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Detección precoz de cambios cognitivos sutiles en el envejecimiento y en la fase preclínica del continuo Alzheimer

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La **Dra. Lorena Rami González**, doctora en Psicología por la Universidad Barcelona y el **Dr. Antoni Rodríguez Fornells**, doctor en Psicología por la Universidad de Barcelona,

CERTIFICAN

Que la memoria titulada "Detección precoz de cambios cognitivos sutiles en el envejecimiento y en la fase preclínica del continuo Alzheimer", presentada por Adrià Tort Merino, ha sido realizada bajo nuestra dirección y consideramos que reúne las condiciones necesarias para ser defendidas frente el Tribunal correspondiente, para optar al grado de Doctor en Biomedicina por la Universidad de Barcelona.

Dra. Lorena Rami González

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Universitat de Barcelona

A l'Àsia

A Verónica

Als meus pares i a la meva germana

"Caminante, son tus huellas

el camino, y nada más"

Antonio Machado

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[...] y nada más. Nada más. A todos los que habéis recorrido este largo camino conmigo es, al fin y al cabo, a quien debo este trabajo. Si de algo estoy seguro, después de todo lo vivido, es que gracias a vosotros ha valido la pena.

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I. LISTADO DE ABREVIATURAS

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$A\beta_{42}$: isoforma de 42 aminoácidos de la proteína beta-amiloide

APOE: apolipoproteina E

AFE-T: Ancient Farming Equipment Test

DCL: deterioro cognitivo leve

DCS: declive cognitivo subjetivo

EA: enfermedad de Alzheimer

FTT: Finger Tapping Test

LCR: líquido cefalorraquídeo

NIA-AA: National Institute of Aging – Alzheimer's Association

OLP: olvido acelerado a largo plazo

PET: tomografía por emisión de positrones

PET-FDG: PET de fluorodeoxyglucosa

P-tau: tau fosforilada

RM: resonancia magnética

T-tau: tau total

II. INTRODUCCIÓN

INTRODUCCIÓN

La enfermedad de Alzheimer (EA) comprende una larga fase asintomática (o preclínica) que comienza décadas antes de la aparición de los primeros síntomas, en la que se desencadenan los procesos fisiopatológicos característicos de la enfermedad. El estudio detallado y la detección precoz de los primeros cambios cognitivos en esta etapa, son de vital importancia para predecir la conversión a las posteriores fases sintomáticas de la enfermedad. Asimismo, existe una necesidad creciente de identificar y comprender los diferentes factores que influyen en el rendimiento cognitivo, tanto en esta fase preclínica como en el envejecimiento sano, para aplicar las intervenciones más óptimas de la forma más temprana.

1. Conceptualización actual y epidemiología de la EA

La EA es una enfermedad neurodegenerativa que conlleva un deterioro gradual y progresivo de las funciones cognitivas. En la actualidad, se define como una entidad clínico-biológica y es concebida como un continuo que transcurre desde una fase asintomática conocida como fase preclínica, en la que se desencadenan los procesos fisiopatológicos propios de la enfermedad, hasta una etapa de demencia (Jack et al., 2018). Las características neuropatológicas distintivas de la EA son el depósito extracelular de la proteína β-amiloide (placas neuríticas) y el depósito intracelular de tau hiperfosforilada (ovillos neurofibrilares), cuyo acúmulo conduce a la muerte neuronal y el subsiguiente deterioro cognitivo. Las primeras manifestaciones cognitivas de la EA aparecen en la denominada fase de deterioro cognitivo leve (DCL) e incluyen, en las formas típicas de la enfermedad, la alteración de la memoria episódica, desorientación, cambios en el estado de ánimo y/o en la personalidad, dificultades de expresión y comprensión del lenguaje y disminución de la atención, entre otras.

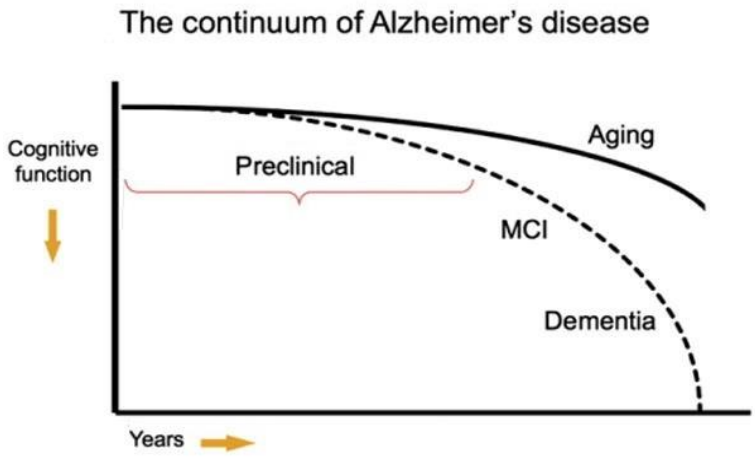

Figura 1. Conceptualización de la EA como un continuo. Tomada de "Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging and the Alzheimer's Association workgroup", por R. A. Sperling et al., 2011. *Alzheimer's and Dementia*, *7*, p. 283.

En 2015, el número total de personas con demencia en todo el mundo se estimó en aproximadamente 46,8 millones de casos y se calculó que este número podría duplicarse cada 20 años, alcanzando así los 74,7 millones de casos en 2030 y los 131,5 millones en 2050. Por otro lado, su incidencia a nivel mundial se estimó en más de 9,9 millones de nuevos casos cada año (Prince et al., 2015). De entre todas las demencias, se calcula que la EA podría representar entre el 60 y el 80% de los casos (Alzheimer's Association, 2020). En España, la prevalencia de la EA podría oscilar entre el 5.5% y el 5.8% en personas mayores de 65 años, con una incidencia de 9,2 por cada 1000 personas / año (Garré-Olmo, 2018).

2. Evolución de los criterios diagnósticos y de investigación de la EA

Históricamente, la EA ha sido definida como una entidad clínico-patológica caracterizada por un fenotipo clínico determinado. Los primeros criterios diagnósticos publicados, establecían que el diagnóstico definitivo de la EA requería de confirmación histopatológica post-mortem (McKhann et al., 1984). En sus primeras aproximaciones, el diagnóstico de la EA implicaba la presencia de un deterioro cognitivo lo

suficientemente severo para afectar la funcionalidad del paciente en sus actividades de la vida diaria, cumpliendo así con los criterios sindrómicos de demencia (American Psychiatric Association, 2000; World Health Organization, 1994). Más adelante, se publicaron los primeros criterios de investigación para la EA en los que se introduce la presencia de biomarcadores específicos para su identificación (Dubois et al., 2007, 2014). En 2011, el *National Institute of Aging* y *la Alzheimer's Association* (NIA-AA) establecen las nuevas guías diagnósticas para las etapas de DCL (Albert et al., 2011) y demencia (McKhann et al., 2011) de la EA. Asimismo, gracias al progreso en el conocimiento de los biomarcadores de la EA, se constituye por primera vez un marco conceptual y unas recomendaciones específicas para el estudio de la fase preclínica de la enfermedad (Sperling et al., 2011). Por último, con la voluntad de unificar los criterios publicados en 2011 y formalizar de este modo la concepción de la EA como un continuo clínico-biológico, se publican en 2018 las vigentes guías de investigación en el marco del estudio de la EA (Jack et al., 2018).

3. Biomarcadores de la EA y los marcos conceptuales de 2011 y 2018

Para el desarrollo de los trabajos incluidos en la presente tesis doctoral, se siguieron las recomendaciones del NIA-AA disponibles en el momento de publicación de cada uno de los estudios. El primer trabajo, publicado en 2017, siguió las guías de investigación de 2011 (Sperling et al., 2011); por otro lado, el resto de trabajos fueron publicados entre 2019 y 2020 y siguieron las recomendaciones de 2018 (Jack et al., 2018). Antes de exponer las características de las diferentes guías de investigación, es necesario definir los biomarcadores centrales de la EA. Actualmente, se conciben tres grandes grupos de biomarcadores: los biomarcadores de amiloide, los biomarcadores de tau patológica y los biomarcadores de neurodegeneración o daño neuronal.

Biomarcadores de amiloide

El acúmulo extracelular de β-amiloide (placas neuríticas) es uno de los procesos patológicos distintivos de la EA. La hipótesis de la cascada amiloide (Hardy & Higgins, 1992), ha sido el modelo dominante de patogénesis de la EA y se basa en el depósito gradual de amiloide, que desencadena la formación de ovillos neurofibrilares y la muerte neuronal. El depósito de placas de amiloide es un proceso insidioso que ocurre décadas antes de que se manifiesten los primeros síntomas de la enfermedad (Jack et al., 2013; Villemagne et al., 2013). Los niveles cerebrales de placas de amiloide pueden obtenerse de manera indirecta mediante la extracción de líquido cefalorraquídeo (LCR) o de manera directa mediante técnicas de Tomografía por Emisión de Positrones (PET; del inglés, *Positron Emission Tomography*) con trazadores específicos de la proteína amiloide. En la obtención de los niveles de amiloide en LCR, la isoforma de 42 aminoácidos de la proteína β-amiloide ($A\beta_{42}$) es uno de los péptidos más utilizados para fines diagnósticos así como un indicador válido de la patología cerebral subyacente a la EA (Blennow, Mattsson, Schöll, Hansson, & Zetterberg, 2015). Diferentes estudios han demostrado que los niveles reducidos de $A\beta_{42}$ en LCR correlacionan de manera inversa con la carga amiloide cerebral tanto en autopsia (Strozyk, Blennow, White, & Launer, 2003; Tapiola et al., 2009) como *in vivo*, mediante técnicas de PET (Fagan et al., 2006; Grimmer et al., 2009).

Biomarcadores de tau patológica

El acúmulo intracelular de tau hiperfosforilada es la segunda de las características neuropatológicas distintivas de la EA. Los denominados biomarcadores de tau patológica hacen referencia expresa al proceso fisiopatológico subyacente a la EA (i.e., el depósito de ovillos neurofibrilares). De este modo, los niveles cerebrales de tau

patológica se pueden medir *in vivo* mediante PET con trazador de proteína tau o de forma indirecta mediante el análisis de los niveles de tau fosforilada en LCR (P-tau). En cuanto a la obtención de los niveles de tau mediante el análisis de LCR, es importante destacar que únicamente los niveles de P-tau se consideran un biomarcador específico de la EA mientras que los niveles de tau total (T-tau) se consideran un biomarcador de neurodegeneración. Esta diferenciación se sostiene por la evidencia de que los ovillos neurofibrilares están compuestos principalmente de tau hiperfosforilada (Bakota & Brandt, 2016; Lee, Balin, Otvos, & Trojanowski, 1991). Asimismo, la especificidad de la alteración de los niveles de P-tau en la EA queda reflejada en el hecho de que la mayoría del resto de enfermedades neurodegenerativas presentan niveles elevados de T-tau mientras que los niveles de P-tau suelen ser normales (Blennow, Hampel, Weiner, & Zetterberg, 2010).

Biomarcadores de neurodegeneración o daño neuronal

Actualmente, se conciben tres biomarcadores de neurodegeneración en la EA: los niveles de T-tau en LCR, el PET de Fluorodeoxyglucosa (PET-FDG) y la atrofia en resonancia magnética (RM).

Los niveles aumentados de T-tau en LCR son indicadores de daño neuronal y no son un biomarcador específico para la EA ya que, como se ha comentado, suelen estar alterados en la mayoría de las enfermedades neurodegenerativas. En la EA, estudios recientes sugieren que los niveles de T-tau podrían ser un factor clave en la predicción de la progresión de la enfermedad tanto en sujetos cognitivamente sanos (Roe et al., 2013) como en pacientes con DCL (Ferreira et al., 2014; Petersen et al., 2013).

El PET-FDG es una técnica de neuroimagen que refleja el nivel de actividad sináptica mediante la medición del metabolismo de glucosa cerebral. En la EA, el patrón

característico del PET-FDG muestra un hipometabolismo bilateral de los lóbulos temporal y parietal. El PET-FDG tiene una buena sensibilidad en la detección de la disfunción sináptica en la EA (Anchisi et al., 2005; Drzezga et al., 2003) y en el seguimiento de la progresión de la enfermedad (De Leon et al., 2001; Johnson, Fox, Sperling, & Klunk, 2012).

La atrofia cerebral en RM estructural es otro biomarcador de neurodegeneración que, en el momento del diagnóstico de la EA, suele mostrar un patrón característico que involucra los lóbulos temporales mediales y las cortezas paralímbicas y temporoparietales (Dubois et al., 2007). De entre diferentes estructuras cerebrales implicadas en la EA, se ha sugerido que tanto la atrofia del lóbulo temporal medial como la del hipocampo son buenos indicadores de la progresión a demencia (Risacher et al., 2009).

3.1 El marco conceptual de 2011

Las recomendaciones del NIA-AA de 2011 (Sperling et al., 2011) emergen tras el auge del conocimiento de los biomarcadores, con la intención de proporcionar un lenguaje común para el estudio y clasificación de sujetos cognitivamente sanos con niveles anormales de biomarcadores de la EA. Estas guías fueron concebidas para que su aplicación se restringiera al ámbito de la investigación y no como criterios diagnósticos que pudieran utilizarse en la práctica clínica diaria. Una limitación de las guías de 2011 fue la agrupación de los biomarcadores en dos categorías: biomarcadores de amiloide y biomarcadores de neurodegeneración. De este modo, lo que actualmente conocemos como biomarcadores de tau patológica y biomarcadores de neurodegeneración se clasificaban en la misma categoría. En las recomendaciones de 2011, se propusieron tres etapas sucesivas para la fase preclínica de la EA:

- **Etapa 1:** Caracterizada por la presencia en solitario de amiloidosis, detectable por los niveles (reducidos) de $A\beta_{42}$ en LCR o por alteración de PET de amiloide.

- **Etapa 2:** Presencia de amiloidosis y neurodegeneración. Como medidas de neurodegeneración se incluían el PET-FDG y/o RM funcional, la RM estructural (atrofia de hipocampos y/o reducción del grosor cortical) y la alteración (indistinta) de los niveles de t-tau y P-tau en LCR.

- **Etapa 3:** Amiloidosis, neurodegeneración y "declive cognitivo sutil". En esta etapa se introduce el concepto "declive cognitivo sutil", definido como el conjunto de cambios cognitivos y/o conductuales sutiles, insuficientes para cumplir criterios de DCL y detectables: 1) en estudios longitudinales, por la reducción del rendimiento cognitivo de un sujeto en comparación con su propio rendimiento basal; o 2) en estudios transversales, mediante nuevas pruebas cognitivas más demandantes que los tests neuropsicológicos estándar.

3.2 El marco conceptual vigente

Las guías del NIA-AA de 2018 (Jack et al., 2018), constituyen un cambio histórico en la conceptualización de la EA, que deja de concebirse como una entidad clínico-patológica para entenderse como un continuo clínico-biológico cuyo sustrato neuropatológico subyacente es detectable en vida. Se abandona la concepción sindrómica de la EA para disociar, por un lado, entre la enfermedad con sus características biológicas y, por otro lado, el síndrome o estado cognitivo que la acompaña (clasificable como cognición normal, DCL o demencia). Es también en este contexto en el que se descarta la idea de una enfermedad formada por tres entidades clínicas separadas (EA preclínica, DCL y demencia) para formalizar la concepción de la enfermedad como un continuo. Esta

nueva concepción surge a raíz de diferentes estudios que señalan que la EA se desarrolla a través de un largo periodo de tiempo (Monsell et al., 2014; Resnick et al., 2010; Wilson, Leurgans, Boyle, Schneider, & Bennett, 2010) y que la progresión de los niveles de biomarcadores es asimismo un proceso continuo que empieza años antes de la aparición de los síntomas (Bateman et al., 2012; Fagan et al., 2014; Villemagne et al., 2013). Otra de las diferencias fundamentales entre las recomendaciones de 2011 y las de 2018, es que en las primeras los biomarcadores se agrupaban únicamente en biomarcadores de amiloide y de neurodegeneración, mientras que en las segundas se implementa la denominada clasificación A / T / N (Jack et al., 2016):

- **A**: biomarcadores de amiloide (niveles de $A\beta_{42}$ en LCR o PET de amiloide). La alteración de este biomarcador determina si un individuo está o no en el continuo Alzheimer. Sin embargo, la alteración o positividad en solitario de los biomarcadores de amiloide no es suficiente para hablar de EA.

- **T**: biomarcadores de tau patológica (niveles de P-tau en LCR o PET de tau). Los biomarcadores de tau patológica determinan si alguien que está en el continuo Alzheimer tiene EA. Así, para hablar de EA es necesaria la alteración conjunta de los biomarcadores de amiloide y tau patológica.

- **N**: biomarcadores de neurodegeneración (niveles de T-tau, PET-FDG y RM). Son biomarcadores inespecíficos de la EA y están relacionados con la severidad de la enfermedad.

Por último, cabe destacar que del mismo modo que en las recomendaciones de 2011, la aplicación del marco conceptual vigente se limita al ámbito de la investigación.

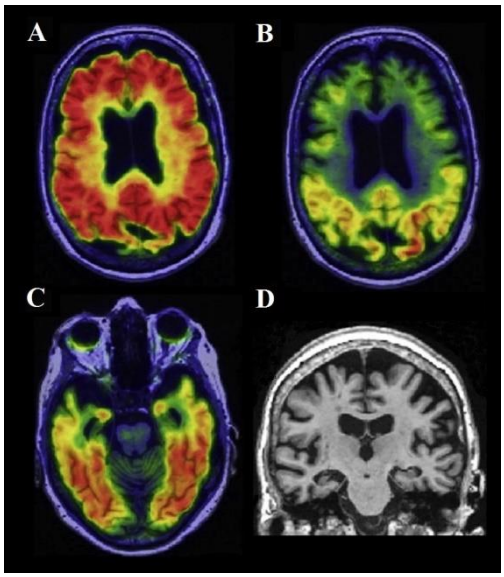

Figura 2. Ejemplo de un perfil de biomarcadores A+ T+ N+. PET de amiloide alterado o positivo (panel A); PET de tau alterado (paneles B y C); y atrofia en RM (panel D). Adaptada de "NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease", por C. R. Jack et al., 2018. *Alzheimer's and Dementia*, *14*, p. 539.

4. El estudio cognitivo del envejecimiento sano y de la fase preclínica del continuo Alzheimer

El depósito de proteínas, la atrofia cerebral y/o la disminución del rendimiento cognitivo son variables que correlacionan de forma significativa con la edad. De hecho, la edad representa el principal factor de riesgo para el desarrollo de la EA (Alzheimer's Association, 2013). Sin embargo, es importante señalar que la EA no es una parte más del proceso normal de envejecimiento y que, para la correcta caracterización de ambos procesos, hay que tener en cuenta diferentes variables.

El envejecimiento sano

La aparición de los biomarcadores no solo ha permitido un mayor conocimiento de la EA, sino que ha abierto la puerta al estudio del envejecimiento sano en ausencia de patología de tipo Alzheimer. En la actualidad, es posible estudiar muestras de sujetos cognitivamente sanos con niveles normales de biomarcadores de EA (i.e., fuera del

continuo Alzheimer). Esto es importante dado que la mayoría de publicaciones sobre la cognición en el envejecimiento sano no incluyen datos biológicos de los sujetos estudiados, aumentando de este modo la posibilidad de incluir en sus muestras a individuos situados dentro del continuo Alzheimer.

Los cambios cognitivos asociados al envejecimiento sano han sido ampliamente documentados en la literatura científica. Si bien hay capacidades cognitivas, como el vocabulario y el conocimiento general, que muestran estabilidad e incluso mejoría con la edad; otras como la memoria, el lenguaje, la velocidad de procesamiento y la atención muestran una clara disminución (Fjell et al., 2014; Harada et al., 2013). Sin embargo, existe una necesidad creciente de identificar y estudiar las diferentes variables relacionadas con estos cambios cognitivos. En primer lugar, existen variables demográficas como la edad, los años de escolaridad y la reserva cognitiva (Kawas, Gray, Brookmeyer, Fozard, & Zonderman, 2000; Stern, 2009, 2012) que tienen un impacto significativo en la cognición a lo largo de la vida. Un estudio prospectivo que incluyó una muestra de 2509 individuos cognitivamente sanos mostró que tanto la edad como el nivel educativo eran predictores del mantenimiento de la función cognitiva durante un período de 8 años (Yaffe et al., 2009). En segundo lugar, algunas variables neuropsicológicas han sido identificadas como predictoras de deterioro cognitivo. Por ejemplo, el deterioro de la memoria se ha considerado como el principal factor de riesgo cognitivo para desarrollar patologías relacionadas con la edad, como el DCL o la EA (Petersen et al., 2001). Por último, se ha sugerido que el mantenimiento de la función cognitiva en el envejecimiento está relacionado principalmente con el mantenimiento del cerebro, es decir, la ausencia relativa de cambios cerebrales estructurales y funcionales (Nyberg, Lövdén, Riklund, Lindenberger, & Bäckman, 2012). En línea con esto, un estudio longitudinal que evaluó 125 participantes durante un periodo de 3 años,

mostró que aquellos sujetos con un mayor volumen del lóbulo temporal medial (LTM) al inicio del estudio, tenían más probabilidades de mantener su función cognitiva a lo largo tiempo (Dekhtyar et al., 2017).

La fase preclínica del continuo Alzheimer

La caracterización integral de la fase más temprana del continuo Alzheimer es esencial para la identificación de individuos que, pese a exhibir un rendimiento cognitivo normal, presentan evidencia del proceso patológico subyacente a la enfermedad. Existen diferentes variables, así como factores de riesgo, que hay que tener en cuenta en el estudio de la fase preclínica del continuo Alzheimer y que se han visto relacionados con el rendimiento cognitivo.

En primer lugar, el impacto de los biomarcadores de la EA en la cognición de sujetos situados dentro del continuo Alzheimer ha sido ampliamente documentado (Dumurgier et al., 2017; Ho & Nation, 2018; Pettigrew et al., 2015; Tijms et al., 2018). Estudios longitudinales han demostrado una relación clara entre la carga de amiloide y el declive de la memoria, sugiriendo que el depósito de β-amiloide en individuos cognitivamente sanos precede y se asocia al futuro deterioro cognitivo (Doraiswamy et al., 2012; Hedden, Oh, Younger, & Patel, 2013). La positividad de los biomarcadores de amiloide también se ha asociado con puntuaciones de memoria más bajas en poblaciones cognitivamente sanas (Harrington et al., 2017; Jansen et al., 2018). Sin embargo, algunos estudios recientes han sugerido que los niveles de tau podrían estar más estrechamente relacionados con los cambios cognitivos que los biomarcadores de amiloide, específicamente con el declive de la memoria (Glodzik et al., 2011; Nelson et al., 2012; Rolstad et al., 2013).

La fase preclínica del continuo Alzheimer también se ha visto relacionada con una serie de cambios estructurales y funcionales a nivel cerebral. Diferentes estudios longitudinales han demostrado que existen áreas estratégicas relacionadas con la memoria, como el hipocampo o estructuras adyacentes del lóbulo temporal medial (i.e., córtex entorrinal), que muestran una pérdida de volumen a nivel basal y una mayor tasa de atrofia entre 5 y 10 años antes del diagnóstico (Bernard et al., 2014; Younes, Albert, & Miller, 2014). Sin embargo, además del lóbulo temporal medial se ha observado que existen regiones prefrontales (Rugg & Vilberg, 2013; Wolk & Dickerson, 2011) y estructuras como el cerebelo (Kim, Uğurbil, & Strick, 1994; Rosenbloom, Schmahmann, & Price, 2012; Stoodley & Schmahmann, 2018) relacionadas con el rendimiento en tareas de memoria. También se ha sugerido que los individuos cognitivamente sanos situados dentro del continuo Alzheimer, muestran cambios sutiles a nivel funcional que preceden a los síntomas clínicos y cognitivos de la EA (Rami et al., 2014; Sheline et al., 2010).

A nivel cognitivo, es ampliamente aceptado que la pérdida de memoria es generalmente el cambio cognitivo más temprano en la EA (Albert, Moss, Blacker, Tanzi, & McArdle, 2007; Howieson et al., 2008; Petersen et al., 1999). Estudios longitudinales sugieren que la evaluación de la memoria episódica representa una de las medidas más sensibles en la predicción de la futura progresión a fases sintomáticas de la EA (Caselli et al., 2014; Pietrzak et al., 2015; Schmid, Taylor, Foldi, Berres, & Monsch, 2013). Sin embargo, hay estudios que también destacan la alteración temprana de otras funciones cognitivas (Baker et al., 2017; Grober et al., 2008) y proponen que la afectación de otros dominios, como las funciones ejecutivas, podría ser anterior a la de la memoria episódica en el continuo de la EA (Harrington et al., 2013). De hecho, se ha sugerido que se podrían detectar diferencias entre controles y sujetos con EA preclínica en una gran variedad de

habilidades cognitivas como la atención, memoria de trabajo, función visuoespacial, velocidad de procesamiento y/o razonamiento verbal y no verbal (Ritchie et al., 2017). Por otro lado, también se ha investigado el papel de la función motora como un posible predictor de deterioro cognitivo. Algunos estudios han sugerido que la disfunción motora podría ser un marcador sensible de la fase asintomática de la EA (Albers et al., 2015; Buchman & Bennett, 2011).

Por último, existen diferentes factores que se han visto relacionados con un mayor riesgo de desarrollar deterioro cognitivo y EA. A nivel genético, el gen de la apolipoproteina E (APOE) representa el mayor factor de riesgo para la EA (Yu, Tan, & Hardy, 2014). Los sujetos portadores de una (heterocigotos) o dos (homocigotos) copias del alelo ε4 tienen un riesgo mayor de desarrollar EA en comparación con los no portadores (Corder et al., 1993; Farrer et al., 1997). La frecuencia estimada de la EA es del 91% en homocigotos ε4/ε4, con una edad media de inicio de los síntomas de 68 años; del 47% en heterocigotos ε3/ε4, con edad de inicio de 76 años; y del 20% en no portadores, con inicio a los 84 años (Liu CC, Kanekiyo T, Xu H, 2013). Estudios longitudinales sugieren que el genotipo APOE ε4 podría estar relacionado con un mayor deterioro de la memoria en sujetos cognitivamente sanos (Caselli et al., 2009; Wilson et al., 2002) y con mayores tasas de atrofia del lóbulo temporal medial en la EA (Filippini et al., 2009; Manning et al., 2014). El estudio de sujetos cognitivamente sanos homocigotos o heterocigotos para APOE ε4, ofrece la oportunidad de investigar los primeros cambios cognitivos relacionados con la EA. Por otro lado, en cuanto a factores psicológicos que se han visto relacionadas con el futuro desarrollo de deterioro cognitivo, se ha sugerido que el declive cognitivo subjetivo (DCS) podría representar una de las primeras manifestaciones de la EA (Jessen, 2014; Mitchell, Beaumont, Ferguson, Yadegarfar, & Stubbs, 2014; Reisberg et al., 2008). El DCS ha sido definido

como la disminución autopercibida de las capacidades cognitivas en sujetos cuyo rendimiento cognitivo se sitúa dentro de la normalidad (Jessen et al., 2014). Esta autopercepción de la disminución del rendimiento cognitivo viene dada por la perspectiva subjetiva y persistente del propio sujeto en relación a un estado de normalidad cognitiva previo y no requiere de la confirmación de otros individuos. Asimismo, no esta percepción no está relacionada con ningún evento súbito o agudo que pudiera explicarla (Jessen et al., 2020). Estudios longitudinales recientes muestran que los sujetos con DCS y evidencia de biomarcadores de EA tienen un mayor riesgo de desarrollar deterioro cognitivo (Van Harten et al., 2013; Wolfsgruber et al., 2017). De este modo, el estudio de sujetos con DCS que se encuentran dentro del continuo Alzheimer podría representar uno de los escenarios más óptimos para la identificación de las primeras manifestaciones cognitivas relacionadas con el EA. Para la evaluación y cuantificación del DCS en los estudios incluidos en la presente tesis doctoral, se ha utilizado el *Subjective Cognitive Decline Questionnaire* (SCD-Q; Rami et al., 2014). El SCD-Q evalúa el grado de DCS en un período de tiempo de dos años. Incluye preguntas que abordan la percepción de cambio (o disminución del rendimiento cognitivo) en diferentes actividades de la vida diaria. Evalúa diferentes dominios cognitivos, incluyendo la memoria, el lenguaje y las funciones ejecutivas. Contiene dos formularios paralelos con las mismas preguntas: el formulario "MiCog" es respondido por el sujeto de estudio y el formulario "SuCog" es respondido por un informante (generalmente un familiar). El cuestionario ha sido validado, mostrando una alta validez convergente, consistencia interna y poder discriminante para distinguir entre sujetos con y sin DCS (Rami et al., 2014). En un estudio reciente, se ha observado una correlación entre las puntuaciones del SCD-Q y los biomarcadores de EA en LCR (Valech et al., 2015). El SCD-Q ha mostrado una alta especificidad para la fase preclínica del continuo

Alzheimer, especialmente aquellos ítems relacionados con el lenguaje y las funciones ejecutivas (Valech et al., 2018).

5. Detección precoz de cambios cognitivos sutiles

La identificación de cambios cognitivos sutiles en individuos cognitivamente sanos que se encuentran dentro del continuo Alzheimer es crucial para predecir la progresión hacia las posteriores fases sintomáticas de la EA. Sin embargo, la mayoría de los estudios con un diseño transversal no han podido encontrar una clara relación entre el rendimiento cognitivo en pruebas neuropsicológicas estándar y la evidencia de biomarcadores de EA en individuos cognitivamente sanos (Aizenstein et al., 2008; Mormino et al., 2009; Storandt, Mintun, Head, & Morris, 2009; Villemagne et al., 2011). En este contexto, parece necesario el desarrollo de nuevas pruebas neuropsicológicas más demandantes, que aporten mayor sensibilidad para la detección de dificultades cognitivas sutiles en la fase preclínica del continuo Alzheimer.

En los últimos años, se han desarrollado nuevas tareas neuropsicológicas altamente exigentes para la detección de los primeros cambios cognitivos en el continuo de la EA (Rentz et al., 2013). Son ejemplos de pruebas altamente demandantes el *Face Name Associative Memory Exam* (Rentz et al., 2011), el *Short-Term Memory Binding test* (Parra et al., 2010) o el *Memory Capacity Test* (Rentz et al., 2010), entre otros. Hasta la fecha, el uso de este tipo de medidas más exigentes ya ha mostrado resultados prometedores en individuos cognitivamente sanos, incluyendo muestras de sujetos con biomarcadores alterados de EA (Rentz et al., 2011), portadores presintomáticos de mutaciones de EA familiar (Parra et al., 2010) e individuos con DCS (Sanabria et al., 2018). Incluso se han encontrado asociaciones entre el depósito de β-amiloide cerebral

en áreas estratégicas y el rendimiento neuropsicológico en sujetos cognitivamente sanos (Rentz et al., 2011).

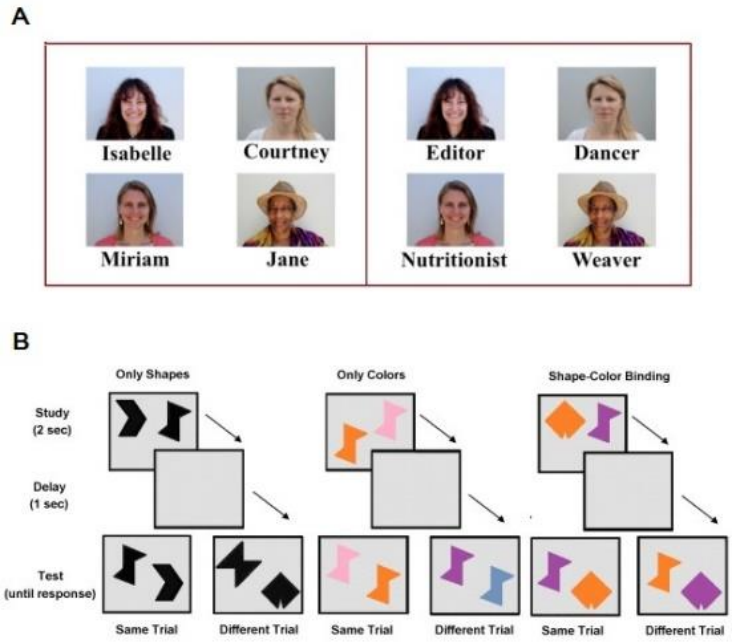

Figura 3. Ejemplos de estímulos del *Face Name Associative Memory Exam* (panel A) y el *Short-Term Memory Binding test* (panel B). Adaptada de "Promising developments in neuropsychological approaches for the detection of preclinical Alzheimer's disease: a selective review", por D. M. Rentz et al., 2013. *Alzheimer's research and Therapy*, *5 (58)*, p. 3-4.

Para el desarrollo de los trabajos incluídos en la presente tesis doctoral, se diseñaron dos nuevas tareas cognitivas: el *Ancient Farming Equipment Test* (AFE-T) *y el Finger tapping test* (FTT).

Ancient Farming Equipment Test

El AFE-T es innovadora tarea de aprendizaje asociativo basada en el paradigma *Ancient Farming Equipment* (Laine & Salmelin, 2010). Este paradigma fue originalmente diseñado para examinar el aprendizaje de nuevas palabras en la adquisición de un nuevo lenguaje e involucra el sistema de memoria declarativa para asociar adecuadamente nombres desconocidos a objetos que son igualmente novedosos. Los nombres de los objetos son pseudopalabras (e.g., Gorsi, Folute) y los objetos son imágenes en blanco y negro de maquinaria agrícola antigua, de origen finlandés. La necesidad de asociar

estímulos novedosos, desprovistos de contenido semántico previo, hace que la tarea sea especialmente exigente, sobretodo en comparación con tareas clásicas de memoria episódica que únicamente requieren de la memorización de palabras u objetos existentes.

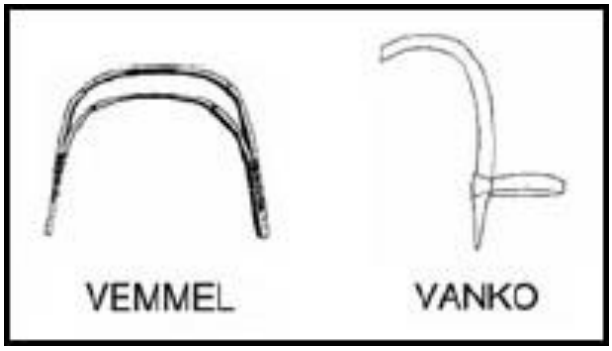

Figura 4. Ejemplo de estímulos del paradigma *Ancient Farming Equipment*. Adaptada de "Neurocognition of New Word Learning in the Native Tongue: Lessons From the Ancient Farming Equipment Paradigm", por M. Laine y R. Salmelin, 2010. *Language learning, 60 (Suppl. 2)*, p. 28

En general, los modelos biológicos de memoria que han estudiado las bases neurales de la adquisición de palabras, diferencian entre contribuciones hipocampales y neocorticales (O'Reilly & Norman, 2002). En primer lugar, la codificación inicial de una nueva palabra y su asociación con una nueva imagen dependería del hipocampo (Davis, Di Betta, Macdonald, & Gaskell, 2009; Mestres-Missé, Càmara, Rodriguez-Fornells, Rotte, & Münte, 2008; Rodriguez-Fornells, Cunillera, Mestres-Missé, & de Diego-Balaguer, 2009). Un estudio reciente de RM funcional en el que se aplicó el paradigma AFE en un paciente afásico y en controles sanos, mostró una clara implicación de esta estructura durante el período de aprendizaje (Tuomiranta et al., 2015). En segundo lugar, la posterior integración del aprendizaje en el léxico mental involucraría sistemas neorcorticales, incluyendo áreas del lóbulo temporal izquierdo (Raboyeau et al., 2004), lóbulo parietal inferior izquierdo (Breitenstein et al., 2005) y región frontal inferior izquierda (James & Gauthier, 2004).

Hasta la fecha, el paradigma AFE se ha utilizado principalmente para estudiar la adquisición de nuevas palabras en individuos sanos (Cornelissen et al., 2004; Grönholm, Rinne, Vorobyev, & Laine, 2005) y en dos estudios de pacientes con DCL y EA (Grönholm-Nyman, Rinne, & Laine, 2010; Grönholm, Rinne, Vorobyev, & Laine, 2007). Grönholm *et al.* (2007) estudiaron los correlatos neuroanatómicos del paradigma AFE en sujetos con DCL mediante PET. En comparación con controles de la misma edad, el grupo de pacientes con DCL mostró una mayor activación de la corteza cingulada anterior. En un estudio posterior, los mismos autores mostraron diferencias de aprendizaje y recuerdo a largo plazo entre controles, sujetos con DCL y pacientes con EA utilizando el mismo paradigma (Grönholm-Nyman et al., 2010).

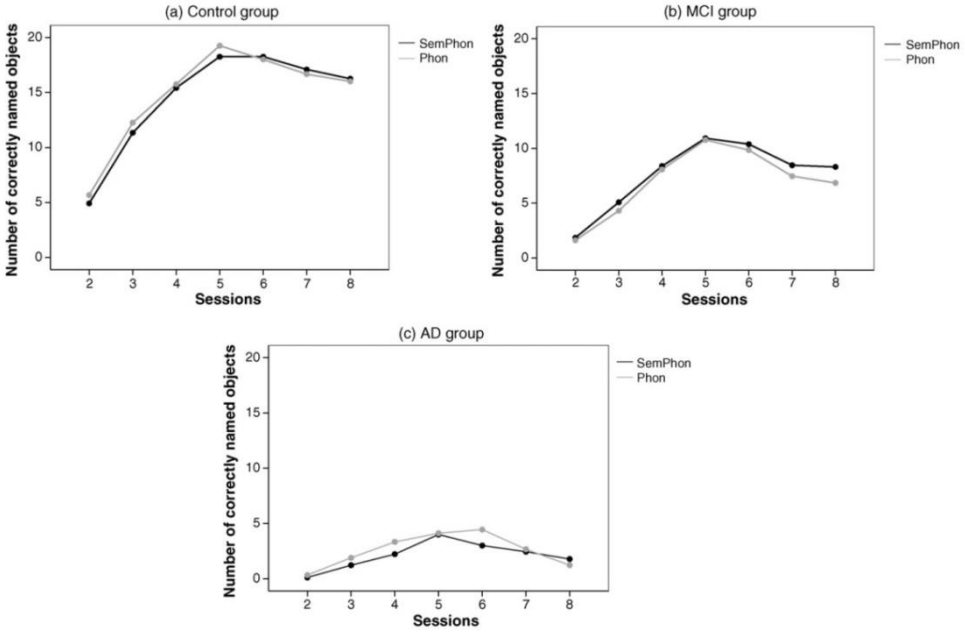

Figura 5. Resultados del estudio de Grönholm-Nyman, Rinne y Laine (2010), en el que se utilizó el paradigma *Ancient Farming Equipment*. Los gráficos muestran las curvas de aprendizaje y olvido de tres grupos de sujetos: Controles (a), DCL (b) y EA (c). Las sesiones de aprendizaje (sesiones 2-5) se completaron dentro de un periodo máximo de dos semanas. Las sesiones de recuerdo se llevaron a cabo una semana (sesión 6), un mes (sesión 7) y dos meses (sesión 8) tras la última sesión de aprendizaje. Las líneas representan el rendimiento al asociar parejas de objeto/nombre para las cuales se proporcionaba tanto el nombre del objeto como su definición (líneas oscuras) o únicamente el nombre (líneas claras). Adaptada de "Learning and forgetting new names and objects in MCI and AD", por P. Grönholm-Nyman, J. O. Rinne y M. Laine, 2010. *Neuropsychologia*, *48*, p. 1084.

