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Brain Structure and Phenotypic Profile of Superagers

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Universidad Autónoma de Madrid | Escuela de Doctorado

Brain Structure and Phenotypic Profile of Superagers

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Madrid, 2023

RESUMEN

Llegar a la vejez con una memoria episódica comparable a la de una persona 30 años más joven es posible, y esta capacidad de recordar experiencias pasadas como si fuéramos más jóvenes se ha conceptualizado bajo el nombre de superanciano. El estudio de los superancianos es una estrategia alternativa a los numerosos esfuerzos dedicados a frenar el deterioro patológico de la memoria episódica que caracteriza a la enfermedad de Alzheimer. En esta tesis se han llevado a cabo cuatro estudios complementarios sobre una amplia muestra de superancianos y adultos mayores típicos de la misma edad seleccionados de la cohorte longitudinal del Proyecto Vallecas. En el primer estudio, un examen transversal y longitudinal de la sustancia gris revela un mayor volumen en el lóbulo temporal medial, el prosencéfalo basal y el tálamo motor, así como una tasa de atrofia más lenta en los superancianos. Un enfoque de aprendizaje automático muestra que una mayor velocidad de movimiento y una mejor salud mental son los factores más diferenciadores de los superancianos. El segundo estudio explora transversal y longitudinalmente la microestructura de la sustancia blanca utilizando medidas de difusión, y revela que los cambios asociados a la edad se suceden a un ritmo más lento en el cerebro de los superancianos en comparación a los adultos mayores típicos. En el tercer estudio se midió la longitud de los telómeros en las células sanguíneas y los superancianos muestran una longitud menor en comparación con los adultos mayores típicos. El cuarto estudio correlaciona la estructura cerebral con la tasa de aprendizaje episódico en la muestra cognitivamente sana de la primera visita del Proyecto Vallecas y amplía este análisis mostrando que los superancianos tienen una tasa de aprendizaje más rápida con relación a los adultos mayores típicos. Esta tesis supone un avance en el campo del envejecimiento de la memoria episódica al identificar a una gran muestra de superancianos que disponen de datos de neuroimagen con un seguimiento anual de hasta 5 años y un amplio número de variables demográficas, clínicas y de estilo de vida. En conjunto, esta tesis aporta valiosos conocimientos sobre la estructura cerebral de los superancianos y su mecanismo de envejecimiento, así como de los factores asociados a este fenotipo, ofreciendo nuevas perspectivas sobre cómo preservar la función de la memoria episódica a edades avanzadas.

ABSTRACT

Reaching old age with an episodic memory comparable to that of a person 30 years younger is possible, and this ability to remember past experiences as if we were younger has been conceptualised as superageing. The study of the superager phenotype is an alternative strategy to the numerous efforts devoted to curbing the pathological deterioration of episodic memory that characterises Alzheimer's disease. In this thesis, four complementary studies have been carried out on a large sample of superagers and typical older adults of the same age selected from the longitudinal cohort of the Vallecas Project. In the first study, a cross-sectional and longitudinal examination of the grey matter reveals a larger volume in the medial temporal lobe, the basal forebrain and the motor thalamus, as well as a slower rate of atrophy in superagers. A machine learning approach shows that higher movement speed and better mental health are the most differentiating factors in superagers. The second study replicates the cross-sectional and longitudinal examination for white matter microstructure using diffusion measures and reveals that age-related changes in the brains of superagers occur at a slower rate than in typical older adults. In the third study, telomere length was measured in blood cells and superagers show shorter telomere length compared to typical older adults. The fourth study correlates brain structure with episodic learning rate in the cognitively healthy sample from the first visit of the Vallecas Project and extends this analysis by showing that superagers have a faster learning rate relative to typical older adults. This thesis advances the field of episodic memory ageing by identifying a large sample of superagers who had neuroimaging data with up to 5 years of annual follow-up and a wide range of demographic, clinical and lifestyle variables. Overall, this thesis provides valuable insights into the brain structure of superagers and their ageing mechanism, as well as the factors associated with this phenotype, offering new perspectives on how to preserve episodic memory function at advanced ages.

ABREVIATIONS

Aβ	amyloid β
APOE	apolipoprotein E
ATR	anterior thalamic radiation
BMI	body mass index
BOLD	blood-oxygen-level-dependent
DNA	deoxyribonucleic acid
FA	fractional anisotropy
FCSRT	free and cued selective reminding test
FDR	false discovery rate
fMRI	functional magnetic resonance imaging
FOV	field of view
FWE	family-wise error
GFAP	glial fibrillary acidic protein
GLM	general linear model
GWAS	genome wide association analysis
HT Q-FISH	high-throughput quantitative in-situ hybridisation
IFO	inferior fronto-occipital fasciculus
ILF	inferior longitudinal fasciculus
IQR	interquartile range
Kbp	kilobase pairs
MAP2K3	mitogen-activated protein kinase kinase 3
MD	mean diffusivity
MTL	medial temporal lobe
MMVT	multimodal analysis and visualisation tool
MNI	Montreal neurological institute
NfL	neurofilament light polypeptide
NFT	neurofibrillary tangles
PCR	polymerase chain reaction
PET	positron emission tomography
p-Tau181	tau phosphorylated at threonine 181
ROI	regions of interest
SD	standard deviation
SE	standard error
SE-EPI	spin echo echo planar imaging
Simoa	single molecule array
SLF	superior longitudinal fasciculus
SNPs	single nucleotide polymorphisms
TBSS	tract-based spatial statistics
TE	echo time
TFCE	threshold free cluster enhancement
TI	inversion time
TIV	total intracranial volume
TR	repetition time
t-Tau	total tau

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

The old stories of the elders exemplify how important is to remember our life events for healthy ageing. Memories of past events are referred to as episodic memory and this function enable us to remember our past, to learn though experiences and to navigate the future, in sum, it builds our identity as individuals. Like most functions of our body and mind, episodic memory is expected to decline slightly with age (Glisky, 2007; Greene et al., 1996; Nilsson et al., 1997). The effects of ageing cannot be explained solely by the passage of time, but by a complex interaction between genetic make-up, stochastic life factors and the passage of time. Therefore, the older the age of a population group, the greater the variability in the performance of vital functions. Human episodic memory ageing in most people manifests as a slight decline that does not limit daily life, a second group that suffers a more severe decline leading to a diagnosis of dementia, and a third group who has an episodic memory as good as that of a young adult (Gefen et al., 2015; Harrison et al., 2012; Josefsson et al., 2012; Sun et al., 2016; Zacks et al., 2000). In the current context of progressively increasing life expectancy and the consequent growth of the ageing population (World Health Organization, 2023), the number of people suffering from pathological alterations of episodic memory is rising and the first treatments capable of altering the course of memory impairment have only recently been approved (van Dyck et al., 2023). This increase in the ageing population may be a double-edged sword: at the same time as the incidence of dementia is rising, the number of people with a successful episodic memory for their age is also increasing, and now the study of this rare phenotype can be systematised with the aim to prevent the pathological deterioration of episodic memory. The study of elders with youthful episodic memory will unravel how successful episodic memory ageing can occur naturally. Their brain signature can provide unexplored targets for brain stimulation and their lifestyle can inform of potential protective factors that could be included in intervention trials to evaluate its beneficial effect.

The aim of the present PhD thesis is to advance conceptually the field of successful episodic memory ageing through a comprehensive phenotyping of a cohort of superagers —elderly people with an episodic memory as good as a healthy person 20-30 years younger— in relation to a group of typical older adults from the Vallecas Project cohort. This conceptual advance involves the identification of the dominant ageing mechanism in the brain of superagers by a cross-sectional and longitudinal study of their brain structure, and the association of this phenotype with demographic, clinical and lifestyle factors, neurodegenerative blood biomarkers and telomere length.

The first chapter will introduce the concept of episodic memory, how this cognitive function can be examined in human cohorts and what its neural substrates are. The vulnerability of episodic memory to age and age-related pathologies will then be explored. Finally, previous research on successful episodic memory ageing will be reviewed, including the brain signature from neuroimaging to histology studies, demographic, lifestyle, clinical, molecular, and genetic factors. Subsequently, a general methods chapter will describe recruitment characteristics of the Vallecas Project cohort and follow-up protocols, as well as the neuropsychological criteria applied to select the group of superagers and typical older adults that are used across all empirical chapters. The first of the four empirical chapters investigates the cross-sectional and longitudinal signature of the brain grey matter volume of superageing together with a multivariate approach to study which demographic, lifestyle and clinical factors are associated with this phenotype. In the next chapter, the brain white matter of superagers is phenotyped cross-sectionally and longitudinally with diffusivity measures. In the third empirical chapter, measures of telomere length as a biological marker of age are examined cross-sectionally in superagers and typical older adults. In the last analysis, we explore the episodic learning rate in the whole cognitively healthy sample of the Vallecas Project cohort at baseline and compare this rate between superagers and typical older adults. Finally, the observed results are assessed together in the context of previous research in the field and their usefulness for the design of intervention studies aimed at the prevention of episodic memory loss is discussed.

