not investigate the hippocampal sub-regions.

The antipsychotic treatment produces clear structural changes in the caudate nucleus depending on the type of treatment (Chakos et al., 1994; DeLisi et al., 1995; Chakos et al., 1998). In our patients we cannot exclude possible effects of the medication on hippocampal gray matter reductions. A recent MRI study (McClure et al., 2006) reported no effects of the treatment status and antipsychotic type in the volume of the hippocampus measured by ROI's procedures. However, VBM data of these patients showed a significant effect for time and interaction between time and type of medication. Atypical antipsychotics produce an increase of the left hippocampal gray matter but typical antipsychotics have no effects.

To summarize, our results suggest that the hippocampal reduction may partly explain the memory deficits in schizophrenia and gives support to the hypothesis of bilateral structural deficits, albeit with clear left posterior predominance. Voxel based morphometry seems to be more sensitive to subtle changes than volumetric approaches.

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All authors contributed to and have approved the final manuscript.

Conflicts of interest

None of the authors have financial or other conflicts of interest related to the material in the manuscript.

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PAPER II

A voxel-based diffusion tensor imaging study of temporal white matter in patients with schizophrenia

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Abstract

Diffusion tensor imaging (DTI) is a relatively new technique used to detect changes in the anisotropic diffusion of white matter. The study of the disruption of brain connectivity may increase our understanding of cognitive deficits associated with schizophrenia. Here we analysed DTI data in 25 patients with DSM-IV schizophrenia and 24 healthy controls. Two complementary measures, fractional anisotropy and apparent diffusion coefficient, were considered and analysed using voxel-based morphometry analysis of the whole brain. Declarative memory functions were also investigated and their associations with DTI data were analysed. Fractional anisotropy was significantly reduced and apparent diffusion coefficient increased in the left sub-gyral white matter of the temporal lobe, which involves the posterior part of the fornix. In the schizophrenic group, females had lower FA than males in the genu of the corpus callosum. Memory functions correlate with FA values. These data provide further evidence for the disruption of white matter connectivity in the left medial temporal lobe, and for its contribution to the declarative memory deficit in schizophrenia.

1. Introduction

The heterogeneity of the cerebral dysfunctions associated with schizophrenia requires comprehensive investigation involving the use of several neuroimaging techniques. The best technique for investigating white matter (WM) dysfunctions and structural brain connectivity deficits is probably diffusion tensor imaging (DTI). DTI allows study of the structural integrity of neuronal connections sensitizing the MR signal to the three-dimensional diffusion of water (Basser et al., 1994). The diffusion-weighted images are obtained by introducing strong magnetic field gradient pulses into an imaging pulse sequence. Anisotropy is the property of being directionally dependent, as opposed to isotropy, which means homogeneity in all directions. In the brain, in diffusion anisotropy the movement of the water molecules is parallel, rather than perpendicular, to the axons. The motion of water diffusion becomes abnormal as it is impeded by the presence of structural barriers in tissue such as white matter tracts. Fractional anisotropy (FA) is a measure of the magnitude of diffusion

anisotropy (Basser and Pierpaoli, 1996), expressed as a numerical value between 0 and 1 without a unit. A higher FA value implies a larger degree of anisotropic motion of water molecules. The apparent diffusion coefficient (ADC) is a measure of the average intravoxel diffusion of water molecules in all directions (Ardekani et al., 2005).

The DTI findings are of great clinical relevance because they provide information on WM tissue to complement the data obtained from T1 and T2 images (Le Bihan et al., 1992).

The results of studies using DTI in schizophrenia are not always consistent (Kanaan et al., 2005; Kubicki et al., 2007). Some report WM reductions in several brain regions (Lim et al., 1999; Agartz et al., 2001; Ardekani et al., 2005; Mitelman et al., 2006), while others report no differences compared to controls (Steel et al., 2001; Foong et al., 2002; Price et al., 2005).

Few studies have focused on the hippocampal fibers. Decreased FA values have been reported in the fornix of schizophrenic patients (Kubicki et al., 2005; Kuroki et al., 2006).

Memory dysfunctions in schizophrenia have been attributed mainly to gray matter reductions (Delisi et al., 1991; Rametti et al., 2007), but it is possible that white matter changes may play a role in such cognitive impairments. On the basis of PET and other physiological studies, Friston et al., (1995) proposed a model of fronto-temporal dysfunctions to explain the pathophysiology of schizophrenia. They concluded that the abnormalities in specific regions cannot explain the clinical and cognitive symptoms of the patients. An abnormal cerebral functional connectivity is probably the cause of the psychopathology of these patients. The association between the impairment in connectivity reflected by DTI abnormalities and the cognitive deficits of schizophrenia is still unclear. A few studies have reported correlations between neuropsychological performance and DTI findings. FA values of the cingulum correlated with executive functions (Kubicky et al., 2003, 2005; Nestor et al., 2004) and verbal associative memory functions with the uncinate fasciculus (Kubicky et al., 2002, Nestor et al., 2004). Recently, a significantly correlation between reduced FA values of the fornix and scores of declarative episodic memory has been reported (Nestor et al., 2007). These studies were performed using a priori regions of interest (ROI analysis). It is possible that other as yet unexamined white matter regions may contribute to memory deficits. The objective of the present study was to investigate abnormal white matter morphology involving the medial temporal region and its relationship with declarative memory impairment.

2. Methods

2.1 Subjects

Twenty-five patients (12 men and 13 women) with schizophrenia and 24 healthy controls (11 men and 13 women) were recruited for the study.

The subjects were matched in pairs with regard to gender, age and parental educational status. The mean (SD) age was 32.2 (± 6.8) years for the schizophrenia subjects and 31.8 (± 7.0) for controls. Table 1 summarizes the demographic and clinical characteristics of these samples.

Schizophrenic patients were diagnosed according to DSM-IV-TR criteria (American Psychiatric Association, 1994), by the consensus of two psychiatrists (R.C. and M.B.) who had conducted the Structural Clinical Interview (SCID) for the DSM-IV. All patients were receiving stable doses of antipsychotic medication. All but two patients were on atypical antipsychotic medication. The mean of chlorpromazine equivalents was 205.94 mg/day (± 142.79).The patients were recruited from the Psychiatry Service of the Hospital Clinic, Barcelona.

The healthy comparison subjects were screened using the Structured Clinical Interview for DSM-IV, Non Patient version (First et al., 1998), by a trained psychologist in order to rule out possible psychopathologies. The healthy comparison subjects had never been mentally ill, nor did they have any first-degree relatives with a psychotic disorder. None of the patients or controls had a history of neurological or significant medical illness, or recent substance abuse. After a complete description of the study to the subjects, informed consent was obtained.

2.2 MRI acquisition and preprocessing

MRI data were obtained on a GE Signa 1.5 T scanner (NV/Cv1 8.4 General Electric, Milwaukee, WI). A high resolution whole brain T1-weighted fast spoiled gradient-recalled three dimensional sequence (time

of repetition/time of echo = 12.1/5.2 ms, time of inversion = 300, FOV = 24 x 24 cm, 256 x 256 matrix, flip angle 20°) was acquired in an axial plane yielding contiguous slices of thickness 1.5 mm.
Diffusion tensor images (DTI) were acquired in the axial plane with a pulsed gradient, single spin echo, EPI sequence (TR/TE= 10.000/83.1ms, 96 x 96matrix, field of view 24 cm x 24 cm, slice thickness= 3 mm). The diffusion sensitizing gradients were applied along twenty-five non-collinear directions at a gradient strength corresponding to b value of 1000 s/mm^2. This scan also provides two T2-weighted volumes (B0 images).
Maps of the diffusion tensor elements, FA and ADC, were computed from the 25 DTI volumes for each subject using the Functool Program 2.6.6 (GE, Milwaukee,WI), prior to any image analysis. The program allows removal EPI distortions (scaling, translation and shearing) from the raw images.

Table 1 Demographic and clinical characteristics of the sample

	Patients with Schizophrenia		**Normal Comparison Subjects**	
Number of subjects	25		24	
Gender (Male/Female)	12/13		11/13	
Handedness (right/left)	24/1		24/0	
Schizophrenia subtype:				
- **paranoid**	21			
- **disorganized**	1			
- **undifferentiated**	2			
- **residual**	1			
	Mean	**SD**	**Mean**	**SD**
Age (years)	32.2	6.8	31.8	7.0
Education (years)	12.2	2.4	15.1	1.6
Parental education (years)	10.7	4.0	9.4	4.2
Duration of illness (years)	10.42	5.7		
PANSS score:				
- **positive**	16.88	10.1		
- **negative**	22.52	9.5		
- **general**	38.40	13.6		
- **total**	77.84	29.8		

Note. PANNS: Positive and Negative Syndrome scale (Kay et al., 1987)

2.3 Image analysis
VBM. T1, T2, FA and ADC images were analysed using the voxel-based morphometry approach by SPM2 software (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, University College London, UK, http://www.fil.ion.ucl.ac.uk/spm), running in Matlab 6.5 (MathWorks, Natick, Massachusetts, USA).

For the image preparation, a single investigator performed the prior manual steps (line determination of the anterior and posterior commissures and image reorienting) for all images T1, T2, FA and ADC. Each B0 image was coregistered to the T1 structural image and the coregistration parameters obtained were applied to the corresponding FA and ADC maps. Next, T1 images were automatically segmented into three tissue types: gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF).
For the normalization step, a MNI template obtained from the normalization of T1 images was used to put FA and ADC maps

into a standard space. Moreover an a priori SPM5 white matter mask was used in order to include only the voxels that corresponds to WM.

The normalized FA and ADC maps were smoothed with a 12-mm isotropic Gaussian kernel. The choice of such kernel depends of the image quality. Our DT-MRI data included 25 non-collinear directions producing high noise level. By increasing the size of the smoothing kernel we were able to reduce the noise level. At present there is no consensus on the width of the Gaussian filter when DT-MRI data are analysed with the VBM approach; the only rule of thumb is that the full with at half maximum (FWHM) must be 2-3 times the voxel dimension (Jones et al., 2005).

Non-brain areas on the smoothed normalized fractional anisotropy (SWFA) and smoothed normalized apparent diffusion coefficient (SWADC) images were removed automatically using an explicit brain mask. Those final images were compared to identify differences between the two groups.

We performed ROI (region of interest) analyses using the WFU-Pickatlas toolbox software for SPM version, version 1.02 (Joseph Maldjian, Wake Forest University Baptist, Medical Center, Department of Radiology, Winston-Salem, NC). For this purpose, in normalized images a sphere 10-mm diameter sphere was placed at the centre of mass of each region of interest (ROI).

Table 2 Diffusion indices in fornix using VBM and manual ROIs

		Group				t	*P*
		Patients		Controls			
		Mean	SD	Mean	SD		
VBM	FA[a]	0.46	0.09	0.55	0.05	-3.86	<0.001
	ADC[a] x 10-3mm2/sec	1.04	0.07	0.96	0.03	4.40	<0.001
ROIs	Body FA[b]	0.47	0.03	0.54	0.02	-6.32	<0.001
	Body ADC[b] x 10^{-3} mm^2/sec	1.03	0.26	0.86	0.06	3.00	=0.004
	Pillars FA[b]	0.37	0.04	0.42	0.03	-3.92	<0.001
	Pillars ADC[b] x 10^{-3} mm^2/sec	1.07	0.18	0.97	0.10	2.35	=0.023

Note. [a] VBM analysis; [b] Manual ROIs analysis.