Como se ha podido comprobar, los diferentes estudios que han utilizado el paradigma *Ancient Farming Equipment* incluyen diseños complejos. En particular, el AFE-T, empleado para el deasarrollo de cuatro de los cinco trabajos que constituyen la presente tesis doctoral, incluye: 1) una fase de aprendizaje inicial, con dos sesiones de aprendizaje en dos días consecutivos de una lista de 24 estímulos (lista A) con un ensayo final de recuerdo inmediato facilitado, 2) una fase de recuerdo a largo plazo (evocación libre, facilitada y reconocimiento a la semana, 3 meses y 6 meses desde el aprendizaje inicial), 3) una fase de reaprendizaje a los 6 meses (re-administración de la lista A) y 4) un seguimiento a los 18 meses con la administración de una nueva lista (lista B). Las diferentes fases de esta prueba perminten una evaluación exhaustiva de la funciones de aprendizaje y memoria, lo cual resulta particularmente importante en el estudio de sujetos cognitivamente sanos a riesgo de desarrollar EA. Específicamente, la fase de recuerdo a largo plazo permite la evaluación del denominado olvido acelerado a largo plazo (OLP). El OLP es un fenómeno cognitivo que se caracteriza por la pérdida rápida de información en periodos de días o semanas a pesar de una capacidad de aprendizaje inicial intacta (Butler, Gilboa, & Miller, 2019). Recientemente, el OLP se ha postulado como un marcador potencial de las fases asintomáticas de la EA. Dos estudios recientes han identificado OLP en un período de retención de 1 semana en una cohorte de sujetos presintomáticos con EA familiar (Weston et al., 2018) y en individuos asintomáticos con riesgo genético aumentado de EA (Zimmermann & Butler, 2018). El primero de los dos estudios, mostró OLP mediante el uso de tres pruebas cognitivas estándar (recuerdo de una lista de palabras, evocación de una figura y recuerdo de una historia) en sujetos presintomáticos con EA familiar (Weston et al., 2018). Por otro lado, Zimmerman y Butler (2018) identificaron OLP en sujetos asintomáticos portadores de APOE ε4. Evaluaron 60 participantes (20 homocigotos para

ε3, 20 heterocigotos para ε3 y ε4, y 20 homocigotos para ε4) con una prueba de aprendizaje de una lista de 15 palabras. En sus análisis, el haplotipo APOE ε4 no mostró efectos sobre la capacidad de aprendizaje o el recuerdo a corto plazo, pero se asoció con OLP en el periodo de una semana.

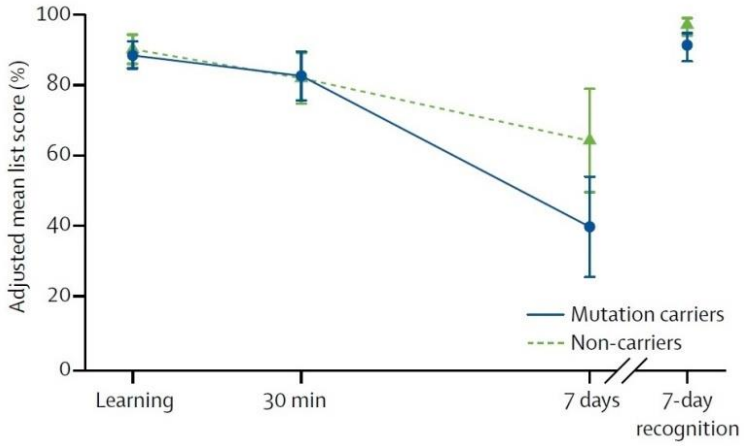

Figura 6. Resultados del estudio de Weston et al. (2018). El gráfico muestra como un grupo de sujetos presintomáticos con EA familiar presentó un rendimiento similar al del grupo control al aprender una lista de palabras, pero rindió significativamente peor al tratar de recordar las palabras al cabo de 7 días. Adaptada de "Accelerated long-term forgetting in presymptomatic autosomal dominant Alzheimer's disease: a cross-sectional study", por P. S. J. Weston et al., 2018. *The Lancet Neurology, 17*, p. 127.

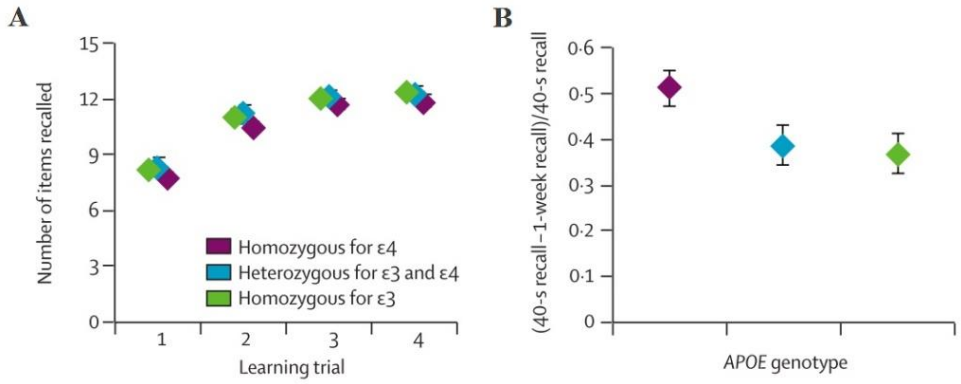

Figura 7. Resultados del estudio de Zimmermann & Butler (2018). El panel A muestra como los tres grupos de estudio rindieron de manera similar al aprender una lista de 15 palabras. El panel B muestra como los grupos de sujetos portadores del alelo APOE ε4 obtuvieron mayores tasas de olvido que el grupo de no-portadores. Adaptada de "Accelerated long-term forgetting in asymptomatic APOE ε4 carriers", por J. F. Zimmermann y C. R. Butler, 2018. *The Lancet Neurology, 17*, p. 395.

Finger Tapping Test

Por útlimo, para el tercero de los trabajos de la presente tesis doctoral, se desarrolló una versión computarizada de la tarea clásica Finger Tapping Test (Reitan, 1985). Esta nueva versión del FTT consiste en presionar repetidamente y lo más rápido posible la barra espaciadora de una computadora con el dedo índice mientras se mira un punto de fijación. La tarea proporciona dos indicadores sensibles de rendimiento motor que son: 1) la velocidad de tapping y 2) la variabilidad intra-sujeto. La velocidad de tapping es el número de veces que se prensiona la barra espaciadora. La variabilidad intra-sujeto hace referencia a la inconsistencia en el rendimiento de un mismo sujeto entre los diferentes ensayos de la tarea (Hultsch, Strauss, Hunter, & MacDonald, 2008). Aunque la mayor parte de la literatura se ha centrado en el análisis de la velocidad motora (Buracchio, Dodge, Howieson, Wasserman y Kaye, 2010; Camicioli, Howieson, Oken, Sexton y Kaye, 1998; Del Campo et al., 2016), algunos estudios recientes han sugerido que la variabilidad intra-sujeto también podría ser un marcador específico de las primeras fases de la EA (Verghese et al., 2008; Verghese, Wang, Lipton, Holtzer y Xue, 2007).

III. OBJETIVOS

OBJETIVOS

La fase preclínica del continuo Alzheimer comienza décadas antes de la aparición de los primeros síntomas de la enfermedad. Los procesos fisiopatológicos característicos de la enfermedad se desencadenan a lo largo de esta fase en ausencia de deterioro cognitivo objetivo. En la actualidad, existe una necesidad creciente de identificar y comprender los diferentes factores que influyen en el rendimiento cognitivo tanto en la fase preclínica del continuo Alzheimer como en el envejecimiento sano. Dado que los tests neuropsicológicos estándar no permiten una evaluación detallada de la función cognitiva en estas poblaciones, es necesario desarrollar nuevas herramientas más exigentes, sensibles y específicas. En este contexto, mediante el desarrollo y uso de dos nuevas tareas cognitivas, el objetivo general de la presente tesis doctoral ha sido estudiar las funciones de aprendizaje y olvido a largo plazo, así como la función motora fina, tanto en el envejecimiento sano como en la fase preclínica del continuo Alzheimer.

Asimismo, de los diferentes trabajos desarrollados derivan los siguientes objetivos específicos:

1. Estudiar las funciones de aprendizaje y olvido a largo plazo, a través del *Ancient Farming Equipment Test*, en sujetos cognitivamente sanos situados dentro del continuo Alzheimer y evaluar su posible relación con los biomarcadores de enfermedad de Alzheimer en líquido cefalorraquídeo.

2. Investigar la relación de diferentes variables biológicas, neuroanatómicas y neuropsicológicas con los cambios cognitivos más tempranos en el envejecimiento sano, es decir, en individuos cognitivamente sanos situados fuera del continuo Alzheimer.

3. Explorar la función motora fina, mediante el *Finger Tapping Test*, en sujetos situados dentro del continuo Alzheimer y su posible relación con los niveles de biomarcadores de enfermedad de Alzheimer en líquido cefalorraquídeo.

4. Determinar el potencial del olvido acelerado a largo plazo como marcador de disfunción cognitiva sutil en individuos con riesgo genético aumentado de desarrollar enfermedad de Alzheimer y examinar la posible relación entre el olvido acelerado a largo plazo y los biomarcadores de enfermedad de Alzheimer en líquido cefalorraquídeo.

5. Evaluar las funciones de aprendizaje y olvido a largo plazo, mediante el *Ancient Farming Equipment Test*, en sujetos con deterioro cognitivo subjetivo y explorar su posible relación con los biomarcadores de enfermedad de Alzheimer en líquido cefalorraquídeo.

IV. PUBLICACIONES

Trabajo número 1:

Early detection of learning difficulties when confronted with novel information in preclinical Alzheimer's disease stage 1

Tort-Merino A, Valech N, Peñaloza C, Grönholm-Nyman P, León, M, Olives J, Estanga A, Ecay M, Fortea J, Martínez-Lage P, Molinuevo JL, Laine M, Rodríguez-Fornells A, Rami L

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Early Detection of Learning Difficulties when Confronted with Novel Information in Preclinical Alzheimer's Disease Stage 1

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Abstract. We employed a highly demanding experimental associative learning test (the AFE-T) to explore memory functioning in Preclinical Alzheimer's Disease stage 1 (PreAD-1) and stage 2 (PreAD-2). The task consisted in the learning of unknown object/name pairs and our comprehensive setup allowed the analysis of learning curves, immediate recall, long-term forgetting rates at one week, three months, and six months, and relearning curves. Forty-nine cognitively healthy subjects were included and classified according to the presence or absence of abnormal CSF biomarkers (Control, $n = 31$; PreAD-1, $n = 14$; PreAD-2, $n = 4$). Control and PreAD-1 performances on the experimental test were compared by controlling for age and education. These analyses showed clear learning difficulties in PreAD-1 subjects ($F = 6.98$; $p = 0.01$). Between-group differences in long-term forgetting rates were less notable, reaching statistical significance only for the three-month cued forgetting rate ($F = 4.83$; $p = 0.03$). Similarly, relearning sessions showed only statistical trends between the groups ($F = 3.22$; $p = 0.08$). In the whole sample, significant correlations between CSF $A\beta_{42}$/tau ratio and the AFE-T were found, both in the total learning score ($r = 0.52$; $p < 0.001$) and in the three-month cued forgetting rate ($r = –0.38$; $p < 0.01$). Descriptive subanalyses involving PreAD-2 suggested greater learning and recall difficulties in these subjects when compared with the PreAD-1 group. The present results suggest that explicit learning difficulties when binding information could be one of the earliest signs of the future emergence of episodic memory difficulties on the Alzheimer's disease continuum. Our findings indicate that the AFE-T is a sensitive test, capable of detecting subtle memory difficulties in PreAD-1.

Keywords: Alzheimer's disease, biomarkers, cognitive aging, memory, neuropsychology

[1]These authors contributed equally to this work.

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INTRODUCTION

The identification of subtle cognitive changes in preclinical Alzheimer's disease (Pre-AD) has long been considered critical for predicting progression toward later clinical AD stages. Within the Pre-AD phase, three preclinical stages were defined by the National Institute of Aging and Alzheimer's Association (NIA-AA): stage 1 with abnormal amyloid-β (Aβ) levels, stage 2 with both amyloidosis and neurodegeneration (including elevated levels of CSF tau or brain atrophy), and stage 3 with the onset of subtle cognitive decline [1, 2]. The usefulness of the NIA-AA staging has been demonstrated in recent reports involving Pre-AD subjects [3–6]. These studies on the different Pre-AD stages support the idea that the co-occurrence of Aβ deposition and neurodegeneration (i.e., Pre-AD-2) accelerates cognitive decline in cognitively healthy individuals and is needed for the emergence of subtle cognitive difficulties. Another important measure in Pre-AD studies is the apolipoprotein E (APOE) ε4 genotype. In that line, Lim et al. [7] analyzed the cognitive performance of 144 healthy older adults classified as APOE ε4 carriers ($n = 61$) and APOE ε4 noncarriers ($n = 83$). They found a moderate negative relationship between cerebral Aβ and episodic memory performance only in APOE ε4 carriers.

A cognitive feature of Pre-AD subjects is that all of them, regardless of staging, have scores within the normal range on standard neuropsychological tests. Thus, most studies using a cross-sectional design have failed to find a relationship between cognitive performance on standard neuropsychological tests and biomarker evidence of AD in clinically asymptomatic at-risk individuals [8–11]. However, studies such as the conducted by Rentz et al. [12] found associations between a high demanding face-name associative memory test and Aβ accumulation in brain regions associated with memory systems. Other later studies including Pre-AD staging, reported group differences between PreAD-2 subjects and controls but failed to find cross-sectional difficulties in PreAD-1 [4, 5], and only a posterior report showed that a demanding memory test (the free recall subtest of the Memory Capacity Test; MCT) managed to discriminate between PreAD-1 subjects and controls [6]. The MCT is a high demanding associative memory task consisting on binding a total of 32 words (distributed in two lists) with a semantic cue to improve encoding and recall. The test includes free and cued immediate recall and free and cued delayed recall at 30 minutes. Therefore, it seems mandatory to develop more sensitive cognitive measures to detect subtle cognitive difficulties at Pre-AD stages, especially in PreAD-1.

In the present study, we adapted an innovative associative learning task based on the Ancient Farming Equipment (AFE) paradigm [13] to assess learning, recall, and relearning in Pre-AD subjects. This task, originally devised to examine the early stages of learning new words in one's native tongue, engages the declarative memory system in order to properly associate unfamiliar names to objects that are equally novel. The fact that participants need to create a new associative link between the representations at the lexical (new-word trace) and at the visual-conceptual level (new-object) makes this task highly demanding, especially compared to classical episodic memory tasks that require solely the memorization of words or existing objects. Task difficulty is further enhanced by the fact that AFE performance is measured by spontaneous oral production of the novel word. Based on the influential Complementary Learning Systems model (CLS) [14], it has been hypothesized that the initial encoding of a new word and its associative link to a new picture (concept) engages medial-temporal lobe regions (e.g., hippocampal and parahippocampal cortices) [13, 15–18]. There is evidence showing that these regions are affected early in AD [19–21].

To date the AFE paradigm has been mainly used to study acquisition of new words in healthy individuals [22–24] and in two studies concerning mild cognitive impairment (MCI) and AD [25, 26]. A recent fMRI study using the AFE paradigm in an aphasic patient and in healthy controls showed a clear involvement of medial temporal lobe regions during the learning period [21]. In a later study, Grönholm et al. [25] studied the neural correlates of the AFE paradigm in MCI subjects using positron emission tomography (PET). Compared to age-matched controls, MCI subjects showed increased activation in the anterior cingulate cortex, suggesting that the naming of newly learned objects imposed additional executive and attentional demands. The behavioral results of this study showed learning differences between controls and MCI since the first training run, indicating that initial learning measures were sensitive to MCI. The same authors reported learning and forgetting differences between MCI, AD, and controls in a later study using the same paradigm [26]. These results showed that both learning and forgetting performances were significantly impaired in the MCI subjects in comparison

to controls but all groups showed similar forgetting patterns, and that the MCI group benefited less from phonological cueing than controls.

Because of the novelty of the learning materials in the test (here coined as the AFE-Test, or AFE-T) and the high demands of its outcome measure (spontaneous naming), participants need multiple runs in order to be able to learn the set of new object/word pairs. It has been suggested that learning across multiple trials may provide the most sensitive index for initial diagnosis of MCI [27]. Besides, a crucial difference between this test and other standard memory tests is the longer time span for assessing forgetting (which comprised three time-points: one week, 3 months, and 6 months after initial learning). Previous memory tasks used in MCI and in early detection of people at risk of AD have usually evaluated delayed memory recall or recognition only after a 20-30-min delay from the encoding phase [28]. Importantly for the present research, recent studies have suggested that longer-term follow-up is crucial for tracking forgetting rates, including both recognition and recall, in order to obtain a level of sensitivity able to detect subtle memory difficulties [29, 30]. In addition, we included a phonological cueing test in the long-term follow-up in order to evaluate recall processes in more detail. Finally, the AFE-T also includes an additional *relearning task* carried out six months after the initial learning. Some studies suggest that information that has become inaccessible in recall or recognition tests can be reactivated by relearning tasks [31–33]. In sum, the information provided by the present comprehensive AFE-T includes detailed learning curves (learning rate), short and long-term forgetting measures, and a relearning curve.

We expected that the AFE-T, being a highly demanding learning and memory test, would enable us to detect subtle difficulties in learning and/or recall in Pre-AD subjects that cannot be detected by standard neuropsychological tests. Moreover, we aimed to evaluate the possible relationship between learning and memory performance and CSF proteins in Pre-AD subjects.

MATERIAL AND METHODS

Participants

Forty-nine cognitively normal subjects were included in the present study between 2013 and 2015. They were recruited from longitudinal ongoing projects at three Spanish memory centers: Hospital Clinic (HC), Hospital de la Santa Creu i Sant Pau (HSP) in Barcelona, and the CITA-Alzheimer Foundation (CITA) in San Sebastian. All subjects were bilingual (Catalan-Spanish for HC and HSP, and Basque-Spanish for CITA participants). The ethics committee of the Hospital Clinic of Barcelona approved the study, and all participants provided signed, informed consent before undergoing the neuropsychological assessment, MRI, and the lumbar puncture. All subjects had to meet the following inclusion criteria: a) at least three years of formal education, so as to exclude mental retardation or congenital learning disability, b) Mini-Mental State Examination (MMSE) [34] score >24, and c) objective cognitive performance within the normal range (cutoff 1.5 SD from normative mean) in all tests on a specific neuropsychological battery (see below). The following exclusion criteria were applied: a) presence of any neurological diagnosis, b) presence of a serious or unstable medical condition, c) diagnosis of a major psychiatric disorder including schizophrenia, major depression or substance abuse, and d) presence of a CSF pattern compatible with suspected non-amyloid pathology (SNAP). In accordance with the guidelines proposed by the National Institute on Aging and the Alzheimer's Association (NIA-AA) for defining Pre-AD for research purposes [1], healthy subjects were classified into three groups: control (CTR, $n = 31$), preclinical Alzheimer disease stage 1 (PreAD-1, $n = 14$) and preclinical Alzheimer's disease stage 2 (PreAD-2, $n = 4$). CSF tau and p-tau levels and MRI imaging (evaluated by an expert neurologist in order to exclude cases with brain structural damage or hippocampal atrophy) were used to classify Pre-AD subjects into PreAD-1 or PreAD-2.

Determination of biological and CSF biomarkers

All subjects underwent a lumbar puncture between 9 a.m. and 12 p.m. In the extraction, 10 ml of CSF was collected. The samples were centrifuged and stored in polypropylene tubes at –80°C within the first hour after extraction. CSF $A\beta_{42}$ levels, total tau (tau), and phosphorylated tau at threonine-181 (ptau) were measured by enzyme-linked immunosorbent assay kits (Innogenetics, Ghent, Belgium). Cut-off values of abnormality for each CSF biomarker were defined according to previous work [35, 36]: a) $A\beta_{42} \leq 550$ pg/ml, b) tau ≥ 350 pg/ml for subjects younger than 50 years, ≥ 400 pg/ml for subjects between 50–70 years old, and ≥ 450 pg/ml for subjects older than 70 years, and c) ptau ≥ 75 pg/ml. The time lapse between

the lumbar puncture and the AFE-T assessment was 2.25 (1.4) years. Both, the AFE-T administrator and the study participants were blind to the CSF results.

Apolipoprotein E analysis

Genomic DNA was extracted from peripheral blood of probands using the QIAamp DNAblood minikit (Qiagen AG, Basel, Switzerland). ApoE genotyping was performed by polymerase chain reaction amplification and HhaI restriction enzyme digestion. The study participants were blind to the ApoE results.

Neuropsychological battery and psychological assessment

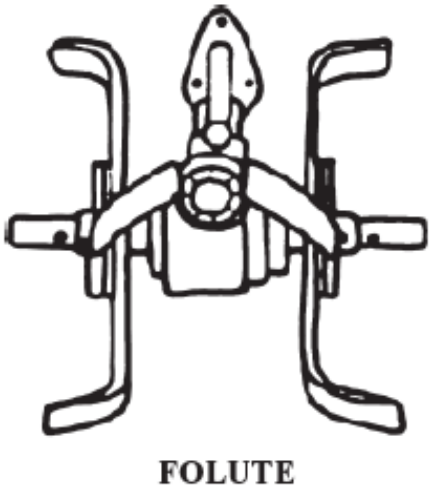
All participants were assessed with a comprehensive neuropsychological battery, administered by a trained neuropsychologist blind to the CSF results. The battery encompassed four cognitive domains. The memory domain included the Free and Cued Selective Reminding Test [37], the language domain comprised of the Boston Naming Test [38] and Semantic fluency [39]; the visual perception domain contained the number location subtest of the VOSP battery [40], and the executive functions domain consisted of the Trail Making Test [41], the Stroop Test [42], the Symbol Digit Modalities Test [43], and the Digit Span test of the WAIS [44]. Global cognition was assessed with the MMSE [34]. Premorbid intelligence was assessed with the Spanish word accentuation test [45]. The average time lapse between the neuropsychological assessment and the AFE-T administration was 1.21 (0.2) months.

Word and pseudoword spans

At the end of the one-week recall session of the AFE-T, two word and pseudoword verbal span tests were administered. We developed these experimental tests to assess verbal working memory in the context of both familiar and unknown words, and to ensure that learning performance between the groups in the AFE-T was not influenced by different working memory capacities. In both tests, words or pseudowords appeared one at a time for 3 s on a white background on a computer screen. The participants were asked to read each word aloud and try to remember them in the exact order. After the items were presented, an image of a microphone appeared on the screen and the participants were asked to repeat all the items just presented in exactly the same order. Points were given for fully correct responses (i.e., when the exact words were pronounced in the exact order of presentation). When a correct response was given on at least one out of the three sequences of a given span length, the next series with a higher length was presented. The task was initiated with two-item sequences and ended with a maximum of eight items for the word span and six for the pseudoword span. The task was interrupted if the participant was unable to repeat any of the three sequences of a given span. The total score corresponded to the maximum span that the participant was able to repeat correctly.

The ancient farming equipment test (AFE-T)

The task was to learn to orally name new object/name pairs. The objects were 24 black-and-white images of ancient farming equipment taken from the AFE paradigm [13]. These objects are unknown today (see an example of a novel picture in Fig. 1), and subjects' unfamiliarity with the object was confirmed in a pre-training screening test. In this screening test, the objects were presented one by one and the participant was requested to indicate whether they knew them. Each object was paired with a pseudoword, that is, a non-existing word that follows the phonotactic rules of Spanish [46]. The object names consisted of 14 bisyllabic and 10 trisyllabic pseudowords that did not exist in the Spanish dictionary (e.g., *gorsi, folute*; see the complete list in Appendix 1). All the stimuli were presented on a computer screen against a white background using the E-prime 2.0 (Psychology Software Tools, Inc., PA,



FOLUTE

Fig. 1. Example of a novel picture and a novel word in the AFE-T (see Appendix 1 for a full list of materials used).

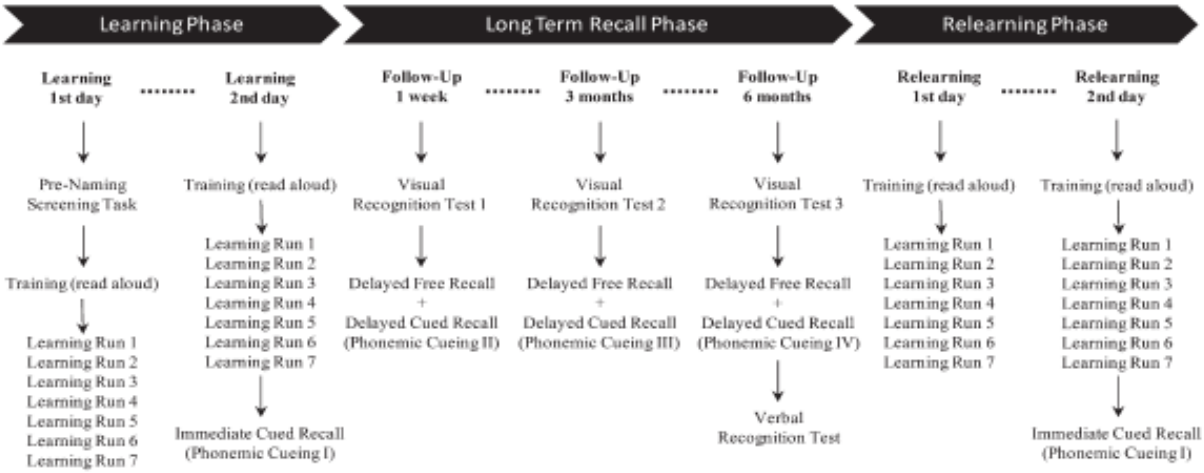


Fig. 2. Schematic design of the phases of the AFE-T.

USA). In order to thoroughly explore learning and forgetting in Pre-AD, the test design included two consecutive learning sessions, one immediate cued recall, three long-term delayed recall/recognition sessions, and two relearning sessions. The AFE-T had a total duration of six months. All the phases are explained in detail below (see Fig. 2 for a schematic description of the overall design used).

Initial learning sessions (LS)

Two learning sessions were performed on two consecutive days (LS1 for the first day, and LS2 for the second day). Each learning session included a total of seven runs and took approximately 45 min. Before starting the learning phase, each of the 24 object/name pairs was displayed for 7 s with a 500 ms pause between them. The participants were asked to read aloud the name of the object printed below, and to try to learn each object/name pair. After the presentation, the seven learning runs were performed. In each run, the participants were presented with the objects one at a time, and were asked to spontaneously say its name aloud. They were given a maximum of 7 s to recall the name of each object. After this, the correct name of the object appeared below the object for 4 s, regardless of whether the participant was able to say the correct name. The following object was presented after a 500 ms interval. The order of presentation of the objects in each run was randomized.

Immediate Cued Recall (ICR)

After the last run of the LS2, an immediate cued-recall test was administered. In this task, each object was presented one at a time. When the object appeared, the experimenter verbally provided the first syllable of the object's name (phonemic cue). The participant then had a maximum of 7 s to provide the complete correct name. In this run, the administrator did not give the whole correct name.

Long-term recall

Long-term recall was examined at one week, three months, and six months after the initial learning phase. Each session took 10–15 min and began with a visual recognition task. The visual recognition task required the participant to identify the 24 trained objects among 24 foils (maximum score of 48). The stimuli were presented one by one for 7 s, with a 500 ms interstimulus interval, in a pseudo-randomized order. The participant had to verbally respond "YES" or "NO" to indicate whether the object had been among the 24 trained items. The recognition task was followed by free recall. Here, each trained object appeared on the screen in a randomized order, and the participant was asked to name it orally. A maximum of 7 s was given to name each object. When the participant could not provide the correct response, the experimenter provided the first syllable of the name (*delayed cued recall*). The same procedure of cued recall was repeated one week, three months, and six months after the initial learning. At the end of the 6-month period, a picture-word matching task was administered in order to further explore the participants' word acquisition through recognition memory. In this task, three pseudowords, the target and two foils sharing the

same initial syllable, were presented beside each object. The participants were requested to choose the correct alternative for each stimulus (maximum score of 24).

Relearning phase (RL)

Relearning started immediately after the end of the 6-month session, following the verbal recognition memory task. Relearning was also performed on two consecutive days, and followed exactly the same procedure as in the learning phase.

Scoring system

All verbal responses were recorded for offline scoring. Following the scoring procedure of the AFE paradigm, a response was considered correct (score = 1) when: (a) the participant recalled the exact name of the object, or when (b) the name recalled differed by a single phoneme from the original name. Under (b), the following cases were considered: the substitution, addition, or omission of a single phoneme at any given position of the word, or a change in position of an otherwise correct phoneme. This criterion was applied for all runs. Thus, for each run, the range for the scores was 0–24.

Statistical analyses

Statistical analyses were performed using the SPSS (v.22.0) package for Windows. In all analyses, a $p < 0.05$ was considered to be significant. The main analyses were performed comparing the CTR and the PreAD-1 groups. Demographical data, levels of CSF $A\beta_{42}$, CSF tau, and CSF ptau, and APOE ε4 frequencies were compared using Student *t*-tests for independent samples and χ^2 analyses when appropriate.

AFE-T learning and relearning scores were analyzed using mixed-model analyses of variance, controlling for age and years of education. In these analyses, the within-group learning curves (runs), overall group differences (group), and the interaction between learning and group (run × group) were explored. Additionally, analyses of covariance (ANCOVA) controlling for age and years of education with *post-hoc* Bonferroni corrections were performed to analyze the specific runs in which the scores differed significantly between the two groups.

Forgetting rates for CTR and PreAD-1 groups were determined to analyze the delayed recall scores relative to the learning scores of each group. The forgetting rate was defined as one minus the ratio between each delayed session score and the score obtained on the last learning run (e.g., 1-(one-week free recall score/LS2 run 7 score), for one-week free forgetting rate). In this way, the forgetting rate represents the mean percentage of object/name items previously learned that were forgotten. In the delayed recall sessions, the free and cued raw scores, the forgetting rates and the recognition scores were compared between the groups using ANCOVAs adjusted for age and education. Finally, to explore the possible relearning benefits (i.e., the reactivation of stored information that cannot be voluntarily recalled), paired *t*-tests were run comparing the within-group scores of the first runs of the learning versus relearning sessions.

Due to the fact that the present AFE-T version has not been used before, our study is essentially explorative. Following the recommendations by Armstrong [47], we applied Bonferroni corrections because here "a large number of tests are carried out without pre-planned hypothesis in an attempt to establish any results that may be significant" (op.cit., p. 505).

Using the whole sample, Pearson bivariate correlations were calculated to assess the associations between the CSF $A\beta_{42}$ levels and the following AFE-T scores: total learning and three-month cued forgetting rate. Total learning, operationalized as the total score in the last run of the learning sessions, was hypothesized to reflect mainly acquisition. It has been suggested that the latter trials in repetitive tests of memory are more strongly related to the integrity of medial temporal lobe structures, whereas early trials correlate more strongly with inferior parietal, middle frontal gyrus, and temporal pole regions of interest [48]. Three-month cued forgetting rate was hypothesized to represent consolidation in long-term memory. The cued nature of recall allows for a more specific measure of consolidation, minimizing the role of executive components in recall [49]. Cued recall at three months was hypothesized to be the best measure to capture the longer term forgetting rate while avoiding floor effect.

Finally, even though the subjects had to be within the normal range on all the neuropsychological tests administered to be eligible, we ran t tests to explore possible between-group differences on these tests. For these analyses, the scaled scores (i.e., Neuronorma) of each test were used.

Table 1
Demographics, biological data, and CSF biomarker levels of CTR and PreAD-1 groups

Parameters	CTR ($n=31$)	PreAD-1 ($n=14$)	t	p value
Demographics				
Gender (% women)	70.9%	78.6%	0.29[a]	0.59
Age	64.8 ± 6.4 [49–77]	67.8 ± 7.1 [58–78]	1.39	0.17
Years of education	11.6 ± 3.7	10.8 ± 3.9	–0.69	0.49
Biological data and CSF				
APOE ε4 (% positive)	6.5%	57.1%	14.34[a]	<0.0001**
$A\beta_{42}$	801.6 ± 211.2	414.5 ± 82.9	–8.81	<0.0001**
Tau	233.6 ± 81.8	240.8 ± 99.3	0.25	0.80
Ptau	51.7 ± 14.1	46.2 ± 16.4	–1.15	0.26

Data are presented as means ± standard deviation. CSF, cerebrospinal fluid; $A\beta_{42}$, amyloid-beta isoform 42; tau, total tau; ptau, phosphorylated tau. [a]Pearson Chi-Square; **$p<0.0001$.

RESULTS

Sample characteristics

Demographical and biological data of the CTR and PreAD-1 groups are shown in Table 1. Age ranged between 49 and 86 years, and educational level ranged between 3 and 20 years. There were no significant differences in age ($p=0.17$) or years of education ($p=0.49$) between the CTR and PreAD-1 groups. Gender distribution was also similar ($\chi^2=0.29$; $p=0.59$) with women accounting for more than 70% in both CTR and PreAD-1. Regarding the AD biomarkers, CSF $A\beta_{42}$ was significantly lower in the PreAD-1 group ($t(43)=-8.81$; $p<0.001$). There were no significant group differences in the levels of CSF tau ($t(43)=0.25$; $p=0.80$) or ptau ($t(43)=-1.15$; $p=0.26$). The APOE-ε4 allele was significantly more frequent in the PreAD-1 group than in CTR ($\chi^2=14.34$; $p<0.001$), with a frequency of 57.1% versus 6.5% of carriers, respectively.

Descriptive characteristics of the PreAD-2 subjects ($n=4$)

The PreAD-2 subjects ($n=4$) had a mean age of 77.8 (6.9) years [range 70.9–86.1], compared to a mean age of 67.8 years in the PreAD-1 [range 58.2–78.3]. Their mean length of education was 11 years [range 3–18], compared to 10.8 [range 6–20] in PreAD-1 group. In PreAD-2, 50% were women, compared to 79% in PreAD-1. APOE-4 carriers represented 25% of the PreAD-2 sample compared to 55.5% in the PreAD-1. Regarding the biological data, PreAD-2 group had a mean CSF $A\beta_{42}$ of 341.6 (124.1) pg/ml [228.5–512.2], CSF tau of 486 (128) pg/ml [389–666], and CSF ptau of 89.8 (17.4) pg/ml [75.2–114], compared to 414.5 (82.9) pg/ml, 240.8 (99.3) pg/ml, and 46.2 (16.4) pg/ml in the PreAD-1 group, respectively.

AFE-T performance in PreAD-1

Initial learning phase

The ANCOVA on correct spontaneous naming responses showed a significant main effect for run in the whole sample ($F(13,533)=5.3$; $p<0.001$), indicating an overall increase of naming performance throughout the learning sessions in both groups (see Fig. 3). Furthermore, the run x group interaction term was significant ($F(13,533)=4.7$; $p<0.001$), reflecting the steeper learning curve of the CTR group and their better overall performance ($F(1,41)=6.9$; $p<0.01$) (see Fig. 3). Specifically, the CTR group showed a mean learning progression of 16.7 (4.9) items ($t(30)=18.7$; $p<0.001$) (i.e., the difference between the first and the last learning run scores), whereas the PreAD-1 group showed a mean value of 12.1 (6.0) items ($t(13)=7.52$; $p<0.001$). The main statistical comparisons are shown in Table 2. When looking at the scores for each run, ANCOVA revealed significant between-group differences in runs 1, 2, 6, and 7 on the first learning day and in all the runs of the second learning day (see Fig. 3).

Immediate cued recall

In the immediate cued-recall performed at the end of the second learning day, the mean for the CTR group was 21.6 (2.4) points, and the PreAD-1 group a mean of 17.3 (4.5) points. The ANCOVA showed that this group difference was statistically significant ($F(1,40)=15.4$; $p<0.001$; see Fig. 3).

Forgetting rate (one-week, three-month, and six-month delayed sessions)

Forgetting rates (one minus the ratio between each delayed session score and the score obtained on the

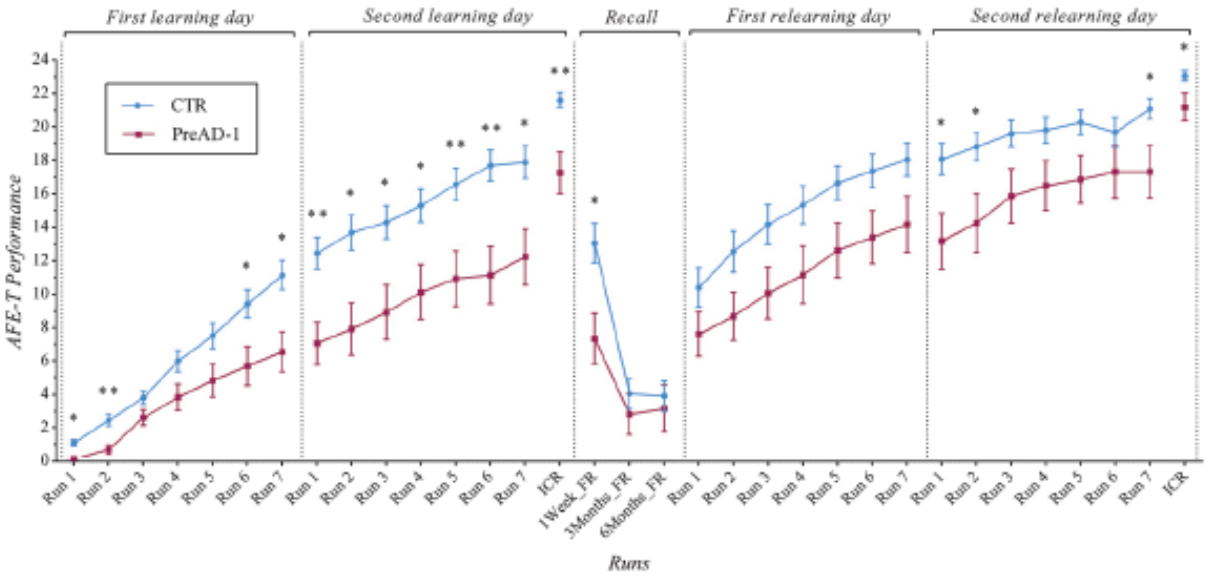


Fig. 3. AFE-T learning, free recall, and relearning scores of CTR and PreAD-1 groups. ICR, immediate cued recall; FR, free recall. Group differences in each point are indicated by an asterisk (*$p<0.05$, **$p<0.01$).