1.1 HUMAN EPISODIC MEMORY AND METHODS OF STUDY

The concept of episodic memory was coined by Endel Tulving in 1972 and referred to it as the feeling of remembering past events and being able to associate these

memories with a particular place and time (Tulving, 1972). Episodic memory is one of many labels that have been used to compartmentalise the memory systems for study purposes. There are many classification schemes that are constantly being revised, here we present the classification scheme of human memory systems proposed by Tulving (Tulving, 1991): *procedural memory, perceptual priming, short-term memory, semantic memory* and *episodic memory*. These systems are not independent and rather their constant interaction is responsible for the overall cognitive response (Tulving, 1991), although there are unique characteristics that motivate the classification into these five categories. Procedural memory retains abilities learned through the effects of practice in perceptual-motor tasks, also encompasses simple conditioning and it is the only one of the five that does not involve cognition. Perceptual priming describes the enhanced identification of an object or a word when it has been exposed to it prior to retrieving that memory. Short-term memory is often referred to as working memory (Baddeley, 1986) and represents information that is highly accessible for a short period of time after the stimulus presentation. Semantic memory stores knowledge about the world, allowing one to operate with those ideas mentally. Finally, episodic memory, as mentioned above, stores personal experiences from the past, providing the capacity to place the event in a subjective time and place, to recall the timeline of events and to mentally travel backwards and forwards in time.

There is controversy about episodic memory as to whether the overlap with semantic memory is such that they cannot be considered two separate entities. This controversy is based on the notion that both memory systems share a common process of encoding information in long-term storage (Tulving and Markowitsch, 1998). Tulving, the same author who gave episodic memory an entity of its own, considers episodic memory to be a unique extension of semantic memory, because it depends on the capabilities of semantic memory but extends over it (*i.e.*, episodic memory implies semantic knowing, but a semantic memory does not require episodic remembering) (Tulving, 1984, 1985). The uniqueness of episodic memory is the conscious awareness of recollection —also called *autonoetic consciousness* (Tulving, 1985)— implying knowledge of the context in which the episodic memory was acquired, whereas this is not the case with semantic memory. Other classification schemes of human memory include episodic and semantic memory under the umbrella term *declarative memory* (Cohen, 1984; Squire, 1992). Empirical attempts to elucidate whether semantic and episodic memory are two separate entities have tested the ability to learn new semantic information in amnesic patients (*i.e.*, patients whose ability to recall personal

experiences was compromised after brain lesions). There is evidence that some amnesic patients are able to learn new facts (Kitchener et al., 1998; Tulving et al., 1991; Verfaellie et al., 2000), although it remains uncertain whether this might be due to a residual capacity related to the extent of the lesions causing the amnesia or even whether the new semantic information was acquired unconsciously (Bayley and Squire, 2002).

Beyond controversies in the theoretical conceptualisation of episodic memory, a practical challenge for memory evaluation is to control for interpersonal variability in its processing (Buschke, 1984). Individuals learn in slightly different ways due to differences in the level and focus of attention or in the strategy used to retrieve learned information (Buschke, 1984). Different working definitions have been proposed to control memory processing in order to facilitate its systematic study. The three main paradigms for the assessment of human episodic memory are reviewed below.

Interviews or self-report are used to assess episodic memory (Addis and Schacter, 2008; Audrain et al., 2022; Levine et al., 2002; Setton et al., 2022), although this approach can be problematic when assessing specific groups, such as children or dementia patients, due to the strong dependence on language competences (Cheke and Clayton, 2013). The second paradigm is The What-Where-When test proposed by Clayton and Dickinson (Clayton and Dickinson, 1998) based on the original definition of episodic memory as the ability to store temporal and spatial references from past experiences (Tulving, 1972). The same authors later argued that the recollection of the elements *what*, *where* and *when* must be integrated into a single representation to resemble episodic memory (Clayton et al., 2003). This paradigm has been used in animal experiments, from birds, to rodents and to great apes, due to the language independence (Amodio et al., 2021; Babb and Crystal, 2006; Clayton and Dickinson, 1998; Martin-Ordas et al., 2010), and also in humans (Cheke and Clayton, 2013; Craig et al., 2016; Holland and Smulders, 2011). Lastly, The Free-Recall paradigm was designed considering the type of cues that trigger memory retrieval, external (cued recall) or internal (free recall). Memories cued internally are easier to recall episodically than with an external cue (Tulving, 1985) and that is the motivation for assessing episodic memory with tests of free-recall of items previously presented in a word list. The Free-Recall paradigm is part of many standardised neuropsychological evaluations in clinical and research settings. Examples of free-recall episodic memory tests are the Rey Auditory Verbal Learning Test (Schmidt, 1996), the California Verbal Learning Test (Delis D.C, 2000) or the Free and

Cued Selective Reminding Test (FCSRT) (Buschke, 1984), among others. The FCSRT is the memory test used in the sample selection of the analyses covered in this thesis and, for illustrative purposes, the following describes how the FCSRT test is administered in the Vallecas Project.

The FCSRT in the Vallecas Project is administered using standard procedures (Peña-Casanova et al., 2009a). Participants are presented with cards containing four words and are asked to identify the word corresponding to a specific semantic category (*e.g.*, clothing, animals, etc.), going through all four words, on four different cards (16 words in total). The words presented are not the most obvious member of each semantic category. Following the presentation phase, participants are asked to recall as many words as possible in three consecutive recall trials each one followed by 20 s of interference counting backwards. For each trial, participants are asked to freely recall as many words as possible with a time limit of 90 s, then examiners provide the semantic category clue for the forgotten items. These three free and cued recalls constitute the three immediate recall trials of the task. This immediate recall phase is followed by a 30 min delay, after which the delayed phase of the test starts. Participants are then asked on a single trial to freely recall as many words as possible otherwise cues are provided. The score in the delayed free recall score is typically use as a proxy for episodic memory performance.

Despite the widespread use of all three paradigms, they assess memory function in settings that differ in two main respects from what memory function would be like in everyday life. Everyday life episodic memories 1) are encoded incidentally in a complex context with many stimuli and 2) are retrieved unexpectedly, *i.e.,* at the time of encoding it is unknown whether the information will need to be retrieved. In conducting these tests, 1) the person's attention is totally driven to items being tested, and 2) the person is informed that their memory is being evaluated (Cheke and Clayton, 2013). There is evidence that deliberately encoded memories may benefit memory performance compared to unexpected tests (Craig et al., 2016; Holland and Smulders, 2011), as well as deliberately encoded memories might be stored as semantic memories rather than episodic (Zentall et al., 2001; Zentall et al., 2008). Although the equivalence among the different episodic memory paradigms is not complete, indicating that different tests might be based on different episodic memory strategies (verbal *vs.* visuospatial), they all seem to recruit the episodic memory system. (Cheke and Clayton, 2013; Holland and Smulders, 2011; Pause et al., 2013; Plancher et al., 2010).

1.2 NEURAL SUBSTRATES OF EPISODIC MEMORY

Medial temporal lobe

The modern era of memory research began with the studies of patient H.M. in the late 1950s, which became instrumental in understanding the importance of the medial temporal lobe (MTL) in the memory system, consisting of the hippocampus and adjacent cortices grouped under the term parahippocampal region, which includes the parahippocampal, perirhinal and entorhinal cortices (Squire and Zola-Morgan, 1991). H.M. was a young man with refractory epilepsy who underwent bilateral MTL resection extending into the hippocampus and adjacent cortices to control his seizures (Milner et al., 1968; Scoville and Milner, 1957). The epileptic seizures disappeared after the surgery, but H.M. suffered from a severe anterograde amnesic syndrome that affected his ability to learn new information about life events and world facts (Milner et al., 1968; Scoville and Milner, 1957). His retrograde amnesia was less severe and, despite he had problems recalling past experiences from the years before the surgery, he retained remote memories from childhood (Milner et al., 1968; Scoville and Milner, 1957). His ability to store long-term declarative memories (Squire, 1992) was impaired after the MTL resection, however, he preserved some levels of perceptual priming (Postle and Corkin, 1998), and procedural memory, as his performance in a motor task improved after practise (Milner, 1972; Milner et al., 1968). Scrutiny on the mind of H.M. revealed the existence of several memory systems that do not share neural substrates, and thus the modern era of memory research definitively abandoned the idea of a unitary memory system.

Since H.M.'s studies, evidence has been accumulating in favour of the idea that lesions of the MTL are necessary to cause the amnesic syndrome and, by extension, the MTL is necessary for episodic memory function (Clark and Squire, 2010; Lad et al., 2019; Rempel-Clower et al., 1996; Scoville and Milner, 1957; Spiers et al., 2001). The role of the MTL in episodic memory is also supported by activation studies, from the earliest event-related functional magnetic resonance imaging (fMRI) evidence of MTL activation specifically during successful memory encoding in humans (Brewer et al., 1998) to later studies (Kao et al., 2005; Katsumi and Dolcos, 2020; Kirchhoff et al., 2000). Recollection of memories depends on the hippocampus (Fortin et al., 2004; Sauvage et al., 2008) as well as the recollection of the context of a specific experience (Clayton and Dickinson, 1998; Day et al., 2003). The hippocampus integrates spatial and non-spatial information

arriving via segregated pathways from different association cortices (*i.e.*, prefrontal, parietal and temporal) and parahippocampal regions (Dickerson and Eichenbaum, 2010; Witter et al., 2000) (Figure 1A). From association cortices to the hippocampus, the spatial information (*where*) is processed through the parahippocampal cortex and the medial entorhinal cortex, and the non-spatial information (*what*) is processed by the perirhinal cortex and the lateral entorhinal cortex (Burwell and Amaral, 1998; Suzuki and Amaral, 1994; Witter et al., 2000). During memory retrieval, cues can activate the object-context representation in the hippocampus by projections of the hippocampus to association areas via these two parahippocampal pathways (Dickerson and Eichenbaum, 2010; Eichenbaum et al., 2007).