To perform the gray matter analysis of the hippocampus and parahippocampus, we applied the standard VBM protocol to the image data, as reported by Mechelli et al. (2005). The T1 MP-RAGE images of all subjects were normalized using the standard MNI template and linear transformation functions (12 parameter using affine transformations explicitly excluding non-linear functions). The normalized scans were then segmented into grey (GM) and white matter (WM), cerebrospinal fluid (CSF), applying the algorithms implemented in SPM2 based on the ones developed by Ashburner and Friston (2000) and Ashburner and Friston (2001). Applying this iterative segmentation procedure to each individual volume produces a probability value for each voxel's belonging to either WM or GM. This probability, usually referred to as density, provides a local estimate of GM or WM volume within the particular voxel. The resulting grey matter images were finally smoothed with the 8 mm FWHM and used for statistical evaluation.

Manual ROIs. To test our SPM2-VBM analysis, we performed two different manual Region of Interest analyses (ROIs) in the fornix: one for the body and one for the posterior pillars.

DTI measurements of fornix were taken using the FMRIB Software Library package (http://www.fmrib.ox.ac.uk/fsl). We positioned the ROI of the body of the fornix with a method similar to that used by Kuroki et al. (2006). Specifically, the body of the fornix was limited from the hippocampal commissure until the anterior commissure. To optimize our ROI placement we first detected scan slice as represented in the atlas of Mori et al. (2005). Then we placed the ROI, combining the FA and ADC maps to verify the right location. We compared the fibers that were visualized with the fiber bundles described in the atlas of Mori et al.

We also performed a separate ROI analysis for the posterior pillars of the fornix following the landmarks used in Bilir et al. (1998). We considered the limits as follows: the posterior pillars arise from the alveus on the ventricular surface of the hippocampus and then accumulate as the fimbria along the medial edge of the hippocampus. To further establish the posterior border we used the description provided by Copenhaver et al. (2006): as the fornix approaches the hippocampus, the two structures merge together to form the fimbria, making them indistinguishable at the point at which they connect. To establish a reliable posterior border, the coronal traces were terminated at the point where the crus of the fornix can be seen in its entirety, extending inferior-laterally to join the hippocampus. This typically occurs when the pulvinar of the thalamus is still visible, and the fornix is seen as a white matter tract passing laterally to the pulvinar. The pillars of the fornix curve gradually along the splenium of the corpus callosum until the hippocampal commissure. When the pillars join a bundle is formed, this was considered as the body of the fornix.

We repeated the analysis two times to test the reliability. The Intraclass Correlation Coefficient (ICC) showed the inter-rater reliability to be 0.76 for FA values and 0.84 for ADC values.

2.4 Memory assessment

We used Rey's Auditory Verbal Learning test (RAVLT) test to explore verbal memory performance. Due to its freedom from associative context, this test is one of the most sensitive for investigating verbal memory. To test visual memory we used the Face Recognition I and II subtests of Wechsler Memory Scale (WMS) which allows the study of immediate and delayed visual recall. To state general mental ability we used the Vocabulary subtest of Wechsler Adult Intelligence Scale (WAIS-III) to obtain a premorbid estimate of IQ. The Vocabulary scores provide the best estimates of the general premorbid ability level (Lezak et al., 2004).

In the RAVLT procedure, during five consecutive presentations, we recorded the sum of number of words learned in each trial (learning measure) and the number of words recalled after a 20-minute delay. The percentage of words recalled after delay with respect to the last trial was taken as a measure of forgetting. The words recognized from the list containing previously presented words mixed randomly with new words were taken as a measure of recognition.

In the WMS-III face recognition subtest, we recorded the number of faces recognized from forty-eight photos of human faces (maximum score= 48) and the variable scores after 30-minute delay (long term recognition). Non verbal memory impairment is well documented in schizophrenia (Aleman et al., 1999), and face recognition is more sensitive to right hippocampal dysfunctions than other tests such as reproduction of Rey's complex figure (Glosser et al., 1998).

2.5 Statistical analysis

Between groups comparison of demographic variables and neuropsychological performance were examined using *t* tests for independent samples from SPSS 12.0. Voxel-based morphometry (VBM) analysis was carried out on SPM2. VBM compared FA and ADC images using statistical parametric mapping to identify and make inferences about regionally specific differences. We analyzed FA and ADC performing a group comparison using a one-sided *t* test with two different contrasts: patients>controls and patients<controls.

Initially, to assess the differences between patients and controls we used the corrected false discovery rate (FDR) p value ($p<0.05$). We later performed a student's t-test with significance defined by a cluster-level of $p<0.001$.

We used an extent threshold of 20 contiguous voxels to exclude small cluster. This selection prevents cluster emerging by chance (Wilke et al., 2001). After resampling, the cluster was 2x2x2 mm3. Therefore the minimal cluster volume was (2x2x2) x20= 160 mm3.

To investigate the effect of gender on the structure for each parameter (FA, ADC), we performed a group comparison using a one sided t test with two different contrasts, male>female and male<female, in schizophrenic and control groups. We also conducted a separate analysis looking for gender differences in FA and ADC, considering patients and controls together.

In hippocampal gray matter analysis, the normalized smoothed segmented data were analysed using statistical parametric mapping in the context of the General Linear Model. Regionally specific between-groups differences in GM density were statistically assessed using a two-tailed contrast. The significance level was set at p

< 0.05 corrected for multiple comparisons by the false discovery rate (FDR). We selected two ROI (hippocampus and parahippocampus) from the WFU Pickatlas toolbox software.

Manual ROIs. The differences between patients' and controls' FA and ADC values calculated from manual ROIs in the white matter tracts were analysed by student's t test (SPSS 12.0).

Correlations. The relationship between FA, ADC and memory scores (RAVLT learning, delayed recall, recognition and forgetting; WMS Face Recognition I and II) was also investigated. In a further step we calculated the mean FA and ADC values for each subject from

the fornix ROI. For this calculation a 10-mm diameter sphere was placed at the centre of the mass of each volume of interest (VOI). These individual values were correlated with the scores of memory performance using Pearson's correlation analysis from SPSS 12.0. Moreover, we performed a "simple regression" SPM2 analysis correlating the FA and ADC maps with the scores of the schizophrenic patients on the memory tests. The results of these analyses were thresholded at P<0.001(uncorrected). We also performed the correlation between the fornix FA values obtained by manual ROIs and memory scores using SPSS 12.0. Following the Bonferroni correction for multiple comparisons, the level of significance was established at P equal or inferior to 0.008.
The relationship of FA and ADC values with pharmacological treatment was assessed by a simple regression SPM2 analysis.

3. Results

VBM. Voxel-based analysis revealed a significant FA reduction in the white matter in the left temporal lobe in the schizophrenic subjects compared to healthy controls (Fig. 1). The cluster size was 219, the MNI coordinates for the voxel of the maximum significance were -36 -12 -14, and *t* statistic = 3.27. The opposite contrast, i.e. schizophrenics greater than controls, did not show any significant difference. On the other hand, the ADC values demonstrated an increase in the same region in the patient's group (Fig. 2): the cluster size was 529, the MNI coordinates for the voxel of the maximum significance were -36 -10 -14; and *t* statistic = 3.27. The opposite contrast, i.e. schizophrenics smaller than controls, did not show any significant difference. We also found increased ADC in left inferior longitudinal fasciculus and in both side of middle temporal gyrus.
Applying a ROI analysis in a 10.0 mm radius sphere centered at -36 -12 -14 MNI coordinates, we obtained a p<0.03 for FA and p< 0.01 for ADC, both corrected using the family wise error (FWE)-corrected procedure P value (P < 0.05). The FWE value is the multiple comparison family-wise error type I error, which eliminates false-positives. Figure 3 shows the FA values of the all 49 subjects.

There was a significant effect of gender in patients' group. Female subjects showed lower fractional anisotropy values in the genu of the corpus callosum than males (cluster size= 202; t= 3.48; p< 0.001). When we performed the analysis of gender irrespective of subject status the results were similar, with a smaller cluster size (cluster size= 85; t= 4.93; p< 0.001). In control group we found no significant differences between male and female in FA and ADC values.

Gray matter ROIs analysis revealed lower GM densities in schizophrenics compared with controls in hippocampus (-10 -6 -16 MNI coordinates; t= 4.08) and parahippocampus (-10 -6 -18 MNI coordinates; t=3.85). No areas presented greater GM density in patients than in controls.

Manual ROIs. In the manual analysis of the fornix ROI, we found a significant FA decrease (p<0.001; t = -6.3) and ADC increase (p= 0.004; t = 3.0) of the body of fornix and a significant FA decrease (p<0.001; t = -3.9) and ADC increase (p= 0.02; t = 2.3) of the posterior pillars of fornix in patients compared to controls (see Table 2).

Memory scores. Significant group differences were found in memory test performance. In all cases, the schizophrenics displayed impairment relative to the healthy subjects, as shown by scores on the Rey's and visual WMS subtest (the results are summarized in Table 3).

Table 3. Results of memory scores

	Group					
	Patients		**Controls**		**t**	**P**
	Mean	**SD**	**Mean**	**SD**		
Rey's Learning	47.36	7.52	56.50	9.19	3.81	<0.001
Rey's Delayed Recall	9.20	3.26	12.79	2.58	4.25	<0.001
Rey's Recognition	14.36	1.15	14.96	0.20	2.50	0.016
Rey's Forgetting	24.81	16.12	10.45	10.92	-3.63	0.001
WMS Recognition I	35.60	5.52	41.75	3.75	4.53	<0.001
WMS Recognition II	37.32	5.06	42.04	4.01	3.60	0.001

Correlations. When we correlated memory scores with the fornix FA values obtained by VBM, we found a significant correlation with delayed recall (r= 0.44; p=0.027).
We also observed significant positive correlations for face recognition I and II between the body of the fornix and FA and ADC values obtained by fornix manual ROIs. There was no significant correlation between DTI values and the posterior pillars of the fornix. The correlations are shown in Table 4.
For medication, there were no correlations between chlorpromazine equivalents and FA or ADC values.

Table 4. Pearson's correlations between memory scores and fornix DTI values for patients with schizophrenia.

	Fornix [1]		Body [2]		Pillars [2]	
	FA	**ADC**	**FA**	**ADC**	**FA**	ADC
Learning [a]	0.15	-0.25	0.31*	- 0.34*	0.26	**-0.06**
Delayed recall [a]	**0.40****	-0.18	0.32*	-0.24	0.23	**-0.14**
Recognition [a]	-0.02	-0.13	0.22	-0.21	0.17	**-0.07**
Forgetting [a]	-0.31	0.11	-0.31*	0.28*	-0.20	**0.23**
Face Recognition I [b]	-0.06	0.36	**0.37****	-0.05	0.25	**-0.10**
Face Recognition II [b]	0.06	0.31	**0.38****	-0.09	0.13	**-0.09**

Note. [a]Rey's memory variable (RAVLT); [b] Weschler memory variables (WMS);
[1] VBM analysis; [2] Manual ROIs analysis; ***P*<0.01, **P*<0.05.
In bold the P values that achieved statistical significance after Bonferroni's correction.