Table 2
ANCOVA of learning, recall and relearning AFE-T scores between CTR and PreAD-1 group (only the first and last run of each learning and relearning session are shown)

	CTR ($n=31$)	PreAD-1 ($n=14$)	F	p value
LS1_R1	1.2 ± 1.1	0.2 ± 0.6	7.39	0.01*
LS1_R7	11.2 ± 4.8	6.6 ± 4.4	6.73	0.01*
LS2_R1	12.5 ± 5.2	7.1 ± 4.8	7.80	<0.01**
LS2_R7	17.9 ± 5.4	12.3 ± 6.2	6.99	0.01*
ICR	21.6 ± 2.4	17.3 ± 4.5	15.37	<0.01**
1 Week_FFR	0.27 ± 0.3	0.47 ± 0.3	3.75	0.06
1 Week_CFR	0.16 ± 0.1	0.29 ± 0.2	4.09	0.05
3 Months_FFR	0.77 ± 0.2	0.83 ± 0.2	0.23	0.64
3 Months_CFR	0.46 ± 0.2	0.66 ± 0.2	4.83	0.03*
6 Months_FFR	0.79 ± 0.2	0.81 ± 0.3	0.01	0.99
6 Month_CFR	0.50 ± 0.2	0.61 ± 0.3	0.35	0.55
RLS1_R1	10.5 ± 6.5	7.7 ± 4.8	0.79	0.38
RLS1_R7	18.1 ± 5.1	14.2 ± 6.0	2.93	0.10
RLS2_R1	18.1 ± 5.2	13.2 ± 6.0	5.32	0.03*
RLS2_R7	21.1 ± 3.4	17.4 ± 5.6	5.35	0.03*
RL_ICR	23.1 ± 1.7	21.2 ± 2.9	5.21	0.03*

Data are presented as means ± standard deviation. LS1, 1st learning day; LS2, 2nd learning day; R, run number; ICR, immediate cued recall; FFR, free forgetting rate; CFR, cued forgetting rate; RL, relearning. *$p<0.05$; **$p<0.01$.

last learning run) were analyzed in each group. In this way, the forgetting rate represents the mean percentage of object/name items previously learned that were forgotten. At the one-week free recall session, the CTR had a forgetting rate of 0.27 (0.3) while the PreAD-1 group had a forgetting rate of 0.47 (0.3). ANCOVA revealed that this difference was not statistically significant ($F(1,36)=3.7$; $p=0.06$). When including the phonemic cue, the forgetting rate decreased to 0.16 (0.1) in the CTR and 0.29 (0.2) in the PreAD-1; the difference showed a marginal significant trend ($F(1,36)=4.1$; $p=0.05$). At three months, the CTR group showed a forgetting rate of 0.77 (0.2) in the free recall and the PreAD-1 showed a forgetting rate of 0.83 (0.2). When the cue was administered, the CTR got a forgetting rate of 0.46 (0.2) whereas the PreAD-1 decreased only to 0.66 (0.2). The latter difference was statistically significant ($F(1,36)=4.8$; $p=0.03$). At six months, both CTR and PreAD-1 once again had similar forgetting rates in the free recall: 0.79 (0.2) and 0.81 (0.3), respectively. When including the cues, the between-group differences did not reach statistical significance ($F(1,36)=0.4$; $p=0.55$), with forgetting rates of 0.50 (02) and 0.61 (0.3) in the CTR and PreAD-1 groups, respectively. Both groups benefited significantly from the phonemic cues in each of the delayed sessions (see Supplementary Figure 1).

Delayed recognition

Recognition scores were based on correctly recognized objects and correctly rejected foils (24 for each, total score = 48). The ANCOVA showed a significant group difference in the one-week delayed visual recognition scores ($F(1,41)=5.5$; $p=0.02$), although the difference between the means was only 0.5 points (CTR = 47.9 ± 0.4, PreAD-1 = 47.4 ± 1.4). No significant group differences in visual recognition were observed at three months or six months. Neither did the picture-word matching task at six-month post

learning reveal any group difference in recognition memory.

Relearning phase

The ANCOVA on correct spontaneous naming responses showed a significant main effect of run ($F(13,533) = 11.7$; $p < 0.001$) in the whole sample, reflecting an overall significant increase of naming performance throughout the relearning sessions in both groups (see Fig. 3). However, an contrary to the learning phase, similar steepness of the relearning curves between the CTR and the PreAD-1 groups were found (Time x Group interaction, $F(13,533) = 0.7$; $p = 0.75$). The results showed significant overall differences in performance across groups ($F(1,41) = 4.4$; $p < 0.05$). When looking at the scores for each run, ANCOVA revealed significant between-group differences in runs 1, 2, and 7 on the second relearning day. Significant differences were observed by the ANCOVA on the immediate cued recall ($F(1,39) = 5.2$; $p = 0.03$), with the CTR scoring 23.1 (1.7) compared to 21.2 (2.9) in the PreAD-1 group.

Descriptive analyses of the AFE-T performance in PreAD-2

The PreAD-2 group learned a total of 7.8 (6.9) object/name pairs on the AFE-T, with a range between 0 and 14 on the last learning run (see Fig. 4). This was below the mean in the PreAD-1

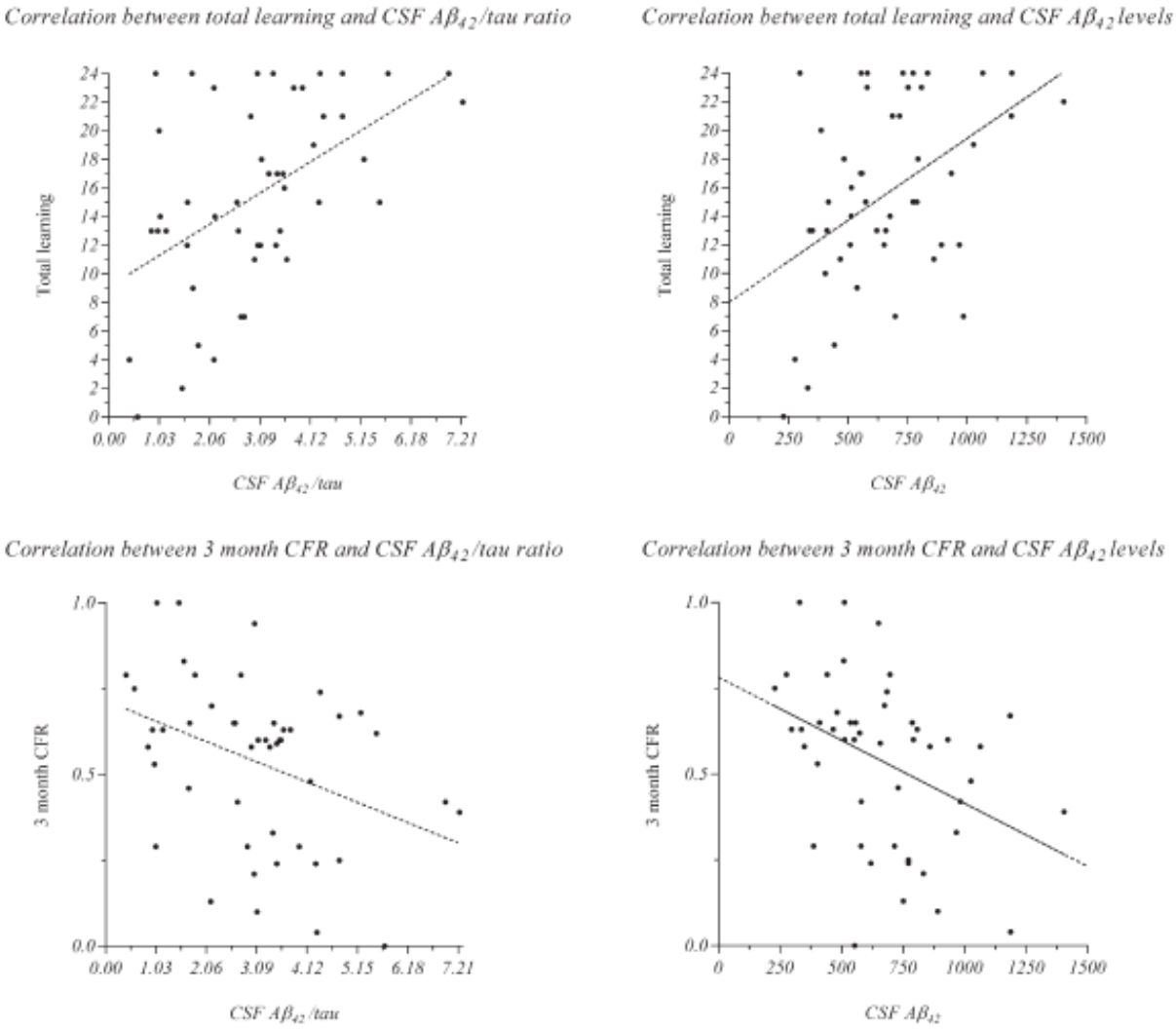


Fig. 4. Correlations between total learning and 3 month forgetting rate scores of the AFE-T and CSF Aβ42 and CSF Aβ42/tau ratio in the whole sample. CFR, cued forgetting rate.

group (12.3), while the standard deviation was similar in the two groups (6.2 and 6.9 for PreAD-1 and PreAD-2, respectively). As regards the forgetting rates, at the one-week free recall the PreAD-2 subjects forgot 0.4 (0.2) of the acquired items, compared to 0.5 (0.3) in the PreAD-1 group. When the cue was administered, the PreAD-2 rate remained at 0.4 (0.3) compared to 0.3 (0.2) in the PreAD-1. At three months, the PreAD-2 subjects had a mean index of 1 (0) in free-recall, decreasing to 0.8 (0.2) when the cue was included. The PreAD-1 group, in comparison, had scores of 0.8 (0.2) in the free forgetting rate and 0.7 (0.2) in the cued forgetting rate.

CSF $A\beta_{42}$ levels and AFE-T scores in the whole sample

Total learning was defined as the score obtained on the last run of the learning sessions. A significant positive correlation was found between the total learning score and CSF $A\beta_{42}$ ($r = 0.37$; $p = 0.01$). To explore the relationship of CSF $A\beta_{42}$ and recall, the correlation between the biomarker and the three-month cued forgetting rate was calculated, showing a negative correlation ($r = –0.34$; $p = 0.03$; see scatter plots in Fig. 4).

We also measured the association between the AFE-T and the ratio $A\beta_{42}$/tau. Results showed a significant positive correlation between the total learning score and the ratio ($r = 0.52$; $p < 0.001$). Regarding the three-month cued forgetting rate, a significant negative correlation was found with $A\beta_{42}$/tau ($r = –0.38$; $p < 0.01$; Fig. 4).

Standard neuropsychological tests in PreAD-1

There was no significant difference in global cognition between the CTR and the PreAD-1 group ($t(43) = –0.2$; $p = 0.9$), as assessed by the MMSE [34]. Nor was there a significant difference on the verbal intelligence score ($t(43) = 0.6$; $p = 0.5$). Crucially to the present research, no single test of the standard neuropsychological battery showed significant differences between the groups, with p values ranging from 0.07 to 0.95 (see Table 3). Regarding the word and pseudo-word span tasks included in the AFE-T (see Supplementary Table 1), significant group differences were found for the pseudo-word span ($F(1, 38) = 7.7$; $p < 0.01$) with better performance in the PreAD-1 group than in the CTR. No significant differences were observed in the word span task ($F(1, 38) = 0.1$; $p = 0.7$). Finally, the mean scores on the standard neuropsychological tests of PreAD-1 group and the descriptive scores of the PreAD-2 subjects showed that the four PreAD-2 participants had higher scaled scores on all the tests administered.

Table 3
T-test of the standard neuropsychological scores between CTR and PreAD-1 groups

Parameters	CTR ($n = 31$)	PreAD-1 ($n = 14$)	t	p value
Global Cognition				
MMSE[a]	28.1 ± 1.6	28.1 ± 1.6	–0.15	0.88
WAT[a]	24.9 ± 4.6	23.9 ± 4.7	0.63	0.53
Memory				
FCSRT – FR	12.4 ± 2.8	11.1 ± 2.6	1.42	0.16
FCSRT – TR	12.5 ± 2.7	12.2 ± 3.0	0.37	0.71
FCSRT – DFR	12.5 ± 2.7	11.6 ± 3.0	0.97	0.34
FCSRT – DTR	13.7 ± 4.3	12.6 ± 4.0	0.81	0.42
Language				
BNT	11.5 ± 1.8	11.1 ± 1.9	0.66	0.51
Sem-Flu	11.0 ± 2.0	10.2 ± 2.9	1.06	0.27
Perception				
VOSP – Numbers	13.1 ± 4.6	13.0 ± 4.9	0.06	0.95
Executive functions				
TMT-A	11.1 ± 2.2	10.5 ± 2.6	0.80	0.43
TMT-B	10.3 ± 2.3	9.4 ± 2.5	1.11	0.27
Stroop-W	11.3 ± 2.0	10.7 ± 1.6	0.97	0.34
Stroop-C	11.1 ± 1.8	9.9 ± 2.2	1.76	0.09
Stroop-CW	11.7 ± 2.3	10.3 ± 1.8	1.91	0.07
SDMT	12.2 ± 2.8	11.1 ± 3.7	0.99	0.33
Digits-F	10.8 ± 2.6	11.6 ± 2.8	–0.99	0.33
Digits-B	12.4 ± 2.1	12.0 ± 2.7	0.54	0.59

Data correspond to scaled scores of the standard neuropsychological tests and are presented as means ± standard deviation. MMSE, Mini-Mental State Examination; WAT, Word Accentuation Test; FCSRT-FR, Free and cued selective reminding test free recall; FCSRT-TR, total recall; FCSRT-DFR, delayed free recall; FCSRT-DTR, delayed total recall; BNT, Boston naming test; Sem-Flu, Semantic fluency; VOSP, visual object and space perception battery; TMT-A, Trail making test A; TMT-B, Trail making test B; Stroop-W, Stroop test words; Stroop-C, Stroop test color; Stroop-I, Stroop test color-word; SDMT, Symbol digit modality test; Digits-F, WAIS Span-digit forward; Digits-B, WAIS span-digit backward. [a]Raw scores.

DISCUSSION

This study searched for evidence for subtle learning and/or recall difficulties in Pre-AD by employing a highly demanding associative word learning test, the AFE-T. The test had to be particularly sensitive as these cognitive difficulties are too mild to be detected by standard neuropsychological tests. Moreover, we explored the possible associations between learning and memory performance and CSF proteins in Pre-AD subjects. The results observed were very conclusive in showing initial learning difficulties in our PreAD-1 subjects, while their long-term

recall and relearning were relatively preserved. Additionally, we found that CSF $A\beta_{42}$ levels correlated significantly with the total learning score. Our findings suggest that the AFE-T is a promising tool for characterizing the cognitive profile of PreAD-1, being sensitive enough for detecting incipient episodic memory difficulties in this population.

The usefulness of the NIA-AA staging has been demonstrated in recent reports involving Pre-AD subjects [3–6]. Mormino et al. [4] studied 166 cognitively normal individuals divided into preclinical groups for a mean of 2.1 years. PreAD-1, control, and SNAP showed improvement in performance over time (due to task repetition effects) while PreAD-2 subjects declined, suggesting that the co-occurrence of Aβ deposition and neurodegeneration (i.e., PreAD-2) accelerates cognitive decline in cognitively healthy individuals. In a recent study by Soldan et al. [5], 222 cognitively healthy subjects were followed up for a mean of 11.0 years and classified into preclinical stages. Only PreAD-2 subjects showed worse cognitive performance both at baseline and longitudinally compared with the other biomarker groups, whereas controls, PreAD-1 and SNAP groups did not differ. The authors concluded that baseline and longitudinal cognitive decline is only detected in PreAD-2 subjects. However, it should be borne in mind that these studies used standard neuropsychological tests and memory composites to evaluate the cognitive performance of Pre-AD subjects. Otherwise, Papp et al. [6] studied 260 clinically normal older adults grouped in preclinical stages using a highly demanding associative memory test (the MCT). The authors found decrements in PreAD-1 subjects' free recall score when compared with Controls.

The present study investigated the cognitive performance of PreAD-1 subjects using a highly demanding associative memory test. Unlike most memory tests used to assess Pre-AD, the AFE-T requires learning, binding and storing novel information. Forming new associations or binding unrelated information with previous semantic knowledge is thought to set high demands on cognitive processing [12]. This kind of learning may depend on the formation of new neural connections in brain areas specifically related to the acquisition of new knowledge [13] which show incipient changes in Pre-AD, such as the hippocampus and adjacent medial temporal lobe structures [19, 20]. Probably due to these higher cognitive demands related to the associative learning, the AFE-T was able to find consistent learning difficulties in PreAD-1 subjects when compared to controls. This important finding suggests that the AFE-T is sensitive enough to identify differences between controls and Pre-AD subjects, even at the first stage of the Pre-AD phase. Furthermore, considering the fact that the PreAD-1 group performed better in the pseudo-word span, their impaired initial learning in the AFE-T could not be due to impairments in attention or working memory, factors that are strongly linked to episodic memory [50, 51]. However, it is important to note that the idea that episodic (associative) memory is the first memory system to be affected in AD has been questioned [52]. Moreover, the fact that item-based and associative memory systems are independent also remains unclear [53].

One of the main strengths of the present study is the long-term follow-up of participants, which allowed a comprehensive assessment of delayed memory and forgetting rates. Since most of the time intervals in standard neuropsychological tests range between 20 and 30 minutes [28], the assessment of longer term (days or months) forgetting rates in Pre-AD remains a field to be explored. Our analysis involving PreAD-1 subjects and controls showed differences in their raw scores at one-week free and cued recall, and at three-month cued recall. Nevertheless, these group differences were influenced by the initial learning performance, since analysis of forgetting measures showed that the PreAD-1 subjects and controls presented similar forgetting rates. These findings suggest that the initial consequences of amyloid deposition affect initial learning and encoding processes more than posterior recall and retrieval processes. Only the three-month cued forgetting rate showed significant differences between PreAD-1 subjects and controls. Though weak, the greater benefit from the phonological cueing in the CTR group than in the PreAD-1 suggests that the poorer performance exhibited by the PreAD-1 group in this long-term recall session should not be attributed merely to a "tip-of-the-tongue" effect, but to a subtle information loss. Similar results were presented in a previous study using the AFE paradigm in which MCI patients benefited less from phonological cueing than controls [26]. Regarding the secondary analysis, the small group of PreAD-2 subjects presented a similar performance in the free recall but a lower benefit from the cue. These results are in agreement with a recent report which indicated that while PreAD-1 subjects showed subtle reductions in free recall, a decline in the cued recall may represent progression to PreAD-2 stages [46]. Albeit collection of long-term forgetting rates with free and cued recall is cumbersome, these

findings suggest that they can provide valuable information for identifying memory difficulties in Pre-AD.

Another innovative memory assessment included in the AFE-T was Relearning, which was included to investigate previous learning influence. Information that cannot be remembered in a free or cued recall or recognition tests can be reactivated and detected by relearning tasks [31–33]. Relearning in the Pre-AD phase has not previously been studied. After the floor effect present in both groups at the 6-month recall session, we further explored whether there were existing but inaccessible memory traces that could be reactivated during relearning. As shown by the similar positive relearning curves, both groups were able to benefit from this intervention. Interestingly, between-group differences during this relearning phase were lower than those observed during the initial learning phase. Again, these results might suggest that initial learning is the most powerful cognitive feature for discriminating PreAD-1 subjects from controls. Clinically, this makes the testing paradigm more viable as delayed testing could be avoided. Regarding the usefulness of standard neuropsychological tests, several reports in recent years have failed to find group differences between normal aging and clinically normal at-risk subjects [8–11]. In agreement with these results, and as expected considering the inclusion criteria of the present study, we did not find any significant cross-sectional difference between our two groups in a comprehensive battery of standard neuropsychological tests. Thus, currently available standard neuropsychological tests do not seem to have sufficient sensitivity to differentiate cognitively healthy individuals with decreased CSF $A\beta_{42}$ levels from controls [12, 54].

In the present study, we also examined the association between CSF $A\beta_{42}$ levels and AFE-T performance in the PreAD-1 group. The relationship between amyloid and cognition in Pre-AD has been studied in recent years, but most cross-sectional studies have not found a relationship between amyloid levels and memory performance using standard neuropsychological tests [8, 55–58]. Only longitudinal studies have shown stronger associations between amyloid burden and future memory impairment, indicating that amyloid burden in cognitively normal individuals precedes cognitive impairment and is associated with a higher risk of future cognitive deterioration [59, 60]. In the present study, the highest statistically significant association between the CSF $A\beta_{42}$ levels and the AFE-T performance was found in the total learning score. This finding supports the view that episodic memory decline is more closely related to amyloid levels [60], and that this link may be seen only with a highly demanding associative learning task. In line with this, an association between amyloid accumulation in the frontal cortex and cognitive performance was described in a previous study in which a highly challenging face-name associative test was administered, indicating that in addition to the medial temporal lobe and related structures, frontal regions are also critical in associative memory encoding and recall [12]. This also concurs with a previous PET study which showed that the AFE paradigm engages executive and attentional functions [23].

One notable limitation of the present study is its small sample size, limiting the power of the statistical analyses. For instance, although no standard neuropsychological test showed significant differences between CTR and pre-AD, the probability of type II error in these analyses was high. Regarding AFE-T, the comprehensive long-term assessment procedure of the AFE-T can be considered to safeguard against spurious results that may hamper these kinds of studies. With regards to the delayed recall of the AFE-T, it may appear surprising that both the CTR and PreAD-1 group showed poor performance after six months of learning. There is probably a tradeoff between learning runs and the length of maintenance, and a shorter interval might have shown a difference between the groups. Previous studies in MCI patients [26] used more training days, and those participants had better maintenance of information at six months. Another potential limitation concerns the multiple comparisons problem that arises from the large number of statistical comparisons performed. This was dealt with *post-hoc* Bonferroni corrections, albeit this is an admittedly conservative method. Lastly, although the AFE-T as a whole is not fully suitable for use in the clinical setting, it allows characterization of the different processes involved in learning and memory function in the preclinical phase of AD. In the light of the present results which identify initial learning as the most sensitive area for detecting cognitive difficulties in the PreAD-1, we are now designing and validating a shortened version of the AFE-T for use in the clinical setting.

In conclusion, using a new, highly demanding, comprehensive associative memory test, we identified significant incipient learning difficulties together with a relative preservation of the recall processes in PreAD-1 subjects. Our findings suggest that the AFE-T is a promising tool for characterizing the cognitive profile of PreAD-1 and that it is sensitive

enough to detect incipient episodic memory difficulties in this population.

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SUPPLEMENTARY MATERIAL

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Appendix 1. Stimuli used in the AFE-T (novel pictures and novel words)

Supplementary Material

Supplementary Table 1. ANCOVA of all the AFE-T scores (CTR and PreAD-1 groups).

	CTR (n=31)	PreAD-1 (n=14)	F	P value
LS1_R1	1.2±1.1	0.2±0.6	7.39	0.01*
LS1_R2	2.5±1.9	0.8±1.0	7.68	<0.01**
LS1_R3	3.9±2.2	2.7±1.7	1.34	0.25
LS1_R4	6.0±3.6	3.9±2.9	2.07	0.16
LS1_R5	7.6±4.3	4.9±3.7	2.19	0.15
LS1_R6	9.5±4.7	5.8±4.4	4.17	0.04*
LS1_R7	11.2±4.8	6.6±4.4	6.73	0.01*
LS2_R1	12.5±5.2	7.1±4.8	7.80	<0.01**
LS2_R2	13.7±5.8	8.0±5.8	6.92	0.01*
LS2_R3	14.4±5.5	9.0±6.1	6.39	0.01*
LS2_R4	15.4±5.5	10.2±6.1	5.39	0.02*
LS2_R5	16.6±5.3	11.0±6.1	7.43	<0.01**
LS2_R6	17.7±5.1	11.2±6.4	10.81	<0.01**
LS2_R7	17.9±5.4	12.3±6.2	6.99	0.01*
ICR	21.6±2.4	17.3±4.5	15.37	<0.01**
1Week_ViRe	47.9±0.4	47.4±1.4	5.52	0.02*
1Week_FR	13.1±6.6	7.2±5.7	5.40	0.02*
1Week_FFR	0.27±0.3	0.47±0.3	3.75	0.06
1Week_CR	18.3±4.3	12.9±5.3	9.66	<0.01**
1Week_CFR	0.16±0.1	0.29±0.2	4.09	0.05
3Months_ViRe	46.3±1.7	45.1±2.9	2.32	0.14
3Months_FR	4.1±5.0	2.9±4.4	0.12	0.73
3Months_FFR	0.77±0.2	0.83±0.2	0.23	0.64
3Months_CR	11.9±5.6	6.5±4.0	7.63	<0.01**
3Months_CFR	0.46±0.2	0.66±0.2	4.83	0.03*

6Months_ViRe	44.8±5.1	44.3±3.4	0.14	0.72
6Months_FR	4.0±5.0	3.3±4.8	0.01	0.97
6Months_FFR	0.79±0.2	0.81±0.3	0.01	0.99
6Months_CR	11.0±5.9	7.2±5.8	1.50	0.23
6Month_CFR	0.50±0.2	0.61±0.3	0.35	0.55
6Months_VeRe	21.4±2.5	20.4±3.0	0.06	0.82
RLS1_R1	10.5±6.5	7.7±4.8	0.79	0.38
RLS1_R2	12.6±6.8	8.8±5.2	1.84	0.18
RLS1_R3	14.2±6.6	10.2±5.5	2.18	0.15
RLS1_R4	15.4±6.4	11.2±6.2	2.27	0.14
RLS1_R5	16.7±5.6	12.7±5.9	2.83	0.10
RLS1_R6	17.4±5.6	13.5±5.7	2.74	0.11
RLS1_R7	18.1±5.1	14.2±6.0	2.93	0.10
RLS2_R1	18.1±5.2	13.2±6.0	5.32	0.03*
RLS2_R2	18.9±4.7	14.3±6.3	5.19	0.03*
RLS2_R3	19.6±4.4	15.9±5.8	3.43	0.07
RLS2_R4	19.9±4.4	16.5±5.3	2.84	0.10
RLS2_R5	20.3±4.0	16.9±5.2	3.56	0.07
RLS2_R6	19.7±5.0	17.4±5.7	0.76	0.39
RLS2_R7	21.1±3.4	17.4±5.6	5.35	0.03*
RL_ICR	23.1±1.7	21.2±2.9	5.21	0.03*

Data are presented as means ± standard deviation. Key: LS1, 1st learning day; LS2, 2nd learning day; R, learning run number; ICR, immediate cued recall; ViRe, visual recognition; FR, free recall; FFR, free forgetting rate; CR, cued recall; CFR, cued forgetting rate; VeRe, verbal recall; RL, re-learning.

*** *p*< 0.05**

**** *p*< 0.01**

Supplementary Table 2. ANCOVA of word span and pseudo-word span between CTR and PreAD-1 groups

	CTR (n=31)	PreAD-1 (n=14)	F	*P* value
Word span	4.6±1.1	4.5±0.7	0.14	0.71
Pseudo-word span	3.0±0.5	3.5±0.7	7.71	<0.01**

Data are presented as means ± standard deviation.
****p< 0.01**

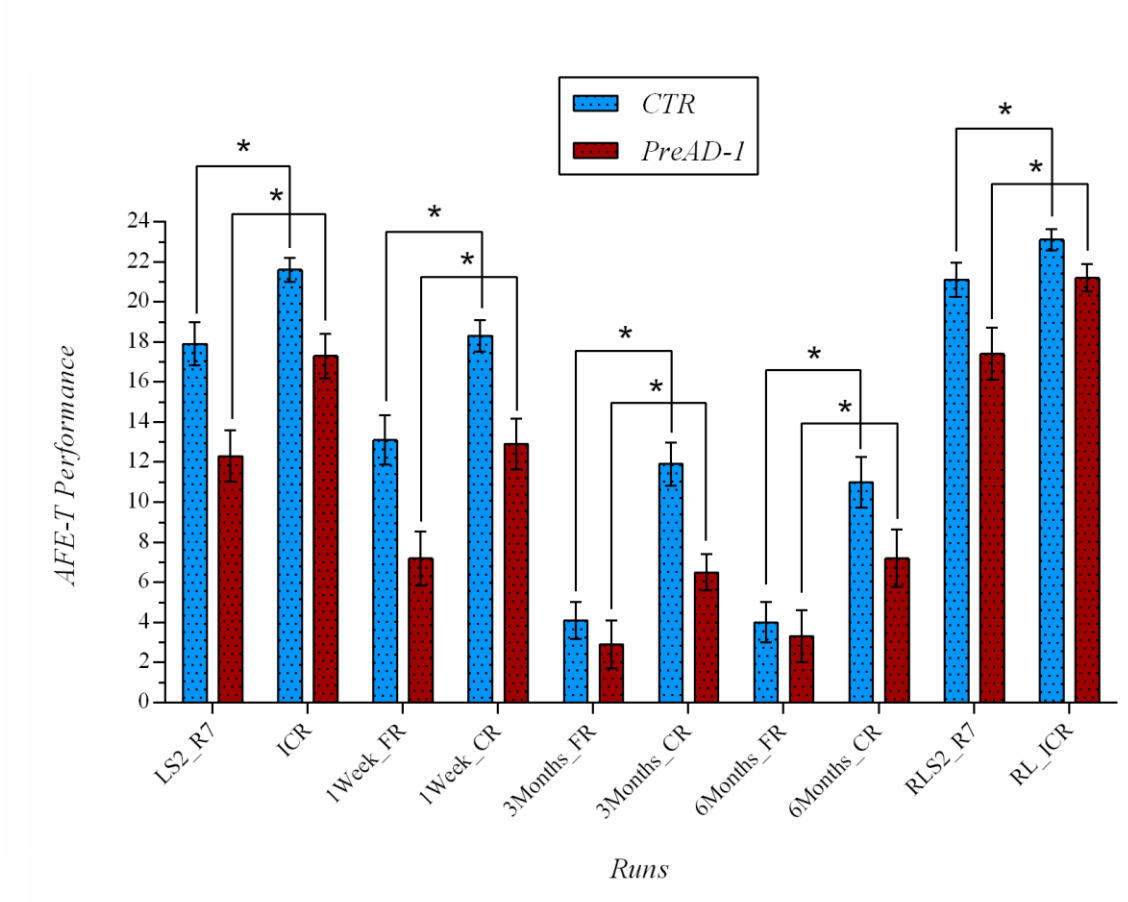
Supplementary Table 3. Comparison of the standard neuropsychological scores between PreAD-1 and PreAD-2 groups.

Parameters	PreAD-1 (n=14)	PreAD-2 (n=4)
Global Cognition		
MMSE[a]	28.1±1.6	28.3±0.9
WAT[a]	23.9±4.7	27.0±3.2
Memory		
FCSRT – FR	11.1±2.6	13.5±1.7
FCSRT – TR	12.2±3.0	17.0±1.4
FCSRT – DFR	11.6±3.0	13.0.±1.4
FCSRT – DTR	12.6±4.0	15.3±4.0
Language		
BNT	11.1±1.9	13.0±3.0
Sem-Flu	10.2±2.9	11.5±2.4
Perception		
VOSP – Numbers	13.0±4.9	13.3±4.9
Executive functions		
TMT-A	10.5±2.6	11.5±1.3
TMT-B	9.4±2.5	10.0±0.0
Stroop-W	10.7±1.6	11.0±1.0
Stroop-C	9.9±2.2	12.3±2.9
Stroop-CW	10.3±1.8	13.0±2.0
SDMT	11.1±3.7	11.3±1.2
Digits-F	11.6±2.8	13.3±2.2
Digits-B	12.0±2.7	15.6±2.2

Data correspond to scaled scores of the standard neuropsychological tests and are presented as means ± standard deviation. Key: MMSE, Mini-Mental State Examination; WAT, Word Accentuation Test; FCSRT-FR, Free and cued selective reminding test free recall; FCSRT-TR, total recall; FCSRT-DFR, delayed free recall; FCSRT-DTR, delayed total recall; BNT, Boston naming test; Sem-Flu, Semantic fluency; VOSP, visual object and space perception battery; TMT-A, Trail making test A; TMT-B, Trail making test B; Stroop-W, Stroop test words; Stroop-C, Stroop test color; Stroop-I, Stroop test color-word; SDMT, Symbol digit modality test; Digits-F, WAIS Span-digit forward; Digits-B, WAIS span-digit backward.

[a] Raw scores

Supplementary Fig. 1. Comparison between AFE-T free and cued recall scores in CTR and PreAD-1 group.



Key: ICR, immediate cued recall; FR, free recall; CR, cued recall; RL, re-learning.
***p< 0.01**

Trabajo número 2:

Tau protein is associated with longitudinal memory decline in cognitively healthy subjects with normal Alzheimer's disease cerebrospinal fluid biomarker levels

Tort-Merino A, Olives J, León, M, Peñaloza C, Valech N, Santos-Santos MA, Càmara E, Grönholm-Nyman P, Martínez-Lage P, Fortea J, Molinuevo JL, Sánchez-Valle R, Laine M, Rodríguez-Fornells A, Rami L

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Tau Protein is Associated with Longitudinal Memory Decline in Cognitively Healthy Subjects with Normal Alzheimer's Disease Cerebrospinal Fluid Biomarker Levels

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Abstract.
Background: We investigated a sample of cognitively healthy subjects with normal Alzheimer's disease (AD) cerebrospinal fluid (CSF) biomarker levels to identify the earliest variables related to longitudinal memory changes.
Objective: Employing a new highly demanding learning and memory test (the Ancient Farming Equipment Test; AFE-T), we aimed to investigate whether a biomarker related to neurodegeneration (i.e., CSF tau) was associated with longitudinal memory decline.

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Methods: Thirty-two cognitively and biologically normal (CBN) subjects underwent MRI, neuropsychological assessment, and the AFE-T at baseline and 18 months later. To explore the relationship between cognitive performance and relevant factors, a linear model was set up. For a secondary analysis that further explore the effect of tau, the subjects were divided into CBN-Tau$^{\downarrow}$ (tau < 228.64 pg/ml; $n = 16$) and CBN-Tau$^{\uparrow}$ (tau > 228.64 pg/ml; $n = 16$). We also performed voxel-based morphometry (VBM) to identify regions of grey matter volume that would predict both baseline and longitudinal cognitive performance.

Results: Our main finding was an association between CSF tau and longitudinal memory decline measured with AFE-T ($B = -0.17$, $p < 0.05$; $r = -0.414$; $p < 0.01$), and further analyses showed different evolvement between subgroups, with an accelerated decline in individuals with higher tau ($F(1,31) = 8.37$; $p < 0.01$). VBM results suggested that AFE-T performance is related to grey matter volume in a medial temporal, middle frontal, and posterior cerebellar network at baseline, and that there are strategic brain areas driving the longitudinal cognitive changes.

Conclusions: The present findings provide evidence for structural and biological markers linked to cognitive aging by highlighting the role of tau, a marker of neurodegeneration, which can be related with the earliest memory changes in healthy subjects.

Keywords: Aging, biomarkers, cognition, early detection, memory decline, tau, voxel-based morphometry

INTRODUCTION

Understanding the factors underlying age-related changes in cognition has long been a challenge. Since cognitive changes in normal aging and in incipient pathological processes (e.g., preclinical Alzheimer's disease; AD) are closely related, there is a need to identify the earliest factors driving the cognitive decline in both populations in order to ensure an early detection of the pathological processes. While there is extensive literature on the aging process in large cohorts of cognitively impaired and unimpaired subjects, we have very little information on biomarkers of age-related cognitive changes in cognitively healthy subjects with a confirmed normal AD cerebrospinal fluid (CSF) biomarker profile.

The cognitive profile in normal aging has been well-documented in the scientific literature [1–3]. However, one important question that remains open concerns the factors related to these observed cognitive changes. First, demographic variables such as age, years of education, and cognitive reserve [4–6] are known to have an impact on the trajectory of cognition over the life span. A prospective study involving a large sample of 2,509 cognitively healthy elderly adults reported that both age and educational level predicted maintenance of cognitive function over an 8-year period [7]. Second, some neuropsychological variables have been identified as predictors of cognitive decline. Memory decline has been considered as the major cognitive risk factor for developing age-related pathologies, such as mild cognitive impairment (MCI) or AD [8]. Third, the maintenance of cognitive functioning in aging is thought to be primarily related to brain maintenance, that is, relative lack of structural and functional brain changes [9]. In line with this, a recent report suggested that subjects with larger medial temporal lobe (MTL) volumes at baseline were more likely to maintain their cognitive function over time [10]. Fourth, biological markers on cognition have been a topic of intensive research during the last few years. The most studied AD CSF biomarkers are the tau protein levels and the 42 amino acid form of amyloid-β ($A\beta_{42}$). Some studies have suggested that CSF tau levels rather than Aβ pathology are more closely related to cognition, specifically to memory decline [11–13].

Due to the proximity of cognitive changes related to normal aging versus incipient pathological processes, there is an increasing need to develop more sensitive tests for an early detection of subtle cognitive difficulties in populations at risk. For that reason, new highly demanding neuropsychological tests such as the Face Name Associative Memory Exam [14–16] or the Short-Term Memory Binding test [17] have been developed. Using previous research on the neural mechanisms involved in language learning and memory, we recently evaluated a highly demanding learning and memory test called the *Ancient Farming Equipment Test* (AFE-T) (for a review, see Laine & Salmelin [18]). This task engages the declarative memory system in learning to associate unfamiliar names (new labels or words) to completely new objects. To date, the AFE paradigm has been used to study acquisition of new words in groups of healthy adults [19, 20] and, in two studies, in MCI and AD patients [21, 22]. A fMRI study using the AFE paradigm in an aphasic patient and in healthy controls showed a clear involvement

of MTL regions during the learning phase [23]. In our recent study, we employed the AFE-T to detect subtle cognitive difficulties in preclinical AD subjects. The AFE-T was found to be a promising tool for characterizing the cognitive profile of preclinical AD, being sensitive enough to detect initial learning difficulties in our at-risk population. Furthermore, the AFE-T was associated with the CSF $A\beta_{42}$/tau ratio [24].

Since there is recent literature regarding the relationships between cognitive function and AD CSF biomarkers in cognitively normal subjects [25–29], the present research pushed further by focusing on a specific sample of cognitively healthy subjects with normal AD CSF biomarker levels. The aim of the present study was to examine and follow up this well-characterized sample in order to detect demographical, structural and biological variables related to the earliest cognitive changes in aging. Employing the sensitive AFE-T cognitive measure, we specifically wanted to investigate whether a biomarker related to neurodegeneration (i.e., CSF tau) is associated with the earliest longitudinal decline of learning and memory in cognitively and biologically normal aging.