Although the MTL is necessary for episodic memory function, it does not act as an isolated structure, and instead, memory function can only be explained by the interaction of the MTL with a complex network of cortical and subcortical regions (Mesulam, 1990; Nyberg et al., 2000). In the following three sections, it will be reviewed how the interaction between the MTL, and other regions drives episodic memory functions, covering first the connections of Papez circuit, then the interaction between the MTL and prefrontal regions, and finally the effects of neuromodulators on episodic memory.

Papez circuit

The Papez circuit was proposed in 1937 as a serial circuit connecting the hippocampus, mammillary bodies, anterior thalamic nuclei, cingulate cortices, and parahippocampal cortices originally in the context of emotion processing (Papez, 1937). Decades later it became highly relevant to episodic memory following reports of amnesia in lesion studies affecting key areas of the circuit and correlations of the structural integrity of these areas with episodic memory performance (Aggleton and Brown, 1999, 2006; Frank et al., 2022; Ji et al., 2020; Takano et al., 2018; Tsivilis et al., 2008; Valenstein et al., 1987; Wang et al., 2018).

This serial view of Papez circuit begins in the hippocampus which projects via the fornix to the mamillary bodies. The mamillary bodies, via the mammillothalamic tract, reaches to the anterior thalamic nuclei which connect via the anterior thalamic radiation (ATR) to the retrosplenial cortex, part of the posterior cingulate cortex. From there, the cingulum fibres continue to the entorhinal cortex, which projects back to the hippocampus (Shah et al., 2012) (Figure 2). With the current knowledge from axonal tracing work, this

unidirectional circuit is an oversimplification. Only two of the connections in the original Papez circuit are unidirectional –fornix and mammillothalamic tract (Aggleton et al., 2022; Bubb et al., 2017). The new view on the still relevant Papez circuit (Frank et al., 2022; Ji et al., 2020; Wang et al., 2020) is that of a more complex interactive network than a serial circuit in which the hippocampal-dominated hierarchy is diluted in favour of functions of the anterior thalamic nuclei and the cingulate cortex over memory processing (Aggleton et al., 2022) (Figure 1B).

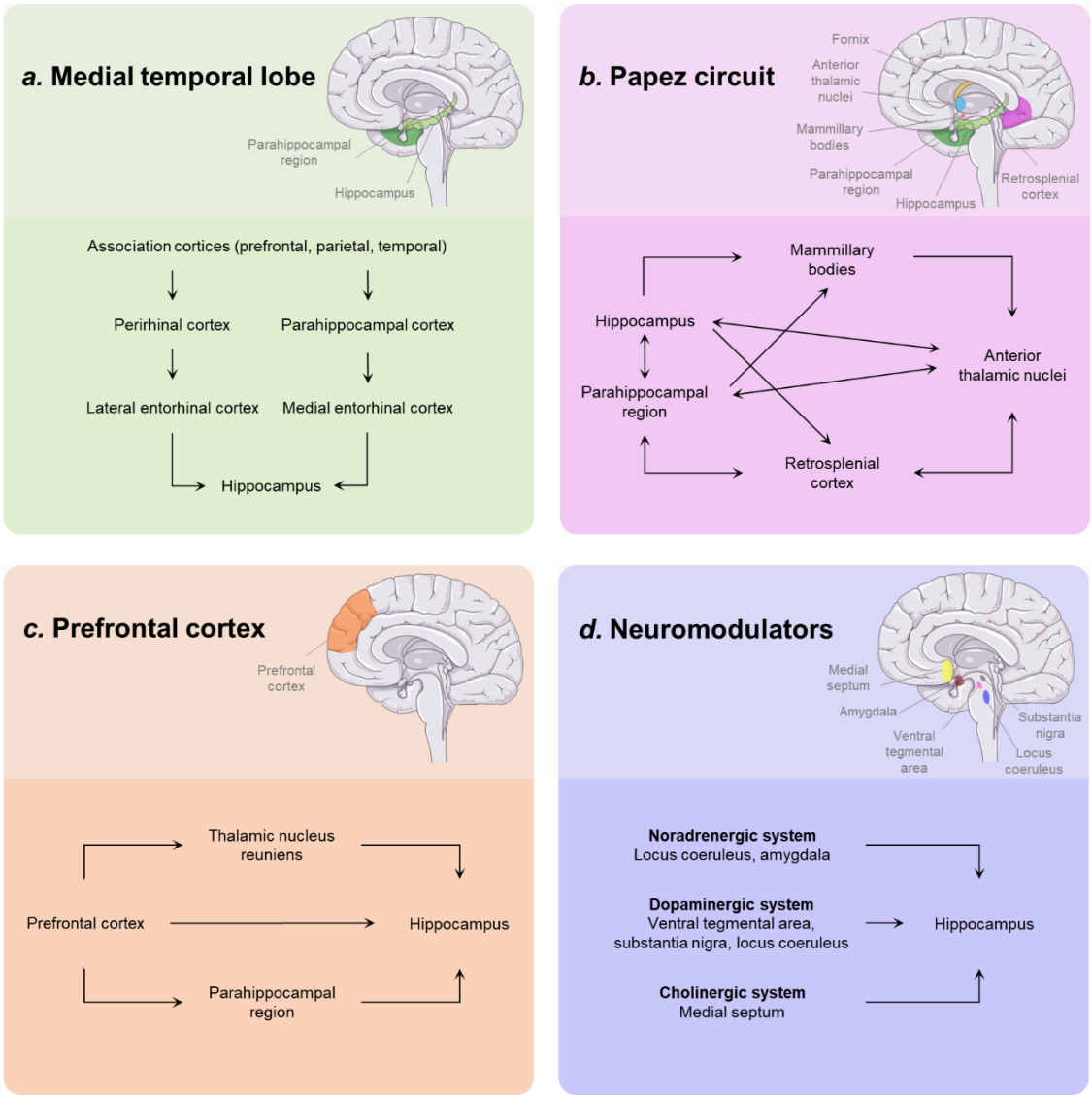

Figure 1. Simplified schematic of neural substrates and their connections involved in episodic memory function. A. The hippocampus integrates spatial and non-spatial information from different association cortices and parahippocampal regions via segregated pathways. **B.** The original serial conception of the Papez circuit has been extended with a more complex network (modified from (Aggleton et al., 2022)). **C.** The top-down control from the prefrontal cortex to the hippocampus is exerted via direct and indirect pathways. **D.** Episodic memory is modulated by noradrenergic, dopaminergic, and cholinergic projections to the hippocampus.

The anterior thalamic nuclei contributes to hippocampal functions in spatial navigation tasks (Aggleton et al., 1995; Clark and Harvey, 2016; Mitchell and Dalrymple-Alford, 2006) but also in episodic memory processes manifested in temporal discriminations, context processing and attentional mechanisms (Nelson, 2021; Wolff and Vann, 2019). The retrosplenial cortex is part of the posterior cingulate cortex and corresponds to Brodmann areas 29 and 30 (Brodmann, 1909). It has an important contribution to spatial navigation (Epstein, 2008; Maguire, 2001; Vann et al., 2009) and episodic memory (Svoboda et al., 2006; Vann et al., 2009), and has been associated with the retrieval of more recent episodic memories than remote ones (Maguire, 2001; Osawa et al., 2006; Valenstein et al., 1987).

Interaction between prefrontal cortex and medial temporal lobe

Processing of episodic memory function goes beyond the interaction between the MTL with Papez circuit nodes, as the MTL interacts strongly with association cortices, and one of particular interest is the prefrontal cortex. The prefrontal-MTL interaction complements the hippocampus in episodic memory function (Miller and Cohen, 2001), and although prefrontal lesions may have consequences for memory function, they do not produce an amnesic syndrome as severe as lesions in the MTL (Davidson et al., 2006; Janowsky et al., 1989; Szczepanski and Knight, 2014). The interaction between the prefrontal cortex and hippocampus can follow different pathways, a direct connection (Hoover and Vertes, 2007; Jay et al., 1989) or indirect connections via the parahippocampal region (Agster and Burwell, 2009; Apergis-Schoute et al., 2006; Dickerson and Eichenbaum, 2010) or via the thalamic nucleus reuniens (Cassel et al., 2013; Ketz et al., 2015; Vertes et al., 2007) (Figure 1C). These interactions are involved in both the encoding and recall process, although some authors argue that they play a more crucial role in memory recall (Cansino et al., 2002; Eldridge et al., 2000; Maril et al., 2003). The prefrontal cortex exerts a top-down control over the MTL by comparing retrieval cues with higher-level representations stored in the cortex and verifying the cues (Dobbins et al., 2002; Simons and Spiers, 2003; Szczepanski and Knight, 2014). Thus, the prefrontal cortex controls the retrieval of appropriate memories and the suppression of inappropriate memories (Anderson et al., 2016; Tomita et al., 1999). This prefrontal-MTL interaction may be asymmetric and left-dominant if memories are verbal in nature (Brewer et al., 1998; Kelley et al., 1998; Simons et al., 2001).