4. Discussion

The present study shows the existence of white matter impairment in the temporal lobe in schizophrenic patients. Using a voxel-based method we found a reduction in FA and an increase in ADC in the left posterior pillars of fornix. Manual region of interest analyses of the fornix found a significant decrease in the posterior pillars as well as in the body.
Both FA and ADC reflect impairment of white matter integrity. Fractional anisotropy (FA) is a measure of the fraction of the magnitude of the tensor ascribed to the anisotropic diffusion (Kubicki et al., 2007). Decreased FA values indicate lower anisotropic diffusion and suggest that the fibers are more disrupted. For their part, ADC values describe the rate of diffusion, and an increase reflects greater mobility of water due to the low organization of the surrounding white matter.
Decreased FA values, along with increased diffusivity within prefrontal and temporal lobes and abnormalities within the fiber bundles connecting these regions are the most frequent positive findings in schizophrenia studies (Kubicki et al., 2007). The exact cause of the FA decreases and ADC increases in schizophrenia is not known but there is growing evidence that the oligodendrocytes are involved. Hakak et

al. (2001) showed an abnormal expression of myelin-related genes, suggesting a disruption in the oligodendrocyte function. Although the degree of fiber myelination may be an explanatory factor for FA and ADC alteration, other white matter alteration cannot be ruled out. The properties of white matter fiber tracts, including fiber density, average fiber diameter, the thickness of the myelin sheaths, and the directionality (or coherence) of the fiber in each voxel, all affect the diffusion of the water molecules (Kubicki et al., 2007).

The region where we found white matter changes corresponds partially to the fornix, a bundle of fibers which connect the hippocampus with the mammiliar bodies, the nucleus accumbens and septal nuclei (Andersen et al., 2007). Patients presented increased ADC in the middle temporal gyrus, hypothetically due to a loss of gray matter volume in this region.

We also found a significant reduction in gray matter density in the left hippocampus and parahippocampus in schizophrenics compared with controls. This result agrees with previous MRI studies reporting a left lateralization of the temporal lobe in schizophrenia (see for review Shenton et al. 2001). In an earlier study we also found a predominantly left-sided reduction in gray matter density in the hippocampus in schizophrenics compared to controls (Rametti et al., 2007). Decreased gray matter in this region may contribute to the reduced connectivity of the frontal and medial temporal regions. Several MRI methods have produced strong evidence of structural impairment of the medial temporal region; it has been systematically reported in volumetric studies (for review see Shenton et al. 2001), studies of hippocampal shape (Shenton et al., 2002) as well as VBM studies (Honea et al., 2005). In general, the MRI studies focus on gray matter alterations (Shenton et al. 2001) and largely neglect white matter. White matter alterations in medial temporal lobe of the

Fig. 1 Sagital, coronal and axial MRI images showing decreased FA in the left temporal white matter in patients. The cluster size was 219 voxels ($p<0.001$ uncorrected).

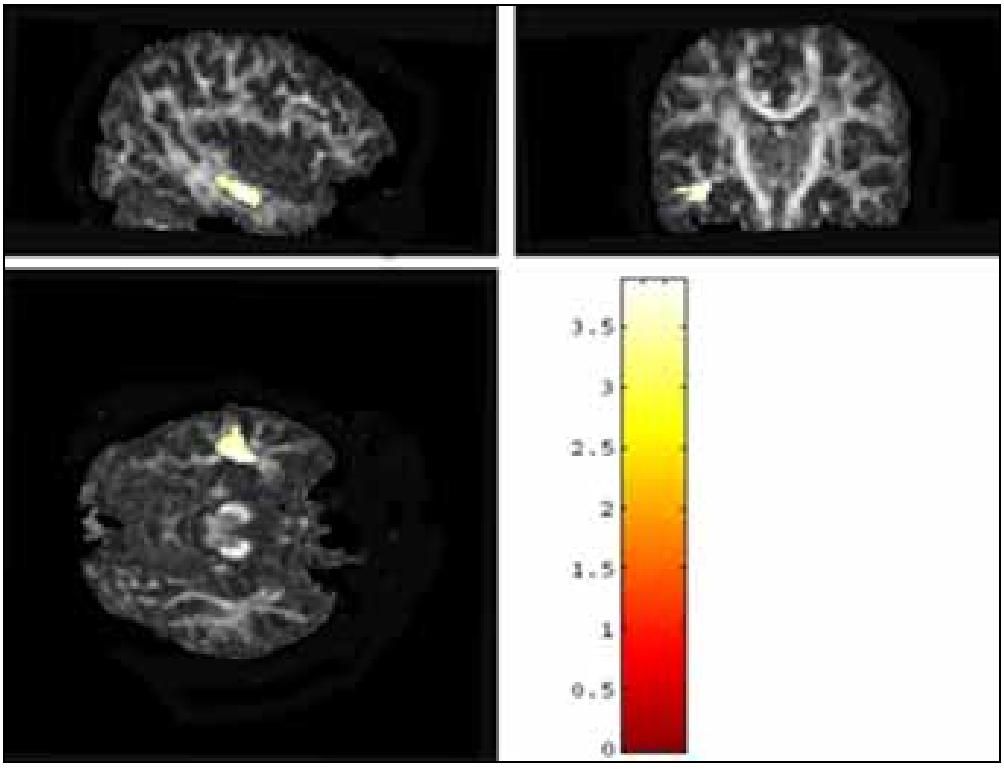
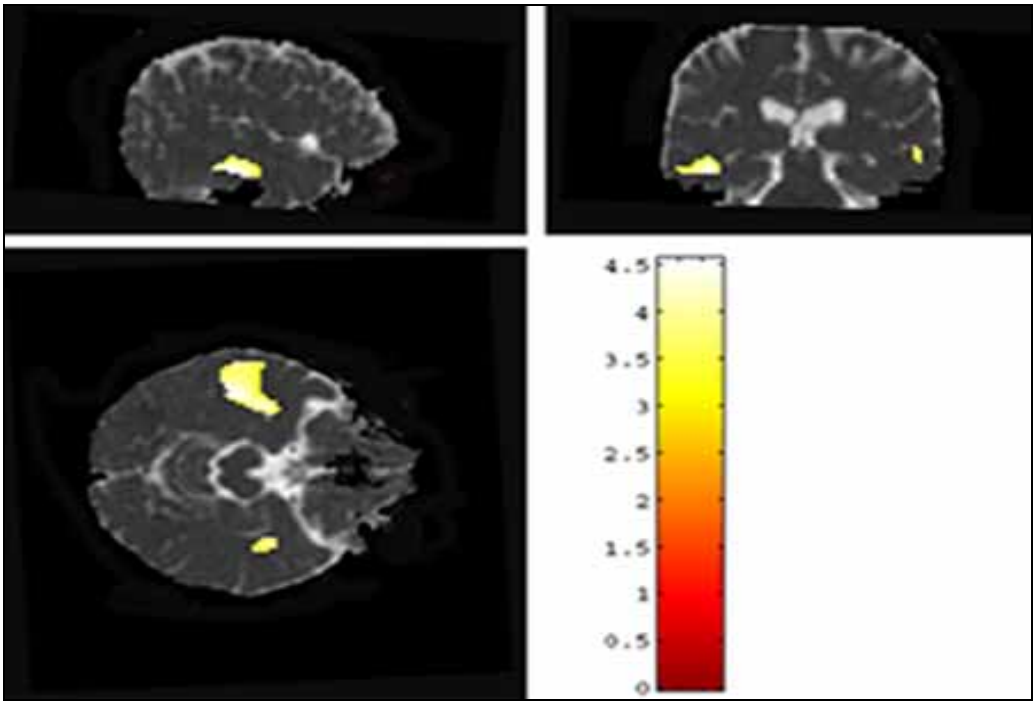


Fig. 2 Sagital, coronal and axial MRI images showing increased ADC in the temporal white matter in schizophrenic patients compared to controls. The left cluster (529 voxels) achieved statistical significance at level P<0.001 uncorrected.



schizophrenic patients were reported using VBM (Sigmudsson et al., 2001) and segmentation procedures (Okugawa et al, 2002; Mitelman et al., 2003). In addition to the temporal lobe findings, white matter reductions were also observed in the frontal lobe (Paillere-Martinot et al. 2001; Sigmudsson et al., 2001). Interestingly, Breier et al., (1992) reported a correlation between prefrontal white matter reduction and amygdala-hippocampal complex volume reduction, suggesting the potential importance of fronto-temporal interaction in schizophrenia. Since the fornix is the major pathway linking the hippocampus with the frontal lobe, our results are in agreement with this hypothesis.

In the hippocampus, FA values in adult schizophrenic patients did not differ from controls (Begré et al., 2003), but ADC values were bilaterally increased (Ardekani et al., 2005). On the other hand, Kalus et al. (2004) found significant alterations of anisotropy using the coherence inter-voxel parameter in the posterior region. Significantly decreased anisotropy was reported in the left hippocampus in a group of adolescent schizophrenic patients compared with age matched controls (White et al. 2007). In the present study we found DTI alterations in the hippocampus and in the fornix fibers, which are the main system of connectivity between the hippocampus and the frontal lobes. The fornix carries signals from and to the hippocampus (Goldman-Rakic et al., 1984). Our results agree with two previous studies that reported FA decrease in schizophrenic patients (Kubicki et al. 2005; Kuroki et al., 2006). These alterations may reflect neuroanatomical changes. Few neuropathological studies of the fornix have been performed. In a postmortem study, Chance et al. (1999) reported a lower fiber density in the fornix of the male subjects, but found no differences in cross-sectional area or total fiber number, suggesting a sex-related effect of schizophrenia on myelination. No significant differences in volume were found between patients and controls (Zahajszky et al., 2001).

Few studies have correlated DTI with neuropsychological measures. Kubicki et al. (2003, 2005) found correlations between FA values in the cingulum bundle and performance on attention and working memory measures of the Wisconsin Card Sorting Test (WCST). Moreover, the same group (Kubicki et al., 2002) found significant correlations between right-sided FA values and visual attention, as well as between left-sided FA and verbal memory. Nestor et al. (2004) showed two different relationships: namely, between left uncinate

fasciculus DTI measures and declarative-episodic memory on the one hand, and between the left cingulum bundle and perseverative errors on the WCST on the other.

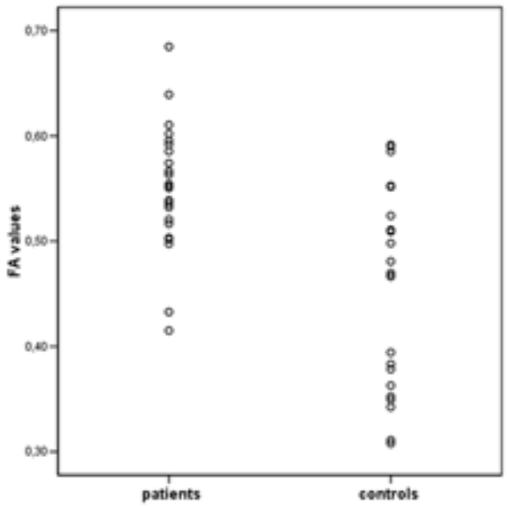


Fig.3 Representation of individual FA values of all subjects. Left: schizophrenic patients' values; right: controls' values. Thirteen patients have FA values below 0.50.