MATERIAL AND METHODS

Participants

The present participants represent a sub-sample of Tort-Merino et al. [24], and thus the present methods description follows that paper. Thirty-two cognitively healthy subjects with normal AD CSF biomarker levels were included in the present study and followed up for 18 months. The participants were recruited at three Spanish memory centers: Hospital Clinic (HC) and Hospital de la Santa Creu i Sant Pau (HSP) in Barcelona, as well as the CITA-Alzheimer Foundation (CITA) in San Sebastian. Due to the nature of this recruitment, some of the subjects included in the study presented memory complaints. The ethics committee of the Hospital Clinic of Barcelona approved the study, and all participants provided a signed, informed consent before undergoing the neuropsychological assessment, MRI and the lumbar puncture. All subjects had to meet the following inclusion criteria: a) at least three years of formal education, b) Mini-Mental State Examination (MMSE) score >24, and c) Clinical Dementia Rating (CDR) score = 0 and objective cognitive performance within the normal range (cutoff 1.5 SD from normative mean) in all tests on a neuropsychological battery (see below). The following exclusion criteria were applied: a) presence of any neurological diagnosis, b) presence of a serious or unstable medical condition, c) diagnosis of a major psychiatric disorder including schizophrenia, major depression or substance abuse, and d) presence of any abnormality in CSF $A\beta_{42}$, tau, and/or phosphorylated tau at threonine-181 (ptau) levels.

For a secondary analysis that further explored the effect of CSF tau in normal aging, we divided the present sample into CBN-Tau$^{\downarrow}$ and CBN-Tau$^{\uparrow}$ subgroups according to their CSF tau levels. These secondary analyses were done in order to visualize the main findings of the study. The mean of the CSF tau values (228.64 pg/ml) of the 32 subjects was used as a cut-off point to ensure an equal distribution between groups. Subjects with CSF tau levels below 228.64 pg/ml were classified as CBN-Tau$^{\downarrow}$ ($n = 16$) and subjects with CSF tau above 228.64 pg/ml were included in the CBN-Tau$^{\uparrow}$ group ($n = 16$).

Determination of biological and AD CSF biomarkers

All subjects underwent a lumbar puncture between 9 a.m. and 12 p.m. to collect 10 ml of CSF. The samples were centrifuged and stored in polypropylene tubes at –80°C within the first hour after extraction. CSF $A\beta_{42}$ levels, tau, and ptau were measured by enzyme-linked immunosorbent assay kits (Innogenetics, Ghent, Belgium). Cut-off values of abnormality for each AD CSF biomarker were defined according to previous work [30]: a) $A\beta_{42}$ $\leq$550 pg/ml, b) tau $\geq$400 pg/ml for subjects between 50–70 years old, and $\geq$450 pg/ml for subjects older than 70 years, and c) ptau $\geq$75 pg/ml. As noted in the inclusion criteria, all subjects included in the study presented normal levels for all AD biomarkers. The AFE-T administrator and the participants were blind to CSF results.

Apolipoprotein E analysis

Genomic DNA was extracted from peripheral blood of probands using the QIAamp DNAblood minikit (Qiagen AG, Basel, Switzerland). *APOE* genotyping was performed by polymerase chain reaction amplification and HhaI restriction enzyme digestion. Both the AFE-T administrator and the study participants were blind to the *APOE* results.

Neuropsychological assessment

All participants were assessed both at the baseline and at the follow-up session with a comprehensive neuropsychological battery, administered by a trained neuropsychologist blind to the CSF results. The battery encompassed four cognitive domains. The memory domain included the Free and Cued Selective Reminding Test (FCSRT) [31], the language domain comprised of the Boston Naming Test [32], and a Semantic Fluency Task [33]; the visual perception domain contained the Number Location subtest of the VOSP battery [34]; and the executive functions domain consisted of the Trail Making Test [35], the Stroop Test [36], the Symbol Digit Modalities Test [37], and the Digit Span test of the WAIS [38]. Global cognition was assessed with the MMSE [39]. Premorbid intelligence was assessed with the Spanish Word Accentuation Test [40]. Subjective memory complaints were measured by the Subjective Cognitive Decline Questionnaire (SCD-Q) [41]. The average time lapse between the baseline neuropsychological assessment and the baseline AFE-T (list A) was +0.4 (SD 0.6) months and the time lapse between the follow-up neuropsychological assessment and the follow-up AFE-T (list B) was –0.4 (SD 0.4) months.

The Ancient Farming Equipment Test

The AFE-T called for learning of two lists of new object/name pairs. For both lists, the objects were 24 black-and-white images of ancient farming equipment taken from the AFE paradigm [18]. Each object was paired with a pseudoword, that is, a non-existing word that follows the phonotactic rules of Spanish [42]. The object names consisted of 14 bisyllabic and 10 trisyllabic pseudowords that do not exist in the Spanish dictionary. All stimuli were presented on a computer screen against a white background using the E-prime 2.0 version (Psychology Software Tools, Inc., PA, USA).

The study had a total duration of 18 months and included a baseline testing session and an 18-month follow-up. List A was administered at the baseline assessment and list B at the 18-month follow-up. The assessments are explained in detail below.

Initial learning sessions, total learning score, and immediate cued recall

List A was administered in two initial learning sessions that were performed on two consecutive days. Each learning session included a total of seven runs and took approximately 45 min. Before starting the learning phase, each of the 24 object/name pairs was displayed for seven seconds with a 500 ms pause between the pairs. The participants were asked to read aloud the name of the object printed below, and to try to learn each object/name pair. After the presentation, the seven learning runs were performed. In each run, the participants were presented with the objects one at a time, and were asked to spontaneously say its name aloud. They were given a maximum of 7 s to recall the name of each object. After this, the correct name appeared below the object for 4 s, regardless of whether the participant had been able to produce the correct name. The following object was presented after 500 ms. The order of presentation in each run was randomized. For each run, the range of scores was 0–24.

After the last run of the second learning day (i.e., *total learning score*), the *immediate cued recall* (ICR) was administered. In this test, each object was presented one at a time. When the object appeared, the experimenter verbally provided the first syllable of the object's name (phonemic cue). The participant then had a maximum of 7 s to provide the correct name. This time feedback (i.e., the correct name) was not provided after the response.

Follow-up session at 18 months

The 18-month follow-up included exactly the same procedure as at the baseline except that list B was administered.

Scoring system

All verbal responses were recorded for offline scoring. Following the scoring procedure of the AFE paradigm, a response was considered correct (score = 1) when (a) the participant recalled the exact name of the object, or when (b) the name recalled differed only by a single phoneme from the original name. Under (b), the following cases were considered: substitution, addition, or omission of a single phoneme at any given position of the word, or a change in position of an otherwise correct phoneme. This criterion was applied for all runs.

Validation study

A validation study with 30 young adults was conducted to confirm that the word lists A and B had comparable difficulty. We ran independent *t*-tests to explore possible between-group differences between word lists A and B. In addition, we compared the per-

formances between the participants of the validation study (younger adults) and the subjects included in the study.

Neuroimaging

Acquisition

For each participant, two T1-weighted, high-resolution, MPRAGE structural MRI (echo time [TE] 2.98 ms, repetition time [TR] 2300 ms, inversion time 900 ms, flip angle 9°, bandwidth 240 Hz/pixel, matrix 256 × 256, 240 axial slices, isometric voxel size 1/4 1.0 mm^3) scans were acquired at the IDIBAPS's Imaging core facilities with a 3T whole-body MRI scanner (Siemens Magnetom Trio; Hospital Clínic, Barcelona).

The mean time between scans was 1.9 (SD 0.2) years. Three participants were excluded due to severe motion artifacts.

Processing and analyses

Image processing was performed using the unified segmentation procedure [43], DARTEL toolbox [44], and Pairwise Longitudinal Registration [28] toolbox implemented in SPM12 (Welcome Trust for Neuroimaging) according to standard procedures. In the whole sample, we performed whole brain voxel-based morphometry (VBM) analyses evaluating which regions of grey matter volume significantly predicted the *total learning score* and the *immediate cued recall* of the AFE-T at baseline (cross-sectional VBM, $n = 29$), as well as their longitudinal change (longitudinal VBM), using multiple regression within the SPM12 environment controlling for age and total intracranial volume.

In the longitudinal VBM analysis, we only included subjects whose scores declined ($n = 20$ for *total learning score*; $n = 23$ for *immediate cued recall*), because our focus was on identifying the brain regions associated with impairment over time. We chose these two measures as they are most representative of total learning and cued recall, respectively. We reported results at a threshold of $p < 0.001$ uncorrected for multiple comparisons and used a threshold of $p < 0.005$ uncorrected for multiple comparisons for visualization purposes (http://www.nitrc.org/projects/mricron).

Statistical analyses

Statistical analyses were performed using the SPSS (v. 22.0) package for Windows. Following the recommendations of the American Physiological Society [46] and in order to avoid type I errors, alpha value of $p < 0.01$ was considered to be significant for all the comparative analyses.

Whole sample analyses

For the whole sample, demographical data, levels of CSF $A\beta_{42}$, CSF tau, and CSF ptau, and *APOE* ε4 frequencies were calculated. Regarding the longitudinal change in both the AFE-T and the standard neuropsychological tests, repeated-measures analyses of variance (ANOVA) were run in order to compare baseline and follow-up performances.

Pearson bivariate correlations were calculated to assess overall associations between the demographic (age, years of education, and cognitive reserve) and biological (AD CSF biomarkers) data and the difference between baseline and follow-up score in the *immediate cued recall* of the AFE-T (*immediate cued recall difference score*). The difference between baseline and follow-up in the *immediate cued recall difference score* was considered to be the best AFE-T outcome measure, as it is the final score of the test (end of the 2nd learning day).

To explore the relationships between the longitudinal memory performance (in both the AFE-T and a standard neuropsychological test) and relevant factors, a linear model was set up. The first analysis included the AFE-T *immediate cued recall difference score* as the dependent variable and the second one included the *FCSRT cued recall difference score*. For the second analysis, the *FCSRT cued recall difference score* was used as a homologous variable of the *immediate cued recall difference score* of the AFE-T (final cued recall output). Age, the SCD-Q score, CSF $A\beta_{42}$, and CSF tau were included as covariates in both analyses.

Further analyses: differences between CBN-Tau$^{\uparrow}$ and CBN-Tau$^{\downarrow}$

Demographic data, levels of CSF $A\beta_{42}$, CSF tau, and CSF ptau, and *APOE* ε4 frequencies were compared using *t*-tests for independent samples and *Chi*-square analyses when appropriate. We ran ANOVAs to explore possible cross-sectional differences between CBN-Tau$^{\uparrow}$ and CBN-Tau$^{\downarrow}$ on the AFE-T at the baseline and at the follow-up assessments.

Regarding the longitudinal change, dependent samples *t*-tests were run to compare within-group differences between baseline and follow-up scores of the AFE-T in both CBN-Tau$^{\uparrow}$ and CBN-Tau$^{\downarrow}$. Follow-

up between-group differences were analyzed using mixed-model ANCOVA controlling for age, years of education, and CSF $A\beta_{42}$ levels with *post-hoc* Bonferroni corrections.

RESULTS

Sample characteristics

Thirty-two cognitively and biologically normal (CBN) subjects were included in the present study. Age ranged between 53 and 78 years, and educational level between 5 and 18 years. Female/male ratio was 62.5/37.5. Regarding the AD CSF biomarker levels, the mean CSF $A\beta_{42}$ was 824.1 (SD 210.9) pg/ml [557.5–1405.0], CSF tau was 228.6 (SD 72.3) pg/ml [83.5–364.2], and CSF ptau was 50.9 (SD 13.5) pg/ml [23.5–71.0]. Only 2 subjects (6.2%) were *APOE* ε4 positive (see Table 1).

Variables associated with longitudinal cognitive decline

Pearson correlations were run in order to find overall associations between demographic and biological data and cognitive changes. The *immediate cued recall difference score* (i.e., the difference between the follow up and baseline score on the final cued recall output) was used as an indicator of longitudinal cognitive decline. Demographic data such as age ($r = -0.24$; $p = 0.20$), years of education ($r = 0.19$; $p = 0.29$) and cognitive reserve ($r = 0.16$; $p = 0.41$) were not associated with the *immediate cued recall difference score*. Neither did the biological variables of CSF $A\beta_{42}$ ($r = -0.04$; $p = 0.82$) and CSF ptau ($r = -0.24$; $p = 0.18$). However, we found a significant negative correlation between CSF tau and the immediate cued recall difference score ($r = -0.414$; $p < 0.01$; see scatter plot in Fig. 1), indicating worse recall associated to higher CSF tau levels.

CSF tau driving longitudinal memory changes

The first linear model analysis showed that CSF tau ($B = -0.17$; $p < 0.05$) predicted the performance in the *immediate cued recall difference score* of the AFE-T. Age ($B = -0.029$; $p = 0.705$), the SCD-Q score ($B = -0.107$; $p = 0.155$), and CSF $A\beta_{42}$ ($B = 0.002$; $p = 0.317$) did not predict change on this cognitive variable. The second model, with the *FCSRT cued recall difference score* as the dependent variable, did not reveal any statistically significant predictors [age ($B = -0.089$; $p = 0.381$), SCD-Q score ($B = -0.130$; $p = 0.188$), CSF $A\beta_{42}$ ($B = -0.003$; $p = 0.327$), and CSF tau ($B = 0.014$; $p = 0.148$)].

Performance in the AFE-T

Validation study

Thirty healthy younger adults were recruited and randomly divided into two groups (group A, for word list A; and group B, for word list B). Age ranged between 17 and 29 years (mean 20.27; SD = 2.57) and all subjects were college students. There were no significant differences between the validation groups on age or educational level.

Importantly for the present purposes, independent samples t-tests revealed no significant differences between the validation groups A and B in any of the learning runs or in the *immediate cued recall* (with *p*-values ranging between 0.20 and 0.86; see Fig. 2). As expected, when comparing the overall performances in lists A and B between the validation subjects and the study participants, significant differences were found in favor of the younger validation subjects in both list A ($t(14) = 3.74$, $p < 0.01$) and list B ($t(14) = 3.98$, $p < 0.01$).

The AFE-T in the whole sample

We compared baseline and 18-month follow-up scores of the AFE-T in the whole sample (Table 2; Fig. 2). ANOVAs showed significant differences with a better performance in the baseline session in comparison with the follow-up in the runs 6 ($F(1, 31) = 7.13$; $p < 0.01$) and 7 ($F(1, 31) = 18.19$; $p < 0.01$) of the first learning day, and in the runs 1 ($F(1, 31) = 13.09$; $p < 0.01$), 2 ($F(1, 31) = 7.20$; $p < 0.01$), 7 (i.e., *total learning score*; $F(1, 31) = 7.81$; $p < 0.01$) and *immediate cued recall* ($F(1, 31) = 7.61$; $p < 0.01$).

Standard neuropsychological tests in the whole sample

Comparisons of baseline and follow-up scores on standard neuropsychological tests are shown in Supplementary Table 1. There was no significant difference in global cognition between baseline and follow-up scores ($F(1,30) = 1.37$; $p = 0.25$), as assessed by the MMSE. Nor was there a significant difference on the verbal intelligence score ($F(1,30) = 4.12$; $p = 0.51$). Most of the scores obtained at the follow-up assessment were higher than at the baseline, even reaching statistical significance for the free recall subtest of the FCSRT ($F(1,30) = 12.25$; $p < 0.01$).

Table 1
Demographics, biological data, and AD CSF levels of the whole sample

Demographics		Biological data & AD CSF levels	
Female/male ratio	62.5/37.5	*APOE* ε4 (% positive)	6.2%
Age	64.6 (SD 6.2) [53–78]	$A\beta_{42}$	824.1 pg/ml (SD 210.9)
Years of education	11.4 (SD 3.8) [5–18]	Tau	228.6 pg/ml (SD 72.3)
CRQ	16.6 (SD 4.5) [6–22]	ptau	50.9 pg/ml (SD 13.5)

Data are presented as means (SD; standard deviation) and [range]. CSF, cerebrospinal fluid; $A\beta_{42}$, amyloid-β isoform 42; Tau, total tau; ptau, phosphorylated tau; CRQ, Cognitive Reserve Questionnaire.

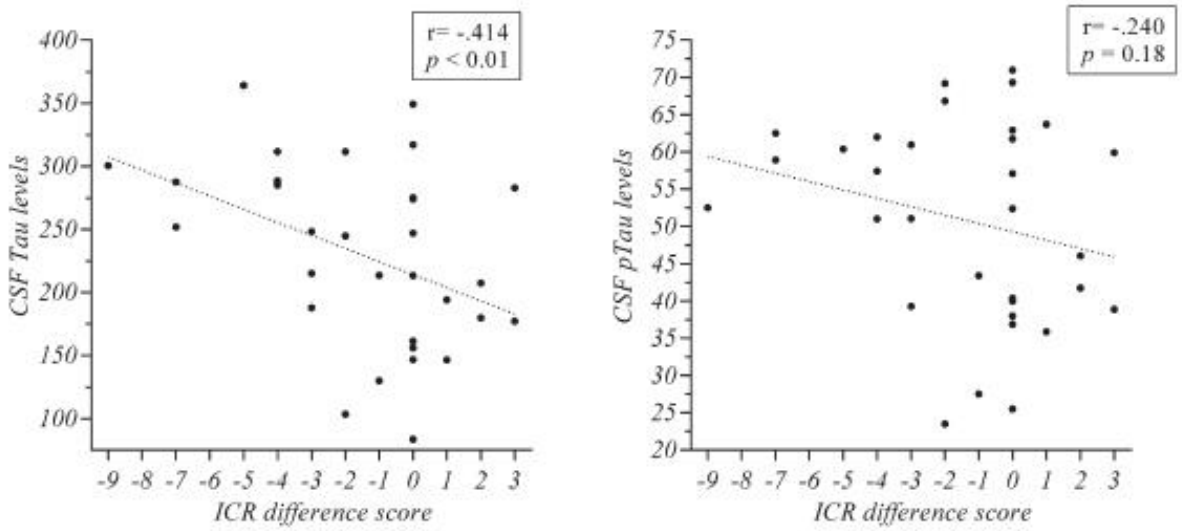

Fig. 1. Correlations in the whole sample between CSF tau and CSF pTau levels and the *immediate cued recall* difference score of the AFE-T. ICR, immediate cued recall of the AFE-T (difference between baseline and follow-up scores).

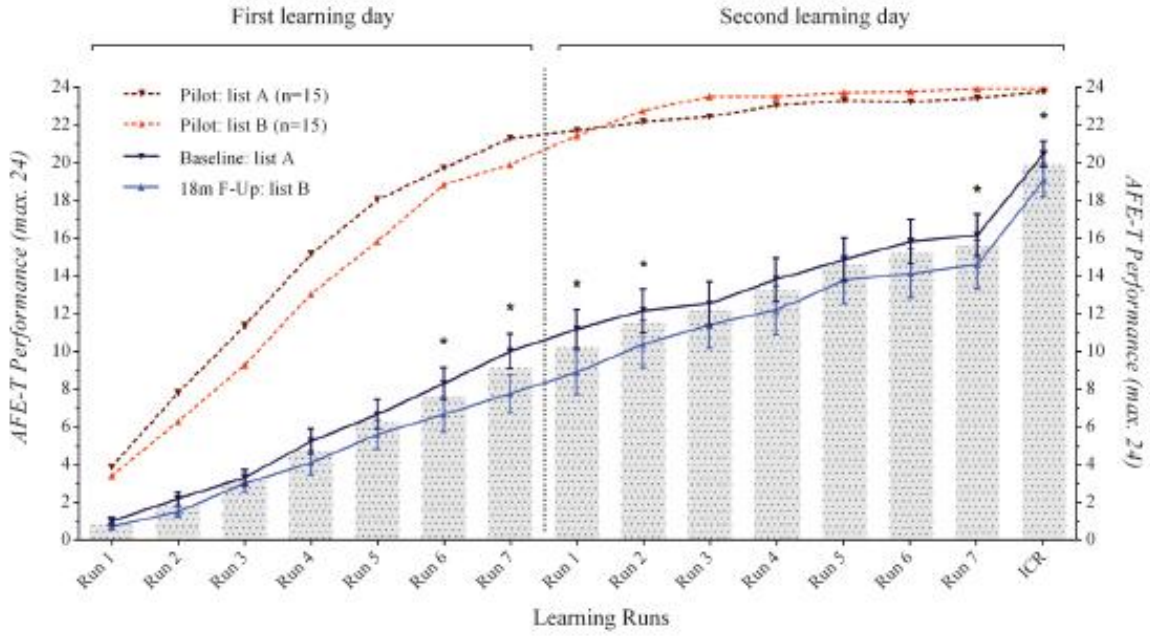

Fig. 2. AFE-T baseline and follow-up performance in the whole sample. ICR, immediate cued recall, $^{*}p < 0.01$.

Table 2
ANOVA of the AFE-T baseline and follow-up scores in the whole sample

Runs	List A (N = 32) Baseline	List B (N = 32) 18 month F-up	*F*	*p*
LS1_R1	1.0 (SD 1.2)	0.7 (SD 0.9)	1.55	0.222
LS1_R2	2.2 (SD 2.0)	1.5 (SD 1.6)	4.15	0.050
LS1_R3	3.3 (SD 2.3)	3.0 (SD 2.7)	0.35	0.557
LS1_R4	5.2 (SD 3.7)	4.1 (SD 3.6)	4.46	0.043
LS1_R5	6.6 (SD 4.3)	5.6 (SD 4.3)	3.22	0.083
LS1_R6	8.3 (SD 4.8)	6.6 (SD 5.3)	7.13	0.009*
LS1_R7	10.0 (SD 5.1)	7.7 (SD 5.6)	18.19	0.001**
LS2_R1	11.2 (SD 5.7)	8.9 (SD 6.5)	13.09	0.001**
LS2_R2	12.1 (SD 6.5)	10.4 (SD 6.9)	7.20	0.009*
LS2_R3	12.5 (SD 6.6)	11.4 (SD 6.9)	2.70	0.111
LS2_R4	13.8 (SD 6.4)	12.1 (SD 7.3)	5.08	0.031
LS2_R5	14.8 (SD 6.4)	13.7 (SD 7.2)	2.46	0.127
LS2_R6	15.8 (SD 6.7)	14.1 (SD 7.2)	6.46	0.016
LS2_R7	16.1 (SD 6.3)	14.5 (SD 7.3)	7.81	0.009*
ICR	20.4 (SD 3.8)	19.0 (SD 4.9)	7.61	0.009*

Data are presented as means (SD; standard deviation). LS1, 1st learning day; LS2, 2nd learning day; R, learning run number; LS2_R7, *total learning score*; ICR, *immediate cued recall*. *$p < 0.01$; **$p < 0.005$.

Further analyses: comparisons between CBN-Tau$^{\downarrow}$ and CBN-Tau$^{\uparrow}$ groups

Group characteristics

Demographic and biological data for both CBN-Tau$^{\downarrow}$ and CBN-Tau$^{\uparrow}$ groups are shown in Table 3. No differences between CBN-Tau$^{\downarrow}$ and CBN-Tau$^{\uparrow}$ groups were found in age ($t(30) = 0.80$; $p = 0.428$), years of education ($t(30) = 0.09$; $p = 0.928$), cognitive reserve ($t(26) = -1.03$; $p = 0.312$), or CSF $A\beta_{42}$ levels ($t(30) = 2.04$; $p = 0.039$). There were no significant differences between groups in terms of gender distribution ($\chi^2 = 0.35$; $p = 0.554$) or in *APOE* ε4 allele frequency ($\chi^2 = 1.88$; $p = 0.170$). Significant differences were found in CSF tau ($t(30) = 9.32$; $p < 0.01$) and CSF ptau ($t(30) = 6.31$; $p < 0.01$).

Differences between CBN-Tau$^{\uparrow}$ and CBN-Tau$^{\downarrow}$ groups

ANOVAs were run to determine whether CBN-Tau$^{\uparrow}$ and CBN-Tau$^{\downarrow}$ groups showed cross-sectional differences at baseline and/or follow-up performance in the AFE-T.

There were no significant differences between groups in either baseline or follow-up performance (with p values ranging from 0.184 to 0.960 and 0.596 to 0.967, respectively).

Regarding the longitudinal change within each group, dependent samples t-tests were run in order to examine differences between baseline and follow-up learning runs. For CBN-Tau$^{\downarrow}$, learning runs 5 and 7 of the first learning day of the follow-up were significantly lower than at the baseline. In the CBN-Tau$^{\uparrow}$ group, these differences were found in the runs 6 and 7 (i.e., *total learning score*) and in the *immediate cued recall* of the second learning day (Table 4; Fig. 3). When ANCOVAs were run to analyze longitudinal between-group differences, the performance difference between the baseline and follow-up *immediate cued recall* scores between the CBN-Tau$^{\downarrow}$ and CBN-Tau$^{\uparrow}$ groups revealed that the CBN-Tau$^{\uparrow}$ had a larger difference in the *immediate cued recall difference score* than the CBN-Tau$^{\downarrow}$ subjects did ($F(1,31) = 8.37$; $p < 0.01$). The differences in the rest of the learning runs showed no significant differences between groups (see Supplementary Table 2; Fig. 3).

Neuroimaging analyses

We evaluated which regions of grey matter volume predicted the *total learning score* and *immediate cued recall* of the AFE-T at baseline, as well as their longitudinal change (*total learning score and immediate cued recall difference scores*).

Cross-sectional VBM

At baseline, both *total learning score* and *immediate cued recall* showed positive correlations with grey matter volume in a similar network including the left and right posterior cerebellar lobes, right and left medial temporal regions, and the right middle

Table 3
Demographics, biological data, and AD CSF levels of the CBN-Tau$^{\downarrow}$ and CBN-Tau$^{\uparrow}$ groups

Parameters	CBN-Tau$^{\downarrow}$ ($n = 16$)	CBN-Tau$^{\uparrow}$ ($n = 16$)	t	p
Demographics				
Gender (% women)	68.8%	56.3%	0.35[a]	0.554
Age	63.7 (SD 6.7) [53–78]	65.5 (SD 5.6) [56–73]	0.80	0.428
Years of education	11.3 (SD 3.3) [6–18]	11.4 (SD 4.4) [5–18]	0.09	0.928
CRQ	17.7 (SD 4.2) [9–22]	15.9 (SD 4.8) [6–22]	–1.03	0.312
Biological data & CSF				
APOE ε4 (% positive)	0%	12.5%	1.88[a]	0.170
$A\beta_{42}$	747.7 pg/ml (SD 231.5)	900.5 pg/ml (SD 161.0)	2.04	0.039
Tau	167.3 pg/ml (SD 39.2)	290.0 pg/ml (SD 35.1)	9.32	0.001*
ptau	40.8 pg/ml (SD 11.1)	60.9 pg/ml (SD 6.1)	6.31	0.001*

Data are presented as means (SD; standard deviation). CSF, cerebrospinal fluid; CBN, cognitively and biologically normal; $A\beta_{42}$, amyloid-β isoform 42; Tau, total tau; ptau, phosphorylated tau; CRQ, cognitive reserve questionnaire. [a]χ^2 statistic; *$p < 0.01$.

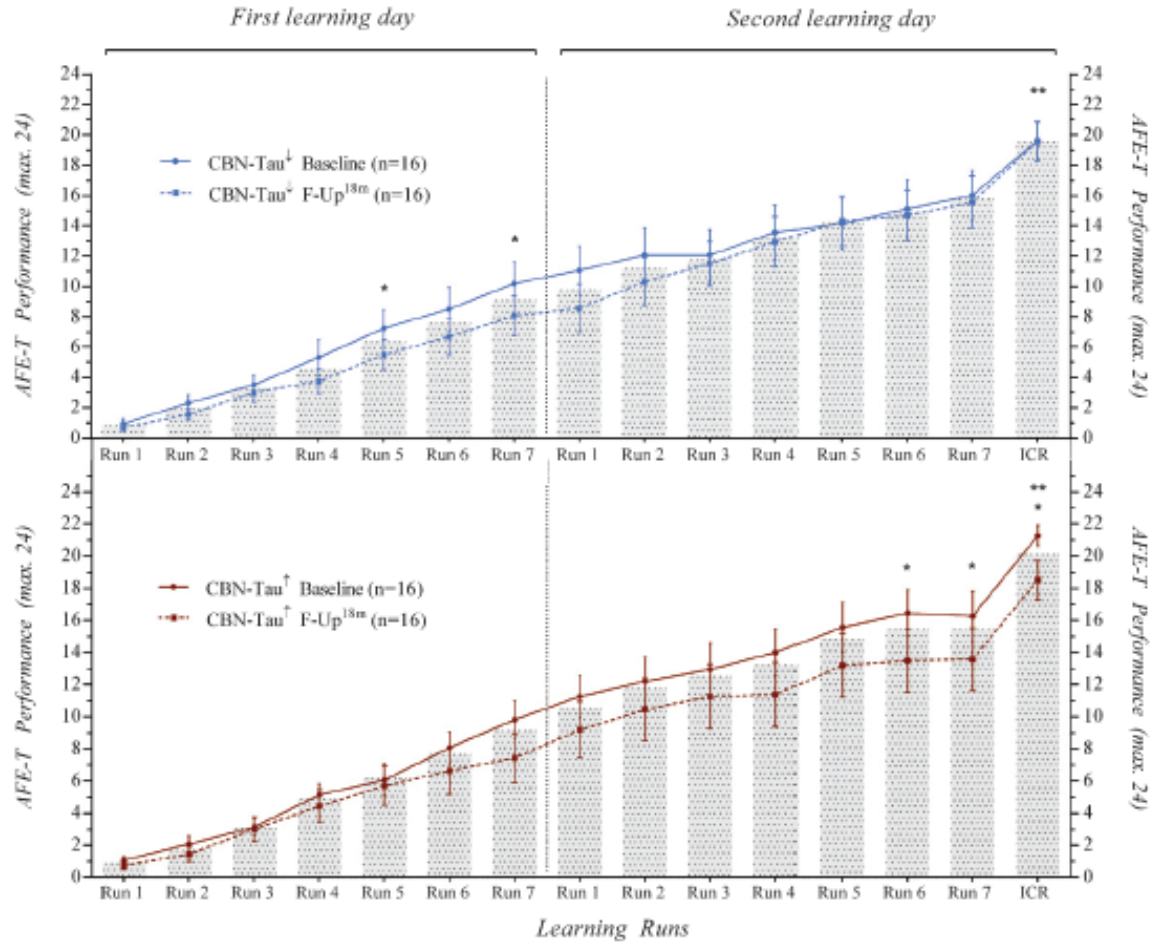

Fig. 3. Comparison of the AFE-T baseline and follow-up performance in the CBN-Tau$^{\downarrow}$ and CBN-Tau$^{\uparrow}$ groups. CBN, cognitively and biologically normal; ICR, immediate cued recall; *$p < 0.01$ (within-group differences); **$p < 0.01$ (between-group difference).

occipital gyrus (Fig. 4). For the total *learning score*, an additional left middle frontal cluster was a significant predictor. No grey matter regions showed a significant negative correlation with either score.

Longitudinal VBM

The longitudinal decline in both scores correlated with the rate of grey matter volume reduction in the right posterior cerebellar lobe (Fig. 4). Volume

Table 4
Dependent samples *t*-tests for the AFE-T baseline and follow-up scores of the CBN-Tau$^{\downarrow}$ and CBN-Tau$^{\uparrow}$ groups

Runs	CBN-Tau$^{\downarrow}$ ($n=16$)				CBN-Tau$^{\uparrow}$ ($n=16$)			
	List A Baseline	List B 18 m F-Up	*t*	*p*	List A Baseline	List B 18 m F-Up	*t*	*p*
LS1_R1	0.9 (SD 1.4)	0.6 (SD 0.9)	0.77	0.451	1.0 (SD 0.9)	0.7 (SD 0.9)	0.96	0.352
LS1_R2	2.3 (SD 2.1)	1.6 (SD 1.5)	1.96	0.068	2.0 (SD 2.0)	1.4 (SD 1.8)	1.01	0.289
LS1_R3	3.5 (SD 2.6)	3.0 (SD 2.4)	0.67	0.510	3.1 (SD 2.0)	3.0 (SD 3.0)	0.16	0.873
LS1_R4	5.3 (SD 4.6)	3.7 (SD 3.2)	2.33	0.034	5.1 (SD 2.7)	4.4 (SD 4.0)	0.82	0.423
LS1_R5	7.2 (SD 4.8)	5.5 (SD 3.9)	3.36	0.004*	6.1 (SD 3.9)	5.7 (SD 4.8)	0.35	0.728
LS1_R6	8.5 (SD 5.7)	6.7 (SD 4.8)	2.83	0.013	8.1 (SD 3.8)	6.6 (SD 5.8)	1.36	0.194
LS1_R7	10.2 (SD 5.7)	8.1 (SD 5.2)	3.29	0.005*	9.8 (SD 4.6)	7.4 (SD 6.1)	2.77	0.014
LS2_R1	11.1 (SD 6.4)	8.6 (SD 6.3)	2.64	0.018	11.3 (SD 5.2)	9.2 (SD 6.9)	2.39	0.030
LS2_R2	12.0 (SD 7.1)	10.3 (SD 6.3)	2.28	0.037	12.2 (SD 6.0)	10.4 (SD 7.7)	1.62	0.127
LS2_R3	12.0 (SD 6.7)	11.5 (SD 5.8)	0.59	0.562	13.0 (SD 6.5)	11.2 (SD 8.0)	1.69	0.112
LS2_R4	13.6 (SD 7.2)	12.9 (SD 6.6)	0.62	0.543	14.0 (SD 5.7)	11.4 (SD 8.0)	2.61	0.019
LS2_R5	14.1 (SD 6.7)	14.3 (SD 6.6)	–0.21	0.834	15.6 (SD 6.1)	13.2 (SD 8.0)	2.35	0.032
LS2_R6	15.1 (SD 7.6)	14.7 (SD 6.7)	0.55	0.588	16.4 (SD 5.7)	13.5 (SD 7.9)	2.95	0.009*
LS2_R7	16.0 (SD 6.6)	15.6 (SD 6.8)	0.79	0.437	16.2 (SD 6.2)	13.5 (SD 7.8)	2.96	0.009*
ICR	19.6 (SD 4.7)	19.6 (SD 5.2)	0.14	0.884	21.2 (SD 2.5)	18.5 (SD 4.9)	3.40	0.004*

Data are presented as means (SD; standard deviation). CBN, cognitively and biologically normal; LS1, 1st learning day; LS2, 2nd learning day; R, learning run number; LS2_R7, *total learning score*; ICR, immediate cued recall. *$p<0.01$.

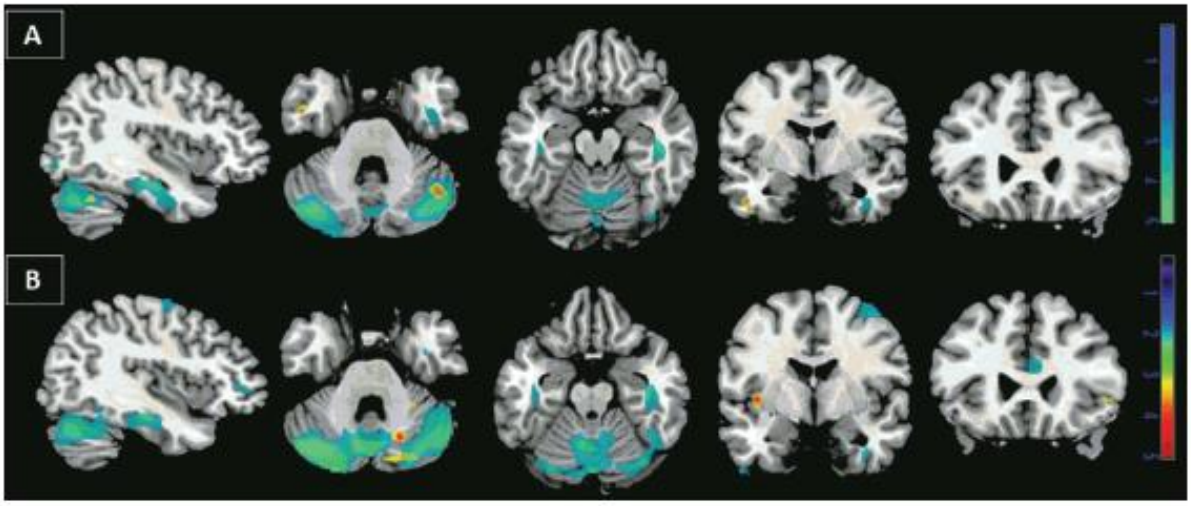

Fig. 4. Grey matter volume correlates of the Immediate Cued Recall (A) and Total Learning Score (B) of the AFE-T. Grey matter volume correlates of the *immediate cued recall* (A) and *total learning score* (B) performance on the AFE-T. Green represents cross-sectional performance at baseline. Hot color represents the correlates of longitudinal change. Results are displayed at a $p<0.005$ threshold for visualization purposes.

reduction in additional clusters in the left insula for the *total learning score,* and the left anterior inferior temporal lobe for the *immediate cued recall*, correlated with decline in memory performance. All grey matter volume correlates of cross-sectional and longitudinal performance on the AFE-T are shown in Supplementary Table 3.

DISCUSSION

We conducted the first study examining a specific sample of cognitively healthy subjects with a normal pattern of AD CSF biomarkers using a highly demanding learning and memory test and VBM, with the aim to identify the earliest biological and structural variables related to longitudinal cognitive decline in cognitively and biologically normal aging. Our main finding showed that CSF tau is associated with longitudinal cognitive changes in this population. Furthermore, we found different longitudinal cognitive patterns by dividing our cohort into two subgroups based on their CSF tau levels. The VBM results also suggested that performance on the AFE-T is related to grey matter volume in a medial tempo-

ral, middle frontal, and posterior cerebellar network at baseline, and that there are strategic brain areas driving the observed longitudinal cognitive changes. Our findings suggest that there are biological and structural markers reflecting normal cognitive aging and highlight the critical impact of tau as a good predictor of the earliest memory decline in healthy subjects with normal AD CSF biomarkers. Taken together, since both CSF tau and anatomic MRI are considered biomarkers of neurodegeneration in the current ATN classification [47], our results point the presence of neurodegeneration as a potential predictor of cognitive change.

In the last few years, the relationships between biomarkers and cognition have been intensively studied [25–29, 48] and some studies have suggested a stronger association between cognition and tau than between cognition and Aβ [11, 13, 49]. According to the literature, Aβ accumulates first in the neocortex and then in subcortical areas [50]. Instead, tau usually concentrates first in the medial temporal areas [51] that are closely related to encoding of new information, learning processes and memory. The main finding of the present study was to find of an association between CSF tau and the longitudinal cognitive decline in the AFE-T in a sample of subjects with normal AD CSF biomarker levels. Some longitudinal studies have found associations between cognition and tau [12, 49] in cognitively healthy subjects. Hessen et al. [49] studied 122 subjects with subjective cognitive decline (SCD) and found that a subgroup presenting memory decline during the study period had significantly higher CSF tau levels at baseline. However, it has been suggested that SCD population could have a somewhat higher risk of developing incipient cognitive decline than controls [52]. Furthermore, a study by Glodzik et al. [12] found that elevated p-tau$_{231}$ levels were related to both decreased memory function and MTL atrophy in a group of normal elderly subjects that experienced longitudinal memory decline. Nevertheless, in this study it is important to note that 1) CSF p-tau$_{231}$ is a specific marker of neurofibrillary pathology and 2) the group with decreased memory function presented lower levels of the $A\beta_{42}/A\beta_{40}$ ratio indicating a noticeable contribution of amyloid deposition. In the present study, our statistical model showed that SCD (measured by the SCD-Q) and CSF $A\beta_{42}$ were not associated with the *immediate cued recall* difference score of the AFE-T, and allowed the identification of a unique contribution of CSF tau into predicting the longitudinal memory decline.