Attention has focussed on the prefrontal-MTL interaction because of its particular importance, although there are other association cortices involved in episodic memory. The parietal and temporal areas also have a role in episodic memory processing (Dickerson and Eichenbaum, 2010; Tulving et al., 1994; Uncapher et al., 2006; Wagner et al., 2005). Many of these association cortices are part of the so-called default mode network (posterior cingulate cortex/precuneus, lateral inferior parietal lobe, lateral and medial temporal lobe, and medial prefrontal cortices) that is active when no other perceptual, attentional or language task is being performed, and this network processes internal information, such as episodic memory (Buckner et al., 2008; Buckner et al., 2013; Raichle et al., 2001).

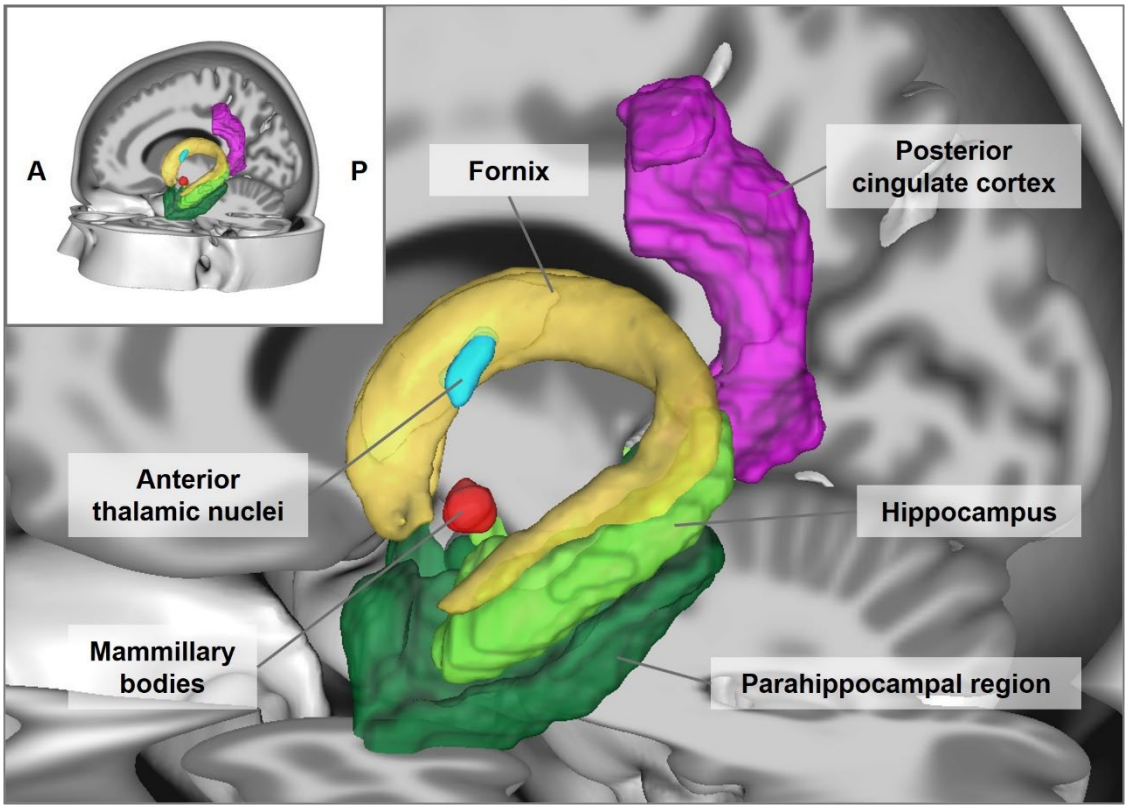

Figure 2. Anatomy of the central substrates of Papez circuit. The classical serial view of Papez circuit begins in the hippocampus which projects via the fornix to the mamillary bodies. The mamillary bodies, via the mammillothalamic tract (not shown), reaches to the anterior thalamic nuclei which connect via the anterior thalamic radiation (ATR) (not shown) to the retrosplenial cortex, part of the posterior cingulate cortex. From there, the cingulum fibres (not shown) continue to the entorhinal cortex, which projects back to the hippocampus (Shah et al., 2012). A, anterior; P, posterior.

Neuromodulators of episodic memory

An additional layer of complexity can be added to the neural substrates of episodic memory in terms of neuromodulation via noradrenergic, dopaminergic, and cholinergic systems (Figure 1D). The noradrenergic system enhances episodic memory of emotional events, and this effect is mediated by the amygdala (Cahill et al., 1994; LaBar and Cabeza, 2006; Strange and Dolan, 2004; Strange et al., 2003). The locus coeruleus, the major source of noradrenaline in the brain (Glowinski and Iversen, 1966), is engaged in goal-directed movement and recent findings have established that action/movement enhances episodic memory encoding mediated by noradrenergic projections from the locus coeruleus (Yebra et al., 2019). Dopamine signals rewarding, novelty and salient information (Berridge, 2007; Horvitz, 2000; Matsumoto and Hikosaka, 2009; Schultz, 2000) and different empirical data indicates that memory of rewarding, novel and salient events can be boosted by dopaminergic projections from the ventral tegmental area and substantia nigra to the hippocampus (Lisman and Grace, 2005; Shohamy and Adcock, 2010). The locus coeruleus also has dopaminergic projections to the hippocampus that enhance memory function (Kempadoo et al., 2016; McNamara et al., 2014; Takeuchi et al., 2016). A recent combined assessment of the noradrenergic and dopaminergic system shows their specific contribution on episodic memory: noradrenaline plays a dominant role in boosting memory for emotionally arousing information, whereas dopamine is more influential in modulating selectivity bias and boosting memories of rewarding and salient information (Hauser et al., 2019). Taken together, this helps to select the small amount of information to be stored in long-term memory relative to the constant flow of information we receive. Acetylcholine modulates episodic memory function via projections from basal forebrain regions, specifically from the medial septum through the bundle of the diagonal band of Broca that terminates in the hippocampus (Amaral and Kurz, 1985; Lewis et al., 1967; Lynch et al., 1977). These cholinergic projections modulate the hippocampal firing pattern, including the theta rhythm, as demonstrated by pharmacological blockage of septal cholinergic neurons (Bolding et al., 2020; Koenig et al., 2011; Rawlins et al., 1979) and optogenetic stimulation (Dannenberg et al., 2015; Mamad et al., 2015). A reduction in hippocampal theta frequency and power is observed along with memory impairment following medial septum cooling in a rat model (Petersen and Buzsaki, 2020), and this theta rhythm may mediate memory function in the hippocampus (Buzsaki, 2002; Eichenbaum, 2017; Staudigl and Hanslmayr, 2013).

1.3 EPISODIC MEMORY VULNERABILITY TO AGE AND AGE-RELATED PATHOLGOGIES

Episodic memory is a memory system of recent evolution, late development and early deterioration (Tulving, 2002), more vulnerable than other memory systems (Tulving, 2002) and other cognitive functions to age-related changes (Bäckman et al., 2000; Nilsson et al., 1997; Nyberg et al., 2003; Ronnlund et al., 2005) with an estimated onset of decline between 60 and 65 years of age (Josefsson et al., 2012; Ronnlund et al., 2005; Schaie, 2005). Global reductions in brain volume occur with ageing (Enzinger et al., 2005; Hedman et al., 2012; Scahill et al., 2003), although the frontal and temporal lobes are particularly affected (Fjell et al., 2009; Pfefferbaum et al., 2013; Raz et al., 2005; Raz and Rodrigue, 2006). Age-related structural changes account for the decline in episodic memory, as the integrity of the MTL, and specifically that of the hippocampus, has been associated with episodic memory performance in the older (Gorbach et al., 2017; Head et al., 2008; Hedden et al., 2016; Persson et al., 2012). In functional terms, the brain experiences with ageing a global decline in task-related activity and resting-state activity (Oschmann and Gawryluk, 2020; Persson and Nyberg, 2006; Salami et al., 2014; Staffaroni et al., 2018). Prefrontal-MTL and parietal-MTL connectivity (Salami et al., 2014) and connectivity within the default mode network (Staffaroni et al., 2018) decreases with ageing and this decrease correlates with poor episodic memory performance. Exceptionally, evidence has been found for an increase in resting-state hippocampal activity with ageing, but this increase correlates with lower activity during a memory task along with poorer memory performance (Salami et al., 2014). This increased resting-state hippocampal activity may be due to decreased inhibition exerted by the prefrontal cortex (Nyberg, 2017).