A recent study showed that episodic memory and executive functioning correlated significantly with structural measures of the hippocampus and fornix (Nestor et al., 2007). In our study, long term memory correlated with the fornix FA values of patients. Our result is consistent with the neurofunctional system of memory, since the fimbria-fornix fiber system provides the major conduit for afferent and efferent connections from the hippocampus to the frontal lobe, which are regions involved in declarative memory (Andersen et al, 2007). Our results suggest that, in addition to the previously reported dysfunctions in the cingulum bundle and the uncinate fasciculus, a dysfunction in the fornix may contribute to the declarative memory deficit in schizophrenia.

As regards sex, we found decreased FA in the genu of corpus callosum in female schizophrenics compared to male. The results agree with previous DTI and neuropathological studies. A postmortem study demonstrated a reduction in axonal density of the corpus callosum of the females compared to males (Highley et al., 1999) and lower FA values of the corpus callosum in females than in males with a first episode of the illness (Price et al., 2007). Our findings stress the importance of considering gender differences in schizophrenia.

We also examined possible correlations between FA and ADC values and antipsychotic medication, but found no association. The effect of neuroleptics on axon water diffusion is not clear, however, neuroleptic treatment may affect the number or diameter of axons connecting frontal and striatal centers (Buchsbaum et al., 1998).

Our results could be attributed to the possible effect of duration of antipsychotic treatment and the relative young age of the patients. Previous DTI studies have found no effect of medication on DTI parameters (Foong et al. 2000; Buchsbaum et al., 1998). Only one study (Minami et al., 2003) showed a significant correlation between higher FA in left frontal white matter and higher dosage of antipsychotic medication. Therefore the effect of the antipsychotic on the change in axon water diffusion remains to be fully elucidated, although our findings do not indicate any influence of antipsychotics on fornix integrity.

As far as methodology is concerned, we chose to perform VBM before the ROI analysis in order to study the entire brain and bearing in mind that fiber tracts present higher variability. The subsequent implementation of ROI was able to test the results and improve the sensitivity of the multiple comparison correction method. The following ROI approach took the advantage of testing the results, improving the sensitivity of the multiple comparison correction method. The decision to smooth the image at 12 mm Gaussian kernel may mean that smaller differences are missed, but improves the detection of differences in the analysis of brain as a whole.

In conclusion, fornix integrity appears to be compromised in our sample of schizophrenic patients. This finding suggests that the abnormalities of the fronto- temporal circuitry may contribute to the declarative memory deficits usually found in schizophrenic patients.

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PAPER III

Hippocampal underactivation in an fMRI study of word and face recognition in schizophrenia

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Key words: hippocampus; word recognition; face recognition; schizophrenia.

Abstract

Schizophrenia is a major mental disorder which is characterized by several cognitive deficits. In declarative memory, one of the most impaired functions, the alteration involves both encoding and recognition. However, almost all studies on recognition have used only verbal stimuli. Investigations of the neural basis of memory dysfunctions using structural and functional neuroimaging techniques suggest that the hippocampus plays an important role in this impairment. The goal of this study was to investigate possible dysfunctions in cerebral activation in schizophrenic patients during both word and face recognition memory tasks. We tested 22 schizophrenics and 24 controls matched by gender, age, handedness and parental socioeconomic status. Compared to healthy volunteers, patients with schizophrenia showed decreased bilateral hippocampal activation during word and face recognition tasks. The whole brain analysis also showed a pattern of cortical and subcortical hypoactivation for both verbal and non-verbal recognition. This study provides further evidence of hippocampal involvement in declarative memory impairments of schizophrenia.

1. Introduction

Schizophrenia is a syndrome characterized by several cognitive dysfunctions, one of the most important of which is memory disturbance. Declarative memory, usually divided into episodic (personal events) and semantic (facts), is primarily affected (Weiss and Heckers, 2001). By breaking down the memory process into its components – encoding, storage and retrieval – we can broaden our knowledge of the circuitry of memory in schizophrenia. All processes of declarative memory are impaired in schizophrenic patients, but recognition is the aspect that is least affected (Aleman et al., 1999; Cirillo y Seidman 2003; Pelletier et al., 2005). To date, non-verbal memory has not been widely investigated. In a meta-analysis of 70 studies, only 8 reported data on recognition of non-verbal stimuli (Aleman et al., 1999). However, studies that have assessed non-verbal memory have also found it to be impaired (Tracy et al., 2001), and a recent meta-analysis of 84 studies of recognition memory in schizophrenia found a greater impairment in figural than verbal recognition (Pelletier et al., 2005). In that meta-analysis, data on face memory recognition were reported in only 3 studies.

The understanding of the origin of memory impairment in schizophrenia has been greatly enhanced by the development of structural and functional neuroimaging techniques (Weiss and Heckers, 2001). Declarative memory impairment in schizophrenia appears to involve abnormal connectivity between the prefrontal cortex and three regions that are important in normal learning and memory: the hippocampus, the thalamus, and the cerebellum (Weiss and Heckers 2001). Structural MRI studies have obtained

significant correlations of memory performance and prefrontal and medial temporal regions (Antonova et al., 2004). Functional studies using SPECT, PET and MRI show a pattern of hypoactivation in the hippocampus and both hypoactivation and hyperactivation in the prefrontal cortex (Weiss and Heckers, 2001). Beneath the apparent heterogeneity of the published findings on schizophrenia and memory, a consistent, robust pattern of group differences in memory processes is observed. Like neuropsychological studies, functional studies have mainly been performed using verbal tasks. In a meta-analysis by Achim and Lepage (2005) only one study used face encoding, and one other used object encoding. Interestingly, though the majority of the studies were performed with verbal stimuli, the right side of the hippocampus was more activated than the left.

In normal subjects, evidence from positron emission tomography (PET) studies showed that encoding of episodic memory involves the left prefrontal cortex, whereas retrieval is accompanied by enhanced right-sided prefrontal activity (Fletcher, Frith and Rugg, 1997). In the hippocampus, encoding activates the rostral portion and retrieval the caudal portion (Lepage et al 1998). In a review of 275 PET and fMRI studies, Cabeza and Nyberg (2000) concluded that there is a clear effect of material lateralization. Objects, faces and spatial stimuli recruited the right temporal medial regions more, and verbal stimuli the left.

The patterns of verbal memory recognition in schizophrenics have been obtained with paradigms contrasting encoding and retrieval processes. The results have shown both increases and decreases in various cerebral regions. Jessen et al. (2003) observed smaller bilateral hippocampal activation in patients compared to controls, but they did not analyse the cortical regions. Using a whole brain analysis in unmedicated patients during an acute episode of schizophrenia, Hofer et al (2003) found hypoactivation in the bilateral dorsolateral prefrontal and anterior cingulate cortices and increased activation in other prefrontal regions, but did not observe hippocampal differences. Increased activation was also reported in medicated patients; in an event-related fMRI study, Ragland et al. (2004) found that during word recognition, patients showed low left dorsolateral prefrontal cortex activation, but also greater activation in the right prefrontal cortex than controls.

Regarding non-verbal stimuli, in a paired object recognition task Lepage et al. (2006) found that patients had hypoactivation in the left dorsolateral prefrontal and right inferior prefrontal regions but did not differ from controls in the hippocampal activation.

Table 1 Demographics and clinic characteristics of the sample

	Patients with Schizophrenia		***Normal Subjects***	
Number of subjects	22		24	
Gender (Male/Female)	11/11		12/12	
	Mean	**SD**	**Mean**	**SD**
Age (years)	31.7	6.6	31.7	7.0
Education (years)	5.0	1.2	6.5	0.7
Parental education (years)	4.1	1.7	4.4	1.8
Duration of illness (years)	10.0	5.7		
PANSS score:				
- **positive**	12.2	4.8		
- **negative**	21.0	5.9		
- **general**	32.5	9.6		
- **total**	65.9	17.3		

Note: Positive and Negative Syndrome Scale (PANSS).

Eyler Zorrilla et al. (2002) examined the activation in four cerebral regions: the hippocampus, parahippocampal gyrus, inferior prefrontal cortex and fusiform gyrus during presentation of novel and repeated pictures, finding that repeated pictures produced more activations than the novel ones. Face recognition has mainly been used to investigate visual perception (Quintana et al. 2003) or working memory processing (Barch et al., 2002; Yoo and Choi, 2005). In epileptics and traumatic brain injury patients, face recognition is the best test for identifying right hemisphere hippocampal dysfunctions (Morris et al., 1995; Ariza et al. 2006).

Voxel based morphometry studies showed that the medial temporal lobe is the most affected region in schizophrenics, and although the left side predominates, the reductions are usually bilateral (Arnold et al., 1997; Rametti et al. 2007). It is possible that reductions in the gray matter of the right hippocampus contribute to face recognition deficits in schizophrenic patients.

The purpose of the current study was to elucidate how the declarative memory pattern of cerebral activity may be disrupted in both word and face recognition tasks in schizophrenia. We hypothesized that the left hippocampus may be disrupted predominantly during verbal recognition and the right hippocampus mainly during non-verbal recognition.

2. Methods and Materials

2.1 Participants

Subjects were 24 healthy volunteers (12 male, 12 female) and 22 chronic schizophrenia patients (11 male, 11 female). The patients were recruited from the Psychiatry Service of the Hospital Clinic of Barcelona. The controls were recruited from the community via an advertisement. The patients were diagnosed on the basis of DSM-IV criteria, using the Structural Clinical Interview (SCID), by agreement between two psychiatrists (R.C and M.B.). Clinical symptoms were rated using the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1983) and the Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen, 1983). Schizophrenia subtypes included 18 paranoid, 1 disorganized, 2 undifferentiated and 1 residual. All patients were receiving neuroleptic medication equivalent to a mean of 186.30 mg of chlorpromazine per day. The mean duration of illness was 10.00 years (SD= 5.7). The control group was matched for gender, age, handedness (right) and parental socio-economic status distribution to the group of schizophrenic patients. Healthy comparison subjects had no history of mental illness, nor first-degree relatives with psychiatric disorder. Table 1 summarizes the demographic and clinical characteristic of the samples. After a full explanation of the study, all subjects gave written informed consent to a protocol approved by the ethics committee at the Hospital Clinic of Barcelona.

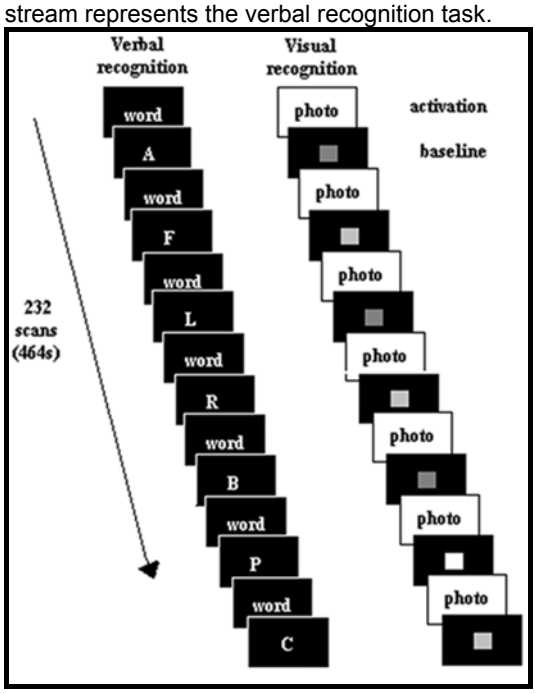
2.2 Experimental design

The fMRI study consisted of two recognition tasks (words and faces) of material previously seen outside the scanner. The Presentation 0.76 version program (Neurobehavioral System, USA) was used to develop the stimuli task.