An important issue was to further investigate our main finding regarding the CSF tau impact on the cognitive performance of cognitively healthy subjects. In this regard, we identified distinct patterns of cognitive decline in the AFE-T between the two groups that were established according to their CSF tau levels. Our findings are enhanced by the fact that the study groups showed a marginal difference in the CSF $A\beta_{42}$ levels in favor of the group with higher tau levels. This rejects the possibility that Aβ was contributing to the observed cognitive differences and, again, argues in favor of a unique effect of CSF tau in the memory function. As seen in Fig. 3, the group with higher tau levels performed worse in more runs of the follow-up assessment when compared with the group exhibiting lower levels of tau. Furthermore, in their last runs, the lower tau group reached their baseline performance level, whereas the group with higher levels of tau remained below their baseline level throughout the whole follow-up testing. These results suggest that subjects with lower tau levels exhibited practice effects in comparison with those with higher tau. Importantly, the lack of learning effects has been recently identified as a cognitive marker of subtle cognitive changes [53], highlighting its importance as a predictive variable of future cognitive decline. In addition, the change between the baseline and the follow-up *immediate cued recall* showed a significant difference in favor of the lower tau group. Taken together, our results suggest that CSF tau levels could have an impact on the learning and memory functions of cognitively healthy individuals, and that highly demanding memory tests such as AFE-T could detect this pathological process even in this population.

Our VBM results also suggest that AFE-T performance is related to grey matter volume in a medial temporal, middle frontal, and posterior cerebellar network at baseline (cross-sectional VBM, $n = 29$), and that there are strategic brain areas driving the observed longitudinal cognitive changes (longitudinal VBM, $n = 20$ for *total learning score* and $n = 23$ for *immediate cued recall*). We were able to identify grey matter regions the volume of which predicted performance on the AFE-T at baseline, as well as those in which the longitudinal change in volume predicted the amount of decline in the studied measures. Cross-sectional performance at baseline in both total learning and cued recall measures was associated with grey matter volume in a medial temporal and posterior cerebellar network. For the *total learning score*, an additional left middle frontal cluster was

a significant predictor, perhaps reflecting a larger executive function component than that involved in cued recall. These results are in line with extensive previous literature showing that the medial temporal and prefrontal regions are related to performance on a variety of memory tasks [54–56]. The predominant brain region involved in both cross-sectional and longitudinal performance was the posterior cerebellum. This finding was unexpected. However, the role of the posterior cerebellum in a variety of cognitive tasks including language, learning, memory and especially executive functions such as working memory and planning is well established [57–61]. The large extension of the cerebellar clusters with respect to the cortical clusters may be explained by the healthy cognitive and biological state of our cohort. In normal aging, the posterior cerebellar lobes show accelerated volume reduction with respect to other structures [62], and this reduction may be more relevant to cognitive performance than typically shown by studies of diseased patients with cortical lesions. In line with our results, other recent studies including similarly aged and cognitively healthy cohorts [63–65] show involvement of the cerebellum in memory and executive function tests. Taken together, our findings showed structural correlates of cognitive performance in a well-characterized sample of subjects with normal cognition and normal AD CSF biomarker levels, providing evidence on the most vulnerable brain systems related to learning and memory in aging.

Another important topic of the present work concerned the sensitivity of cognitive measures in the assessment of cognitively healthy subjects. Our whole-sample analyses of the standard neuropsychological tests showed improved follow-up performance on most of the tasks, suggesting clear practice effects that are commonly observed in cognitively healthy individuals [53, 66–68]. Practice effects have been explained by several factors such as knowledge about testing procedures, previously learned strategies, and a reduced sense of novelty and nervousness when re-testing [69]. On the other hand, our findings on the AFE-T showed a poorer performance at the follow-up assessment when compared with the baseline, suggesting that the use of a demanding cognitive measure can alleviate practice compound that may mask subtle cognitive changes in cognitively normal subjects. This is crucial for pinpointing age-related cognitive changes that otherwise could become underestimated [70]. Importantly for the present research, our validation study demonstrated that both baseline and follow-up lists of stimuli were equally challenging and therefore comparable, and thus the observed follow-up changes could not be explained by list-related differences. Taken together, our findings support the idea that standard neuropsychological tests are not sensitive enough to detect subtle cognitive changes in a cognitively healthy population [71, 72], and suggest that the AFE-T could be a sensitive task for the early detection of longitudinal cognitive decline.

This study has some limitations. One important issue is its small sample size which limits the strength of the statistical analyses. However, the comprehensive AFE-T protocol allowed for a better and more fine-grained characterization of learning and memory processes. With AFE-T, participants' learning and memory functions were evaluated using 15 learning runs at both baseline and follow-up assessments for both free and cued recall. Regarding the AFE-T, it is also important to note that only the form A was administered at baseline and form B at the 18-month follow-up. Although our pilot study indicated that lists A and B did not differ in terms of level of difficulty, it would have been more appropriate to counterbalance their presentation for the baseline and follow-up. Another potential limitation of the present study concerns the multiple comparisons problem that arises from the large number of statistical comparisons performed. This was dealt with *post-hoc* Bonferroni corrections, albeit this is an admittedly conservative method. Moreover, following recommendations [46] to avoid type I errors, alpha level was set to $p < 0.01$ for all the comparative analyses. Finally, considering the novelty of the present findings and the limited sample size, the results have to be interpreted with caution and replication is called for in further studies involving larger samples of cognitively and biologically normal subjects.

Conclusions

The present study is the first to show that in a sample of cognitively healthy individuals with normal AD CSF biomarker levels, tau, a marker of neurodegeneration, is associated with longitudinal cognitive decline. Furthermore, our results pinpoint critical brain areas related to cross-sectional and longitudinal learning and memory performance in this well-characterized sample. Taken together, our results suggest the presence of neurodegeneration as a potential predictor of cognitive change in healthy individuals. The present findings shed light on the

impact of CSF tau in cognition and provide important knowledge about the relationships between biological status and the earliest age-related cognitive changes.

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SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-190046.

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Supplementary Material

Supplementary Table 1. Mean values and ANOVA results for the baseline vs. follow-up standard neuropsychological measures in the whole sample.

Parameters	1st evaluation Baseline	2nd evaluation 18 month follow-up	*F*	P value
Global Cognition				
MMSE[a]	28.1 (SD 1.6)	28.4 (SD 1.3)	1.37	.250
WAT[a]	24.4 (SD 4.7)	25.6 (SD 3.3)	4.12	.051
Memory				
FCSRT – FR	12.1 (SD 2.3)	13.4 (SD 2.5)	12.25	.001*
FCSRT – TR	12.4 (SD 2.7)	13.4 (SD 2.8)	2.53	.122
FCSRT – DFR	12.1 (SD 2.5)	13.2 (SD 2.7)	4.10	.052
FCSRT – DTR	13.6 (SD 4.3)	15.1 (SD 3.1)	6.64	.015
Language				
BNT	11.6 (SD 1.9)	12.3 (SD 2.1)	7.18	.012
Sem-Flu	11.0 (SD 2.0)	11.6 (SD 2.5)	1.31	.262
Perception				
VOSP – NL	12.7 (SD 4.5)	12.9 (SD 4.1)	0.21	.647
Executive Functions				
TMT – A	11.0 (SD 2.1)	11.4 (SD 2.7)	0.59	.449
TMT – B	10.0 (SD 2.2)	11.0 (SD 2.8)	5.21	.030
Stroop – W	11.3 (SD 2.0)	11.2 (SD 2.2)	0.06	.807
Stroop – C	10.9 (SD 1.5)	10.8 (SD 1.3)	0.79	.781
Stroop – CW	11.6 (SD 2.3)	12.2 (SD 2.2)	3.15	.092
SDMT	11.7 (SD 2.9)	11.1 (SD 1.8)	0.71	.412
Digits – F	10.9 (SD 2.5)	10.8 (SD 2.4)	0.04	.843
Digits – B	12.1 (SD 2.1)	12.8 (SD 2.8)	1.35	.255

Data correspond to scaled scores of the standard neuropsychological tests and are presented as means (SD standard deviation. Key: MMSE, Mini-Mental State Examination; WAT, Word Accentuation Test; FCSRT-FR, Free and cued selective reminding test free recall; FCSRT-TR, total recall; FCSRT-DFR, delayed free recall; FCSRT-DTR, delayed total recall; BNT, Boston Naming Test; Sem-Flu, Semantic fluency; VOSP, visual object and space perception battery; NL: number location; TMT-A, Trail Making Test A; TMT-B, Trail Making Test B; Stroop-W, Stroop test words; Stroop-C, Stroop test colors; Stroop-I, Stroop test color-word; SDMT, Symbol digit modality test; Digits-F, WAIS Digit span forward; Digits-B, WAIS Digit span backward.

[a] **Raw scores**

* ***p*< 0.01**

Supplementary Table 2. ANCOVAs of the differences between AFE-T baseline and follow-up scores between CN-Tau$^{\downarrow}$ and CN-Tau$^{\uparrow}$ groups.

	CN-Tau$^{\downarrow}$ (n=16)		CN-Tau$^{\uparrow}$ (n=16)			
Runs	**List A Baseline**	**List B 18m F-Up**	**List A Baseline**	**List B 18m F-Up**	**F**	**P value**
LS1_R1	0.9 (SD 1.4)	0.7 (SD 0.9)	1.0 (SD 0.9)	0.7 (SD 0.9)	0.13	.720
LS1_R2	2.3 (SD 2.1)	1.6 (SD 1.5)	2.0 (SD 2.0)	1.4 (SD 1.8)	0.03	.862
LS1_R3	3.5 (SD 2.6)	3.0 (SD 2.4)	3.1 (SD 2.0)	3.0 (SD 3.0)	0.41	.525
LS1_R4	5.3 (SD 4.6)	3.7 (SD 3.2)	5.1 (SD 2.7)	4.4 (SD 4.0)	1.43	.243
LS1_R5	7.2 (SD 4.8)	5.5 (SD 3.9)	6.1 (SD 3.9)	5.7 (SD 4.8)	0.70	.410
LS1_R6	8.5 (SD 5.7)	6.7 (SD 4.8)	8.1 (SD 3.8)	6.6 (SD 5.8)	0.52	.821
LS1_R7	10.2 (SD 5.7)	8.1 (SD 5.2)	9.8 (SD 4.6)	7.4 (SD 6.1)	0.26	.613
LS2_R1	11.1 (SD 6.4)	8.6 (SD 6.3)	11.3 (SD 5.2)	9.2 (SD 6.9)	0.64	.428
LS2_R2	12.0 (SD 7.1)	10.3 (SD 6.3)	12.2 (SD 6.0)	10.4 (SD 7.7)	0.48	.494
LS2_R3	12.0 (SD 6.7)	11.5 (SD 5.8)	13.0 (SD 6.5)	11.2 (SD 8.0)	0.12	.728
LS2_R4	13.6 (SD 7.2)	12.9 (SD 6.6)	14.0 (SD 5.7)	11.4 (SD 8.0)	0.63	.434
LS2_R5	14.1 (SD 6.7)	14.3 (SD 6.6)	15.6 (SD 6.1)	13.2 (SD 8.0)	1.43	.241
LS2_R6	15.1 (SD 7.6)	14.7 (SD 6.7)	16.4 (SD 5.7)	13.5 (SD 7.9)	2.23	.147
LS2_R7	16.0 (SD 6.6)	15.6 (SD 6.8)	16.2 (SD 6.2)	13.5 (SD 7.8)	2.50	.125
ICR	19.6 (SD 4.7)	19.6 (SD 5.2)	21.2 (SD 2.5)	18.5 (SD 4.9)	8.37	.007*

Data are presented as means (SD; standard deviation). Key: LS1, 1st learning day; LS2, 2nd learning day; R, learning run number; LS2_R7, *total learning score*; ICR, immediate cued recall.

*** *p*< 0.01**

Supplementary Table 3: Grey matter volume correlates of cross-sectional and longitudinal performance on the AFE-T.

Cross-sectional VBM							
Total learning score							
	x	**y**	**z**	**t-value**	**p(corr)**	**p(uncorr)**	**Region**
Cluster 1 (6134 vox)							
maximum 1	-5	-75	-29	7.44	< 0.001	< 0.001	L Vermis
maximum 2	-36	-75	-30	6.18	< 0.001	< 0.001	L posterior cerebellum
Cluster 2 (241 vox)							
maximum 1	-26	51	24	5.06	0.298	< 0.001	L middle frontal gyrus
Cluster 3 (1985 vox)							
maximum 1	47	-68	-30	5.05	0.302	< 0.001	R posterior cerebellum
maximum 2	54	-53	-30	4.49	0.661	< 0.001	R posterior cerebellum
Cluster 4 (169 vox)							
maximum 1	41	-21	-21	4.32	0.758	< 0.001	R Fusiform
Cluster 4 (90 vox)							
maximum 1	21	-101	5	3.90	0.443	< 0.001	R occipital pole
Immediate Cued Recall							
Cluster 1 (1000 vox)							
maximum 1	-50	-74	-33	5.17	0.214	< 0.001	L posterior cerebellum
maximum 2	-30	-78	-24	3.93	0.939	< 0.001	L posterior cerebellum
Cluster 2 (394 vox)							
maximum 1	21	-99	5	4.78	0.412	< 0.001	R occipital pole
Cluster 3 (1018 vox)							
maximum 1	36	-72	-33	4.66	0.494	< 0.001	R posterior cerebellum
maximum 1	47	-69	-32	4.55	0.573	< 0.001	R posterior cerebellum
Cluster 4 (565 vox)							
maximum 1	-3	-65	-15	4.50	0.609	< 0.001	R Vermis
maximum 2	-2	-77	-27	4.39	0.690	< 0.001	R Vermis
Cluster 5 (217 vox)							
maximum 1	42	-17	-21	4.35	0.713	< 0.001	R Fusiform
Longitudinal VBM							
Total learning score							
Cluster 1 (45 vox)							
maximum 1	44	54	-5	4.00	0.659	< 0.001	R middle frontal gyrus
Cluster 2 (15 vox)							
maximum 1	-17	-50	2	3.64	0.848	< 0.001	L posterior cingulate
Cluster 3 (9 vox)							
maximum 1	-20	-56	69	3.85	0.757	< 0.001	L superior parietal
Immediate Cued Recall							
Cluster 1 (10 vox)							
maximum 1	41	14	36	3.70	0.860	< 0.001	R middle frontal gyrus
Cluster 2 (14 vox)							
maximum 1	-47	-5	-32	3.67	0.873	< 0.001	L inferior temporal gyrus
Cluster 3 (3 vox)							
maximum 1	38	-57	-30	3.49	0.937	< 0.001	R posterior cerebellum

Key: VBM= voxel-based morphometry.

Trabajo número 3:

Early detection of subtle motor dysfunction in cognitively normal subjects with amyloid-β positivity

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Research Report

Early detection of subtle motor dysfunction in cognitively normal subjects with amyloid-β positivity

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ABSTRACT

Since the current neuropsychological assessments are not sensitive to subtle deficits that may be present in cognitively normal subjects with amyloid-β positivity, more accurate and efficient measures are needed. Our aim was to investigate the presence of subtle motor deficits in this population and its relationship with cerebrospinal fluid (CSF) amyloid-β levels. We adapted the Finger Tapping Task to measure tapping speed and intrasubject variability. Seventy-two right-handed participants completed the study. Subjects were divided into three groups according to their CSF biomarker profile: 37 control participants (negative CSF AD biomarkers, CTR), 20 cognitively normal subjects with amyloid-β positivity (abnormal levels of CSF $A\beta_{42}$, Aβ+) and 15 AD patients. All subjects underwent lumbar puncture for the CSF analysis, apolipoprotein E genotyping and completed the Finger Tapping Task, a neuropsychological battery and cardiovascular risk factor and physical

Abbreviations: CN, Cognitively normal; $A\beta_{42}$, amyloid-beta isoform 42; Aβ+, amyloid-beta positive; FTT, Finger Tapping Task; CoV, Coefficient of Variation.

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activity assessments. An overall difference between groups was found both in tapping speed [$F(2,66) = 19.37$, $p < .01$] and in intrasubject variability [$F(2,66) = 11.40$, $p < .01$]. More specifically, the Aβ+ group showed lower speed [$F(1,52) = 5.33$, $p < .05$] and greater intrasubject variability [$F(1,52) = 8.48$, $p < .01$] than the CTR group, and higher speed than the AD group [$F(1,30) = 13.61$, $p < .01$]. Speed ($\beta = .263$, $p < .05$) and intrasubject variability ($\beta = -.558$, $p < .01$) were significantly associated with CSF amyloid-β levels. The present findings suggest that subtle motor difficulties can be detected in cognitively healthy subjects with amyloid-β positivity and be related to CSF $A\beta_{42}$ levels. An accurate assessment of motor functions could help on identifying individuals at the earliest stage of the Alzheimer's continuum.

1. Introduction

Cognitively normal (CN) subjects with amyloid-β positivity present, by definition, biomarker evidence of amyloid deposition and normal cognition according to existing standard tests until they meet criteria for mild cognitive impairment (MCI) (Jack et al., 2018). Therefore, the early detection of this population is still based on the analysis of the Alzheimer's disease (AD) cerebrospinal fluid (CSF; Shaw et al., 2009) and/or positron emission tomography imaging (PET; Johnson et al., 2013) biomarkers, which are highly-invasive and costly methods.

Since the current neuropsychological assessments are not sensitive to subtle deficits that may be present in CN subjects with amyloid-β positivity, more accurate and efficient measures would be helpful for the development of an initial screening of this population. In this context, the use of accurate computerized neuropsychological testing methods could be more suitable than traditional tests for an early detection of subtle cognitive difficulties at this earliest stage of the disease (Rentz et al., 2013) and recent research has reported that motor dysfunction may be a sensitive marker of preclinical phases of AD (Albers et al., 2015; Buchman & Bennett, 2011). Importantly, although previous literature has mainly focused on analyzing motor speed (Buracchio, Dodge, Howieson, Wasserman, & Kaye, 2010; Camicioli, Howieson, Oken, Sexton, & Kaye, 1998; Del Campo et al., 2016), a few recent studies have suggested that intrasubject variability (i.e., the inconsistency of performance) might also be a relevant marker of early AD (Verghese et al., 2008; Verghese, Wang, Lipton, Holtzer, & Xue, 2007).

In the present study, a novel version of the Finger Tapping Task (FTT) (Reitan, 1985) was developed to explore two indicators of motor function (speed and intrasubject variability; Hultsch, Strauss, Hunter, & MacDonald, 2008) in a group of CN subjects with amyloid-β positivity (Aβ+), a control group (CTR) and a group of AD patients. We hypothesized that the FTT would be able to detect the presence of subtle motor deficits at the earliest stage of the Alzheimer's continuum. The Aβ+ group would exhibit lower speed and greater intrasubject variability than the CTR group and similar response patterns to those of the AD group. We also expected to find an association between tapping performance and the levels of CSF amyloid-β isoform 42 ($A\beta_{42}$).

2. Methods

We report how we determined our sample size, all data exclusions, all inclusion/exclusion criteria, whether inclusion/exclusion criteria were established prior to data analysis, all manipulations, and all measures in the study. Materials and data for the study are publicly archived at https://osf.io/2kje8/. Missing outputs are not included due to confidentiality reasons and are available from corresponding author on request. Researchers who wish to access the data should contact the corresponding author, Lorena Rami. Sufficient data to replicate all results reported in the paper will be released to researchers after completion of a data sharing agreement.

2.1. Study participants

Seventy-two right-handed participants completed the study. The Hospital Clinic's ethics committee approved the study and all participants provided written informed consent prior to enrollment. No part of the study procedures or analyses was pre-registered prior to the research being conducted. Subjects were recruited from three Spanish centers: the Alzheimer's Disease and Other Cognitive Disorders Unit of the Hospital Clinic and the Memory Unit of the Hospital de la Santa Creu i Sant Pau in Barcelona, and the CITA Alzheimer Foundation in San Sebastián. The following exclusion criteria were applied: significant psychiatric and medical conditions, motor and/or joint problems, parkinsonian signs, essential tremor, suspected non-Alzheimer's pathology, severe cerebral atrophy or white matter hyperintensities. The sample consisted of 57 CN individuals and 15 patients with AD. Normal cognition was defined as a score falling within the normal range (1.5 standard deviations from normative means) in every test of an exhaustive neuropsychological battery. Following the recommendations of the 2018 NIA-AA research framework (Jack et al., 2018), the 57 CN subjects were further divided into two groups according to their CSF biomarker profile: a control group (normal CSF $A\beta_{42}$ levels; CTR; n = 37) and a group of CN subjects with amyloid-β positivity

(abnormal CSF $A\beta_{42}$ levels; Aβ+; n = 20). From the Aβ+ group, 3 subjects presented abnormal ptau levels and therefore met criteria for preclinical AD. Due to the nature of the study recruitment, some of the CN subjects were healthy volunteers (n = 28) and some others (n = 29) presented cognitive complaints. The 15 AD patients also met criteria for AD (Jack et al., 2018) and 8 of them presented a Global Deterioration Scale (GDS; Reisberg, Ferris, de Leon, & J. Crook, 1982) score of 3 and 9 a GDS score of 4.

2.2. *Finger Tapping Task (FTT) design and procedure*

E-Prime 2.0 (Psychology Software Tools Inc., Pittsburgh, PA) was used to create a modified computerized version of the standard (and manually-administered) FTT. Participants were instructed to tap repeatedly and as fast as they could on the computer keyboard's spacebar with their index finger while looking at a fixation point, until a STOP sign appeared on the screen. Instructions were given verbally by the experimenter and also displayed on the monitor. The participants sat in front of the monitor at a distance of approximately 60 cm, with the palms of their hands facing downwards and their fingers extended on a table. The test included six different blocks of 10s each (three right-hand blocks and three left-hand blocks, in alternating order). Participants were asked to begin tapping with their right index finger and were allowed to rest for 30s between each 10s block. The FTT was administered using an Intel Core computer connected to a 19-inch LCD monitor (HP Compaq LA 1956X Monitor, 75 Hz) in a quiet room. The experimental session lasted approximately 10 min. The measures of interest were the tapping rate (i.e., the total number of taps) and intrasubject variability, which was obtained after calculating the coefficient of variation (CoV) (Hultsch et al., 2008). CoV is the standard measure of intrasubject variability and is computed by dividing the individual's standard deviation (SD) by his/her mean (SD/mean). Higher CoV indicated more inconsistent performance across trials.

2.3. *Functional, cognitive and risk factors for cardiovascular disease procedures*

Before performing the FTT, all subjects underwent a comprehensive neuropsychological battery. The same battery was used in all three recruitment centers. The memory domain included the Free and Cued Selective Reminding Test (FCSRT; Grober & Buschke, 1987), the language domain comprised of the Boston Naming Test (Kaplan, Goodglass, & Weintraub, 1983) and a Semantic Fluency Task (Roth, 2011); the visual perception domain contained the Number Location subtest of the VOSP battery (Warrigton & James, 1991), and the executive functions domain consisted of the Trail Making Test forms A and B (TMT-A/B; Reitan, 1958), the Stroop Test (Stroop, 1935), and the Symbol Digit Modalities Test (SDMT; Smith, 1968). Furthermore, as cardiovascular risk factors may have a significant impact on motor functioning, the clinical histories of the participants were reviewed to record information on the presence or absence of hypertension, diabetes, hyperlipidemia, smoking history and history of stroke or myocardial infarction. Body mass index was calculated from height and weight measurements. Frequency of involvement in physical activities was assessed by responses to questions from a leisure activities questionnaire (Karp et al., 2006).

2.4. Apolipoprotein E (APOE) analysis and determination of CSF biomarkers

Genomic DNA was extracted from peripheral blood of probands using the QIAamp DNAblood minikit (Qiagen AG, Basel, Switzerland). APOE genotyping was performed by polymerase chain reaction amplification and HhaI restriction enzyme digestion.

All subjects underwent a lumbar puncture between 9 a.m. and 12 p.m. to collect 10 ml of CSF. The samples were centrifuged and stored in polypropylene tubes at −80 °C within the first hour after extraction. CSF $A\beta_{42}$ levels, tau and p-tau were measured by enzyme-linked immunosorbent assay kits (Innogenetics, Ghent, Belgium). Cut-off values of abnormality for each CSF biomarker were defined according to previous work (Valech et al., 2015): a) $A\beta_{42} \leq 550$ pg/ml, b) tau ≥ 400 pg/ml for subjects between 50 and 70 years old, and ≥450 pg/ml for subjects older than 70 years, and c) p-tau ≥ 75 pg/ml. Both the FTT administrator and the study participants were blind to the *APOE and* CSF results.

2.5. *Statistical analyses*

As the distribution of tapping intrasubject variability was positively skewed, we applied a logarithmic transformation (log10; Tabachnick & Fidell, 1996) in order to obtain normality so as to be able to use parametric analyses (e.g., regression models). Since Group × Hand interactions were not significant all the analyses of tapping performance included data from both hands. Statistical significance was set at $p < .05$.

Analyses of variance (ANOVA), Fisher's exact tests, and T-Tests were carried out to compare demographic and clinical data, and CSF levels of biomarkers in the CTR, Aβ+, and AD groups. Analyses with FTT and cognitive measures were performed controlling for the effects of the demographic variables (age, gender and educational level) with *post-hoc* Bonferroni corrections. Analyses of covariance (ANCOVA) were performed to compare cognitive performance in the CTR and the Aβ+ group, and tapping speed and intrasubject variability performances across the three groups.

Multiple regression analyses were performed with either intrasubject variability or speed as the outcomes, and including AD biomarkers as independent variables. A series of models examined $A\beta_{42}$, tau, and p-tau alone (models 1–3), in combination (model 4), and in interaction with each other (models 5–6). Also, a logistic regression model including both tapping speed and intrasubject variability as dependent variables and the group factor (CTR *vs* Aβ+ group) as the independent variable was also performed. Potential confounders such as age, gender, education, APOE status, the time interval between FTT and lumbar puncture, and the GDS score were included in all the regression models.

Receiver Operating Characteristic (ROC) curves were used to compare the diagnostic accuracy of the FTT measures in discriminating between Aβ+ and AD groups from controls and between Aβ+ and AD groups. Finally, correlations were employed to evaluate the relationship between FTT

performance and the cognitive test battery in CN subjects and correlations between FTT measures and cardiovascular risk factors were also studied.

3. Results

3.1. Demographics and clinical characteristics

There were no statistically significant differences in terms of age, years of education and gender between the CTR, Aβ+ and AD groups. No differences on SCD frequency were found ($\chi^2 = 1.46$, $p = .23$) between CTR (56%) and Aβ+ (40%) groups. As expected, the CSF $A\beta_{42}$ levels were significantly higher in the CTR group than in the Aβ+ and the AD groups. The CTR and the Aβ+ groups showed no differences in tau and p-tau levels, and differed significantly from the AD group. Percentage of APOE ε4 carriers was significantly higher in Aβ+ (40%) and AD (53%) groups, compared to CTR (11%) group. Specifically, the frequency of APOE ε4 carriers was significantly higher in the Aβ+ when compared with the CTR group ($\chi^2 = 6.655$, $p < .05$). Demographics, CSF AD biomarker levels and APOE ε4 allele distributions are shown in the Table 1.

3.2. Finger tapping performance

An overall difference between groups was found both in tapping speed [$F(2,66) = 19.37$, $p < .01$] and in intrasubject variability [$F(2,66) = 11.40$, $p < .01$]. More specifically, the Aβ+ group showed lower speed [$F(1,52) = 5.33$, $p < .05$] and greater intrasubject variability [$F(1,52) = 8.48$, $p < .01$] than the CTR group, and higher speed than the AD group [$F(1,30) = 13.61$, $p < .01$]. The Aβ+ and AD groups did not differ in terms of intrasubject variability [$F(1,30) = 2.65$, $p = .114$]. At the same time, the AD group showed lower speed [$F(1,47) = 35.72$, $p < .01$] and greater intrasubject variability [$F(1,47) = 19.01$, $p < .01$] than the controls (see Fig. 1A, Table 2).

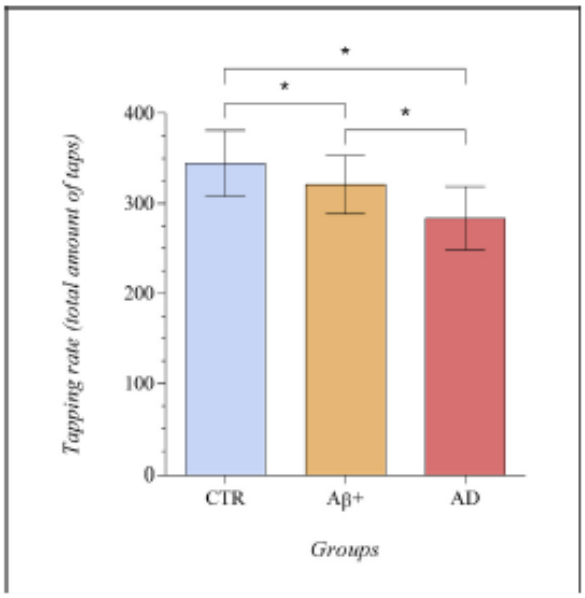

Fig. 1 – Group comparisons of tapping rate for each of the three groups. *p<0.05.

3.3. The relationship between CSF AD biomarkers and tapping performance

Multiple regression analyses with speed ($\beta = .263$, $p < .05$) or intrasubject variability ($\beta = -.558$, $p < .01$) as the outcomes revealed statistically significant associations between these measures and CSF $A\beta_{42}$ levels after ruling out potential confounding effects such as age, gender, education, APOE status, the time interval between FTT and lumbar puncture, and the GDS score. These associations remained significant after the inclusion of tau in the regression model ($\beta = .260$, $p < .05$, for speed; and $\beta = -.551$, $p < .01$, for intrasubject variability). The

Table 1 – Demographics, CSF biomarker levels and APOEε4 status.

Parameter	CTR (n = 37)	Aβ+ (n = 20)	AD (n = 15)	ANOVA				
				F	Effect size	p	Post Hoc comparison[b]	p
Age	64.7 (6.4)	66.5 (7.7)	67.3 (8.5)	.836	.024	.438	–	–
Education (years)	11.7 (4.3)	11.2 (4.3)	10.8 (5.1)	.238	.007	.789	–	–
% female	64.8% (24/37)	70.0% (14/20)	60.0% (9/15)	.384[a]	.073	.825	–	–
$A\beta_{42}$ (pg/ml)	822.9 (177.8)	442.9 (103.9)	368.6 (109.2)	70.80	.672*	.001**	CTR versus Aβ+	.001**
							CTR versus AD	.001**
							Aβ+ versus AD	.439
tau (pg/ml)	232.1 (76.0)	232.7 (115.9)	624.1 (217.1)	55.98	.619*	.001**	CTR versus Aβ+	1.00
							CTR versus AD	.001**
							Aβ+ versus AD	.001**
p-tau (pg/ml)	52.6 (12.7)	47.8 (20.2)	94.3 (26.4)	33.41	.492*	.001**	CTR versus Aβ+	1.00
							CTR versus AD	.001**
							Aβ+ versus AD	.001**
% APOEε4	11.7% (4/37)	40.0% (8/20)	57.1% (8/14)[c]	12.70[a]	.423	.002**	CTR versus Aβ+	.013*
							CTR versus AD	.001**
							Aβ+ versus AD	.247

Data are presented as Means (Standard Deviation).
*p < .05. **p < .01.
[a] Chi-square value.
[b] Bonferroni correction to adjust for multiple comparisons.
[c] Missing APOE data for one of the AD subjects.

Table 2 – Motor performance in the Finger Tapping Task and group comparisons.

FTT measures	CTR (n = 37)	Aβ+ (n = 20)	AD (n = 15)	F	Effect size	p[a]	Post-hoc	p[a]
Tapping speed	344.37 (36.6)	320.70 (32.3)	283.53 (35.1)	19.37	.370*	.001	CTR versus Aβ+	.022*
							CTR versus AD	.001**
							Aβ+ versus AD	.001**
Tapping variability[b]	−.75 (.21)	−.57 (.19)	−.46 (.19)	11.40	.257*	.001	CTR versus Aβ+	.003**
							CTR versus AD	.001**
							Aβ+ versus AD	.126

Data are presented as Means (Standard Deviation). Key: Tapping speed: total number of taps; Tapping variability: coefficient of variation (SD/mean, CoV) of the time interval between taps.
*p < .05. **p < .01.
[a] ANCOVA (controlling for gender, age, and educational level).
[b] Log10 scaled.

contributions of tau or p-tau levels on tapping performance were not significant. After the inclusion of the interaction terms between tau and $A\beta_{42}$ biomarkers to the regression model, the contribution of the explained variance was significant (β = −.302, p < .05, for speed; and β = −.375, p < .01, for intrasubject variability). Regression models and β coefficients with 95% CIs are presented in Table 3. A scatter plot including the association between $A\beta_{42}$ and tapping rate is shown in Fig. 1B.

A logistic regression model including both tapping speed and intrasubject variability showed that intrasubject variability made a unique contribution to the prediction of $A\beta_{42}$ abnormality (CTR *vs* Aβ+), over and above the effect of tapping rate (B = 4.115; p < .05).

3.4. ROC curves of tapping rate and intrasubject variability

ROC curves of tapping rate and intrasubject variability for discriminating the Aβ+ group from controls yielded an area under the curve (AUC) of .654 (p = .057) and .731 (p < .01), respectively (see Fig. 2A). Optimal cut-off scores were 342.5 for tapping rate and .76 for intrasubject variability. ROC curves for discriminating the AD group from controls yielded an AUC of .875 and .836 (both p < .01), for rate and intrasubject variability, respectively (see Fig. 2B). In this case, optimal cut-off scores were 309.5 for tapping rate and .70 for intrasubject variability. Finally, ROC curves for discriminating the Aβ+ group from AD yielded an AUC of .768 (p < .01) and .670 (p = .89), for rate and intrasubject variability, respectively (see Fig. 2C). Optimal cut-off scores were 296.5 for tapping rate and .57 for intrasubject variability.

3.5. Functional, cognitive and cardiovascular disease variables

The CTR and the Aβ+ groups did not show significant differences on any of the neuropsychological (functional and cognitive) measures (with p-values ranging from .124 to 928). Performance in FTT was not correlated with any of the cardiovascular risk factors studied (p-values ranging from .200 to 543 for tapping rate and .202 to .906 for intrasubject variability).

3.6. Relationships between FTT measures and neuropsychological tests in CN subjects

Tapping rate and intrasubject variability correlated with standard tests of visuomotor, attention and executive functions, such as TMT-A (r = −.507, p < .01 and r = .352, p < .01,

Table 3 – Multiple regression analyses with variability or speed as the outcomes and CSF AD biomarkers as the independent variables.

Models	CSF factor	Tapping rate			Tapping variability[a]		
		β	(CI 95%)	p	β	(CI 95%)	p
Model 1	$A\beta_{42}$	.263	(.006 – .080)	.023*	−.558	(−.001 – .000)	.001**
Model 2	tau	−.101	(−.080 – .038)	.481	.039	(.000 – .000)	.824
Model 3	p-tau	−.021	(−.475 – .404)	.872	−.094	(−.004 – .002)	.554
Model 4	$A\beta_{42}$	.260	(.002 – .083)	.041*	−.551	(−.001 – .000)	.001**
	tau	−.121	(−.152 – .102)	.692	.085	(−.001 – .001)	.804
	p-tau	−.019	(−.924 – .990)	.946	−.043	(−.007 – .006)	.892
Model 5	$A\beta_{42}$ x tau	.070	(.000 – .000)	.517	−.302	(.000 – .000)	.019*
Model 6	$A\beta_{42}$ x p-tau	.137	(.000 – .001)	.180	−.375	(.000 – .000)	.002**

Key: β: standardized coefficient; CI: Confidence interval.
*Associations that remained significant (p < .05) after including potential confounding effects.
**Associations that remained significant (p < .01) after including potential confounding effects.
[a] Log10 scaled.

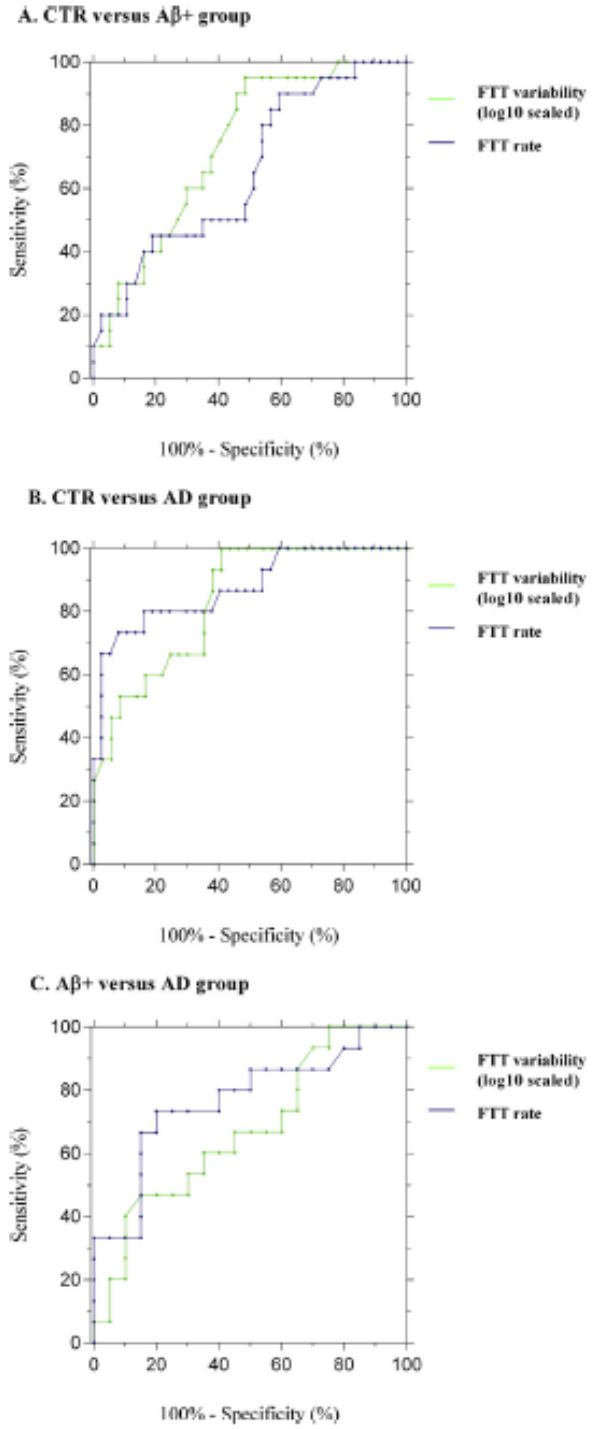

Fig. 2 – ROC curves of tapping rate and variability for the prediction of amyloid abnormality (A) and AD (B) and for discriminating the Aβ+ group from AD (C).

respectively), TMT-B ($r = -.421$, $p < .01$ and $r = .324$, $p < .05$), SDMT ($r = .391$, $p < .01$ and $r = -.433$, $p < .05$) and Stroop Color-Word subtest ($r = .348$, $p < .01$ and $r = -.500$, $p < .01$). Also, FTT rate showed a significant correlation with the BNT ($r = .402$, $p < .01$).