The boundary between age-related episodic memory impairment and pathological impairment is not well defined. Alzheimer's disease is characterised by a primary episodic memory impairment of greater severity than the so-called normal age-related episodic memory impairment as it compromises the patients' independence (Glisky, 2007; Greene et al., 1996). A prodromal state of Alzheimer's disease has been operationalised as the amnestic variant of mild cognitive impairment, which includes subjects who show more severe memory impairment than expected for their age, but do not meet the diagnostic criteria for dementia (Bondi et al., 2014; Flicker et al., 1991; Petersen et al., 1999). This prodromal state is likely to progress to Alzheimer's disease as episodic memory decline in unimpaired ageing cohorts has been associated with Alzheimer's disease conversion (Grober et al., 2000; Small et al., 2000). Brain structure

is affected differentially in normal ageing and Alzheimer's disease, although decline of episodic memory is a characteristic feature of both conditions. Early brain changes in Alzheimer's disease are centred in the MTL before extending to other brain regions, whereas changes in normal ageing are not limited to the temporal lobe and the frontal lobe is also affected (Jack et al., 1997; McKhann et al., 2011; Pini et al., 2016). Other hallmarks of Alzheimer's disease are the presence of amyloid deposition and neurofibrillary tangles (NFT) (DeTure and Dickson, 2019; Khachaturian, 1985; Mirra et al., 1991), however, both markers are found in elderly individuals cognitively unimpaired (Bennett et al., 2006; Erten-Lyons et al., 2009; Kramer et al., 2011) and episodic memory decline in the elderly has been associated with amyloid deposition and NFT in the MTL (Maass et al., 2018; Mormino et al., 2009).

Episodic memory, despite being a cognitive function particularly vulnerable to age and age-related pathologies, can also age successfully, as there are elderly people with excellent episodic memory for their age. The following section reviews the field of research on the successful ageing of episodic memory to provide the necessary context for the analyses conducted in this thesis and presented in subsequent chapters.

1.4 SUCCESSFUL AGEING OF EPISODIC MEMORY

Interindividual variability in cognitive performance increases with ageing and for episodic memory, one the most vulnerable cognitive functions to age (Bäckman et al., 2000; Nilsson et al., 1997; Nyberg et al., 2003; Ronnlund et al., 2005), there is a great range of responses in the elderly (Christensen et al., 1999; Nyberg et al., 2012) that can be categorised into three main groups (McDaniel et al., 2008; Rogalski et al., 2013): 1) normal agers who have experienced a slight episodic memory impairment that does not limit daily functioning, 2) pathological agers whose episodic memory impairment is more severe and life-limiting, such as Alzheimer's disease or related prodromal states, and 3) successful agers. The two first groups have been discussed in the previous section and here we will dissect the literature on the elders ageing with successful episodic memory.

A unified definition for successful ageing of episodic memory does not exist and this phenomenon has been studied via multiple approaches resulting in a heterogeneous literature. The main points of divergence are the age range of the elderly population, the

longitudinal evolution of episodic memory and the benchmark for comparing episodic memory performance. In terms of age range, the existing heterogeneity can be summarised as successful ageing studies from age 60 or from age 80 onwards. The argument to focus on a population over 60 years old is that episodic memory starts to decline around that age (Josefsson et al., 2012; Ronnlund et al., 2005; Schaie, 2005), but the opposing approach argues age 60 might not be old enough to study the ageing mechanisms (*i.e.*, resistance *vs.* resilience) (Rogalski, 2019). In any case, episodic memory ageing trajectories are not linear (Nyberg et al., 2012; Ronnlund et al., 2005) and each time window might manifest a different ageing phase, thus the age range should be considered when comparing studies on successful ageing of episodic memory. The second point of divergence is the longitudinal evolution of episodic memory. Some authors consider as a hallmark of successful ageing the maintenance of a high episodic memory performance (Arenaza-Urquijo et al., 2019; Lin et al., 2017b; Pudas et al., 2013), whereas other authors do not restrict the definition of successful ageing to this aspect and contemplate the possibility that having a successful memory in old age does not imply protection against a decline years later (Dekhtyar et al., 2017; Gefen et al., 2014; Harrison et al., 2018; Rogalski et al., 2018). The last divergence point is the benchmark used to determine successful ageing of episodic memory in contrast to the expected performance for age. Normative values for a cognitive test are generated by assessing a healthy ageing cohort representative for a specific population or country. These normative values are used when successful episodic memory performance in old age is defined by comparison with the performance of a younger adult. This is the seed of the original definition of superagers as elderly subjects whose episodic memory is at least as good as the average of healthy adults 20-30 years younger (Gefen et al., 2015; Harrison et al., 2012). Another possibility for defining successful ageing is to select the subgroup of elderly people with the best episodic memory performance for their age. This is the idea behind the supernormal definition (Lin et al., 2017a; Mapstone et al., 2017; Wang et al., 2019), that avoids comparisons with younger adults.

The heterogeneity of the literature on successful episodic memory ageing invites caution in comparing studies, although these approaches complement each other and help to better understand how to age without pathological episodic memory decline. The remainder of this section will review the main findings in the field of successful episodic memory ageing.

Neuroimaging studies

Structural macroscopic neuroimaging studies have revealed that superagers have a larger total cortical volume than age-matched typical older adults and indistinguishable total cortical thickness values from middle-aged controls 20-30 years younger (Harrison et al., 2012). The volume of the hippocampus was found larger in superagers than age-matched typical older adults (Dekhtyar et al., 2017; Harrison et al., 2018; Sun et al., 2016) and a positive correlation between episodic memory performance and hippocampal volume has been described in these population (Harrison et al., 2018; Sun et al., 2016). Longitudinal studies of brain structure have shown a slower total cortical atrophy rate in superagers compared to age-matched controls (Cook et al., 2017b). Opposing findings show that there is no difference in atrophy rate between superagers and typical older adults in grey and white matter volume, hippocampal volume, and white matter hyperintensities volume (Dang et al., 2019). This discrepancy can be explained by the age difference of the participants in the two studies, while superagers over 80 years old were scanned in the study showing divergent atrophy rates, superagers between 60-80 years old were recruited for the second study where similar group atrophy rates were found. Region-specific structural analyses have revealed a thicker cortex in superagers compared to typical older adults in regions of the default mode network and salience network including the angular gyrus, prefrontal cortex, insula, inferior frontal gyrus and the cingulate cortex (Harrison et al., 2018; Sun et al., 2016) and specifically in the anterior cingulate cortex (Gefen et al., 2015; Harrison et al., 2012; Rogalski et al., 2013)

Connectivity studies have found improved structural and functional markers in the superager brain compared to typical older adults in regions that have already been highlighted by structural analysis such as stronger functional connectivity within the default mode network (Zhang et al., 2020), the salience network (Zhang et al., 2020) and the cingulate cortex (Lin et al., 2017a), and additionally, in the corpus callosum and the right superior longitudinal fasciculus (SLF) (Kim et al., 2020). A longitudinal functional connectivity study shows a set of regions in superagers whose oscillations are resistant to neurodegeneration including the fusiform gyrus, middle frontal gyrus, anterior cingulate cortex, middle temporal gyrus, precentral gyrus, and orbitofrontal cortex (Wang et al., 2019). Other indirect measures of brain activity show that successful elders have higher whole cortex glucose metabolism compared to typical older adults (Baran et al., 2018) and also higher blood-oxygen-level-dependent (BOLD) imaging signal during

encoding in bilateral prefrontal cortex and left hippocampus compared to typical older adults (Pudas et al., 2013). In sum, successful agers show preserved structural and connectivity measures relative to age-matched typical older adults although longitudinal analyses with larger samples sizes are needed to better understand the ageing mechanisms of successful episodic memory ageing.

Histology studies

Most histological studies in the superager field have focused on the pathological features of Alzheimer's disease, which include tau pathology, based on the presence of NFT, and amyloid pathology, based on the presence of amyloid plaques. Tau pathology in Alzheimer's disease spreads from the entorhinal cortex to association areas and the Braak staging accounts for this progression (Braak and Braak, 1995), while amyloid pathology starts in association cortices and extends to other brain regions (Thal et al., 2002). Tau pathology is present in superager samples ranging from Braak stages II-III — presence of NTL in hippocampus and entorhinal cortex and absence in association cortices— (Rogalski et al., 2018), while in normal agers the Braak stage ranged from III-IV (Gefen et al., 2021). Although there are exceptions of superagers with higher Braak stages (Rogalski et al., 2013), they show lower density of NFT compared to typical older adults in the entorhinal cortex (Gefen et al., 2021) and the anterior cingulate gyrus (Gefen et al., 2015). In terms of amyloid pathology, superager samples are free of plaques in the hippocampus and entorhinal cortex whereas moderate plaque density was found in association cortices (Rogalski et al., 2018). In relation with typical older adults, superagers show similar low levels of amyloid plaques in the entorhinal cortex (Gefen et al., 2015) and lower levels in the anterior cingulate cortex (Gefen et al., 2015). Histology findings are consistent with positron emission tomography (PET) studies that have revealed no differences in whole-brain amyloid burden between superagers and typical older adults (Baran et al., 2018; Dang et al., 2019; Dekhtyar et al., 2017; Harrison et al., 2018), although superagers show a lower amyloid burden in the right isthmus cingulate cortex compared to typical older adults (Baran et al., 2018). Taken together these findings, the superager brain does not show advance pathological Alzheimer's disease hallmarks, instead they seem resistant to age-related tau pathology and they show no difference in whole-brain amyloid burden compared to typical older adults (Baran et al., 2018; Dekhtyar et al., 2017; Gefen et al., 2021; Harrison et al., 2018).