During the encoding task, previous to MRI acquisition, the subjects first viewed 2 types of items for the 2 different tasks: 25 words and 25 photos of human faces (duration, 3 sec; intertrial interval (ITI) 0,5 sec). The subjects were not instructed to memorize the items presented but to make a judgment of pleasantness (pleasant/unpleasant?) and to press a response button when the item was considered pleasant. The words were selected from the Lexesp-Corco database, matched for frequency of occurrence in written Spanish (Sebastián Gallés et al. 2000). The face photographs (equivalent number of women and men randomly intermixed) were taken from the AR Face database (Martinez and Benavente, 1998).

Approximately 15 min after this study phase, the two experiments of recognition began: 49 verbal stimuli (25 target and 24 non-studied foils) and 49 face stimuli (25 target and 24 non-studied foils) were respectively presented on a screen via a mirror (duration, 3 sec; ITI, between 1000 and 1500 msec) while fMRI data were collected (see Fig.1). The stimuli were back-projected (by a Sanyo Multimedia Prox-III) onto a screen which subject viewed through a mirror located on the scanner's head coil. Subjects' heads were fixed with foam pads to minimize movement during MRI data acquisition. Subjects were also instructed to remain immobile.

Fig. 1 The experiment design. The left stream represents the visual recognition task. The right stream represents the verbal recognition task.



They had to indicate if the item was the one previously seen (target) by pressing a button with their right hand. The presentation of stimuli (ON) alternated with a low level baseline task (OFF). During baseline of verbal stimuli, white capital letters was shown for 3 s followed by a black screen for 1000/1500 msec. Subjects had to respond by pressing a button when the letter A appeared on the screen. During baseline of non-verbal stimuli, coloured screens were shown for 3 s followed by a black screen for 1000/1500 msec. Subjects had to respond by pressing a button when a white square appeared on a black screen.
A block design was used in which test items were presented in 14 blocks of 7 items each (32 sec/block). Each two blocks presented 2 alternate conditions: 3 target and 4 new stimuli and vice versa. Between each block there was a variable break-time, between 1000 and 1500 milliseconds.

2.3 MRI acquisition

The study was performed in a 1.5-T MR unit (Signa-Lx, General Electric, Milwaukee, WI) using the blood-oxygen level-dependent (BOLD) fMRI signal. A single-shot gradient echo planar imaging sequence (EPI) was used: TR (repetition time) / TE (echo time) = 2000/40 ms; FOV (field of view) = 24 x 24 cm, 64 x 64 pixel matrix; flip angle = 90º; slice thickness 6 mm and 20 axial slices per scan.

Before each time series, seven dummy images were collected to achieve scanner equilibrium. These images were excluded from the following analysis. One run consisting of 232 volumes was acquired during each of the two experiments.
Following fMRI scan, a T1-weighted sequence was selected for the acquisition of anatomical images (TR/TE= 12 /5.2 ms; TI 300 1 nex; FOV = 24 x 24; 256 x 192 pixel matrix; 1.5 mm slice thickness).

2.4 Behavioural data analysis

Three different measures were obtained from both tasks: accuracy (number of correctly identified items), false positives (the number of incorrect “yes” response) and the number of omissions. Reaction time was also measured by calculating the median reaction time (in milliseconds) for target stimuli. The data of the groups were compared with the Student’s t test. All statistical analyses were carried out with the SPSS 12.0 version.

2.5 fMRI data

For image processing Statistical Parametric Mapping (SPM5 Wellcome Department of Cognitive Neurology, London) was used. The images of each subject were corrected for motion and realigned to remove any minor motion-related signal change. All volumes for each subject were normalized into an EPI template supplied with SPM5. During spatial normalization all scans were resampled to 2-mm3 isotropic voxels. Low-frequency noise was removed with a high-pass filter (128 seconds) applied to the fMRI time series at each voxel. Finally, the images were smoothed with an 8mm full-width half maximum (FWHM) Gaussian filter.
Statistical analyses were first performed at a single-subject level: a linear contrast was used to evaluate the effect of performing the recognised item compared to baseline items. SPM maps were calculated for this (recognition>baseline) contrast, which reflected differences in activation between the two different conditions.
Next we performed a group analysis on a second level using the appropriate contrast images from the single-subject analysis. We performed a two-sample t test with two different contrasts: schizophrenics>controls and schizophrenics<controls. The height threshold value was set to false discovery rate (FDR) $p<0.05$ corrected and a cluster extent threshold greater than 20 voxels. First we performed a whole-brain analysis

to test all possible differences in cerebral regions. The anatomical location of the cerebral activated areas was determined by the Montreal Neurological Institute (MNI) global maxima coordinates.
We then conducted a region of interest (ROI) analysis to focus on possible hippocampal differences. We used the WFU-Pickatlas toolbox software for SPM, version 1.02 (Joseph Maldjian, Functional MRI Laboratory, Wake Forest University School of Medicine) to create an ROI including the hippocampus and the parahippocampal structure.

Verbal and non-verbal memory related activity was compared using a 2x2 factorial design with group and memory task as the two factors. The interaction between groups and memory tasks was calculated by a t contrast of the positive and negative effect. The probability threshold was set at 0.005 uncorrected.

To assess the relationship between task-related activation and performance, we performed a "simple regression" SPM5 analysis. Task performance was correlated with changes in scaled pixel intensity for each group. In addition, we conducted a "simple regression" analysis to examine the relationship between clinical symptoms and abnormal brain activation in schizophrenia.

2.6 Voxel-based morphometry

All MRI images were pre-processed according to the Standard VBM protocol (Mechelli et al., 2005) using SPM5 (Statistical Parametric Mapping, The Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College London, UK), running in Matlab 6.5 (MathWorks, Natick, MA). We first reoriented all images according to the anterior-posterior commissure and then we segment them into gray and white matter and cerebrospinal fluid (CSF). This step comprises a new integrated spatial normalization and segmentation routine. The spatial normalization involves registering each of the images onto the SPM T1 template, whereas the segmentation step uses a priori probability maps to segment tissues. Finally, the GM images were smoothed with an 8-mm full-width at half-maximum isotropic Gaussian kernel.

Table 2. Verbal and Visual memory task performance assessed by accuracy, false positives, omissions and mean reaction time (milliseconds)

	Patients with Schizophrenia	***Normal Comparison Subjects***		
	Mean (SD)	***Mean (SD)***	**t**	**P**
Verbal task				
Accuracy	20.95 (5.43)	22.38 (2.48)	-1.14	0.25
False positives	1.32 (0.74)	1.42 (1.64)	-0.24	0.80
Omissions	3.11 (5.29)	1.67 (2.46)	1.18	0.24
Reaction time (ms)	1093.15 (303.15)	1012.91 (143.32)	1.14	0.25
Visual task				
Accuracy	14.37 (4.21)	15.08 (3.81)	-0.58	0.56
False positives	4.21 (3.22)	3.75 (2.62)	0.51	0.60
Omissions	9.63 (4.15)	8.46 (3.93)	0.94	0.34
Reaction time (ms)	1398.88 (289.35)	1183.33 (169.67)	3.02	0.004*

Note. Milliseconds: ms. *P<0.05

We evaluated concentration gray matter differences between groups, using the SPM5 Student's t test group comparison. We used the convention that the group comparison results should survive at the corrected false-discovery rate (FDR) p value ($p < 0.05$). Moreover, only clusters of more than 10 contiguous voxels were considered in the statistical model. We performed ROI (region of interest) analyses using the WFU-Pickatlas toolbox software for SPM version, version 1.02 (Joseph Maldjian, Wake Forest University Baptist, Medical Center, Department of Radiology, Winston-Salem, NC). For this purpose, we selected different ROI corresponding to the brain structures showing hypoactivation in patients during fMRI.

3. Results

3.1 Memory performance

Performance on recognition was similar in schizophrenic patients and healthy controls in the scanner (see Table 2). However, Student's t tests revealed a significant difference in reaction time between patients and healthy subjects in the non-verbal recognition task. Because of software problems, responses were not recorded for three patients.

3.2 fMRI results

Examination of the whole brain response during both recognition tasks revealed several foci of decreased activation in patients compared to controls. The differences were more striking in face recognition than in word recognition. During word recognition tasks, patients showed significantly lower brain activity than controls in the amygdala, basal ganglia, thalamus, and posterior cingulate gyrus (Fig. 2, Table 3).

Non-verbal recognition elicited less activation in patients compared to controls in inferior frontal gyrus, cerebellum, insula, postcentral and precentral gyrus, cuneus and precuneus, superior frontal gyrus and posterior cingulate gyrus (Fig. 3, Table 4).

We selected the hippocampus and parahippocampus as regions of interest because of their involvement in recognition. Bilateral hippocampal activation was significantly lower in schizophrenics than in the control group ($p<0.05$, FDR corrected) in a verbal recognition task (see Fig. 4). The left hippocampus showed a significantly greater hypoactivation than the right side: cluster sizes were 264 and 196 respectively.

For the face recognition task, we found also a significant bilateral decrease hippocampal activation in patients compared to control group ($p<0.05$, FDR corrected). Here, the right hippocampus showed a greater hypoactivation than the left side. The cluster sizes were 329 and 266 respectively. We found no significant differences in the patients>controls contrast, that is, patients did not present any area of increased activity.

Table 3 Regions of decreased cerebral activation in patients compared to controls in verbal recognition. Local maxima of change in cerebral BOLD were presented in the standard Montreal Neurological Institute (MNI) space. The level of significance was presented at $p<0.05$ FDR corrected. The BA was determinated by visual inspection using the stereotaxic atlas of Talairach and Tournoux.

Region	*Local maxima x, y, z*	*Hemisphere*	*Cluster size*	*BA*	t
Amygdala	-20 -2 -10	L	290		5.04
Basal ganglia (caudate)	10 8 2	R	396		5.00
Thalamus	18 -12 8	R			4.96
	-10 -24 16	L			4.67
Cingulate gyrus	-2 -20 32	L	44	23	4.84
	-2 -38 28		37	31	4.36
Basal ganglia (putamen)	22 6 -10	R	56		4.36

Note: BA= Brodmann's area; L= left; R=right.

Table 4 Regions of decreased cerebral activation in patients compared to controls in visual recognition. Local maxima of change in cerebral BOLD were presented in the standard Montreal Neurological Institute (MNI) space. The level of significance was presented at p<0.05 FDR corrected. The BA was determinated by visual inspection using the stereotaxic atlas of Talairach and Tournoux.

Region	*x, y, z*	*Hemisphere*	*Cluster size*	*BA*	t
Inferior frontal gyrus	30 22 -8	R	4750	47	4.86
Cerebellum	-2 -46 -14	L	4265		5.04
Insula	48 -16 14	R	269	41	3.76
	-52 -32 18	L		42	3.63
Precentral gyrus	50 -6 10	R		6	3.54
Postcentral gyrus	60 -10 14	R		43	3.33
Superior frontal gyrus	-16 54 -2	L	100	10	3.99
Cuneus	-10 -82 34	L	173	19	3.98
Precuneus	20 -56 38	R	100	7	3.90
Cingulate Gyrus	-14 30 28	L	50	32	3.82

Note: BA= Brodmann's area; L= left; R=right.