4. Discussion

Our results suggest that motor dysfunction is associated with amyloid pathology and may subtly emerge during the earliest stage of the Alzheimer's continuum. Considering that the standard neuropsychological tests suffer from insufficient sensitivity in healthy controls, several authors suggested that the detection of subtle deficits should be based on the application of more accurate tests (Rentz et al., 2013). In this line of research, our results emphasize the importance of applying adequate measures of specific brain functions to find early AD-related dysfunctions in apparently healthy adults.

A computer-based tapping task such as the one used in the present study may be suitable for detecting subtle motor difficulties in an otherwise asymptomatic population. In this regard, the non-invasive method employed in our study, the tapping task, proved to be easy enough to be quickly understood, brief enough not to generate fatigue, and easy to administer independently of educational level, which is known to be a main confounder in the study of cognitive deficits (Stern, 2009). In contrast with other measures used both in the present and in previous studies, the large amount of data points obtained for the calculation of CoV makes this measure a good candidate for the study of subtle motor deficits in relatively small preclinical sample groups, thus compensating for the intrinsic difficulty of recruiting participants in this kind of population.

The results reported here are in line with recent studies suggesting that motor dysfunction may be a sensitive marker of the preclinical phases of AD (Albers et al., 2015; Buchman & Bennett, 2011). Also, our statistical models show that both amyloid alone and the interaction between amyloid and pathologic tau contributed to tapping performance while tau biomarkers alone did not. These results support the current research criteria (Jack et al., 2018), which suggest that abnormal Aβ biomarkers alone could serve as the defining signature of the AD continuum and that both Aβ and pathologic tau biomarkers together characterize Alzheimer's disease. A plausible interpretation of our findings might be that motor dysfunctions are related to an initial amyloidosis, which is, according to the 'amyloid cascade hypothesis' (Jack et al., 2010), the earliest biomarker at the asymptomatic stage of AD. The mechanisms by which amyloid pathology could lead to the motor dysfunctions in the preclinical phases of AD still remain unknown. However, some neuropathological studies suggest that AD pathology may be early present in many cortical areas including regions that subserve motor functions (Schneider et al., 2006; Suva et al., 1999). This is true not only in AD patients (Arnold, Hyman, Flory, Damasio, & Van Hoesen, 1991) but in CN subjects as well (Giannakopoulos, Hof, Michel, Guimon, & Bouras, 1997). Here, it is also worth mentioning that tapping performance is not merely automated motor activity (Amboni, Barone, & Hausdorff, 2013). Indeed, the internal generation of movement requires executive components such as initiation and sustained attention in order to execute an action and maintain it to achieve the task goals. Consistent with these assumptions, our analyses of correlations between tapping performance and the traditional neuropsychological tests

revealed that the former was especially associated with tests assessing attention and executive functions. These functions are also related to areas where amyloid deposition is early sited (Grothe et al., 2017). Taken together, our results suggest that the presence of AD pathology could lead to the motor dysfunction observed in the Aβ+ subjects.

To our knowledge, very few studies have investigated intrasubject variability in the AD continuum. These previous studies suggested that intrasubject variability might also be a relevant marker of early AD (Bangert & Balota, 2012; Verghese et al., 2008, 2007). For instance, Verghese et al. (2008) reported that individuals with mild AD presented greater intrasubject variability when walking than did healthy adults. Our results are even more consistent with a study showing that a group with AD exhibited greater intrasubject variability during the execution of tapping movements than a group of healthy older adults (Bangert & Balota, 2012). In line with these previous works, we also report an increase in intrasubject variability in the Aβ+ and AD groups suggesting the potential role of this less studied motor measure in distinguishing these populations from controls.

Also, when comparing the role of tapping rate versus intrasubject variability in determining the classification of controls and CN individuals with amyloid-β positivity, intrasubject variability was found to be the only variable that made a unique contribution to the prediction of amyloid abnormality. In this regard, a previous study found that intrasubject variability during a psychomotor task, predicted the classification of MCI versus healthy elders, beyond the effects of speed (Dixon et al., 2007). When comparing the diagnostic accuracy of the FTT measures, both aspects of motor performances had an important role in AD discrimination at different stages of the disease. However, intrasubject variability showed to be more accurate than tapping rate at the earliest stage of the AD continuum.

Importantly, the groups tested in the present study did not differ in terms of cardiovascular risk factor status, and performance in FTT was not associated with any of these factors. However, since we focused on tapping performance, additional motor measures might be necessary to characterize the groups' motor status. Furthermore, considering the cross-sectional nature of our study and that the current research criteria for defining preclinical AD require the presence of both amyloid and tau biomarkers, our findings preclude the possibility of determining whether amyloid-related motor difficulties are also associated with subsequent cognitive impairment due to AD. Also, considering the between-methods variability for measuring CSF amyloid levels and the small sample size of Aβ+ subjects, the Aβ+ group may not be representative of typical amyloid positive CN subjects. Another important issue refers to the extrapolation of the present results to older populations. Here, it is important to take into account the increasing prevalence of cardiovascular risk factors with age and the impact of these variables on motor performance. Although our sample ranged between 50 and 80 years and performance in FTT was not correlated with any of the cardiovascular risk factors, we cannot ascertain that the present results are generalizable to older subjects. Finally, although motor dysfunctions were found in otherwise asymptomatic individuals, these results do not imply that subtle dysfunctions cannot be found in domains other than motor control when they are appropriately tested (perhaps with the use of more sensitive methods). Further research is called for regarding the sensitivity of intrasubject variability in tests for psychomotor and/or cognitive dysfunction in preclinical AD.

In conclusion, the present results suggest that the newly developed version of FTT may be a useful test able to detect subtle motor deficits in CN individuals with amyloid-β positivity and that both speed and intrasubject variability are associated with CSF $A\beta_{42}$ levels. Our findings suggest that an accurate assessment of motor functions could help on identifying individuals at the earliest stage of the Alzheimer's continuum.

Disclosure of conflicts of interests

The authors declare no financial or other conflicts of interests.

CRediT authorship contribution statement

Maria A. Mollica: Methodology, Formal analysis, Investigation, Writing - original draft. **Adrià Tort-Merino:** Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Jordi Navarra:** Methodology, Conceptualization, Funding acquisition, Writing - original draft. **Irune Fernández-Prieto:** Conceptualization, Formal analysis, Writing - original draft. **Natalia Valech:** Formal analysis, Investigation. **Jaume Olives:** Formal analysis, Investigation. **María León:** Formal analysis, Investigation. **Alberto Lleó:** Resources, Writing - review & editing. **Pablo Martínez-Lage:** Resources, Writing - review & editing. **Raquel Sánchez-Valle:** Resources, Writing - review & editing. **José L. Molinuevo:** Methodology, Resources, Writing - review & editing. **Lorena Rami:** Methodology, Conceptualization, Supervision, Funding acquisition, Writing - original draft, Writing - review & editing.

Open practices

The study in this article earned Open Materials badge for transparent practices. Materials and data for the study are available at https://osf.io/2kje8/.

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Trabajo número 4:

Accelerated long-term forgetting over three months in asymptomatic APOE ε4 carriers

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Accelerated long-term forgetting over three months in asymptomatic APOE ε4 carriers

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Abstract

Accelerated long-term forgetting (ALF) refers to a rapid loss of information over days or weeks despite normal acquisition/encoding. Notwithstanding its potential relevance as a presymptomatic marker of cognitive dysfunction, no study has addressed the relationship between ALF and Alzheimer's disease (AD) biomarkers. We examined ALF in APOE ε4 carriers vs. non-carriers, and its relationships with AD cerebrospinal fluid (CSF) biomarkers. We found ALF over three months in APOE ε4 carriers ($F(1,19)=5.60$; $p<0.05$; Cohen's $d=1.08$), and this performance was associated with abnormal levels of the CSF $A\beta_{42}$/ptau ratio ($r=-.614$; $p<0.01$). Our findings indicate that ALF is detectable in at-risk individuals, and that there is a relationship between ALF and the pathophysiological processes underlying AD.

Keywords: accelerated long-term forgetting; biomarkers; memory; early detection

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Introduction

Accelerated long-term forgetting (ALF) has been defined as a rapid loss of information over days or weeks despite normal acquisition and encoding. Recently, the potential usefulness of ALF in the early detection of subtle cognitive difficulties in asymptomatic stages of familial[1] and sporadic[2,3] Alzheimer's disease (AD) has attracted particular attention. Two recent studies showed larger ALF in autosomal dominant disease mutation carriers[1] and asymptomatic individuals with increased genetic risk (APOE ε4 carriers)[3] over a 1-week retention period.

Despite the potential relevance of ALF as a presymptomatic marker of subtle cognitive dysfunction, no study has yet addressed the relationship between ALF and AD biomarkers, or even evaluated ALF over periods beyond one week. To fill this gap, we employed an effortful and cognitively demanding associative memory task to explore ALF during a 6-month follow-up in cognitively healthy carriers vs. non-carriers of the APOE ε4 haplotype. Moreover, we examined the relationship between ALF and AD cerebrospinal fluid (CSF) biomarker levels. The possible findings derived from the present work would be of particular relevance for AD prevention trials, as well as for the assessment and monitoring of cognitively unimpaired populations at risk of AD.

Participants and methods

The present participants represent a sub-sample of Tort-Merino et al.[2], and thus the methods description follows that paper. Amongst all the participants included in this previous work, 11 subjects were APOE ε4 carriers (heterozygous for ε3 and ε4). In the present study, these 11 carriers were age-, sex-, and education-matched with 11 non-carriers (homozygous for ε3). The study was not pre-registered, and thus the present analyses are exploratory. All subjects underwent APOE genotyping, a lumbar puncture to determine CSF amyloid-ß ($A\beta_{42}$), total tau (tau), and phosphorylated tau (ptau) levels,

a standard neuropsychological assessment to ensure that they were cognitively normal, and the cognitively demanding Ancient Farming Equipment Test (AFE-T) to tap learning and long-term retention at 1 week, 3 and 6 months. Participants were blind to APOE status and CSF results. The mean time lapse between the lumbar puncture and the AFE-T assessment was 2.09 (1.4) years. The neuropsychological battery encompassed four cognitive domains (memory, language, perception and executive functions; see Tort-Merino et al.[2] for tests details), and it included the Cognitive Reserve Questionnaire (CRQ) for assessing cognitive reserve[4] and the Subjective Cognitive Decline Questionnaire (SCD-Q)[5] for measuring cognitive concerns.

The AFE-T calls for learning novel object/name pairs: it contains 24 black-and-white images of unfamiliar ancient farming equipment taken from the AFE paradigm[6] which are paired with a pseudoword. The test consists of two initial learning sessions administered on two consecutive days. Each learning session include seven learning runs (range of performance score 0-24 points per run). The AFE-T provides two final learning outcomes: (1) the free learning score (FLS) and (2) the cued learning score (CLS). Long-term recall is examined one week, three months and six months after the initial learning phase, including a visual recognition task, free recall and cued recall (participants are not told in advance that they will be reassessed). Forgetting rates for free and cued recall scores at 1 week, 3 months and 6 months were obtained with comparisons to the initial learning phase. The forgetting rate was defined as one minus the ratio between each delayed score (free or cued) and the score obtained on the FLS (for free forgetting rates) or CLS (for cued forgetting rates) [e.g., 1-(one-week free recall score / FLS), for one-week free forgetting rate; and 1-(one-week cued recall score / CLS), for one-week cued forgetting rate]. Recall rates at 3 months and 6 months were also compared with the 1-week recall session. At the end of the 6-month session, a

verbal recognition task was given. Concerning the APOE carriers, one subject did not obtain the CLS and three subjects refused to complete the 6-month assessment.

In order to mathematically model the forgetting functions, we followed previous recommendations[7–9]. Forgetting curves are characterized by a curvilinear relation that shows a rapid initial decline of information that is followed by a slower and longer decay, thus showing that information is lost in a larger extent after initial encoding. Previous studies on forgetting have shown that these functions (power and logarithmic) are the most accurate ones to describe forgetting curves. Information decay with time in our sample was fitted better using a logarithmic function [(y=a–bln(time)] when compared to a power function. Thus, each data point (4-time data points) for each subject and condition (free and cued recall conditions) was fitted using a non-linear least-squares regression. Fit parameters were calculated based on the residual sum of squares and showing the proportion of data variance accounted for (R^2). The *slope* (parameter *b*) and *intercept* (parameter *a*) were computed separately for each subject and condition. The *slope* captures the forgetting rate of encoded information while the *intercept* represents the estimated initial level of performance (immediately after the last learning run).

Demographical data, levels of CSF Aß$_{42,}$ CSF tau and CSF ptau were compared using Student *t*-tests for independent samples and *Chi*-square analyses when appropriate. Analysis of variance (ANOVA) with post-hoc Bonferroni corrections and effect sizes expressed as Cohen's *d* were conducted to compare the between-group scores on the AFE-T and the standard neuropsychological tests. Magnitude scale for Cohen's *d* has been suggested as: $d = 0.2$, small effect size, $d = 0.5$, medium effect size and $d = 0.8$, large effect size[10]. The AFE-T scores included in the analyses were the two final learning scores (i.e., FLS and CLS) and the forgetting measures (raw scores and

forgetting rates). Pearson's bivariate correlations were used to assess the association between the forgetting measures (including the *slope* parameter from the modeled functions) with AD CSF biomarkers and subjective cognitive ratings.

Results

Group differences between APOE ε4 carriers and non-carriers

We observed no group differences in age, sex, years of education or cognitive reserve. Regarding the AD biomarkers, there were no differences in CSF tau or p-tau levels, but CSF $A\beta_{42}$ levels were significantly lower ($t(20)$=3.98; p<0.01) in the APOE ε4 carrier group (Table 1). There were no differences in any of the standard neuropsychological tests. Neither did the AFE-T exhibit group differences in the learning scores (Table 1).

Modeling of forgetting curves

Figures 1A and 1B show the forgetting curves as well as the decaying information function modeled for the whole group. Importantly, the fit of the logarithmic function to the group data was nearly perfect in both groups, slightly better for the non-carriers (R^2=99% of variance explained) compared to carriers (R^2=97%). Particularly for cued recall (Fig. 1B), the *slope* (forgetting rate parameter) was steeper for the carriers (b=-9.1) compared to non-carriers (b=-6.6). Importantly for the convergent validity of the different forgetting measures used (information decay mathematical model and forgetting rates at each time point, see results below), the 3-month cued forgetting rate and the *cued slope* were strongly correlated (r=-.796; p<0.01).

Forgetting rates at 1-week, 3- and 6-months

We computed forgetting rates as in previous studies of ALF[1,3] (see Figs. 1C and 1D). At 3 months, cued recall score was significantly lower (F(1,19)=4.99; p<0.05; Cohen's d=0.99) in the carriers than in the non-carriers (Table 1; Fig. 1B). Besides, the APOE ε4

carriers evidenced ALF in their 3-month cued forgetting rate ($F(1,19)=5.60$; $p<0.05$; Cohen's $d=1.08$), as well as in their cued forgetting rate between the 1-week and 3-month recall ($F(1,19)=8.70$; $p<0.01$; Cohen's $d=1.32$; Post-hoc power = 0.81) (Table 1; Fig. 1D). No significant group differences were found on any of the free forgetting rates (Table 1; Fig. 1C) or on the recognition scores (Table 1).

Correlational analyses

Correlational analyses in the whole sample showed a significant negative correlation between the 3-month cued forgetting rate and the ratio CSF $A\beta_{42}$/ptau (r=-.614; $p<0.01$; Fig. 2A). This correlation remained significant after controlling for potential confounders such as age (r=-.481; $p<0.05$), years of education (r=-.606; $p<0.01$) or the delay between CSF AD biomarkers acquisition and AFE-T testing (r=-.548; $p<0.05$). Besides, a significant positive correlation between the *cued slope* (from the modeled function in each individual) and the ratio $A\beta_{42}$/ptau (r=.458; $p<0.05$; Fig. 2B) was found, again indicating that higher forgetting was associated to lower (more pathologic) levels of CSF AD biomarkers.

A significant positive correlation was found between the 3-month cued forgetting rate and the SCD-Q score (r=.636; $p<0.01$; Fig. 2C), and a significant negative correlation was found between the *cued slope* and the SCD-Q (r=-.508; $p<0.05$; Fig. 2D), showing that higher forgetting was associated with higher cognitive concerns.

Discussion

By using a new highly demanding associative memory test, AFE-T, we examined ALF in cognitively healthy APOE ε4 carriers and its relationships with AD CSF biomarkers. The results revealed ALF in cued recall over three months in our well-characterized sample of APOE ε3/ε4 heterozygotes. Moreover, this performance was positively associated with core AD CSF biomarkers and subjective cognitive scores.

The effects of APOE ε4 on memory performance in clinically normal adults and its relationship with amyloid burden have been widely studied[11–13]. As expected, our results showed that APOE ε4 carriers presented lower CSF Aβ levels than non-carriers (Table 1). However, data on the link between ALF and AD is still limited. Recent evidence from animal research showed accelerated forgetting with intact learning performance in a study using a model of familial AD (pre-pathological PDAPP mice)[14]. In humans, ALF has been proposed as a potential cognitive marker for asymptomatic stages of AD. In an earlier report, our group provided evidence for subtle learning dysfunction and long-term forgetting in preclinical AD subjects by employing the AFE-T[2]. A more recent study on a cohort of autosomal dominant AD families showed one-week ALF in presymptomatic mutation carriers by using three standard cognitive tests[1]. Moreover, Zimmerman & Butler[3] revealed ALF in asymptomatic APOE ε4 carriers by assessing 60 participants (20 homozygous for ε3, 20 heterozygous for ε3 and ε4, and 20 homozygous for ε4) with a standard memory test. Participants were asked to learn a 15-word list from the Rey Auditory Verbal Learning Test (RAVLT)[15] on four consecutive trials to an 80% accuracy criterion. In their analyses, APOE status had no effects on memory encoding or short-term recall, but was associated with long-term forgetting over one week.

In line with Zimmermann & Butler[3], we found intact learning and ALF in APOE ε4 carriers. However, here we employed AFE-T in order to explore participants' cognition in a more comprehensive way. AFE-T requires forming new associations or binding information without previous semantic knowledge, which is setting high demands on cognitive processing[16], especially compared to other standard episodic memory tasks. Regarding the learning performance, AFE-T allowed a deeper analysis of encoding processes by yielding free and cued recall scores. Another strength is that we did not

predefine any learning accuracy criterion which limits scoring range and thereby the chances for finding between-group differences. The setup with AFE-T also allowed exploration of free and cued recall for a longer time period, tracking ALF over 6 months after the learning phase. While the one-week session appeared to be too close to initial learning, the 3-month session was the optimal time-point to find ALF in our APOE ε4 carriers, and cued recall measures showed higher sensitivity than spontaneous naming. Although the APOE ε4 carriers also performed worse than non-carriers on the free recall, the lack of statistically significant between-group differences in these measures might be related to task difficulty and the floor effects observed beyond the 1-week session. Also, the lack of significant between-group differences in the 6-month session might be due to the missing data in the APOE carriers group, which would also explain the slight improvement of this group compared to its performance at 3 months.

Importantly, both groups fitted the logarithmic function nearly perfect and particularly for cued recall, the APOE ε4 carriers showed a steeper slope (comprehensive forgetting measure) compared to non-carriers, indicating an overall tendency to show larger information loss after the initial learning in the carrier group, especially in between the first week and the 3-month period. It is important to note that the slope parameter from the employed function strongly correlated with the AFE-T forgetting rate at 3 months, demonstrating that both measures are clearly associated and again pointing out to the idea that the information is lost to a larger extent during the initial period after encoding. These results may also explain why the correlations found are larger with the 3-month forgetting rate and the slope. After three months the rate of information loss is very small and therefore it might be futile to evaluate ALF beyond this time point. Taken together, our findings speak for the use of more sensitive measures with longer follow-up designs when studying cognitively healthy at-risk subjects. ALF is emerging

as a cognitive feature of presymptomatic AD, highlighting the utility of long-term recall designs on monitoring cognitive changes in this population.

Similarly to the previous studies[1,3], we found a relationship between self-perceived subjective cognitive decline and ALF. This is an important finding since subjective cognitive concerns have been suggested as a risk factor for cognitive decline and dementia[17,18]. Furthermore, the present results showed a strong correlation between ALF (measured by cued forgetting rates and the slope parameter from the AFE-T) and the CSF $A\beta_{42}$/ptau ratio. Importantly, CSF $A\beta_{42}$ and CSF ptau levels are valid indicators of the abnormal protein deposits underlying AD pathophysiology (i.e., β-amyloid plaques and neurofibrillary tangles, respectively) and define AD as a specific neurodegenerative disease amongst other cognitive disorders[19]. Our finding on the correlation between ALF as measured by AFE-T and the ratio CSF $A\beta_{42}$/ptau provides further evidence for ALF as a promising candidate for a specific marker of AD-related subtle cognitive decline in presymptomatic stages of the disease. Finally, it is important to note that given our relatively small sample size, validation of the present results is called for in future studies including larger samples and more robust power analyses.

In sum, ALF is detectable in at-risk individuals, and there is a relationship between this cognitive measure and the pathophysiological processes underlying AD. We strongly recommend the use of more demanding cognitive tests including long-term forgetting measures for identifying and tracking the earliest cognitive manifestations in presymptomatic AD. The present results show that accelerated forgetting is able to capture cognitive dysfunction earlier in the AD continuum. Thus, they are particularly relevant for AD prevention trials, in which regulatory agencies require a change in cognition /functional status and not only a biomarker change.

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Author Contributions

A.R.F, L.R., M.L., and A.T.M. contributed to the conception and design of the study; all authors contributed to acquisition and analysis of the data; and A.T.M., A.R.F., M.L., and L.R. contributed to drafting a significant portion of the manuscript. All authors have reviewed the manuscript and approved the final version.

Potential Conflicts of Interest

Nothing to report

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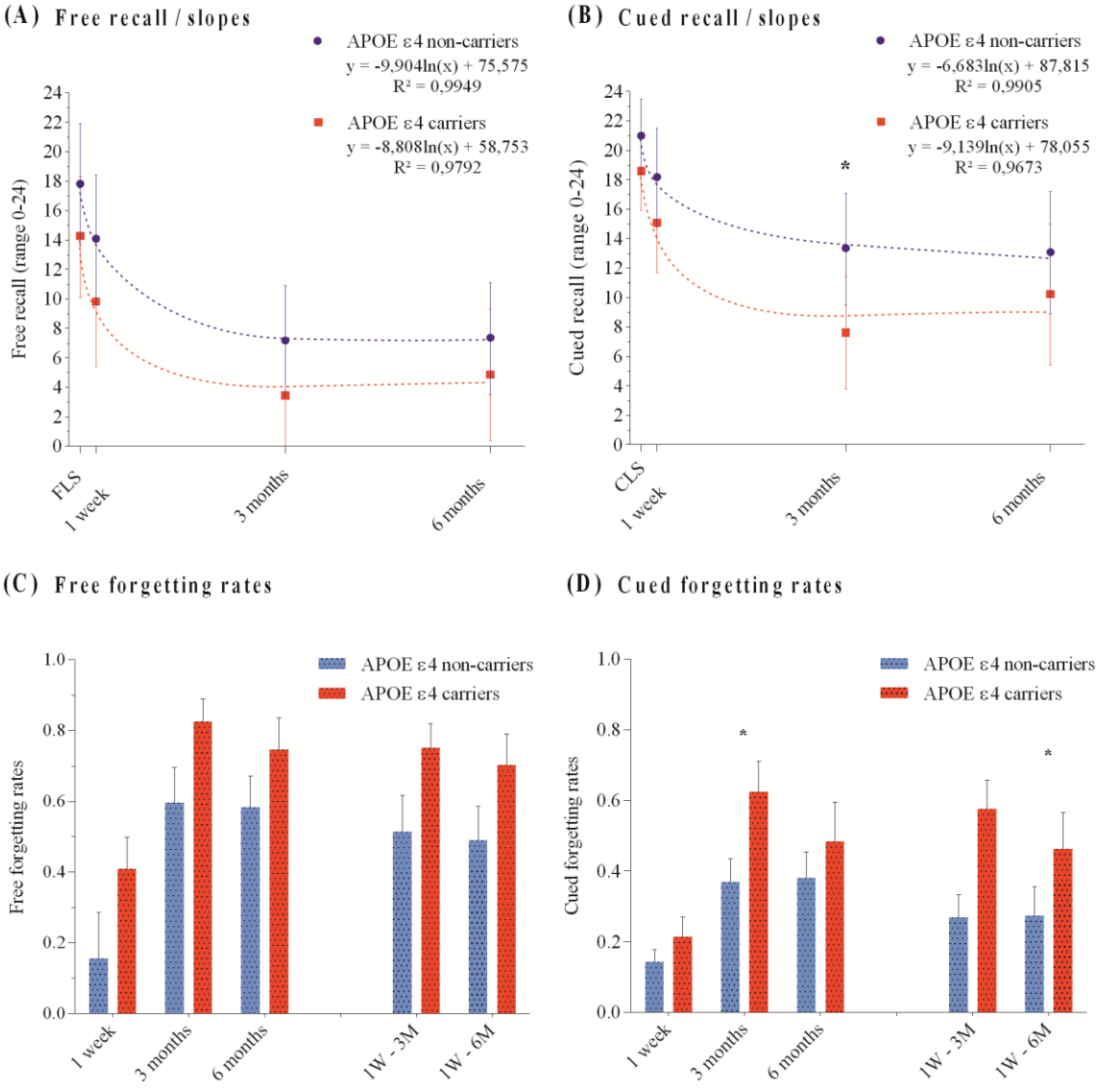
Table 1. Demographics, CSF levels and AFE-T results in the APOE ε4 carriers vs. non-carriers.

Parameters	APOE ε4 non-carriers	APOE ε4 carriers	*F*	*p* [a]
Demographics				
Gender (% women)	72.7%	72.7%	0.00 [b]	1.00
Age	65.1 (SD 6.5) [58-75]	65.8 (SD 6.9) [56-77]	-0.23	.814
Years of education	11.0 (SD 3.3) [8-18]	12.2 (SD 4.3) [8-20]	-0.78	.446
CRQ	16.4 (SD 3.9) [9-22]	15.4 (SD 5.5) [6-22]	0.46	.651
CSF levels				
$A\beta_{42}$	873.9 pg/ml (SD 294.1)	470.5 pg/ml (SD 163.1)	3.98	.001**
Tau	247.4 pg/ml (SD 98.7)	307.1 pg/ml (SD 95.2)	-1.44	.165
Ptau	53.8 pg/ml (SD 14.8)	59.1 pg/ml (SD 15.7)	-0.81	.423
AFE-T				
Free learning score (FLS)	17.8 (SD 6.2)	14.2 (SD 6.8)	1.62	.218
Cued learning score (CLS)	21.0 (SD 3.2)	18.6 (SD 4.8)	1.87	.187
1-week free recall	14.1 (SD 6.5)	9.8 (SD 7.4)	2.05	.168
1-week cued recall	18.2 (SD 4.6)	15.1 (SD 5.9)	1.86	.188
1-week FFR	0.15 (SD 0.4)	0.41 (SD 0.3)	2.54	.127
1-week CFR	0.14 (SD 0.1)	0.21 (SD 0.2)	1.21	.285
3-month free recall	7.1 (SD 6.9)	3.4 (SD 4.6)	2.18	.155
3-month cued recall	13.3 (SD 5.5)	7.6 (SD 6.4)	4.99	.037*
3-month FFR	0.59 (SD 0.3)	0.82 (SD 0.2)	3.76	.067
3-month CFR	0.37 (SD 0.2)	0.62 (SD 0.3)	5.60	.029*
6-month free recall	7.3 (SD 6.2)	4.8 (SD 5.4)	0.81	.380
6-month cued recall	13.1 (SD 5.9)	10.2 (SD 7.2)	0.89	.358
6-month FFR	0.58 (SD 0.3)	0.74 (SD 0.2)	1.61	.222
6-month CFR	0.38 (SD 0.2)	0.48 (SD 0.3)	0.67	.423
1-week to 3-month FFR	0.51 (SD 0.3)	0.75 (SD 0.2)	3.52	.076
1-week to 3-month CFR	0.27 (SD 0.2)	0.57 (SD 0.3)	8.70	.008**
1-week to 6-month FFR	0.49 (SD 0.3)	0.70 (SD 0.2)	2.50	.132
1-week to 6-month CFR	0.27 (SD 0.2)	0.46 (SD 0.3)	2.14	.161
1-week visual recognition	48.0 (SD 0.0)	47.5 (SD 1.5)	1.44	.244
3-month visual recognition	46.3 (SD 1.8)	45.7 (SD 1.6)	0.71	.409
6-month visual recognition	46.0 (SD 1.5)	46.1 (SD 0.8)	0.04	.839
6-month verbal recognition	21.9 (SD 2.3)	22.4 (SD 1.6)	0.26	.616

Data are presented as means (SD; standard deviation) [range]. Key: CRQ, cognitive reserve questionnaire; CSF, cerebrospinal fluid; $A\beta_{42}$, amyloid-β isoform 42; Tau, total tau; ptau, phosphorylated tau; FFR, free forgetting rate; CFR, cued forgetting rate.

[a] *P* values were determined by Student's t-test for demographics and CSF levels; [b] X^2 statistic; * *p*< 0.05; ** *p*< 0.01.

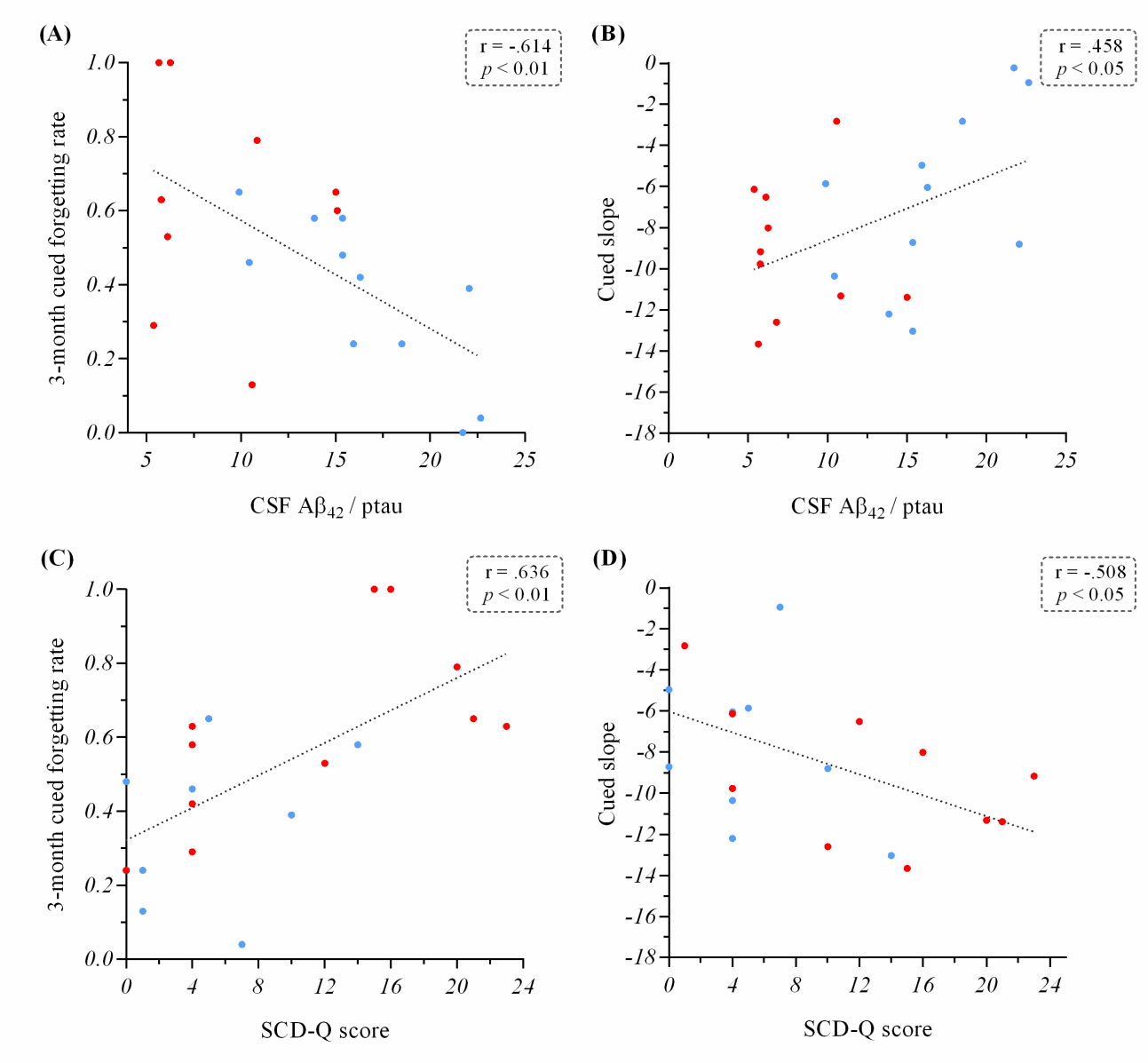
Figure 1. Panel A shows the free recall scores and the free slopes for APOE ε4 carriers and non-carriers. Panel B shows the cued recall scores and the cued slopes. Panels C and D show the free and cued forgetting rates, respectively.



Key: FLS, Free learning score; CLS, Cued learning score; 1W-3M, Forgetting rate between the 1-week and 3-month recall score; 1W-6M, Forgetting rate between 1-week and 6-month recall. Error bars represent 95% CIs.

*** p<0.05**

Figure 2. Panels A and B show the correlation between the ratio CSF $A\beta_{42}$ / phosphorylated tau and the 3-month cued forgetting rate and the cued slope from the AFE-T, respectively. Panels C and D show the correlations between the SCD-Q score and the 3-month cued forgetting rate and the cued slope, respectively.



Key: Blue dots, APOE ε4 non-carriers; Red dots, APOE ε4 carriers; CSF, cerebrospinal fluid; $A\beta_{42}$, Amyloid-β isoform 42; ptau, CSF phosphorylated tau levels; SCD-Q, Subjective Cognitive Decline Questionnaire.

Supplementary Data

Neuropsychological assessment

All participants were assessed with a comprehensive neuropsychological battery, administered by a trained neuropsychologist blind to the CSF and APOE results. The battery encompassed four cognitive domains. The memory domain included the Free and Cued Selective Reminding Test [1], the language domain comprised of the Boston Naming Test [2] and Semantic fluency [3], the visual perception domain contained the number location subtest of the VOSP battery [4], and the executive functions domain consisted of the Trail Making Test [5], the Stroop Test [6], the Symbol Digits Modalities Test [7], and the Digit Span test of the WAIS [8]. Global cognition was assessed with the Mini Mental State Evaluation [9]. Premorbid intelligence was assessed with the Spanish Word Accentuation Test [10].

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Trabajo número 5:

Accelerated long-term forgetting in individuals with subjective cognitive decline and amyloid-β positivity

Tort-Merino A, Valech N, Laine M, Olives J, León M, Ecay-Torres M, Estanga A, Martínez-Lage P, Fortea J, Molinuevo JL, Sánchez-Valle R, Rodríguez-Fornells A Rami L

Submitted

Accelerated long-term forgetting in individuals with subjective cognitive decline and amyloid-β positivity

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Keywords: subjective cognitive decline; early detection; biomarkers; memory; accelerated long-term forgetting.

ABSTRACT

We studied a sample of cognitively unimpaired subjects in order to investigate accelerated long-term forgetting (ALF) in individuals with and without subjective cognitive decline (SCD) and to explore the relationships between objective and subjective cognitive performance and cerebrospinal fluid (CSF) Alzheimer's disease (AD) biomarkers. Fifty-two individuals were included and SCD was quantified through the Subjective Cognitive Decline Questionnaire (SCD-Q), using the validated cutoff of 7 points in the total score to classify subjects as non-SCD (n = 21) or SCD (n = 31). The SCD subjects where further subdivided according to the presence or absence of abnormal levels of CSF $A\beta_{42}$. In order thoroughly explore participants' cognition we employed a new highly-demanding cognitive test: the Ancient Farming Equipment Test (AFE-T). The AFE-T consists on learning novel object/name pairs. Its comprehensive design includes two consecutive learning sessions and a long-term recall phase with free and cued recall at one week, three months, and six months after the initial learning. The SCD group showed a significantly higher free forgetting rate at 3 months compared to the non-SCD ($F(1,44) = 4.72$; $p < 0.05$). When stratifying by amyloid status, SCD/Aβ+ showed a significantly lower performance than SCD/Aβ– on the learning scores and on the 1-week cued forgetting rate and 3-month recall of the AFE-T (all $p < 0.05$). Higher SCD-Q scores predicted higher forgetting rates on the AFE-T. In conclusion, by using more demanding cognitive tasks, it is possible to detect ALF in subjects with SCD, especially in those with abnormal CSF $A\beta_{42}$ levels.

1. Introduction

Subjective cognitive decline (SCD), defined as a self-experienced impairment of cognitive abilities in otherwise cognitively unimpaired subjects, has been suggested to represent an early symptomatic manifestation in Alzheimer's disease (AD) [1]. The identification of subtle cognitive difficulties in cognitively unimpaired individuals within the AD continuum is critical for predicting progression towards later clinical stages. However, given that (by definition) subjects with SCD perform within the normal range on standardized cognitive tests, it seems mandatory to use more sensitive measures in order to explore the earliest cognitive changes in this population.

In recent studies, we employed a new highly-demanding cognitive test (the Ancient Farming Equipment Test; AFE-T) in order to explore accelerated long-term forgetting (ALF) in cognitively unimpaired populations [2, 3]. The concept of ALF – defined as a loss of information over days or weeks despite normal acquisition – has recently emerged in the field of neurodegenerative diseases as a cognitive marker for the presymptomatic stages of AD [4, 5]. To date, the AFE paradigm has been used to study acquisition of new words in groups of healthy adults [6, 7] and in MCI and AD patients [8, 9]. The task engages the declarative memory system in order to associate unknown name/object pairs. Its comprehensive design allows the analysis of learning curves with free and cued measures, and free and cued long-term forgetting rates at one week, three months and six months. In a recent study, we employed the AFE-T to detect subtle cognitive difficulties in the preclinical stage of the Alzheimer's continuum. The AFE-T was found to be a promising tool for characterizing the cognitive profile of preclinical AD and sensitive enough to detect learning difficulties and ALF in this population [3].

Regarding the assessment of SCD, following the Subjective Cognitive Decline Initiative (SCD-I) guidelines [1] we recently developed and validated the Subjective Cognitive Decline Questionnaire (SCD-Q). The SCD-Q has emerged as a potential tool for measuring SCD. In contrast to the pre-existing questionnaires, the SCD-Q explores the perception of decline, as opposed to impairment, in a relatively short period of time (i.e., last 2 years), exploring the self-perceived performance in daily life activities that involve multiple cognitive domains. The questionnaire has been validated, showing high convergent validity, internal consistency, and discriminant power to distinguish between subjects with cognitive impairment and those without [10]. In a previous work, we explored the performance of the SCD-Q in cognitively unimpaired individuals within the Alzheimer's continuum, showing a correlation between SCD-Q scores and cerebrospinal fluid (CSF) AD biomarkers [11]. At the same time, our results have shown high specificity to the preclinical stage of the AD continuum of the SCD-Q items related with language and executive decline [12]. Finally, in a recent work, we explored the associations between gray mater volumes and the SCD-Q scores in a sample of cognitively healthy older adults. Results suggested that the SCD-Q is related to incipient brain changes that may be due to preclinical AD [13] .