Beyond the classical Alzheimer's disease hallmarks, activated microglia in the brain was studied as a neuroinflammatory marker that experiences an age-dependant increase and contributes to neurodegeneration in Alzheimer's disease (Heppner et al., 2015; Luo et al., 2010; Raj et al., 2017; Serrano-Pozo et al., 2011). The density of activated microglia in cortical white matter is lower in the superager brain compared to elderly controls and at similar levels when compared to young adults (Gefen et al., 2019). Additional histological findings in the superager include the density of acetylcholinesterase —enzyme that hydrolyses acetylcholine to terminate its effect on the postsynaptic terminal (Soreq and Seidman, 2001). Lower density is found in cortical pyramidal neurons of post-mortem superager brains (Janeczek et al., 2018), suggesting an enhanced effect of acetylcholine on hippocampal and cortical neurons in superageing. Great interest has received the cingulate cortex and anatomical investigations found that superagers show a higher number of von Economo neurons compared to elderly controls in the anterior cingulate (Gefen et al., 2015; Rogalski et al., 2013). These neurons are mostly found in the anterior cingulate cortex and frontoinsular regions and they seem to play a role in social behaviour (Allman et al., 2010) and a loss of von Economo neurons has been observed in the behavioural form of frontotemporal dementia where social behaviour and emotional processing are impaired (Seeley et al., 2006).

Demographics, genetic, lifestyle and clinical studies

There is no clear demographic profile for successful episodic memory agers, some studies report a higher number of women and highly educated participants among this population (Harrison et al., 2018; Josefsson et al., 2012; Maccora et al., 2021; Pudas et al., 2013), but this profile is not replicated in all cohorts (Dekhtyar et al., 2017; Gefen et al., 2015; Kim et al., 2020; Wang et al., 2019). Genetics have a notable influence on cognitive ageing phenotypes (Nyberg and Pudas, 2019) and apolipoprotein E (*APOE*) gene is of greatest interest in studies on successful episodic memory. Its ε4 allele is the major single genetic risk for sporadic Alzheimer's disease (Corder et al., 1993) and carrying one copy increases in 3-4 times the risk of developing the disease (Farrer et al., 1997). A superager study indicates that ε4 allele is underrepresented in superagers compared to typical older adults (Rogalski et al., 2013), and other cohorts show a similar allelic frequency in both groups (Dekhtyar et al., 2017; Harrison et al., 2018; Spencer et al., 2022; Wang et al., 2019), but larger sample sizes are required to set conclusive results on genetic profiles. On a panel of 31 single nucleotide

polymorphisms (SNPs) associated with Alzheimer's disease, superagers show a similar polygenic risk score compared to typical older adults (Spencer et al., 2022). In a different study, the superager phenotype is associated with three SNPs of the Mitogen-Activated Protein Kinase Kinase 3 (MAP2K3) gene (Huentelman et al., 2018). MAP2K3 protein could be playing a role in beta-amyloid-induced apoptosis in microglial cells (Zhou et al., 2014). A metabolomic study revealed a significantly higher abundance of 12 plasma metabolites that regulate oxidative stress, inflammation, and nitric oxide bioavailability pathways (Mapstone et al., 2017), aspartate is one of these metabolites, likewise N-acetyl aspartate has shown a higher concentration in the posterior cingulate cortex of superagers compared to typical older adults using spectroscopy (de Godoy et al., 2021). Superagers show higher disposition towards new experiences compared to typical older adults (Rogalski et al., 2018), they also have a more positive perception of their relationships compared to elderly controls (Cook et al., 2017a) and living with someone is associated with maintenance of high episodic memory performance (Josefsson et al., 2012). Self-reported physical activity is also associated with maintenance of high episodic memory performance (Josefsson et al., 2012) and, in a different cohort, successful episodic memory agers show greater physical activity measured by energy expenditure compared to typical older adults (Kim et al., 2020). In clinical terms, superagers and typical older adults show similar body mass index (BMI) and similar history of hypertension, hyperlipidaemia, diabetes mellitus, and obesity (Dang et al., 2019; Harrison et al., 2018; Kim et al., 2020; Lin et al., 2017a), although superagers were found to be more resilient than typical older adults to postoperative delirium (Katsumi et al., 2022).

All in all, the field of successful ageing of episodic memory is emerging and new studies are continuously advancing the knowledge on how to age with excellent episodic memory function. The relatively recent emergence of definitions to systematically study this phenomenon implies that there are still opportunities to advance the filed in many meaningful ways. Longitudinal follow-ups of large, well-characterised cohorts of participants is of particular importance to understand the underlying ageing mechanisms and the environmental and genetic factors associated with this phenomenon. This thesis studies successful ageing of episodic memory adopting the superager approach in the context of a longitudinal cohort to select participants over the age of 80 with an episodic memory at or above the average levels of a person 20-30 years younger to phenotype the superager brain longitudinally and demographic, lifestyle, clinical and biological age variables. In the following, the general methods

chapter will describe the characteristics of the Vallecas Project cohort and selection criteria for the group of superagers and typical older adults that are used across the four empirical chapters. The first of the empirical chapters investigates the cross-sectional and longitudinal grey matter brain volume differences between groups and the demographic, lifestyle, clinical factors associated with the superager phenotype. The next empirical chapter explores white matter microstructure cross-sectionally and longitudinally with diffusivity measures. In the third empirical chapter, telomere length was measured cross-sectionally as a biological marker of ageing in the superager and the typical older adult group. The last analysis explores the rate of episodic learning in the whole cognitively healthy sample of the Vallecas Project cohort at baseline and compares this rate between the superagers and the typical older adult group. Finally, the general discussion chapter assesses the results of all empirical chapters as a whole and contextualises them in relation to the previous results in the field of successful ageing of episodic memory.

CHAPTER 2

GENERAL METHODS

A version of the following chapter is published in The Lancet Healthy Longevity:

Garo-Pascual M*, Gaser C*, Zhang L, Tohka J, Medina M, Strange BA. Brain structure and phenotypic profile of superagers compared with age-matched older adults: a longitudinal analysis from the Vallecas Project. The Lancet Healthy Longevity. 2023 Jul 12.

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2.1 THE STUDY DESIGN AND SETTING OF THE VALLECAS PROJECT

Superagers and typical older adults were selected from the single-centre community based Vallecas Project, an ongoing longitudinal cohort established in the south of Madrid (Spain) on October 10th 2011 which was originally designed for one baseline visit and four annual follow-up visits and was subsequently extended for an additional 4 years. The 1,213 participants of the Vallecas Project were all White, community-dwelling individuals between 70 to 85 years-old, independent in activities of daily living with a survival expectancy of at least 4 years and without any neurological or psychiatric disorders (Olazarán et al., 2015). At each yearly visit, demographic and lifestyle parameters were collected, neuropsychological and clinical assessment and

multi-sequence MRI scanning performed, and blood samples collected for measurement of blood dementia biomarkers and genetic analysis. All participants provided written informed consent, and the project was approved by the Ethics Committee of the Instituto de Salud Carlos III.

2.2 PARTICIPANTS

For the studies reported in this thesis, we selected 64 superagers and 55 typical older adults from be Vallecas Project cohort. We based our superager definition on the Northwestern criteria (Harrison et al., 2012), defining a superager as a person 80 years or older with the episodic memory of a healthy person 20-30 years younger; no definition of superager was specified in the original study protocol of the Vallecas Project. Of note, the Northwestern criteria uses reference values for adults aged 50–65 years, whereas we used reference values for adults aged 50–56 years. Furthermore, we used the FCSRT (instead of the Rey Auditory Verbal Learning Test used in the Northwestern criteria) to assess episodic memory, and the Digit Symbol Substitution Test (instead of the Trail Making Test Part B used in the Northwestern criteria) in non-memory assessments.