Group x memory task interactions showed a decrease in activation in verbal task < non-verbal task for the schizophrenic group than for controls in the right hippocampus. No significant group x memory task interactions were found on non-verbal <verbal for both group.

We also examined the differences in hippocampal activation using performance as a covariate. The contrast revealed that for both visual and verbal recognition tasks the analysis yielded an SPM (t) map that is very similar to the map produced without this effect.

In another cycle of analyses, we investigated the relationship between clinical symptoms and fMRI activity. At cluster level there was no significant correlation between the task-related signal changes of the whole brain and both positive and negative symptoms or general psychopathology.

Table 5 VBM results

Region	Local maxima x, y, z	Hemisphere	Cluster size	BA	t
Middle frontal gyrus	-40 50 -14	L	450	11	4.69
Inferior frontal gyrus	-18 12 -20	L		47	3.85
Insula	42 18 4	R	357	13	4.26
Inferior frontal gyrus	50 26 -4	R		47	4.08

Note: BA= Brodmann's area; L= left; R=right. (p<0.05 FDR corrected).

3.3 VBM results

VBM showed a reduced gray matter concentration in the schizophrenic group in the right hippocampus (cluster size = 106; local maxima MNI coordinates = 22 -6 -20, FDR-corrected p value at cluster level < 0.05) and in left hippocampus (cluster size = 54; local maxima MNI coordinates = -20 -6 -22, FDR-corrected p value at cluster level < 0.05). Between-group contrasts also revealed decreased gray matter density in schizophrenic patients in the middle and inferior frontal gyrus and insula (Table 5). We found no significant differences in gray matter concentration in the parahippocampus between groups.

4. Discussion

Our results show altered patterns of cerebral activation in recognition in schizophrenics for both verbal and non-verbal material. In the whole brain analysis, the differences between patients and controls were more striking for face recognition than for word recognition. These differences cannot be attributed to task difficulty, because patients and

controls performed similarly in both tasks. During word
recognition, patients underactivated the amygdala, basal ganglia, thalamus, and posterior cingulate gyrus compared to controls. Similar patterns of activation were found in a PET study by Ragland et al. (2001), who suggested that the strategic processes for organizing encoding and subsequently facilitating retrieval were impaired. Although we selected a task with emotionless human faces in order to focus on memory performance alone, like earlier studies of emotional memory (Schneider et al., 1998; Kosaka et al, 2002) we observed hypoactivity in the amygdala. The basal ganglia are mainly involved in working memory functions (Callicott et al., 1999; Manoach et al., 2000), but our findings suggest that they are also involved in the neural activity underlying declarative memory. Our patients presented hypoactivation in the thalamus and cingulate gyrus compared to controls. Both regions have been previously described as abnormal in schizophrenia (Hazlett et al., 1999; Crespo-Facorro et al., 1999; Ragland et al., 2001; Hofer et al., 2003).

As regards the medial temporal lobe, in the verbal tasks schizophrenics presented lower bilateral hippocampal activation than controls. These results agree with previous fMRI studies: a PET study reported reduced hippocampal activation during conscious recollection of studied word in schizophrenic patients (Heckers et al., 1998), while another fMRI study also found that schizophrenics showed less bilateral

Fig. 2 Views of brain regions showing significant decreases in BOLD activation in patients compared to healthy controls (patients<controls) for word recognition task ($p<0.05$ FDR corrected).

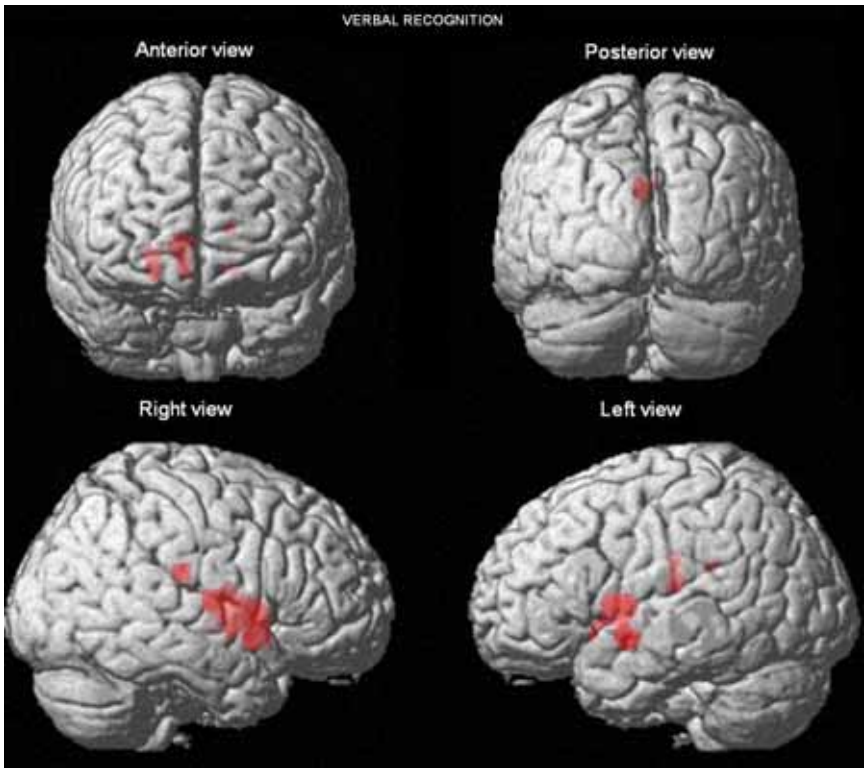
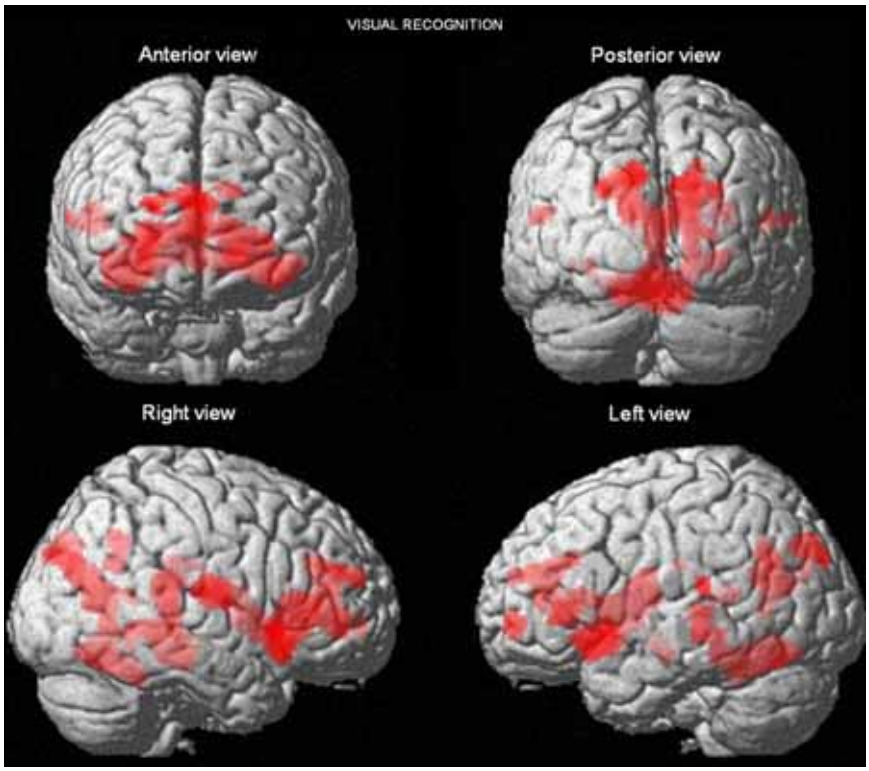


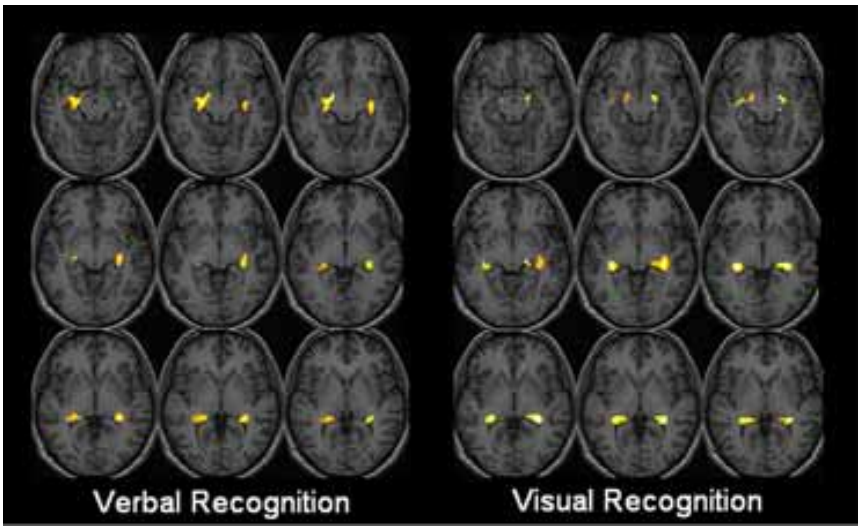
Fig. 3 Views of brain regions showing significant decreases in BOLD activation in patients compared to healthy controls (patients<controls) for facial recognition task (p<0.05 FDR corrected).



hippocampal activation during a verbal task (Jessen et al., 2003).

In our study, recognition-associated activity was not consistently localized to either the anterior or the posterior region of the hippocampus (Greicius et al., 2003). Moreover, the hippocampal activation deficit in patients cannot be attributed to their performance during the task, as both groups had similar results on the tests. These findings suggest that impaired hippocampal activation may be partially compensated by another memory strategy (e. g., familiarity).

Fig. 4 Region-of-interest (ROI) analysis showing the maxima of hippocampal activation difference (schizophrenics<controls) in verbal and non-verbal recognition task (memory activation task>control task) (p<0.05 FDR corrected). The right side of the image corresponds to the right side of the brain. The results are overlapped in a normalized T1 control brain.



As regards hemispheric predominance, though the decrease in hippocampal activation was bilateral, we found greater left hemispheric impairment in word recognition. Verbal memory impairment has been consistently associated with left temporal lobe damage, while non-verbal memory deficits have occasionally been observed in right temporal lobe damage (Pillon et al., 1999). The factor analysis (group x memory task) confirmed a greater decreased in activation in schizophrenic group than in controls in the right hippocampus in verbal<non-verbal memory task contrast. In schizophrenia, decreased activation has been found in the right hippocampus (Heckers et al., 1998), in the left hippocampus (Ragland et al., 2001) and bilaterally (Jessen et al., 2003). From the structural point of view, voxel-based morphometry studies coincided that there is left hemisphere predominance in hippocampal gray matter reduction (Honea et al., 2005). In the non-verbal task, we also found differences between groups in inferior frontal gyrus, cerebellum, insula, postcentral and precentral gyrus, cuneus and precuneus, superior frontal gyrus and posterior cingulate gyrus. At the behavioural level, the good performance of both groups during the non-verbal memory recognition paradigm indicated showing that they were attending to the stimuli. However, patients were slower to process facial recognition, suggesting differences in the levels of mental effort made by the two groups in this situation. Nevertheless, covariating the contrasts between groups for reaction time, the discriminating variable, the results did not change.