In the present study, we explored ALF in subjects with and without SCD. We also aimed to investigate the relationships between subjective and objective cognitive performance considering relevant factors such as AD CSF biomarker levels. We hypothesized that the AFE-T could be highly sensitive to detect possible subtle learning and retention difficulties that would be otherwise undetected by standard neuropsychological tests. We also expected to find higher difficulties in subjects with SCD and Aβ positivity.

2. Methods

2.1. Participants

Fifty-two cognitively unimpaired subjects aged 50 or above were included in the present study. The participants were recruited at three Spanish memory centers: Hospital Clinic and Hospital de la Santa Creu i Sant Pau in Barcelona, and the CITA-Alzheimer Foundation in San Sebastian. The ethics committee of the Hospital Clinic of Barcelona approved the study, and all participants provided a signed, informed consent before undergoing neuropsychological assessment, MRI and a lumbar puncture. All subjects had to meet the following inclusion criteria: a) at least three years of formal education, b) Mini-Mental State Examination (MMSE) [14] score > 24, and c) scores within the normal range (cutoff 1.5 SD from normative mean) in the total recall and delayed total recall scores from the Free and Cued Selective Reminding Test (FCSRT) [15]. The following exclusion criteria were applied: a) presence of any neurological diagnosis, b) presence of a serious medical condition that could affect cognition, c) diagnosis of a major psychiatric disorder including schizophrenia, major depression or substance abuse.

According to the SCD-Q cutoffs established by Rami et al. [10] participants were classified as: 1) non-SCD (n=21): subjects with a SCD-Q score < 7, or 2) SCD (n=31): individuals with a SCD-Q score ≥ 7. Following the recommendations from the NIA-AA [16], SCD subjects were further classified as: (1) SCD with normal CSF $A\beta_{42}$ levels (SCD/Aβ–; n=21) and (2) SCD with Alzheimer's pathologic change (SCD/Aβ+; n=10).

2.2. Determination of biological and AD CSF biomarkers

All subjects underwent a lumbar puncture between 9 a.m. and 12 p.m. to collect 10 ml of CSF. The samples were centrifuged and stored in polypropylene tubes at –80◦C

within the first hour after extraction. CSF $A\beta_{42}$ levels, tau and ptau were measured by enzyme-linked immunosorbent assay kits (Innogenetics, Ghent, Belgium). Cut-off values of abnormality for each AD CSF biomarker were defined according to previous work [3]: a) $A\beta_{42} \leq 550$ pg/ml, b) tau≥400 pg/ml for subjects between 50–70 years old, and ≥450 pg/ml for subjects older than 70 years, and c) ptau≥75 pg/ml. The AFE-T administrator and the participants were blind to CSF results.

2.3. Apolipoprotein E Analysis

Genomic DNA was extracted from peripheral blood of probands using the QIAamp DNAblood minikit (Qiagen AG, Basel, Switzerland). *APOE* genotyping was performed by polymerase chain reaction amplification and HhaI restriction enzyme digestion.

2.4. Neuropsychological assessment

All participants were assessed both at the baseline and at the follow-up session with a comprehensive neuropsychological battery, administered by a trained neuropsychologist blind to the CSF results. The battery encompassed five cognitive domains. The memory domain included the free recall and delayed free recall scores from the FCSRT [15] and the constructional praxis recall subtest from the Consortium to Establish a Registry for Alzheimer' Disease (CERAD) battery [17], the language domain comprised of the Boston Naming Test [18] and a Category Fluency Task [19]; the praxis domain included the constructional praxis subtest from the CERAD battery [17]; the visual perception domain contained the Letters and Number Location subtests from the Visual Object and Space Perception (VOSP) battery [20], and the executive functions domain consisted of the Trail Making Test – Form A [21], the Stroop Test [22], the Symbol Digit Modalities Test [23], and a Letter Fluency Task [24]. Global cognition was assessed with the MMSE [14]. Premorbid intelligence was measured through the Spanish Word Accentuation Test [25].

2.5. The Subjective Cognitive Decline Questionnaire (SCD-Q)

The SCD-Q is a validated questionnaire that follows the SCD-I framework for research of SCD in preclinical AD [1]. It assesses perceived SCD by asking subjects whether their present performance in daily tasks is now worse than two years ago. The questionnaire includes 24 items assessing perceived decline in instrumental activities of daily living that include memory, language, and executive tasks. Subjects must answer if they believe to be performing these activities worse than roughly two years ago, in a Yes/No format. The total score is computed from the 24 items (i.e., YES = 1, and NO = 0), with higher scores indicating greater perceived cognitive decline. For more details, see Rami et al. [10]. All participants in the study answered the SCD-Q.

2.6. The Ancient Farming Equipment Test

The AFE-T request participants to learn a list of new-object/name pairs. The objects were 24 black-and-white images of non-familiar or unknown objects, which were based on ancient farming Finnish equipment [26]. Each object was paired with a new-word (a non-existing word that follows the phonotactic rules of Spanish [27]). The object names consisted of 14 bisyllabic and 10 trisyllabic pseudowords. All stimuli were presented on a computer screen against a white background using the E-prime 2.0 version (Psychology Software Tools, Inc., PA, USA).

2.6.1. AFE-T Design

Learning phase

The learning phase was administered in two initial learning sessions performed on two consecutive days. Each learning session included a total of seven runs and took approximately 45 minutes. Before starting, each of the 24 object/name pairs was displayed for seven seconds with a 500 ms pause between the pairs. The participants were asked to read aloud the name of the object printed below, and to try to learn each

object/name pair. After the presentation, the seven learning runs were performed. In each run, the participants were presented with the objects one at a time, and were asked to spontaneously say its name aloud. They were given a maximum of 7 sec to recall the name of each object. After this, the correct name appeared below the object for 4 sec, regardless of whether the participant had been able to produce the correct name. The following object was presented after 500 ms. The order of presentation in each run was randomized.

Learning indexes: *free learning score (FLS) and cued learning score (CLS)*

The performance (correct naming) during the last run of the second learning day was taking as a final learning index: free learning score (FLS). Afterwards, the *cued learning score* (CLS) was obtained. The CLS was a final cued learning run where after each object appearance the experimenter verbally provided the first syllable of the object's name (phonemic cue). For both scores (i.e., FLS and CLS), the range of scores was 0-24.

Forgetting measures at 1 week, 3 months and 6 months

Each session took 10-15 minutes and began with a free recall run. Here, each trained object appeared on the screen in a randomized order, and the participant was asked to name it orally (free recall). When the participant could not provide the correct response, the experimenter provided the first syllable of the name (cued recall). Free and cued forgetting rates were also examined at 1 week, 3 months and 6 months after the initial learning phase. Forgetting rates were defined as one minus the ratio between each delayed session score and the score obtained on the last learning run [e.g., 1-(one-week free recall score / FLS), for 1-week free forgetting rate; and 1-(3-month cued recall score / CLS score), for 3-months cued forgetting rate], as in previous studies [5, 28]. In

this way, the forgetting rate represents the mean percentage of object/name items previously learned that were forgotten.

Forgetting slopes

In order to mathematically model the forgetting functions, we followed previous recommendations [29–31]. Forgetting curves are characterized by a curvilinear relation that shows a rapid initial decline of information that is followed by a slower and longer decay, thus showing that information is lost in a larger extent after encoding. Although power functions have been encountered to fit better forgetting curves, in the present sample, the logarithmic function [(y = a –b·ln(time)] was even better than the fit observed with power functions. Previous studies on forgetting have shown that these functions (power and logarithmic) are the most accurate ones to describe forgetting curves. Each data point (4-time data points) for each subject and condition (free and cued recall conditions) was fitted using a non-linear least-squares regression. Fit parameters were calculated based on the residual sum of squares and showing the proportion of data variance accounted for (R^2). The slope (parameter *b*) and intercept (parameter *a*) were computed separately for each subject and condition. The slope (b) captures the forgetting rate of encoded information while the intercept (*a*) represents the estimated initial level of performance (immediately after the last learning trial).

2.7. Statistical analyses

Statistical analyses were performed using the SPSS (v. 22.0) package for Windows. An alpha value of $p < 0.05$ was considered to be significant for all the analyses. Demographics, biological and CSF data were compared using t-tests for independent samples and Chi-square analyses when appropriate.

Analyses of covariance (ANCOVA) controlling for age and years of education with post-hoc Bonferroni corrections were performed to explore possible cross-sectional learning and recall differences between SCD and non-SCD groups on the AFE-T. The analyzed learning indexes were the FLS and the CLS. Long-term free and cued recall scores and free and cued forgetting rates were also compared. Finally, group differences on the standard neuropsychological tests were also analyzed trough ANCOVA's adjusted for age and education with post-hoc Bonferroni corrections. The analyses were executed in order to (1) explore possible between-group differences between non-SCD and SCD groups and (2) to further compare SCD subjects' performance according to Aβ status (i.e., SCD/Aβ- vs. SCD/Aβ+).

Pearson bivariate correlations were calculated to assess overall associations between the AFE-T scores, AD CSF biomarker levels and the SCD-Q. To explore the relationships between the subjective and objective cognitive performance and relevant factors, linear regression models were set up. The analyses included the free (model 1) and cued (model 2) slopes from the AFE-T as dependent variables (slopes represents robust measures of long-term forgetting). Age, years of education, CSF $A\beta_{42}$, CSF p-tau and the SCD-Q score were included as independent variables.

3. Results

3.1. Sample characteristics

Demographics, biological and CSF data for the whole sample and the non-SCD and SCD groups are shown in Table 1. Age ranged between 53 and 80 years, and educational level ranged between 3 and 22 years. There were no significant differences in age ($t(50) = 0.04$; $p = 0.97$), years of education ($t(50) = 0.71$; $p = 0.48$) or premorbid intelligence (WAT; $t(50) = 0.01$; $p = 0.99$) between the non-SCD and SCD groups.

Gender distribution was also similar (χ^2 = 1.06; p = 0.38) with women accounting 57% for the non-SCD group and 71% for the SCD group. Regarding the AD biomarkers, there were no significant differences between groups in CSF $A\beta_{42}$ ($t(50)$ = -0.14; p = 0.89), CSF tau ($t(50)$ = -0.98; p = 0.33) or CSF ptau ($t(50)$ = -1.15; p = 0.25). The APOE-ε4 allele was similarly frequent in the non-SCD group than in SCD (χ^2 = 0.12; p = 0.72), with a frequency of 19% versus 16% of carriers, respectively.

Regarding the results of the cognitive testing, there were no significant differences in global cognition, as assessed by the MMSE, between the non-SCD and the SCD groups (28.8±1.4 vs. 28.2±1.5, respectively). Nor was there a significant difference on verbal intelligence (24.8±4.4 vs. 24.8±4.1). No single test of the standard neuropsychological battery showed significant differences between the groups, with p values ranging from 0.10 to 0.95 (see Supplementary Table 1).

3.2. AFE-T performance between non-SCD and SCD groups

3.2.1. Free learning score (FLS) and cued learning score (CLS)

The non-SCD group showed a FLS of 14.9±6.7 named new-words whereas the SCD group showed a mean of 14.3±7.0 new-words. In the CLS, the mean for the non-SCD group was 19.2±5.1 new-words named, and the SCD group obtained a mean of 19.0±4.7 new-words. The ANCOVA showed that these group differences were not statistically significant [($F(1,48)$ = 0.01; p = 0.96) and ($F(1,48)$ = 0.07; p = 0.93), respectively]. Learning indexes (i.e., FLS and CLS) comparisons between SCD and non-SCD groups are shown in Fig. 1A and Table 2.

3.2.2. Long-term recall and forgetting rates

Long-term performance between groups is shown in Fig. 1A (free and cued recall; slopes) and Fig. 1B (free and cued forgetting rates), and in Table 2.

Forgetting rates at 1-week

For the one-week free recall session, the non-SCD group had a forgetting rate of 0.28±0.3 while the SCD group had a forgetting rate of 0.42±0.2. ANCOVA revealed that this difference was not statistically significant ($F(1,46) = 2.53$; $p = 0.12$). When including the phonemic cue to facilitate naming, the forgetting rate decreased to 0.18±0.1 in the non-SCD and 0.22±0.2 in the SCD group ($F(1,46) = 0.71$; $p = 0.40$).

Forgetting rates at 3 months

At three months, the non-SCD group showed a forgetting rate of 0.75±0.2 in the free recall and the SCD showed a forgetting rate of 0.89±0.2. The ANCOVA showed that this group difference was statistically significant ($F(1,44) = 4.72$; $p < 0.05$). When the cue was presented, both groups obtained the same forgetting rate (non-SCD = 0.54±0.3; SCD = 0.54±0.2; $F(1,44) = 0.01$; $p = 0.93$).

Forgetting rates at 6 months

At six months, the non-SCD group had a forgetting rate of 0.75±0.3 while the SCD group had a forgetting rate of 0.87±0.2 ($F(1,42) = 3.26$; $p = 0.08$). When including the phonemic cue, the forgetting rate decreased to 0.53±0.3 in the non-SCD group and 0.56±0.2 in the SCD ($F(1,42) = 0.07$; $p = 0.78$). These differences were not significant.

Forgetting slopes

Curves in Fig. 1 represent the best-fitting function for each group and condition. The fit of the logarithmic function to the group data was nearly perfect in both groups and conditions ($R^2 > 98\%$, in all cases). Function parameters are shown in Fig. 1A.

3.3. AFE-T performance in SCD according to Aβ status

3.3.1. Free learning score (FLS) and cued learning score (CLS)

Within the SCD group, the FLS was significantly lower for the SCD/Aβ+ subgroup compared to the SCD/Aβ– (8.7±4.9 vs. 17.1±6.2, respectively; $F(1,27) = 6.44$, $p <$

0.05). In the CLS, the mean for the SCD/Aβ+ subgroup was 15.1±4.4 vs. 20.8±3.6 points for the SCD/Aβ– subgroup. The ANCOVA showed that this group difference was also statistically significant ($F(1,27) = 7.51$, $p < 0.05$). Learning indexes of SCD subgroups are shown in Fig. 2A and Table 3.

3.3.2. Long-term recall and forgetting rates

The SCD/Aβ+ displayed lower performance in all the long-term memory scores compared to SCD/Aβ–. Significant differences between the SCD/Aβ– and SCD/Aβ+ subgroups were found in the one-week free (SCD/Aβ– = 11.4±7.0 vs. SCD/Aβ+ = 4.6±2.7; $F(1,24) = 4.49$; $p < 0.05$) and cued (SCD/Aβ– = 17.4±5.0 vs. SCD/Aβ+ = 9.8±4.3; $F(1,24) = 7.10$; $p < 0.01$) recall, and in the 3-months free recall (SCD/Aβ– = 10.6±4.7 vs. SCD/Aβ+ = 5.2±3.6; $F(1,24) = 4.27$; $p < 0.05$). Regarding the forgetting rates, the SCD/Aβ– subgroup obtained a free forgetting rate at one week of 0.17±0.1 whereas the SCD/Aβ+ obtained a forgetting rate of 0.35±0.1. This difference was statistically significant ($F(1,24) = 5.13$; $p < 0.05$). Long-term recall and forgetting rates between SCD subgroups are shown in Fig. 2A and B, respectively; and in Table 3.

3.3.3. Standard neuropsychological tests

Regarding the standard neuropsychological assessments, within the SCD group, there were no significant differences in any test of the neuropsychological battery between the Aβ– and Aβ+ subgroups (Supplementary Table 2).

3.4. Correlations between the AFE-T scores, AD CSF biomarkers and the SCD-Q

Significant correlations were found in the whole sample between CSF $A\beta_{42}$ and 1) the FLS ($r = .288$; $p < 0.05$), 2) the TLS ($r = .289$; $p < 0.05$), 3) the 1-week cued forgetting rate ($r = -.465$; $p < 0.01$), and 4) the 3-month cued forgetting rate ($r = -.328$; $p < 0.05$).A

significant correlation was found between the 3-month free forgetting rate of the AFE-T and the SCD-Q (r = .330; $p < 0.05$).

3.5. Models on the association between objective and subjective cognitive performance.

Lineal regression analyses were conducted to explore which variables were associated with the objective cognitive performance in the AFE-T. The models included the free slope (model 1) and the cued slope (model 2) as dependent variables. The AFE-T slopes were selected as dependent variables since they represent the most comprehensive measures of long-term forgetting across all the time period. Age, years of education, CSF $A\beta_{42}$, CSF p-tau and the SCD-Q were included as independent variables.

While age, years of education, CSF $A\beta_{42}$ or CSF p-tau were not associated (all $p > 0.05$) with the free (model 1) and cued (model 2) slopes, the SCD-Q score was associated with the cued slope of the AFE-T [β = -.212 (95% CI -.406 to -.018); $p < 0.05$]. Figure 3 shows the scatterplot on the association between the cued slope of the AFE-T and the SCD-Q score, indicating that higher scores in the SCD-Q predicted faster decrease of information in long-term memory (higher forgetting).

4. Discussion

This study provides evidence for subtle objective memory difficulties in subjects with SCD, especially in those with abnormal CSF $A\beta_{42}$ levels. We thoroughly explored learning and long-term recall processes in a well-characterized sample of cognitively unimpaired subjects with vs. without SCD by means of a new highly-demanding associative memory test. Our findings suggest a clear relationship between SCD ratings and objective memory performance.

Increasing evidence suggests that SCD might be the first symptomatic manifestation in the AD continuum [1, 32, 33]. However, several cross-sectional studies have not been able to show a significantly lower performance in cognitive tests in SCD samples compared to non-SCD, demonstrating that the links between SCD and objective cognitive performance are difficult to find when standard neuropsychological tests are used [34]. The poor cross-sectional correlation typically found between SCD and objective cognitive performance might be due to limitation in sensitivity of the applied neuropsychological tests [35]. To date, the use of more challenging measures has shown promising results in cognitively unimpaired subjects including preclinical AD [36, 37], presymptomatic mutation carriers of familial AD [38] and SCD [39] samples.

The usefulness of the AFE-T for exploring memory function throughout the Alzheimer's continuum has been previously endorsed in several studies including preclinical AD [3], MCI [8] and AD [9]. In the present study, the AFE-T showed subtle memory difficulties in subjects with SCD that were otherwise undetected by our comprehensive neuropsychological battery. Targeting memory function in SCD – especially by using more sensitive cognitive measures – could be of relevant interest as subjective decline on memory capacities are thought to be more related to an increased risk of future cognitive decline [40]. However, this assumption could be skewed as most studies on SCD have concentrated on memory domain and there is scarce evidence [12, 41] focusing on subjective decline in other cognitive functions.

Another important point regarding the nature of the AFE-T and its potential application in SCD concerns to its neural correlates. Recently, the functional and structural correlates of the AFE-T have been identified in cognitively unimpaired subjects [2, 7] as well as in patients [8, 42]. These studies have suggested that performance on this task depends on specific brain regions that are typically affected in AD, such as the medial

temporal lobe (MTL). In this line, cross-sectional studies have shown incipient volume loss in this AD-related brain region in subjects with SCD [43–45].

When comparing performances in the AFE-T between individuals with vs. without SCD, we specifically found accelerated long-term forgetting (ALF) in those subjects with SCD. The concept of ALF – defined as a loss of information over days or weeks despite normal acquisition – has recently emerged in the field of neurodegenerative diseases as a cognitive marker for the presymptomatic stages of AD [4, 5]. A recent study on a cohort of autosomal dominant AD families revealed ALF at one week in presymptomatic mutation carriers [5]. In a similar way, Zimmermann & Butler [28] recently showed ALF in asymptomatic apolipoprotein E (APOE) ε4 carriers. Here, it is important to note that in addition to compute the forgetting rates – which are similar to those reported in these previous studies – we also modeled the participants' forgetting function in order to obtain a comprehensive measure of information decay (i.e., the slope). The modeled function provides an estimate of the intercept and the forgetting slope individually for each subject and allows to compute an overall forgetting measure for the whole period, instead of several measures obtained when calculating a forgetting rate for each time-point. Our results suggest that ALF may be present in subjects with SCD and highlight the need of using more challenging cognitive measures including more specific and sensitive variables when assessing cognitively unimpaired populations.

Interestingly, amyloid status (positive vs. negative) influenced significantly the cognitive performance of SCD subjects. Those individuals with abnormal amyloid levels displayed poorer learning and long-term recall scores than those with normal CSF AD biomarkers. These results are relevant since there are recent longitudinal studies

showing that subjects with SCD and biomarker evidence of AD are at a higher risk of future decline [46, 47]. At the same time, we found a relationship between the participants' amyloid-β levels and cognitive performance on the AFE-T. Also, our regression analyses pointed to cognitive complaints as predictors of long-term memory performance, indicating that higher scores in the SCD-Q are associated with a faster decrease of information in long-term memory (higher forgetting, Fig. 3). Our observations are in line with previous studies suggesting that cognitively unimpaired subjects with evidence of Aβ pathology and SCD undergo objective cognitive decline at a higher rate than individuals with either amyloidosis or SCD alone, and are at a higher risk of rapid cognitive decline [48]. Our findings suggest that subjects with SCD and amyloid-β positivity may be closer to develop the earliest cognitive manifestations in the AD continuum than subjects with only SCD.

Regarding the assessment of SCD, the SCD-Q might be especially suited for the study of the earliest symptomatic manifestations in the AD continuum given it was designed following the SCD-I framework for research of SCD in preclinical AD [10]. Previous studies have shown significantly higher scores in the SCD-Q in samples of preclinical AD subjects when compared to controls [11, 12]. The SCD-Q distinguishes from the pre-existing SCD questionnaires in that it explores the perception of decline in a relatively short period of time, exploring the self-perceived performance in an array of daily life activities that involve multiple cognitive domains, instead of being restricted to memory. These properties increase the likelihood of detecting SCD due to preclinical AD [1]. Taken together, our results suggest the use of more robust and sensitive measures to ensure an exhaustive evaluation of SCD when assessing cognitively unimpaired populations.

Our study has some limitations. First, the relatively small sample size could limit the power of the statistical analyses. Replication of the present results in independent and larger samples is therefore needed. Second, in this study we only included participants from memory clinic and research settings. This is an important consideration since it is well known that recruitment setting (i.e., epidemiological, memory clinic, research) affects studies in SCD [49, 50]. Furthermore, the way by which subjective and objective cognitive outcomes are assessed may also affect these studies. In this sense, it is important to note that the SCD-Q is a validated tool for measuring SCD. Also, regarding the objective assessment of participants' cognitive capacities, the comprehensive AFE-T protocol allowed for a thorough assessment of learning and long-term memory processes. However, validation of the present results is called for in future studies using different assessment methods. Finally, another important limitation concerns the cross-sectional nature of the study. Further assessment is needed to accurately follow-up participants' trajectories of cognitive decline.

In sum, by using comprehensive measures for assessing subjective and objective cognition, we provide evidence for accelerated long-term forgetting in individuals with SCD, especially in those within the Alzheimer's continuum. The presence of significant associations between SCD ratings and AD biomarkers and objective cognitive performance, highlight the contribution of biological and cognitive markers (and its co-occurrence) as potential predictors of the earliest manifestations in Alzheimer's disease.

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Conflicts of interest

The authors declare that they have no conflict of interest

Ethics approval

The study was approved by the Hospital Clinic of Barcelona Ethics Committee and carried out in compliance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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Table 1. Demographics, biological data and CSF levels.

Parameters	**Total (N = 52)**	**non-SCD (n = 21)**	**SCD (n = 31)**	***T***	***p***
Demographics					
Gender (% women)	65.4%	57.1%	70.9%	1.06[a]	.378
Age	67.2±6.7	67.3±6.4	67.2±6.9	0.04	.970
Years of education	11.0±4.3	11.5±4.4	10.6±4.3	0.71	.480
MMSE	28.5±1.5	28.8±1.4	28.2±1.5	1.21	.233
WAT	24.8±4.2	24.8±4.4	24.8±4.1	0.01	.999
Biological & CSF data					
APOE ε4 (% positive)	17.6%	20%	16.1%	0.12[a]	.724
$A\beta_{42}$	706.0±268.9	699.5±225.1	710.4±298.6	-0.14	.888
Tau	254.4±105.5	236.8±84.8	266.3±117.4	-0.98	.328
Ptau	53.3±16.1	50.2±14.4	55.4±17.0	-1.15	.254

Data are presented as means ± standard deviation. Key: WAT: Word Accentuation Test; CSF: cerebrospinal fluid; $A\beta_{42}$: Amyloid-beta isoform 42; Tau: total tau; Ptau: phosphorylated tau.

[a] **Pearson Chi-Square**

Table 2. ANCOVA of learning indexes and long-term forgetting scores of the AFE-T between non-SCD and SCD groups.

Variables	non-SCD (n = 21)	SCD (n = 31)	F	*p*
FLS	14.9±6.7	14.3±7.0	0.01	.965
CLS	19.2±5.1	19.0±4.7	0.07	.934
1-Week FR	11.2±6.8	9.5±6.7	.678	.415
1-Week CR	16.3±5.2	15.4±5.7	.242	.625
1-Week FFR	0.28±0.3	0.42±0.2	2.53	.118
1-Week CFR	0.18±0.1	0.22±0.2	0.71	.404
3-Month FR	3.8±3.8	2.1±3.9	1.92	.172
3-Month CR	9.6±5.9	9.1±4.9	0.14	.708
3-Month FFR	0.75±0.2	0.89±0.2	4.72	.035*
3-Month CFR	0.54±0.3	0.54±0.2	0.01	.929
6-Month FR	4.0±4.3	2.3±4.1	1.57	.217
6-Month CR	9.7±6.3	8.6±5.0	0.26	.610
6-Month FFR	0.75±0.3	0.87±0.2	3.26	.078
6-Month CFR	0.53±0.3	0.56±0.2	0.07	.786

Data are presented as means ± standard deviation. Key: FLS, free learning score; CLS, cued learning score; FR, free recall; CR, cued recall; FFR, free forgetting rate; CFR, cued forgetting rate. *$p < 0.05$

Table 3. ANCOVA of learning indexes and long-term forgetting scores of the AFE-T within the SCD group including Aβ status subgroups.

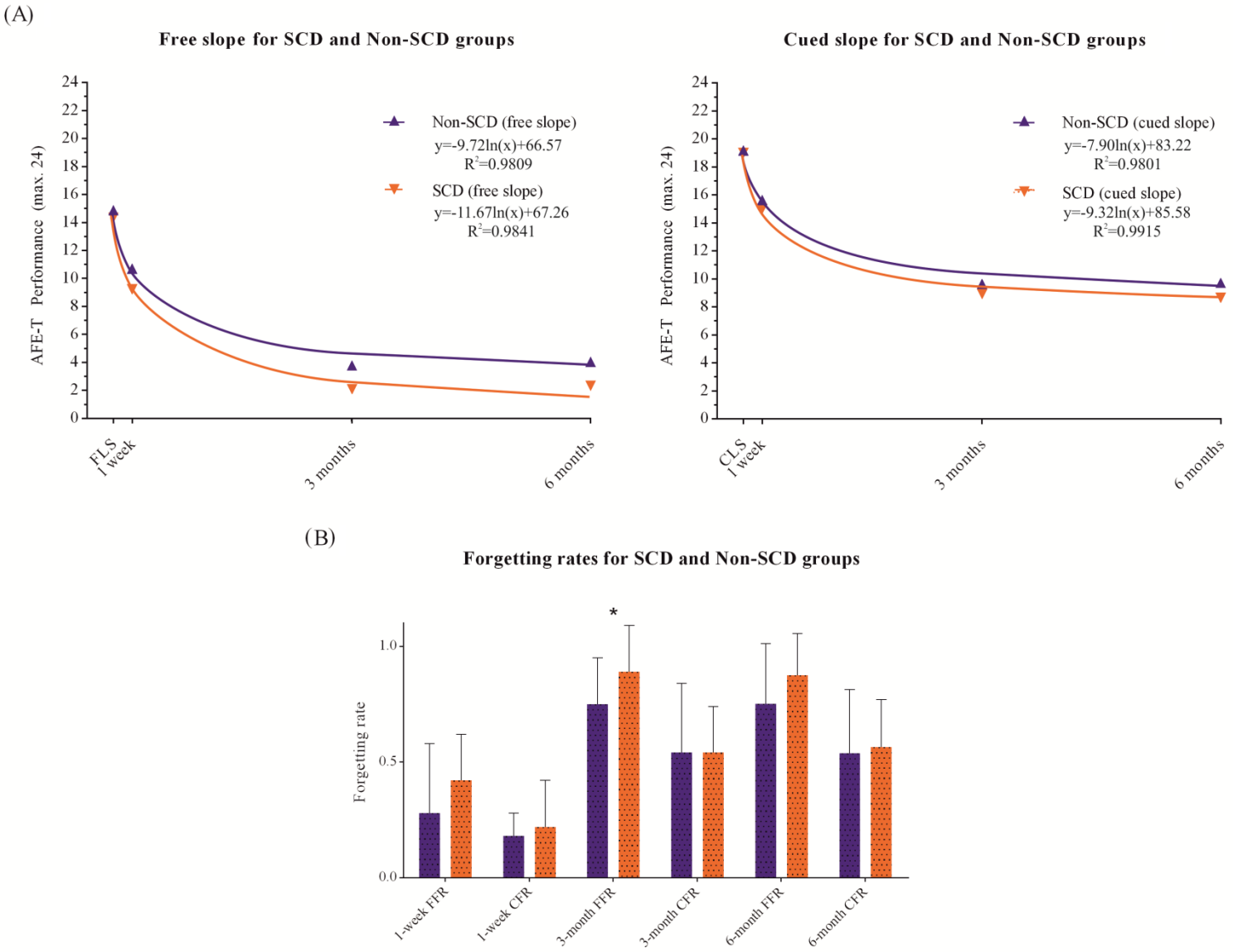
	SCD (n = 31)			
Variables	**Aβ– (n = 21)**	**Aβ+ (n = 10)**	**F**	***p***
FLS	17.1±6.2	8.7±4.9	6.44	.017*
CLS	20.8±3.6	15.1±4.4	7.51	.011*
1-week FR	11.4±7.0	4.6±2.7	4.49	.044*
1-week CR	17.4±5.0	9.8±4.3	7.10	.008**
1-week FFR	0.38±0.2	0.53±0.2	2.54	.123
1-week CFR	0.17±0.1	0.35±0.1	5.13	.032*
3-month FR	2.9±4.4	0.3±0.5	1.08	.308
3-month CR	10.6±4.7	5.2±3.6	4.27	.049*
3-month FFR	0.85±0.2	0.96±0.1	1.45	.239
3-month CFR	0.48±0.2	0.68±0.2	2.76	.110
6-month FR	2.9±4.7	0.8±1.1	0.29	.595
6-month CR	9.9±5.1	5.6±3.4	0.97	.334
6-month FFR	0.86±0.2	0.91±0.1	0.11	.745
6-month CFR	0.53±0.2	0.65±0.2	0.25	.621

Data are presented as means ± standard deviation. Key: FLS, free learning score; CLS, cued learning score; FR, free recall; CR, cued recall; FFR, free forgetting rate; CFR, cued forgetting rate.

*** $p < 0.05$**

**** $p < 0.01$**

Figure 1. AFE-T performance between non-SCD and SCD groups.

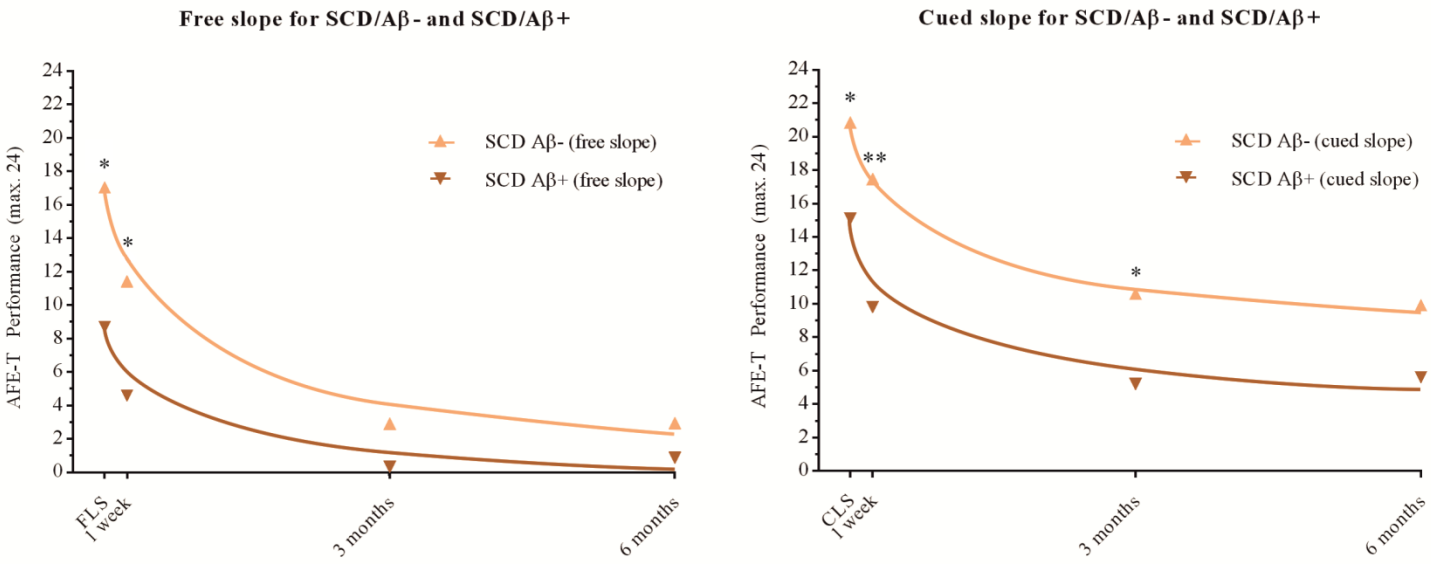


Key: FLS, free learning score; CLS, cued learning score; FFR, free forgetting rate; CFR, cued forgetting rate.

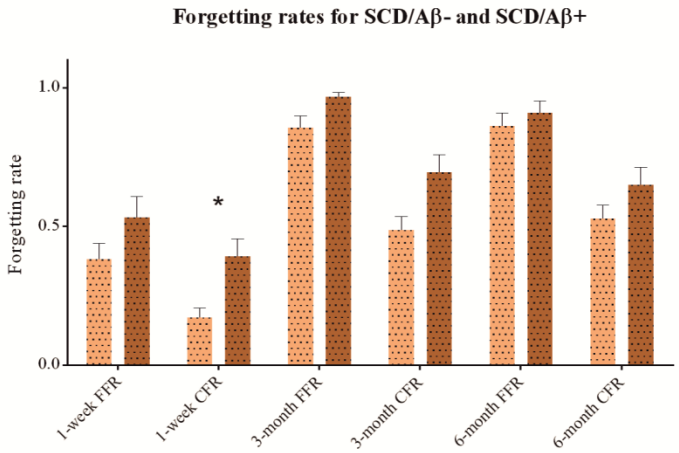
$^{*}p < 0.05$

Figure 2. AFE-T performance in SCD stratifying by Aβ status.

(A)



(B)

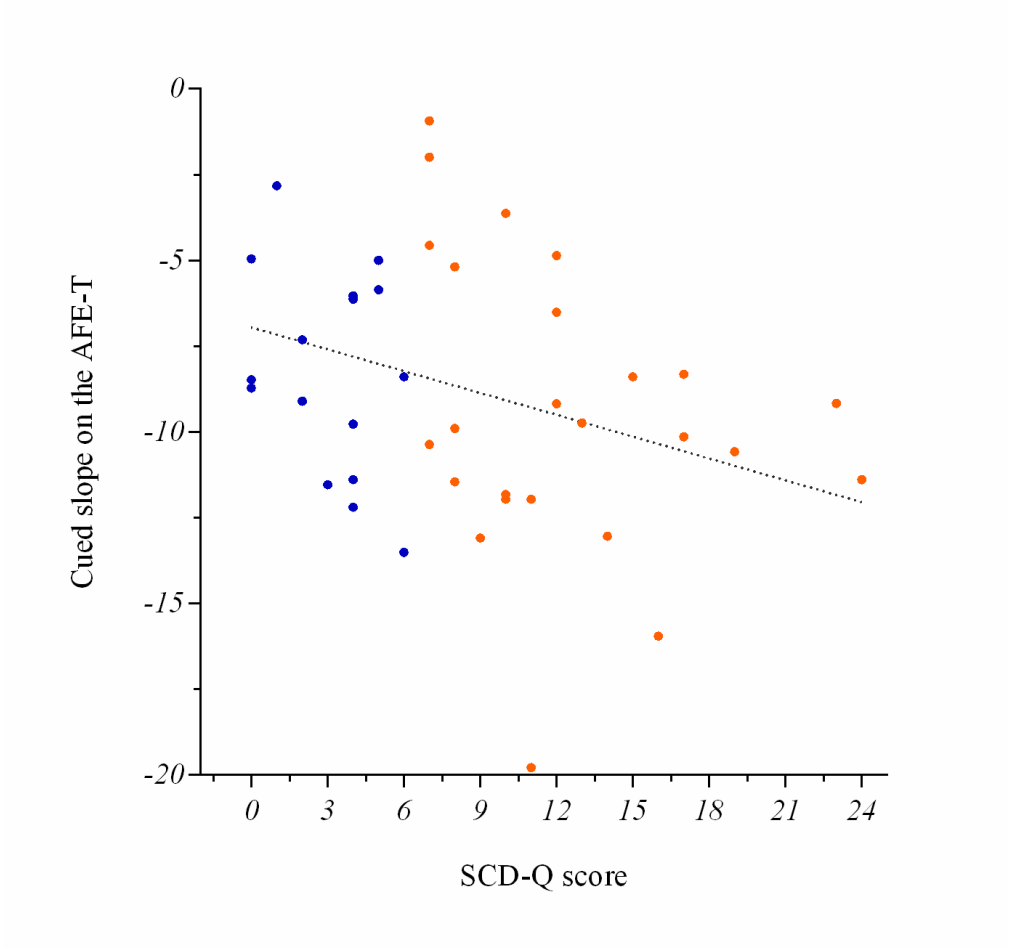


Key: FLS, free learning score; CLS, cued learning score; FFR, free forgetting rate; CFR, cued forgetting rate.

* $p < 0.05$

** $p < 0.01$

Figure 3. Scatterplot on the relationship between the cued slope and the SCD-Q scores



Key: Blue dots: non-SCD subjects; Orange dots: SCD subjects.