Participant selection criteria were grouped into five categories, all of which must be met: age; episodic memory function; cognitive performance in non-memory domains; MRI data availability; and stability of episodic memory (Figure 3). 1) Age: all participants were 79.5 or older. 2) Episodic memory function: superagers were required to perform at or above the mean for adults between 50-56 years old with the same education level on the delayed free-recall score of the Spanish version of the FCSRT (Buschke, 1984). This score was compared with normative values from the Spanish NEURONORMA project (Peña-Casanova et al., 2009a). Typical older adults were required to have a score within one standard deviation (SD) from the mean of a population with the same age and education level. 3) Cognitive performance in non-memory domains: the non-memory domains were assessed with the Spanish version of three different tests, the 15-item Boston Naming Test (Fernández-Blázquez et al., 2012), the Digit Symbol Substitution Test (Wechsler and Psychological, 1997), and the Animal Fluency test (Peña-Casanova et al., 2009b). Participants were compared to normative values for their age and education group (see respective references). Superagers were required to score within or above one SD from the mean of their age in the three tasks. Although the original Northwestern criteria (Gefen et al., 2015; Harrison et al., 2012) required typical older

adults to be within one SD from the mean of their age in all three non-memory tasks, when applied to our sample, this resulted in a too small and thus unbalanced Northwestern-criteria control group compared to the superager group. We therefore also selected a control group without applying non-memory criteria. Neuropsychological and brain imaging comparisons were performed comparing superagers (n = 64) with typical older adults (n = 55) and Northwestern-criteria typical older adults (n = 19). The Random Forest model was not estimated for the Northwestern-criteria typical older adults due to the small sample size. 4) MRI availability: all participants needed a structural MRI at the visit in which they fulfilled the previous criteria. 5) Finally, given the longitudinal nature of the study, stability of episodic memory performance was included as a further selection criterion. Participants from either group who experienced changes in their memory classification (superager or typical older adult) over the course of the study were excluded to avoid the inclusion of subjects whose memory performance improved because of practice, thus preventing participants that met the typical older adult criteria at early visits from meeting the superager criteria at subsequent follow-ups (Figure 3). We elected not to exclude those subjects who later converted to mild cognitive impairment or dementia. None of the 119 subjects at the visit selected as cross-sectional visit had such a diagnosis. Six typical older adults developed mild cognitive impairment the year after they met inclusion criteria. Removing these subjects from grey matter volume analyses (Chapter 3) did not change the group comparison grey matter volume results. Selection criteria were applied from the second to the sixth visit as the 15-item Boston Naming Test was not administered at baseline. The visit used for the cross-sectional analyses reported in Chapter 3-6 was adjusted to match in age the group of superagers and the group of typical older adults.

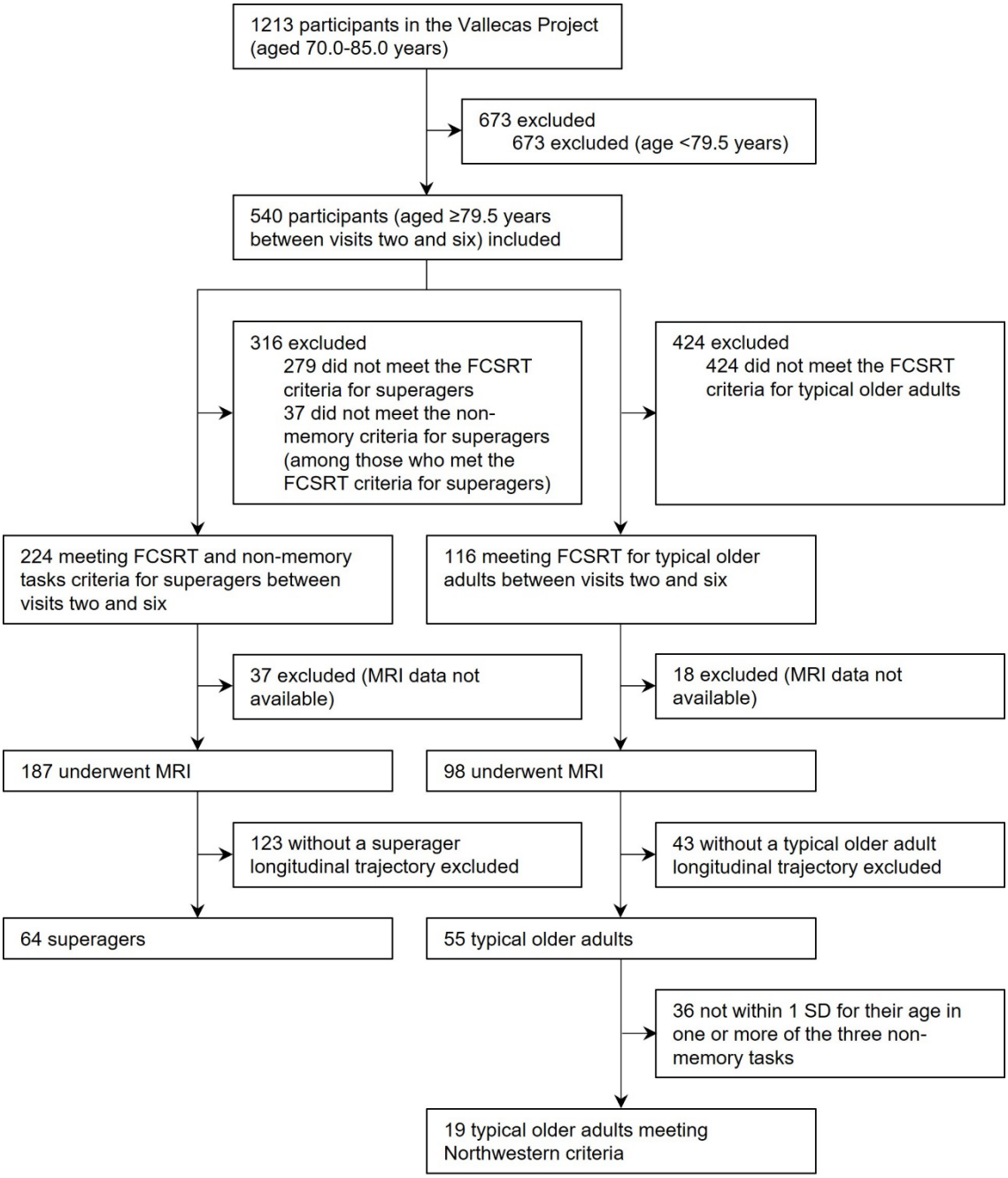

Figure 3. Flow-diagram of the selection criteria of superagers and typical older adults. A final sample of 64 superagers and 55 typical older adults were selected from the Vallecas Project cohort after considering age, superager/typical older adult criteria, magnetic resonance imaging (MRI) availability and longitudinal memory performance trajectory. FCSRT, Free and Cued Selective Reminding Test; SD, standard deviation.

CHAPTER 3

GREY MATTER AND PHENOTYPIC PROFILE

A version of the following chapter is published in The Lancet Healthy Longevity:

Garo-Pascual M*, Gaser C*, Zhang L, Tohka J, Medina M, Strange BA. Brain structure and phenotypic profile of superagers compared with age-matched older adults: a longitudinal analysis from the Vallecas Project. The Lancet Healthy Longevity. 2023 Jul 12.

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3.1 HYPOTHESIS AND OBJECTIVES

Episodic memory —the memory for personal life experiences— is one of the cognitive domains most vulnerable to age-related deterioration (Glisky, 2007). Whereas neurodegenerative diseases such as Alzheimer's disease are often accompanied by a severe decline in episodic memory, some reduction in episodic memory performance is also expected as part of healthy ageing. However, some older adults —often termed superagers— appear to resist this age-associated decline, and instead show an episodic memory that is at least as good as that of healthy adults 20–30 years younger (Cook et al., 2017b; Gefen et al., 2015; Harrison et al., 2018; Harrison et al., 2012; Sun et al., 2016; Wang et al., 2019; Zhang et al., 2020).

A comprehensive characterisation of the mechanisms underlying the preservation of episodic memory function in superagers is currently lacking. One central question is whether superageing reflects a resistance or resilience to dementia or age-related processes. In the typical context of dementia (Arenaza-Urquijo and Vemuri, 2018), *resistance* refers to the avoidance of disease and *resilience* is understood as successful coping with disease. However, if both superager and control groups are negative for neurodegenerative biomarkers, the same framework can be used to describe ageing processes in the absence of neuropathology. Thus, in a healthy ageing context, resistance refers to avoidance of typical age-related memory decline and translates, in brain structural terms, to the preservation of memory-related areas in superagers, whereas resilience refers to successful coping with ageing effects and implies similar brain structure between superagers and normal ageing peers.

Previous neuroimaging studies have shown that superagers have larger hippocampal volumes (Harrison et al., 2018; Sun et al., 2016), thicker anterior cingulate cortices (Gefen et al., 2015; Harrison et al., 2012; Rogalski et al., 2013), and slower cortical atrophy (Cook et al., 2017b) than typical older adults. Prior studies also explored the association between superager status with some lifestyle factors, such as satisfaction with social relations (Cook et al., 2017a). However, most studies had limited sample sizes and were cross-sectional in nature, making it difficult to dissociate long-standing structural differences from differential atrophy rates in superageing *vs*. normal ageing brains. One approach for obtaining larger samples of deeply phenotyped superagers with longitudinal observations is to interrogate large longitudinal ageing cohorts. We applied this approach to the Vallecas Project longitudinal study aiming, first, to characterise the superager cerebral grey matter volume cross-sectionally and longitudinally relative to age-matched typical older adults; and second, to apply machine learning to determine which demographic, lifestyle, and clinical variables are the greatest differentiating factors between superagers and typical older adults. We hypothesised that the superager phenotype is part of the normal ageing spectrum and 1) their levels of Alzheimer's disease and neurodegeneration blood biomarkers would be similar to the typical older adult group both showing resistance to Alzheimer's disease and neurodegeneration, 2) the superager phenotype would be associated with grey matter preservation in memory-related areas of the MTL and adjacent limbic structures, as well as cholinergic nuclei, representing a resistance mechanism against normal memory decline and 3) the superager phenotype would be associated with lifestyle factors that promote better cognitive performance in ageing, for example physical activity,

educational attainment or socioeconomic status (Britton et al., 2008; Holstege et al., 2018; Josefsson et al., 2012; Nyberg and Pudas, 2019).