Our findings agree with reports in the literature which have demonstrated "task-related hypofrontality" in schizophrenia. Abnormal prefrontal cortex activity in schizophrenia during visual recognition paradigms is well documented (Heckers et al., 2000; Lepage et al., 2006). These prefrontal findings are also consistent with a recent meta-analysis of episodic memory which identified the left prefrontal region as the most compromised in schizophrenia during retrieval tasks (Achim and Lepage, 2005). The schizophrenic group showed less activation in the cerebellum than controls. Structural abnormalities of this structure have been related with dysfunctions in motor control and coordination (Andreasen et al., 1999; Nopoulos et al., 1999). In our study, the level of performance was similar for both groups in all variables considered, except for reaction time during visual task, which was slower in patients. The pattern of cerebral and cerebellar hypoactivity may explain the slowness of their performance.

The hypoactivity observed in the insula in the patient group might be interpreted as an effect of the disease. Insular volume reduction has been widely reported (Wright et al., 1999). It has been suggested that signal reductions in bilateral insula are associated to impaired recognition of facial expression (Yoo et al., 2005). The cuneus and precuneus have also been implicated in retrieval success (Fletcher et al., 1997; Dobbins et al., 2003). The cingulate gyrus has been more frequently related to a deficit in visual encoding (Lepage et al., 2006), though its involvement in retrieval processes has also been reported (Achim and Lepage, 2005).

With regard to hippocampal activation during the non-verbal memory task, bilateral hippocampal activity was

significantly lower in patients than in controls. To our knowledge this is the first study of emotionless face recognition memory to show differences in hippocampal activity in schizophrenia. Other studies exploring non-verbal memory recognition have focused more on the prefrontal region (Heckers et al., 2000; Lepage et al., 2006). The difference between patients and controls were more striking for face recognition, this result was also substained by group by task interaction analysis where the right hippocampus was more decreases in verbal <non-verbal task for the schizophrenic group than for controls.

We found no correlation between clinical variables (positive, negative and general symptoms) and abnormal brain activation in the patient group. These findings are consistent with a meta-analysis (Aleman et al., 1999) of memory impairment in schizophrenia which reported that clinical variables, except negative symptoms with frontal lobe dysfunction, did not appear to influence the magnitude of memory impairment.

Because functional brain activity could be related to reduction in the structures considered, a separated voxel-based analysis was conducted to evaluate the possible gray matter density reduction in these regions. The ROI analysis revealed decreased gray matter density in the bilateral hippocampus, middle and inferior frontal gyrus and insula. Evidence of structural abnormalities in these regions has frequently been reported (Arnold et al., 1997; Shenton et al., 2001), though the involvement of these abnormalities in functional hypoactivation remains unclear. In our study, other regions presenting hypoactivation did not show gray matter reduction. In the future it would be particularly interesting to assess the relationship between volumetric and functional imaging analyses.

Regarding memory impairment in schizophrenia, while a verbal recognition deficit has been confirmed in a large meta-analysis (Cirillo and Seidman, 2003), few studies has focused on non-verbal recognition deficit in this population (Tracy et al., 2001; Wood et al., 2002; Brébion et al., 2004). The recognition function is supported by two different processes: recollection and familiarity (Yonelinas, 2002). Recollection is conceived as the retrieval of source information, while familiarity as the feeling experienced during item exposure. In schizophrenia the recollection process seems to be impaired (Achim and Lepage, 2005), though familiarity may be sufficient to support recognition memory.

Regarding neuroanatomical theories, Cirillo and Seidman (2003) claimed that the pattern of memory deficits in schizophrenia could be explained by dysfunctions in two regions: the prefrontal cortex and the hippocampus/parahippocampus. The prefrontal cortex may be involved in attending and organizing information, and the hippocampus in the consolidation of information for later recall. Impaired verbal declarative memory may be due to medial temporal lobe abnormalities (Cirillo and Seidman, 2003). Our results agree with this hypothesis: we found abnormal activity in this region, specifically in the bilateral hippocampus, as well as abnormalities in its structures. In the case of non-verbal declarative memory, most of the studies in normal controls report the involvement of frontal-temporal network in face recognition ability (Kim et al., 1999; McDermott et al., 1999). Schizophrenic patients unsuccessfully recruit the medial temporal lobe but do not constantly show a decreased activation of prefrontal regions (Heckers et al, 2000; Lepage et al., 2006). Our analysis of the activity is in agreed supports this model, revealing the consistent involvement of the frontal-temporal regions in recognition memory deficit of schizophrenia.

To summarize, schizophrenic patients showed abnormal fMRI patterns of hypoactivation in bilateral hippocampus during both verbal and non-verbal memory recognition tasks. There was a slight trend towards left-verbal and right-visual hippocampal hypoactivity in patients. These findings may indicate that in schizophrenia the hippocampal role in memory is relatively specific regarding the type of material.

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DISCUSIÓN GENERAL

Discusión general

Los tres trabajos expuestos en la presente tesis examinan las anomalías en la estructura, la función y la conectividad del hipocampo en relación con el deterioro de memoria declarativa de los pacientes con esquizofrenia. Utilizamos diferentes técnicas de análisis de neuroimagen con el fin de proporcionar una amplia estimación de las disfunciones anatomo-funcionales que afectan a este tipo de pacientes. En el primer trabajo se evidencian diferencias significativas en las regiones anteriores y posteriores del hipocampo, con una predominancia en la reducción estructural de la región posterior izquierda. En el segundo trabajo encontramos una disminución en los valores de anisotropia fraccional y un aumento de los valores del coeficiente medio de difusión en los pacientes respecto a los controles en el fornix. En el tercer trabajo, el resultado más destacable concierne a la hipoactivación bilateral del hipocampo en pacientes esquizofrénicos comparados con controles sanos durante la ejecución de dos tipos de tareas diferentes de reconocimiento de memoria: verbal y no verbal.

El **primer trabajo** demuestra la existencia de reducciones regionales de la sustancia gris del hipocampo en pacientes con esquizofrenia a través de dos técnicas cuantitativas complementarias: la *Voxel-based morphometry* (VBM) y la estereología. La pérdida de la densidad observada en la VBM fue bilateral aunque predominantemente posterior izquierda. Este predominio posterior izquierdo fue observado claramente en el análisis volumétrico llevado a cabo mediante estereología. La reducción volumétrica del hipocampo izquierdo es un hallazgo consistente en la literatura (Csernansky et al., 1998; Wang et al.,

2001; Yamasue et al., 2004). Este resultado es coherente también con otros estudios que refieren un volumen reducido en la parte posterior del complejo amígdala-hippocampal (Bogerts et al., 1993; Becker et al., 1996; Hirayasu et al., 1998; Yamasue et al., 2004). Sin embargo, en algunos estudios previo al nuestro en los que se ha dividido el hipocampo en subregiones anterior y posterior, la reducción se reveló más pronunciada en la parte anterior (Pegues et al., 2003; Szeszko et al., 2003). Esta discrepancia podría ser explicada por el uso de técnicas diferentes en el análisis de las sub-regiones. Nuestros resultados coinciden con un estudio de mapas tridimensionales que encontró reducciones en el volumen posterior del hipocampo así como en su longitud y anchura (Narr et al., 2001), y con otro que demostró reducciones de esta estructura en los dos tercios de la región posterior (Velakoulis et al., 2001). El patrón de reducción de la parte posterior del hipocampo tiene alto interés porque justo es un patrón distinto al de las enfermedades degenerativas. Por ejemplo, tanto en el deterioro cognitivo leve de tipo amnésico como en la enfermedad de Alzheimer en fases iniciales (Alzheimer leve) se observa una atrofia de predominio anterior (Atiya et al., 2003; Chételat et al., 2003). En la enfermedad de Parkinson, en la que también por procesos degenerativos se observa atrofia del hipocampo (Junqué et al., 2005), la atrofia tiene un predominio anterior en los pacientes sin demencia (Ramírez-Ruiz et al., 2005) que pasa a ser anterior y posterior en pacientes dementes (Ibarretxe et al., 2008). En patologías como el daño cerebral adquirido por traumatismos cráneo-encefálicos la atrofia del hipocampo se observa también en la parte anterior (Ariza et al 2006). Es probable que el predominio posterior en la

esquizofrenia sea un patrón de disfunción del neurodesarrollo, en consecuencia distinto al de neurodegeneración.

El estudio neuropsicológico realizado en este primer trabajo mostró que los pacientes manifiestan un deterioro de la memoria verbal en las variables de aprendizaje y olvido. En el análisis de las correlaciones observamos sólo una asociación significativa entre el olvido y la sustancia gris del hipocampo anterior. Según el clásico modelo HERA (Hemispheric Encoding/Retrieval Asymmetry), la parte anterior del hipocampo tiene funciones de codificación y la posterior de evocación (Tulving et al., 1994, 1996, Nyberg et al. 1996, Haxby et al., 1996;).

Según nuestro conocimiento, éste es el primer estudio que pone en relación el volumen regional de la sustancia gris del hipocampo con el rendimiento en memoria en pacientes esquizofrénicos utilizando VBM. La literatura previa, mediante el uso de técnicas volumétricas clásicas, ha referido solamente una asociación entre el hipocampo izquierdo y la memoria verbal (Goldberg et al., 1994; Gur et al., 2000; Sanfilipo et al., 2002), Sin embargo, estos estudios no profundizaron en la investigación de las sub-regiones del hipocampo.

En resumen, los resultados del primer estudio sugieren que la reducción hipocámpica podría explicar en parte los déficits de memoria en la esquizofrenia, apoyando la hipótesis de un déficit estructural bilateral, aunque con un evidente predominio posterior izquierdo.

En el **segundo trabajo**, por medio del análisis de imágenes de difusión, se describe la existencia de un deterioro en la sustancia blanca del lóbulo temporal de pacientes con esquizofrenia. Mediante el uso de la VBM encontramos una reducción en los valores de FA (anisotropia fraccional) y un aumento de los de ADC (coeficiente medio de difusión) en los pilares posteriores izquierdos del fornix. El análisis manual de la región del fornix localiza además una disminución significativa en los pilares posteriores así como en su cuerpo.

Un valor inferior de FA y un aumento de la difusividad en los lóbulos prefrontales y temporales, así como anomalías de las fibras que conectan estas regiones, constituyen los hallazgos más frecuentes en los estudios de difusión en la esquizofrenia (Kubicki et al., 2007). La causa exacta de las reducciones de FA y aumentos de ADC en la esquizofrenia no es todavía bien conocida aunque hay evidencias crecientes de una implicación de los oligodedrocitos. Por ejemplo, Hakak et al. (2001) refirieron una expresión anormal de los genes relacionados con la mielina, sugiriendo una interrupción en la actividad de los mismos.