V. RESUMEN GLOBAL DE RESULTADOS / INFORME DEL DIRECTOR

INFORME DEL DIRECTOR

La presente tesis doctoral ha sido realizada bajo nuestra dirección y consideramos que reúne las condiciones necesarias. Incluye cinco artículos, de los cuales cuatro ya han sido aceptados:

Título de la publicación	**Nombre de la revista y detalles**	**Año**	**IF**
#1 Early detection of learning difficulties when confronted with novel information in preclinical Alzheimer's disease stage 1	Journal of Alzheimer's disease [(58): 855-870] DOI: 10.3233/JAD-161173	2017	3.476
#2 Tau protein is associated with longitudinal memory decline in cognitively healthy subjects with normal Alzheimer's disease cerebrospinal fluid biomarker levels	Journal of Alzheimer's disease [(70): 211-225] DOI: 10.3233/JAD-190046	2019	3.909
#3 Early detection of subtle motor dysfunction in cognitively normal subjects with amyloid-β positivity	Cortex [(121): 117-124] DOI: 10.1016/j.cortex.2019.07.021	2019	4.009
#4 Accelerated long-term forgetting over three months in asymptomatic APOE ε4 carriers	Annals of Clinical and Translational Neurology [Accepted] DOI: 10.1002/acn3.51245	2020	3.660
#5 Accelerated long-term forgetting in individuals with subjective cognitive decline and amyloid-β positivity	Submitted	-	-

El trabajo #3 fue realizado en coautoría, contribuyendo por igual los dos primeros autores. Concretamente, el doctorando participó en el diseño del estudio y aspectos metodológicos, reclutamiento de la muestra, análisis estadístico de los datos, redacción del manuscrito original y revisión y edición de la versión final del artículo.

Firmado,

Dra. Lorena Rami González

Dr. Antoni Rodríguez Fornells

RESUMEN GLOBAL DE RESULTADOS

Trabajo nº 1

Early detection of learning difficulties when confronted with novel information in preclinical Alzheimer's disease stage 1

El objetivo del primero de los trabajos publicados fue estudiar las funciones de aprendizaje y olvido a largo plazo, a través del AFE-T, en sujetos cognitivamente sanos situados dentro del continuo Alzheimer y evaluar su posible relación con los biomarcadores de EA en LCR. Siguiendo las guías de investigación del NIA-AA de 2011, un total de 49 sujetos cognitivamente sanos fueron clasificados como controles (CTR, n = 31), EA preclínica – Fase 1 (EAP-1, n = 14) y EA preclínica – Fase 2 (EAP-2, n = 4). La configuración del AFE-T permitió el análisis de curvas de aprendizaje, tasas de olvido a largo plazo y curvas de reaprendizaje. Los resultados del estudio mostraron dificultades claras de aprendizaje en sujetos EAP-1 ($F = 6.98$; $p < 0.01$) en comparación con los controles. Las diferencias en las tasas de olvido fueron menores, alcanzando la significación estadística la tasa de olvido facilitado a los tres meses ($F = 4.83$; $p < 0.05$). Las curvas de reaprendizaje mostraron leves dificultades entre los grupos que no alcanzaron la significación estadística. Los subanálisis en individuos con EAP-2 mostraron mayores dificultades de aprendizaje y recuerdo en estos sujetos en comparación con los controles y también con EAP-1. En toda la muestra, se encontraron correlaciones significativas entre los biomarcadores de EA en LCR (ratio $A\beta_{42}$/tau) y el AFE-T, tanto en la puntuación final de aprendizaje ($r = 0.52$; $p < 0.01$) como en la tasa de olvido facilitado a los tres meses ($r = –0.38$; $p < 0.01$). Este trabajo sugiere que el AFE-T es una prueba sensible, capaz de detectar dificultades sutiles de aprendizaje y olvido a largo plazo en la fase más temprana del continuo Alzheimer. Las dificultades de aprendizaje podrían ser uno de los primeros signos de pérdida memoria episódica en el continuo Alzheimer.

Trabajo nº 2

Tau protein is associated with longitudinal memory decline in cognitively healthy subjects with normal Alzheimer's disease cerebrospinal fluid biomarker levels

En este trabajo, se estudió una muestra de sujetos cognitivamente sanos con niveles normales de biomarcadores de EA en LCR. El objetivo fue investigar la relación de diferentes variables biológicas, neuroanatómicas y neuropsicológicas con los cambios cognitivos más tempranos en el envejecimiento sano. Treinta y dos sujetos cognitiva y biológicamente normales (CBN) completaron una RM y el AFE-T, al inicio del estudio y tras 18 meses. Para explorar la relación entre las funciones de aprendizaje y memoria y diferentes variables que pueden influir en el rendimiento cognitivo, se estableció un modelo lineal. Posteriormente, se dividió la muestra según los niveles de T-tau de los sujetos, en dos grupos: CBN-Tau↓ (tau < 228.64 pg/ml; n = 16) y CBN-Tau↑ (tau > 228.64 pg/ml; n = 16). Por último, se llevaron a cabo análisis de *voxel-based morphometry* (VBM) para identificar las regiones relacionadas con el rendimiento basal y longitudinal en el AFE-T. Como resultado principal, se encontró una asociación entre los niveles de T-tau en LCR y la disminución del rendimiento en el AFE-T a los 18 meses (B = –0.17, $p < 0.05$; r = –0.41; $p < 0.01$). Asimismo, se observó una evolución diferente de los dos subgrupos, con mayor empeoramiento de los individuos con los niveles de tau más altos (F = 8.37; $p < 0.01$). El rendimiento en el AFE-T correlacionó con el volumen de sustancia gris en áreas del lóbulo temporal medial, lóbulo frontal y cerebelo. Los hallazgos de este estudio sugieren que existen variables biológicas y neuroanatómicas que podrían influir en el empeoramiento cognitivo en el envejecimiento sano y que no están relacionados con los procesos patológicos de la EA. Los resultados obtenidos apuntan a la presencia de neurodegeneración como potencial predictor de los cambios cognitivos más tempranos en el envejecimiento sano.

Trabajo nº 3

Early detection of subtle motor dysfunction in cognitively normal subjects with amyloid-β positivity

El objetivo de este estudio fue investigar la función motora fina en sujetos con niveles alterados de la proteína β-amiloide en LCR (i.e., dentro del continuo Alzheimer) y su posible relación con los biomarcadores de EA en LCR. Mediante una versión computarizada del *finger tapping test* (FTT), se midieron dos indicadores sensibles del rendimiento motor: la velocidad de *tapping* y la variabilidad intra-sujeto. Setenta y dos participantes completaron el estudio. Se establecieron tres grupos según el perfil de biomarcadores de EA en LCR, siguiendo los criterios de investigación vigentes (Jack et al., 2018): 37 controles (CTR), 20 sujetos cognitivamente sanos con niveles de β-amiloide alterados (Aβ+) y 15 pacientes con EA prodrómica o inicial. Todos los sujetos completaron el FTT, una exploración neuropsicológica, cuestionarios de factores de riesgo cardiovascular y actividad física, una punción lumbar y una extracción de sangre para obtener el genotipo de APOE. Se encontró una diferencia general entre los grupos de estudio en velocidad de *tapping* ($F = 19.37$, $p < 0.01$) y en variabilidad intra-sujeto ($F = 11.40$, $p < 0.01$). Específicamente, el grupo Aβ+ mostró menor velocidad de *tapping* ($F = 5.33$, $p < 0.05$) y mayor variabilidad intra-sujeto ($F = 8.48$, $p < 0.01$) que el grupo CTR, y mayor velocidad que el grupo EA ($F = 13.61$, $p < 0.01$). La velocidad de *tapping* ($\beta = .263$, $p < 0.05$) y la variabilidad intra-sujeto ($\beta = .558$, $p < 0.01$) se asociaron significativamente con los niveles de $A\beta_{42}$ en LCR. Este estudio mostró dificultades motoras sutiles en sujetos que se encuentran dentro del continuo Alzheimer y que estas dificultades están relacionadas con los niveles de $A\beta_{42}$ en LCR. Los resultados sugieren que una evaluación precisa de las funciones motoras podría ayudar a identificar individuos en la etapa más temprana de la EA.

Trabajo nº 4

Accelerated long-term forgetting over three months in asymptomatic APOE ε4 carriers

En este trabajo se investigó el papel del olvido acelerado a largo plazo (OLP) como potencial marcador de disfunción cognitiva sutil en individuos con riesgo genético incrementado de desarrollar EA y se examinó la posible relación entre el OLP y los biomarcadores de EA en LCR. Mediante el AFE-T, se examinó la presencia de OLP en sujetos portadores vs. no-portadores del haplotipo APOE ε4. Se incluyeron 22 sujetos cognitivamente sanos: 11 portadores de APOE ε4 (heterocigotos para ε3 y ε4) fueron aparejados por edad, sexo y años de escolaridad con 11 no-portadores de APOE ε4 (homocigotos para ε3). Con el fin de modelar matemáticamente la función de olvido, se aplicó una función logarítmica [(y=a–b·ln(tiempo)]. La pendiente de olvido (parámetro b) y la estimación de la puntuación de aprendizaje final (parámetro a) se obtuvieron por separado para cada sujeto. El SCD-Q fue utilizado para cuantificar el grado de quejas cognitivas subjetivas de los participantes del estudio. No se encontraron diferencias entre portadores y no portadores en ninguna de las pruebas neuropsicológicas estándar. El AFE-T tampoco mostró diferencias grupales en las puntuaciones de aprendizaje. Sin embargo, el recuerdo facilitado a los tres meses del AFE-T fue significativamente menor ($F(1,19) = 4.99$; $p < .05$; Cohen's $d = .99$) en los portadores que en los no-portadores. Particularmente para el recuerdo con pistas, la pendiente (medida de olvido a largo plazo) fue mayor para los portadores ($b = -9.1$) en comparación con los no portadores ($b = -6.6$). Además, se encontraron correlaciones significativas entre la ratio $A\beta_{42}$/P-tau y las medidas de OLP. Estas medidas de olvido también correlacionaron con el grado de DCS. Estos hallazgos indican que es posible detectar OLP en individuos con riesgo genético aumentado de desarrollar EA y que podría existir una relación entre esta variable cognitiva y los procesos fisiopatológicos subyacentes a la EA.

Trabajo nº 5

Accelerated long-term forgetting in individuals with subjective cognitive decline and amyloid-β positivity

En este estudio se examinaron las funciones de aprendizaje y olvido a largo plazo mediante el AFE-T en una muestra de sujetos con deterioro cognitivo subjetivo y se exploró su relación con los biomarcadores de EA en LCR. Se incluyeron 52 individuos y se cuantificó el DCS a través del *Subjective Cognitive Decline Questionnarie* (SCD-Q; Rami et al., 2014), utilizando el punto de corte de 7 puntos en la puntuación final del MiCog para clasificar a los sujetos como DCS o no-DCS (DCS, n = 31; no-DCS, n = 21). Los sujetos con DCS se subdividieron de acuerdo con la presencia o ausencia de niveles anormales de $A\beta_{42}$ en LCR. Todos los sujetos completaron una evaluación neuropsicológica estándar, el AFE-T y una punción lumbar. En el AFE-T, el grupo con DCS mostró una tasa de olvido libre a los 3 meses significativamente mayor que el grupo sin DCS ($F = 4.72$; $p < 0.05$). Al subdividir los sujetos con DCS según los niveles de β-amiloide, se observó que los sujetos con niveles alterados de $A\beta_{42}$ rindieron peor que aquellos con niveles normales tanto en las sesiones de aprendizaje como en las recuerdo a largo plazo del AFE-T. La puntuación del SCD-Q correlacionó significativamente con las tasas de olvido del AFE-T; indicando que cuanto mayor es la queja cognitiva, más alta es la tasa de olvido. Este estudio sugiere que es posible detectar dificultades sutiles de aprendizaje y olvido a largo plazo en sujetos con deterioro cognitivo subjetivo, especialmente en aquellos con niveles anormales de $A\beta_{42}$ en LCR.

VI. DISCUSIÓN

DISCUSIÓN

Los trabajos incluidos en la presente tesis doctoral tienen como objetivo el estudio de las funciones de aprendizaje y olvido a largo plazo, así como de la función motora fina, tanto en el envejecimiento sano como en la fase preclínica del continuo Alzheimer. Se ha investigado la relación de estas funciones con los biomarcadores de la EA y se ha evaluado la contribución de diferentes variables biológicas, neuroanatómicas y neuropsicológicas en los cambios cognitivos más tempranos en el envejecimiento sano. Todo ello aporta información acerca de los procesos subyacentes al empeoramiento cognitivo en estas poblaciones.

El primero de los trabajos, estudió la posibilidad de detectar dificultades sutiles de aprendizaje y/u olvido a largo plazo en sujetos cognitivamente sanos situados dentro del continuo Alzheimer. El AFE-T, una prueba de memoria asociativa altamente exigente, permitió el análisis exhaustivo de curvas de aprendizaje, tasas de olvido a largo plazo y curvas de reaprendizaje. Los resultados obtenidos mostraron dificultades de aprendizaje y olvido a largo plazo en sujetos situados en la etapa más temprana del continuo Alzheimer. Además, se encontraron asociaciones entre los biomarcadores de la EA, especialmente los niveles de $A\beta_{42}$ en LCR, y las puntuaciones del AFE-T.

Mediante el AFE-T se exploró, de forma exhaustiva, la capacidad de aprendizaje en una tarea altamente exigente. A diferencia de la mayoría de pruebas neuropsicológicas estándar, el AFE-T requiere aprender, asociar y almacenar información novedosa. Se ha sugerido que la formación de nuevas asociaciones desprovistas de contenido semántico previo, exige un alto procesamiento cognitivo (Rentz et al., 2011). Este tipo de aprendizaje podría depender de áreas cerebrales relacionadas específicamente con la adquisición de nuevos conocimientos (Laine y Salmelin, 2010), áreas que muestran

cambios incipientes en la fase preclínica del continuo Alzheimer como el hipocampo y estructuras adyacentes del lóbulo temporal medial (Bernard et al., 2014; Younes et al., 2014). Probablemente debido a estas mayores demandas cognitivas, el AFE-T permitió detectar dificultades sutiles de aprendizaje en la fase más temprana del continuo Alzheimer. En línea con estos resultados, Papp et al. (2015) estudiaron 260 sujetos cognitivamente sanos mediante una prueba de memoria altamente exigente, el *Memory Capacity Test* (Rentz et al., 2010). En comparación con el grupo control, los individuos que presentaban niveles alterados del biomarcador de amiloide obtuvieron un rendimiento significativamente más bajo en la puntuación de recuerdo libre.

El AFE-T también permitió una evaluación exhaustiva del olvido a largo plazo, mediante el cálculo de las tasas de olvido. En general, la mayoría de pruebas neuropsicológicas estándar se caracterizan por evaluar la capacidad de retención en un intervalo de tiempo de entre 20 y 30 minutos (Lezak, 1983). De este modo, la evaluación de tasas de olvido a largo plazo (recuerdo diferido a la semana, tres meses y seis meses desde el aprendizaje inicial) representaba un campo por explorar en la fase preclínica del continuo Alzheimer. Las diferencias observadas entre los grupos de estudio, sugiere que el análisis del olvido a largo plazo podría proporcionar datos valiosos para la identificación de dificultades de memoria en la fase más temprana del continuo Alzheimer. Además, el uso de medidas de recuerdo libre y facilitado permitió observar un mayor beneficio de las claves fonéticas por parte grupo control en comparación con los sujetos con niveles alterados de $A\beta_{42}$. Esto sugiere que las dificultades exhibidas no se debían a un déficit de evocación (i.e., efecto de "la punta de la lengua") sino a la pérdida o desvanecimiento de la información previamente codificada. En esta línea, Grönholm et al. (2008) emplearon el paradigma AFE en

pacientes con DCL y observaron que se beneficiaban menos del recuerdo con pistas que los controles.

El reaprendizaje fue otro método de evaluación innovador en el estudio de la fase preclínica del continuo Alzheimer. Diferentes estudios han sugerido que la información que no se puede recordar en una prueba de aprendizaje a través de medidas de recuerdo facilitado o reconocimiento, podría reactivarse mediante tareas de reaprendizaje (Hansen, Umeda, & Mckinney, 2013; MacLeod, 1988; Van der Hoeven & De Bot, 2012). Los resultados obtenidos indicaron que el grupo control y el grupo de sujetos con niveles alterados de $A\beta_{42}$ mostraron curvas de reaprendizaje similares. Las diferencias significativas observadas entre los grupos durante la fase de reaprendizaje fueron menores que las observadas durante la fase de aprendizaje inicial, sugiriendo que el beneficio obtenido al reaprender la tarea por parte del grupo con niveles alterados de $A\beta_{42}$ le restó sensibilidad a la misma para obtener diferencias significativas entre ambos grupos.

En este primer estudio, también se exploraron las posibles asociaciones entre el rendimiento en el AFE-T y los niveles de biomarcadores de EA en LCR. La gran mayoría de estudios con un diseño transversal no ha logrado encontrar asociaciones entre el rendimiento cognitivo en los tests neuropsicológicos estándar y los biomarcadores de EA (Aizenstein et al., 2008; Mormino et al., 2009; Storandt et al., 2009; Villemagne et al., 2011). Únicamente algunos estudios longitudinales han mostrado asociaciones entre los niveles de β-amiloide y el posterior deterioro de la memoria (Clark et al., 2016; Doraiswamy et al., 2012). Mediante el AFE-T se hallaron correlaciones significativas entre el rendimiento en aprendizaje y olvido a largo plazo y los biomarcadores de EA en LCR, especialmente con los niveles de $A\beta_{42}$ en LCR. De

manera similar, Rentz et al. (2011) encontraron una asociación entre el depósito cerebral de amiloide medido mediante técnicas de PET y el rendimiento en una tarea de memoria asociativa altamente exigente, el *Face-Name Associative Memory Exam*. Los hallazgos obtenidos respaldan la idea de que la disminución de la memoria episódica está estrechamente relacionada con los niveles de β-amiloide (Hedden et al., 2013), y que este vínculo puede identificarse únicamente a través de tareas más exigentes que los tests neuropsicológicos estándar. En resumen, el AFE-T se presentó como una herramienta prometedora para la caracterización del perfil cognitivo de la fase preclínica del continuo Alzheimer, siendo significativamente sensible como para detectar dificultades sutiles de aprendizaje y olvido a largo plazo en esta población en comparación con controles. Los hallazgos de este primer trabajo promueven el diseño y uso de nuevas pruebas cognitivas más exigentes para la detección y seguimiento de las primeras manifestaciones cognitivas en el continuo Alzheimer.

El segundo de los trabajos perseguía la identificación de variables neuroanatómicas (i.e., pérdida de volumen de substancia gris) y biológicas relacionadas con el empeoramiento cognitivo longitudinal en el envejecimiento sano (sujetos con niveles normales de biomarcadores de EA). Este estudio mostró que los niveles de tau en LCR, un biomarcador de neurodegeneración, estaban relacionados con el empeoramiento longitudinal de la capacidad de aprendizaje y memoria. Además, mediante análisis de VBM, se observó que el rendimiento basal en el AFE-T está relacionado con el volumen de substancia gris en áreas cerebrales que incluyeron estructuras del lóbulo temporal medial, frontal medio y cerebelo. Asimismo, se identificaron áreas estratégicas (i.e., lóbulo temporal medial y cerebelo) relacionadas con la disminución del rendimiento en el AFE-T a nivel longitudinal en esta población.

En los últimos años, la relación entre los biomarcadores de la EA y la cognición ha sido ampliamente estudiada en sujetos cognitivamente sanos (Donohue et al., 2017; Dumurgier et al., 2017; Ho & Nation, 2018; Pettigrew et al., 2015; Sanabria et al., 2018; Tijms et al., 2018). Algunos estudios han sugerido que los niveles de tau podrían guardar una relación más estrecha con el rendimiento cognitivo que los niveles de β-amiloide (Hessen et al., 2015; Nelson et al., 2012; Rolstad et al., 2013). El principal hallazgo del segundo estudio, fue encontrar una asociación entre los niveles de T-tau en LCR y la disminución del rendimiento en el AFE-T en un periodo de dos años, en una muestra de sujetos cognitivamente sanos con niveles normales de biomarcadores de EA en LCR. En línea con estos resultados, algunos estudios han encontrado asociaciones entre la disminución del rendimiento cognitivo y los niveles de tau en sujetos cognitivamente sanos (Glodzik et al., 2011; Hessen et al., 2015). Hessen et al. (2015) estudiaron una muestra de 122 sujetos con DCS y encontraron que un subgrupo que presentaba niveles de T-tau más elevados al inicio del estudio, mostró una mayor disminución del rendimiento en memoria a los dos años de seguimiento. Sin embargo, los resultados de este estudio podrían estar influenciados por el tipo de muestra evaluada, ya que se ha sugerido que la población con DCS podría tener un riesgo mayor de desarrollar deterioro cognitivo que los controles (Jessen et al., 2014). En otro estudio, Glodzik et al. (2011) encontraron que los niveles elevados de P-tau estaban relacionados tanto con la disminución del rendimiento cognitivo como con la atrofia del lóbulo temporal medial, en un grupo de sujetos cognitivamente sanos que experimentaron una disminución de la memoria en un periodo de dos años. No obstante, es importante destacar que los niveles de P-tau son un marcador específico de patología neurofibrilar y que, además, el grupo que mostró disminución de la memoria también presentaba niveles más bajos de la ratio $A\beta_{42}/A\beta_{40}$ en LCR. Esto sugiere que, en este estudio, hubo

una contribución notable de la patología de tipo Alzheimer en el rendimiento cognitivo de los participantes. Probablemente el aspecto más relevante del segundo trabajo de la tesis doctoral, es que se estudió el envejecimiento sano mediante la evaluación de una muestra con niveles normales de biomarcadores de EA, excluyendo la posibilidad de incluir en el estudio a sujetos dentro del continuo Alzheimer. Se observó una contribución única de los niveles de T-tau en la disminución de la capacidad de aprendizaje y memoria, independientemente del grado de DCS de los participantes o de los niveles de $A\beta_{42}$.

Este trabajo también permitió identificar las regiones de substancia gris relacionadas con el rendimiento en el AFE-T, tanto a nivel basal como longitudinal. El rendimiento basal se vio relacionado con el volumen de substancia gris en estructuras del lóbulo temporal medial, frontal medio y cerebelo. La puntuación de aprendizaje libre se vio asimismo relacionada con áreas específicas del lóbulo frontal, tal vez reflejando una mayor contribución de las funciones ejecutivas en comparación con el recuerdo con pistas. Los resultados coinciden con la literatura existente, que muestra que las regiones temporal medial y prefrontal están relacionadas con el rendimiento en una amplia variedad de tareas de memoria (Eyler, Sherzai, Kaup, & Jeste, 2011; Rugg & Vilberg, 2013; Wolk & Dickerson, 2011). Sin embargo, la región cerebral predominante involucrada tanto en el rendimiento transversal como longitudinal fue el cerebelo. La implicación del cerebelo en tareas de aprendizaje y memoria, lenguaje y funciones ejecutivas, ha sido ampliamente descrita (Desmond & Fiez, 1998; Guell, Gabrieli, & Schmahmann, 2018; Kim et al., 1994; Rosenbloom et al., 2012; Stoodley & Schmahmann, 2018). La contribución predominante del cerebelo frente a otras estructuras podría explicarse por el estado cognitivo y biológico de la muestra estudiada ya que, en el envejecimiento sano, los lóbulos cerebelosos muestran una reducción

acelerada con respecto a otras estructuras (Taki et al., 2013). Los resultados presentados indican que hay áreas del lóbulo temporal medial, frontal medio y cerebelo relacionadas con el rendimiento en aprendizaje y memoria en una muestra bien caracterizada de sujetos con cognición normal y niveles normales de biomarcadores de EA en LCR. Tomados en conjunto, los hallazgos sugieren que existen variables biológicas y neuroanatómicas que podrían influir en el empeoramiento cognitivo en el envejecimiento sano y que no están relacionados con los procesos patológicos de la EA. Dado que tanto los niveles de T-tau en LCR como los cambios en RM se consideran biomarcadores de neurodegeneración (Jack et al., 2018), los resultados obtenidos apuntan a la presencia de neurodegeneración como potencial predictor de los cambios cognitivos más tempranos en el envejecimiento sano.

Más allá del estudio de los dominios cognitivos más típicos (i.e., aprendizaje y memoria, funciones ejecutivas) en la detección de dificultades cognitivas en la EA, se ha sugerido que la evaluación del rendimiento motor podría ser de utilidad en la evaluación de sujetos situados en la fase preclínica del continuo Alzheimer (Albers et al., 2015; Buchman & Bennett, 2011). En esta línea, los resultados del tercer trabajo sugieren que la disfunción motora está asociada con la patología amiloide y que puede ser detectada de forma sutil durante la etapa más temprana del continuo Alzheimer.

En este trabajo se observó que los niveles de $A\beta_{42}$ estaban relacionados con el rendimiento en el FTT. Una interpretación plausible de estos hallazgos es que la disfunción motora podría estar relacionada con el depósito más temprano de proteína amiloide cerebral, que es, según la hipótesis de la cascada amiloide (Hardy & Higgins, 1992), el biomarcador más temprano en la etapa asintomática de la EA. Los mecanismos por los cuales la patología amiloide podría conducir a una disfunción motora en la fase preclínica del continuo Alzheimer todavía se desconocen. Sin

embargo, algunos estudios neuropatológicos sugieren que el acúmulo de β-amiloide puede estar presente de forma temprana en diferentes áreas corticales, incluidas las regiones relacionadas con la función motora (Schneider et al., 2006; Suva et al., 1999). Esto ha sido observado no únicamente en pacientes con EA (Arnold, Hyman, Flory, Damasio y Van Hoesen, 1991), sino también en sujetos cognitivamente sanos (Giannakopoulos, Hof, Michel, Guimon y Bouras, 1997). Asimismo, los modelos estadísticos aplicados mostraron que tanto los niveles de $A\beta_{42}$ como su interacción con los niveles de P-tau contribuyeron al rendimiento en el FTT. Este es un hallazgo importante ya que estos niveles son indicadores válidos de los depósitos de proteínas subyacentes a la fisiopatología de la EA (i.e., placas de β-amiloide y ovillos neurofibrilares, respectivamente) y definen la EA como una enfermedad neurodegenerativa específica entre otros trastornos cognitivos (Jack et al., 2018).

Otro aspecto importante fue investigar diferentes medidas de rendimiento motor como alternativa a los tests neuropsicológicos estándar, los cuales carecieron de nuevo de la sensibilidad necesaria para diferenciar entre los sujetos con niveles alterados de $A\beta_{42}$ y los controles. El FTT se mostró como una tarea no invasiva, breve y fácil de entender y completar independientemente del nivel educativo, lo cual se sabe que es uno de los principales factores de confusión en los estudios sobre la cognición (Stern, 2009). Además, permitió el estudio de un indicador del rendimiento motor menos utilizado: la variabilidad intra-sujeto. La inconsistencia del rendimiento entre los diferentes ensayos del FTT resultó ser un indicador más sensible que la velocidad de *tapping*. Los escasos estudios previos que habían estudiado esta medida, ya sugerían que la variabilidad intra-sujeto podría ser un marcador relevante para la EA temprana (Bangert y Balota, 2012; Verghese et al., 2008, 2007). Verghese et al. (2008) indicaron que un grupo de sujetos con EA inicial presentaba mayor variabilidad al caminar que el grupo control. Otros

estudios han mostrado que los pacientes con EA presentan mayor variabilidad en tareas de *tapping* que los controles (Bangert y Balota, 2012). En línea con estos resultados, el tercer trabajo destaca la potencial utilidad de esta medida motora menos estudiada. Tomados en conjunto, los hallazgos sugieren que la alteración de los biomarcadores de la EA podría conducir a una disfunción motora sutil en sujetos cognitivamente sanos y que una evaluación precisa de las funciones motoras podría ayudar a identificar personas en la etapa más temprana del continuo Alzheimer.

En el cuarto trabajo, se empleó el AFE-T para investigar la presencia de olvido acelerado a largo plazo (OLP) en sujetos cognitivamente sanos con riesgo genético incrementado de desarrollar EA (portadores de APOE ε4), así como la relación entre el OLP y los biomarcadores de EA. En comparación con los no-portadores (homocigotos APOE ε3/ε3), se detectó OLP en el grupo de portadores (heterocigotos APOE ε3/ε4) en el recuerdo con pistas a los tres meses del AFE-T. Este rendimiento se vio relacionado con los biomarcadores de EA en LCR, así como con las puntuaciones del SCD-Q.

Los efectos de APOE ε4 en el rendimiento en memoria de sujetos cognitivamente sanos y su relación con la carga de amiloide han sido ampliamente estudiados (Kantarci et al., 2012; Lim et al., 2013; Mormino et al., 2014). Sin embargo, los datos existentes sobre la relación entre el OLP y la EA son todavía muy limitados. Un estudio reciente de investigación en animales, mostró OLP en ratones *PDAPP*, un modelo de EA familiar presintomática (Beglopoulos et al., 2016). En humanos, el OLP se ha postulado como un potencial marcador cognitivo de las fases asintomáticas de la EA. Un estudio reciente identificó OLP en sujetos presintomáticos con EA familiar en un periodo de una semana (Weston et al., 2018). Utilizando un diseño similar, Zimmerman y Butler (2018) evaluaron 60 participantes cognitivamente sanos (20 homocigotos para ε3, 20 heterocigotos para ε3 y ε4, y 20 homocigotos para ε4) y encontraron OLP en los grupos

portadores de APOE ε4. Los sujetos homocigotos para ε4 olvidaron más información que los heterocigotos y, del mismo modo, los sujetos hereocigotos olvidaron más que los no-portadores. Los resultados del cuarto trabajo, mostraron que los grupos de portadores y no-portadores tenían un rendmiento similar en la capacidad de aprendizaje y codificación. Sin embargo, en comparación con los no-portadores, el grupo de portadores mostró OLP en un periodo de tres meses desde el aprendizaje inicial. El AFE-T permitió un análisis más exhaustivo de los procesos de codificación y olvido, durante un período de tiempo más largo (6 meses) que en los trabajos publicados hasta la fecha. El recuerdo a los 3 meses permitió identificar OLP en sujetos portadores de APOE ε4 y se observó una mayor sensibilidad de las medidas de recuerdo facilitado en comparación con la evocación libre.

En relación al modelado de la función de olvido, ambos grupos se ajustaron adecuadamente a la función logarítmica. Particularmente en el recuerdo con pistas, los portadores de APOE ε4 mostraron una pendiente mayor en comparación con los no-portadores, indicando una mayor pérdida de información después del aprendizaje inicial especialmente entre la primera semana y los 3 meses. Es importante tener en cuenta que, en la función empleada, la pendiente correlacionó significativamente con la tasa de olvido a los 3 meses del AFE-T, demostrando que ambas medidas estaban claramente asociadas. Esto indicó nuevamente que la información se perdió en mayor medida durante el período inicial después de la codificación y que, a partir de los 3 meses, la pérdida de información fue mínima. Esto podría sugerir que el estudio de la función de olvido más allá de los tres meses podría ser menos sensible. En este trabajo, también se encontraron correlaciones significativas entre el grado de DCS y el OLP. Este es un hallazgo importante dado que el DCS se ha sugerido como un factor de riesgo cognitivo para el desarrollo de deterioro cognitivo y demencia (Jessen et al., 2010; Reisberg,

Shulman, Torossian, Leng, & Zhu, 2010). Todavía más relevante es la fuerte correlación observada entre el OLP y la ratio $A\beta_{42}$/P-tau, la cual aporta mayor evidencia para la identificación del OLP como un potencial indicador de dificultades cognitivas sutiles en las fases asintomáticas de la EA. En resumen, es posible detectar OLP en individuos con riesgo genético incrementado de desarrollar EA, y existe una relación entre esta medida cognitiva y los procesos fisiopatológicos subyacentes a la enfermedad. El uso de pruebas cognitivas más exigentes, que incluyan medidas de olvido a largo plazo, podría ser de utilidad en la identificación y seguimiento de las primeras manifestaciones cognitivas en las fases más tempranas del continuo Alzheimer.

El quinto y último trabajo, mostró dificultades sutiles de aprendizaje y olvido a largo plazo en sujetos con DCS, especialmente en aquellos con niveles anormales de $A\beta_{42}$ en LCR. Se exploró la función de aprendizaje y olvido a largo plazo en una muestra bien caracterizada de controles y sujetos con DCS mediante el AFE-T. Los hallazgos sugieren que existe una relación entre el grado de DCS y el rendimiento cognitivo objetivo en las funciones de aprendizaje y olvido a largo plazo.

Cada vez existe mayor evidencia que sugiere que el DCS podría ser una de las primeras manifestaciones sintomáticas en el continuo Alzheimer (Jessen et al., 2014; Mitchell, Beaumont, Ferguson, Yadegarfar y Stubbs, 2014; Reisberg et al., 2008). Sin embargo, la mayoría de estudios transversales no han encontrado un rendimiento significativamente menor en pruebas neuropsicológicas en sujetos con DCS en comparación con controles, lo que sugiere que la relación entre el DCS y el rendimiento cognitivo objetivo es difícil de encontrar cuando se utilizan tests neuropsicológicos estándar (Mark y Sitskoorn, 2013). El AFE-T mostró dificultades sutiles de memoria, en particular olvido a largo plazo, en sujetos con DCS que de otro modo no fueron

detectadas por los tests neuropsicológicos estándar. Recientemente, se ha sugerido que la queja subjetiva de memoria está más estrechamente relacionada con riesgo de deterioro cognitivo futuro (Jessen et al., 2010). Sin embargo, esta suposición podría estar sesgada debido a que la mayoría de trabajos sobre DCS se han focalizado en el estudio de esta función cognitiva y hay pocos trabajos (La Joie et al., 2016; Valech et al., 2018) que se centren en el deterioro subjetivo de otras funciones. Otro punto importante con respecto a la posible aplicación del AFE-T en sujetos con DCS guarda relación con sus correlatos neurales. Recientemente, los correlatos funcionales y estructurales del AFE-T se han identificado tanto en sujetos cognitivamente sanos (Grönholm et al., 2005), como en pacientes con afasia (Grönholm et al., 2007; Tuomiranta et al., 2015). Estos estudios han sugerido que el rendimiento en esta tarea depende de regiones cerebrales específicas que generalmente se ven afectadas en la EA, como el lóbulo temporal medial. En esta línea, estudios transversales han mostrado una pérdida de volumen incipiente en esta región del cerebro en sujetos con DCS (Perrotin et al., 2017; Peter et al., 2014; Saykin et al., 2006).

Uno de los aspectos más interesantes del estudio fue observar que el estado del biomarcador de amiloide (positivo *versus* negativo) influyó significativamente en el rendimiento cognitivo de los sujetos con DCS. Aquellos individuos con niveles alterados de $A\beta_{42}$ mostraron un rendimiento significativamente peor tanto en la fase de aprendizaje como en la de recuerdo a largo plazo del AFE-T, en comparación con aquellos con niveles normales. Estos resultados están en línea con estudios longitudinales recientes que indican que los sujetos con DCS y niveles anormales de biomarcadores de EA tienen un mayor riesgo de deterioro cognitivo (Van Harten et al., 2013; Wolfsgruber et al., 2017). Al mismo tiempo, las observaciones de este trabajo coinciden con estudios previos que indican que los sujetos cognitivamente sanos con

evidencia de patología amiloide y DCS experimentan deterioro cognitivo longitudinal antes que los individuos que presentan únicamente DCS (Vogel et al., 2017).

Con respecto a la evaluación del DCS, el SCD-Q podría ser especialmente adecuado para el estudio de las primeras manifestaciones clínicas en el continuo Alzheimer. Diferentes estudios han mostrado puntuaciones significativamente más altas en el SCD-Q en muestras de sujetos situados dentro del continuo Alzheimer en comparación con controles (Valech et al. 2015; Valech et al. 2018). El SCD-Q se diferencia de otros cuestionarios porque explora la percepción subjetiva de deterioro cognitivo en un período relativamente corto de tiempo (2 años). En lugar de restringirse al ámbito de la memoria, el SCD-Q explora el rendimiento en una serie de actividades de la vida diaria que involucran múltiples dominios cognitivos. Además, el SCD-Q permite recoger la opinión de un informante acerca de la cognición del sujeto de estudio. Estas propiedades aumentan la probabilidad de detectar DCS en el contexto de la fase preclínica del continuo Alzheimer (Jessen et al. 2014). Este trabajo destaca la necesidad de desarrollar y emplear medidas más robustas y sensibles para garantizar una evaluación exhaustiva del DCS. En resumen, mediante el uso de medidas integrales para evaluar la cognición subjetiva y objetiva, es posible detectar dificultades cognitivas sutiles en individuos con DCS, especialmente en aquellos situados dentro del continuo de Alzheimer. Los hallazgos señalan la importancia de estudiar los biomarcadores de la EA y su relación con el DCS en la evaluación de las primeras manifestaciones cognitivas de la fase preclínica del continuo Alzheimer.

VII. CONCLUSIONES

CONCLUSIONES

1. Mediante el uso de nuevas pruebas cognitivas altamente exigentes, como el *Ancient Farming Equipment Test*, es posible identificar dificultades de aprendizaje y olvido a largo plazo en sujetos cognitivamente sanos situados dentro del continuo Alzheimer.

1.1. El rendimiento en el *Ancient Farming Equipment Test* está relacionado con los biomarcadores centrales de la enfermedad de Alzheimer (i.e., biomarcadores de amiloide y tau patológica).

1.2. El *Ancient Farming Equipment Test* es una herramienta prometedora para la caracterización del perfil cognitivo de la fase preclínica del continuo Alzheimer.

1.3. El uso de tests neuropsicológicos estándar para el estudio e identificación de sujetos en la fase preclínica del continuo Alzheimer resulta insuficiente.

2. Los niveles de tau total en líquido cefalorraquídeo, un marcador de neurodegeneración, están relacionados con el empeoramiento cognitivo longitudinal en sujetos cognitivamente sanos situados fuera del continuo Alzheimer.

2.1. El rendimiento en el *Ancient Farming Equipment Test* está relacionado con el volumen de sustancia gris en áreas del lóbulo temporal medial, frontal medio y cerebelo. Un menor volumen de substancia gris en áreas cerebrales estratégicas está relacionado con el empeoramiento cognitivo a nivel longitudinal.

2.2. Los biomarcadores de neurodegeneración estudiados (i.e., niveles de tau total en líquido cefalorraquídeo y atrofia en resonancia magnética estructural) están relacionados con el empeoramiento de la función de aprendizaje y memoria longitudinal en el envejecimiento sano.

3. Los sujetos cognitivamente sanos situados dentro del continuo Alzheimer presentan un rendimiento significativamente peor en el *Finger Tapping Test* en comparación con controles.

3.1. El rendimiento en el *Finger Tapping Test* está relacionado con los biomarcadores específicos de la enfermedad de Alzheimer en líquido cefalorraquídeo.

4. Es posible detectar olvido acelerado a largo plazo en individuos cognitivamente sanos con riesgo genético incrementado de desarrollar EA (portadores del genotipo APOE ε4).

4.1. Existe una relación entre el olvido acelerado a largo plazo y los biomarcadores específicos de la enfermedad de Alzheimer en líquido cefalorraquídeo, que destaca su aplicabilidad como potencial marcador cognitivo en sujetos portadores del genotipo APOE ε4.

5. Es posible detectar dificultades sutiles de aprendizaje y olvido a largo plazo en sujetos con deterioro cognitivo subjetivo, especialmente en aquellos situados dentro del continuo Alzheimer (i.e., con niveles alterados del biomarcador de amiloide).

XIII. BIBLIOGRAFIA

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