La región donde encontramos cambios en la sustancia blanca corresponde en parte al fornix, un haz de fibras que conectan el hipocampo con los cuerpos mamilares, el núcleo accumbens y el núcleo septal (Andersen et al., 2007).
Kalus et al. (2004) encontraron cambios significativos de anisotropía en la región posterior del hipocampo utilizando un parámetro de coherencia intra-voxel. Nuestros resultados concuerdan con dos estudios previos que hallaron

una disminución de FA en pacientes con esquizofrenia (Kubicki et al. 2005; Kuroki et al., 2006). Estas alteraciones podrían reflejar cambios neuroanatómicos.

En este estudio, la memoria a largo plazo correlacionó con los valores de FA del fornix de los pacientes. Nuestros resultados son coherentes con el sistema neurofuncional de la memoria, puesto que el sistema de fibras fimbria-fornix proporciona la vía mayor para las conexiones aferentes y eferentes del hipocampo hacia el lóbulo frontal, que junto con el hipocampo constituyen las regiones más implicadas en la memoria declarativa y que también están alteradas en la esquizofrenia (Andersen et al, 2007).

Respecto al sexo, encontramos valores de FA disminuidos en el genu del cuerpo calloso en las pacientes esquizofrénicas comparados con los de los varones. Los resultados coinciden con estudios de DTI y neuropatológicos previos (Highley et al., 1999; Price et al., 2007). Nuestras conclusiones enfatizan la ya descrita importancia de considerar las diferencias de género en la esquizofrenia (Bryant et al., 1999)

Examinamos también las correlaciones posibles entre los valores de FA y ADC y la medicación antipsicótica. No obstante, no encontramos ninguna asociación. El efecto de los neurolépticos en la difusión axonal del agua no esta claro en los estudios de DTI. No obstante, se ha descrito que el tratamiento con este tipo de fármacos puede afectar al número o al diámetro de los axones que conectan las regiones prefrontales y el estriado (Buchsbaum et al., 1998).

En conclusión, la integridad del fórnix parece estar comprometida en nuestra muestra de pacientes. Este hallazgo abona la hipótesis que las anomalías del circuito fronto-temporal podrían contribuir al déficit de la memoria declarativa encontrado generalmente en los pacientes esquizofrénicos.

Los resultados del **tercer estudio** muestran patrones alterados de activación cerebral en el reconocimiento mnésico de material verbal y no verbal en pacientes esquizofrénicos comparados con controles sanos. En el análisis global de todo el cerebro, las diferencias entre pacientes y controles fueron más evidentes en el reconocimiento de caras que en el de palabras. Estas diferencias no pueden ser atribuidas al desempeño en la tarea, puesto que pacientes y controles realizaron ambas tareas de forma semejante.

Durante el reconocimiento de palabras, los pacientes hipoactivaron la amígdala, los ganglios basales, el tálamo, y el gyrus cingulado posterior en comparación con los controles. Ragland et al. (2001) encontraron patrones semejantes de activación, sugiriendo que ciertos procesos estratégicos de organización y codificación podrían estar alterados.

Cuando consideramos el lóbulo temporal medial mediante la aplicación de análisis de una región de interés (ROI), los esquizofrénicos presentaron en la tarea verbal una activación del hipocampo bilateral inferior a los controles. Estos resultados concuerdan con estudios funcionales previos. Un estudio de PET encontró también una hipoactivación del hipocampo durante el recuerdo consciente de palabras estudiadas en pacientes esquizofrénicos (Heckers et

al., 1998). De forma similar, otro estudio realizado mediante fMRI encontró que los pacientes mostraban, durante el desarrollo de una tarea verbal, una menor activación bilateral del hipocampo (Jessen et al., 2003).

En el análisis de asimetrías, encontramos una mayor hipoactivación del hipocampo izquierdo durante el reconocimiento de palabras. La alteración de memoria verbal se ha asociado coherentemente con el deterioro del lóbulo temporal izquierdo, mientras que los déficits de memoria no verbal se relacionan con el deterioro del lóbulo temporal derecho (Pillon et al., 1999).

En la tarea no verbal (reconocimiento de caras), encontramos también diferencias entre grupos en el gyrus frontal inferior, cerebelo, ínsula, gyrus postcentral y precentral, cuneus y precuneus, gyrus frontal superior y gyrus cingulado posterior. Nuestras conclusiones concuerdan con la literatura que ha demostrado en la esquizofrenia una lateralidad relacionada con el tipo de tarea (Heckers et al., 2000; Lepage et al., 2006).

A nivel de conducta, el rendimiento correcto de ambos grupos durante la ejecución del paradigma de reconocimiento no verbal indicaría que estaban atendiendo a los estímulos. Sin embargo, los pacientes demostraron una mayor lentitud de procesamiento en el reconocimiento facial, sugiriendo diferencias en los niveles de esfuerzo mental necesitados por los dos grupos en la misma situación. No obstante, cuando covariamos los contrastes entre grupos con el tiempo de reacción los resultados no cambiaron.

Respecto a la activación del hipocampo, durante la tarea de memoria no verbal, la activación bilateral del mismo fue sensiblemente inferior en los pacientes que en los controles. Según nuestro conocimiento este es el primer estudio de memoria de reconocimiento de caras sin emoción en manifestar diferencias en la actividad del hipocampo en la esquizofrenia. Otros estudios que exploran el reconocimiento de memoria no verbal se han enfocado más en la región prefrontal (Heckers et al., 2000; Lepage et al., 2006).

Consideramos la posibilidad de que la actividad funcional del cerebro podría estar relacionada con la reducción en sustancia gris de las estructuras estudiadas. Es decir de forma lógica una reducción de una estructura podría suponer menor activación. Para analizar esta hipótesis, llevamos a cabo un análisis de VBM con el fin de evaluar una posible reducción de la densidad de estas regiones. El análisis de ROI reveló una densidad disminuida de sustancia gris en el hipocampo bilateral, en el gyrus frontal inferior y medio y en la ínsula. La evidencia de anomalías estructurales en estas regiones es un dato muy consistente en la literatura de neuroimagen es esquizofrenia (Arnold et al., 1997; Shenton et al., 2001), aunque la contribución de estas anomalías en la hipoactivación funcional no está aún demostrada. Haría falta un análisis simultáneo que contemple estructura y función.

Por lo que concierne las teorías neuroanatómicas, Cirillo y Seidman (2003) propusieron que el patrón de memoria deficitario en la esquizofrenia podría ser explicado por disfunciones en dos regiones: la corteza prefrontal y el hipocampo/parahipocampo. Los resultados de nuestro análisis apoyan este

modelo, revelando una contribución consistente de las regiones fronto-temporales en el déficit de reconocimiento en la esquizofrenia.

En resumen, nuestro trabajo evidencia unas alteraciones estructurales y funcionales que afectan especialmente al hipocampo y que se relacionan específicamente con el deterioro de la función cognitiva mnésica de los pacientes con esquizofrenia.

CONCLUSIONES

Conclusiones

1. Los pacientes con esquizofrenia muestran reducciones en la densidad de la sustancia gris de las regiones anteriores y posteriores bilaterales del hipocampo, con un predominio en la reducción de la región posterior izquierda.

   El déficit de consolidación (olvido) del aprendizaje verbal observado en los esquizofrénicos correlaciona con la sustancia gris del hipocampo anterior.

2. Los pacientes esquizofrénicos muestran una reducción en los valores de anisotropía fraccional y un aumento del coeficiente medio de difusión en la sustancia blanca del cuerpo y los pilares del fórnix. El análisis correlacional muestra una relación significativa entre el recuerdo demorado y los valores de FA en el fórnix.

3. El estudio de la activación cerebral mediante RMf muestra que durante la ejecución de la tarea de reconocimiento verbal, los pacientes esquizofrénicos muestran una hipoactivación difusa de la amígdala, ganglios basales, tálamo y gyrus cingulado posterior. En el análisis de la región de interés se observa que los pacientes presentan una hipoactivación bilateral del hipocampo, con una predomio izquierdo.

4. Durante la ejecución de la tarea de reconocimiento de caras los pacientes esquizofrénicos muestran una hipoactivación difusa del gyrus frontal inferior, cerebelo, ínsula, gyrus postcentral y precentral, cuneus y precuneus, gyrus frontal superior y gyrus cingulado posterior. En el análisis de la región de interés se observa que los pacientes presentan una hipoactivación bilateral del hipocampo, con predomio derecho

5. Los trastornos de memoria declarativa en la esquizofrenia se explican en parte por las alteraciones cerebrales estructurales del hipocampo asociadas a la enfermedad. Tanto la reducción de sustancia gris como la alteración de la conectividad fronto-hipocámpica contribuyen al déficit de memoria.

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RESUMEN

Resumen de la tesis

La literatura científica demuestra diferentes anomalías cerebrales estructurales y funcionales en la esquizofrenia. El propósito de la presente tesis ha sido investigar las anomalías en la estructura, la función y la conectividad del hipocampo en relación con el deterioro de memoria declarativa de los pacientes con esquizofrenia. En el primer trabajo utilizamos dos técnicas cuantitativas complementarias: la *Voxel-based morphometry* (VBM) y la estereología para investigar diferencias regionales en la sustancia gris del hipocampo. La muestra estaba compuesta por 28 pacientes esquizofrénicos comparados con 33 sujetos sanos. Ambas técnicas demostraron una reducción bilateral en la densidad posterior del hipocampo, con una predominancia en la región posterior izquierda en el análisis estereológico. En el análisis de las correlaciones observamos una asociación significativa entre la variable de olvido y la sustancia gris del hipocampo anterior. Los resultados del primer estudio sustentan la hipótesis de una atrofia regional del hipocampo de los pacientes esquizofrénicos que podría explicar en parte los déficits de memoria frecuentemente presentes en esta enfermedad.

En el segundo trabajo se describe, por medio del análisis de imágenes de difusión mediante el uso de VBM y de un análisis manual de región de interés, la existencia de un deterioro en la sustancia blanca del lóbulo temporal de pacientes con esquizofrenia. La muestra estaba constituida por 25 pacientes con esquizofrenia y 24 controles. Encontramos una reducción en los valores de FA (anisotropia fraccional) y un aumento de los de ADC (coeficiente medio de difusión) en el cuerpo y los pilares posteriores del fornix. Hallamos además

diferencias de género en el grupo de pacientes, presentando las pacientes valores de FA disminuidos en el genu del cuerpo calloso. En este estudio, el recuerdo demorado correlacionó con los valores de FA del fornix de los pacientes. Los datos proporcionados por este segundo estudio sugieren una interrupción en la conectividad de la sustancia blanca del lóbulo temporal medial, lo que podría contribuir a elucidar el déficit de memoria declarativa de los esquizofrénicos.

En el tercer trabajo investigamos la activación cerebral en 22 pacientes con esquizofrenia y 24 controles sanos durante la ejecución de dos tareas de memoria de reconocimiento: de palabras y de caras. Los pacientes mostraron en ambas tareas unos patrones cerebrales corticales y subcorticales difusos de hipoactivación respecto a los controles. En particular, en el análisis de región de interés se halló una hipoactivación bilateral del hipocampo con una preeminencia izquierda en el reconocimiento de las palabras y derecha en el caso de las caras. Los resultados del tercer estudio proveen evidencias de una participación funcional del hipocampo en el déficit de memoria declarativa de la esquizofrenia.

En síntesis, nuestros resultados en conjunto evidencian que en la esquizofrenia estarían presentes unas alteraciones estructurales y funcionales del hipocampo especialmente relacionadas con el deterioro de memoria declarativa de estos pacientes.