3.2 MATERIALS AND METHODS

Participants. The sample used for the following analyses was described in the General Methods of Chapter 2.

Neuropsychological variables. In addition to tests used as selection criteria, total score of the Mini Mental State Examination (two typical older adults have missing data) and Functional Activities Questionnaire are reported as estimators for general cognitive performance (one typical older adult has missing data), the delayed recall score of the Rey-Osterreith Complex Figure test as a visuospatial memory measure (39 superagers and 32 typical older adults have missing data), the Lexical Fluency test with letter P and the Spanish version of the National Adult Reading Test for vocabulary and intelligence (8 typical older adults have missing data). The scores pertain to the visits used in cross-sectional analyses.

***APOE* genotyping.** Total deoxyribonucleic acid (DNA) was isolated from peripheral blood following standard procedures (Calero et al., 2009) Genotyping of *APOE* gene polymorphisms (rs429358 and rs7412) was determined by Real-Time polymerase chain reaction (PCR). The failure rate of genotyping was 0.3%.

Blood biomarkers. Blood samples were processed within 1 hour of procurement to obtain serum and plasma fractions, which were then aliquoted and stored at -80ºC until use. Samples were thawed one hour before performing each biomarker analysis. Levels of plasma amyloid β (Aβ40 and Aβ42), total tau (t-Tau) and tau phosphorylated at threonine 181 (p-Tau181), and serum neurofilament light polypeptide (NfL) and glial fibrillary acidic protein (GFAP) were measured by single molecule array (Simoa) using the SR-X platform (Quanterix) using the Neurology 3-Plex kit (Aβ40, Aβ42, t-Tau), Neurology 2-Plex kit (NfL, GFAP), and p-Tau181 kit assays following manufacturer's instructions. Two participants have missing data for Aβ40, Aβ42 and t-Tau (1 superager and 1 typical older adult). Missing data is due to technical errors in the measurement process.

MRI acquisition. T1-weighted images (3D fast spoiled gradient echo with inversion recovery preparation) were all acquired using a single 3 Tesla MRI (Sigma HDxt GEHC, Waukesha, USA) with a phased array 8-channel head coil and the following parameters: repetition time (TR) 10 ms, echo time (TE) 4.5 ms, inversion time (TI) 600 ms, field of view (FOV) 240 mm, matrix 288x288 and slice thickness 1 mm, yielding 0.5x0.5x1mm voxel size.

Whole-brain voxel-wise volume analysis of grey matter volume. Grey matter volume analysis was performed using the CAT12.7 toolbox (https://neuro-jena.github.io/cat/) (Gaser et al., 2022) implemented in SPM12 (https://www.fil.ion.ucl.ac.uk/spm). Using a standard pipeline, T1-weighted images were bias-field corrected, then segmented (Ashburner and Friston, 2005) and spatially normalised using the DARTEL algorithm (Ashburner, 2007). Grey matter volumes were generated after modulation of segmented normalised grey mater images. A longitudinal pipeline for estimating ageing effects in CAT12 was used that additionally considers deformations between time points. The use of deformations between the time points makes it possible to estimate and detect larger changes that usually occur during ageing. CAT12 provides a retrospective quality control framework for the empirical quantification of key image parameters, such as noise (*i.e.*, due to head motion), intensity inhomogeneities and resolution, and combines these values into an overall image quality parameter. We have only included data with an overall image quality of at least grade 3, as recommended by the CAT12 manual. In addition, image outliers were identified by calculating a Spearman's rank correlation coefficient, which depends on the quality of the image processing as well as the anatomical characteristics of each brain. This ensures that the segmentation is of sufficient quality and that the included data are homogeneous (*i.e.*, without outliers). Finally, the segmentations were smoothed with a Gaussian kernel of 6 mm. To guarantee that we only examined grey matter regions, we applied an absolute grey matter threshold of 0.1. For the cross-sectional analysis, total intracranial volume (TIV) was introduced as a covariate; age was introduced as a covariate for the longitudinal model (scans from visit 1 to visit 6 were used). Self-reported sex was considered as a covariate but finally excluded as it did not change the model output. For the longitudinal analysis follow-up loss was handled by the general linear model (GLM) used, age at each visit was included in the model and no covariate was used. The corrected statistical threshold was set to $P < 0.05$ and the Threshold Free Cluster Enhancement (TFCE) approach was implemented (5000 permutations, E = 0.5

and H = 2) employing the TFCE toolbox version r186 for SPM12 (https://www.neuro.uni-jena.de/tfce). The AAL3 atlas was used for mapping thalamic nuclei (Rolls et al., 2020).

Cortical and subcortical volume measurements derived from FreeSurfer and CAT12 for replication purposes. With FreeSurfer, cortical and subcortical volumes were extracted from cross-sectional visits and total cortical volume was extracted from visit 1-6 to calculate atrophy rates. These measurements were obtained using the standard *recon-all* pipeline from FreeSurfer v6.0 (https://surfer.nmr.mgh.harvard.edu/), which includes removal of non-brain tissue, automated Talairach transformation, segmentation of the subcortical white matter and deep grey matter structures (Fischl et al., 2002; Fischl et al., 2004), and intensity normalisation (Sled et al., 1998). All images were visually inspected to ensure adequate quality of the segmentations and manual editing was only performed for the cross-sectional images used for cortical thickness analysis. Cross-sectional whole-brain voxel-wise analysis on cortical thickness was conducted with TIV as a covariate. CAT12 was employed to test cross-sectional regional cortical thickness differences between groups. Cross-sectional whole-brain voxel-wise analysis on cortical thickness conducted in CAT12 used a smoothing Gaussian kernel of 12 mm and TIV as a covariate. Cortical thickness analyses performed with the FreeSurfer and CAT12 pipeline were only conducted for replication purposes (Gefen et al., 2015; Harrison et al., 2018; Harrison et al., 2012; Sun et al., 2016).

Random Forest analysis. Participants were classified into superagers and typical older adults by Random Forest analysis (Breiman, 2001), a supervised learning method for classification, computed with a total of 89 predictors regarding demographics, lifestyle, and clinical variables. Classification trees were constructed using bagging (random sampling with replacement of the training set to avoid overfitting) using the TreeBagger implementation of Matlab (version 2019b). The number of trees in the ensemble was set to 7500. This high number was required to obtain stable predictor importance estimates (Wang et al., 2016). Other parameters were set to their default values, in particular the mtry-parameter was set to sqrt(d), where d is the number of predictor variables. Missing values were imputed by the median value of the variable to avoid the positive bias potentially associated to predictors with many missing entries. The median was computed over all subjects (superagers and typical older adults). Variables with more than 50% of missing values for either superagers or typical older adults were excluded from the model, five in total.

We estimated the test error of the classifier via out-of-bag error. For each sample (x,y) in the training set, out-of-bag predictor aggregates predictions of those classification trees which were not trained using (x,y) due to bagging (Breiman, 2001). Then, the prediction error can be computed based on the out-of-bag predictions. These out-of-bag errors can be computed during training, without the need for re-training (as in cross-validation). Moreover, out-of-bag error estimates are equally or more accurate than cross-validation or holdout error estimates (Breiman, 1996, 2001; Wolpert and Macready, 1999).

We estimated the importance of each predictor by the permutation importance (Breiman, 2001), calculated as the decrease in the model's accuracy after permuting that variable. The "raw" importance measures can be used to rank predictors but are otherwise challenging to interpret. Therefore, we computed a one-sided *p*-value for each predictor's importance by comparing the importance measure to a null distribution (Altmann et al., 2010; Hapfelmeier and Ulm, 2013), obtained by permuting the response variable 1500 times and computing the importance of each predictor in the model.

Statistical methods. The rest of the statistical tests were performed in R 3.5.1 (https://www.r-project.org/). Chi-squared tests and Fisher's exact tests were used for comparisons of categorical data and two-sample t-test and Mann-Whitney U test (two-tailed) were used for continuous variables with significance level set at 0.05. Log transformation was performed on blood biomarker variables to fit a normal distribution. To examine cross-sectional between-group differences in total grey matter volume, an analysis of covariance was conducted using TIV as a covariate. False discovery rate (FDR) was used to correct for multiple comparisons.

Between-group longitudinal differences in grey matter volume were tested with a linear mixed model using the lme4 package, a model that can handle missing data once participants are lost. Grey matter volume was adjusted by TIV and both measurements were extracted from CAT12 pipeline. Group, scaled age and the interaction between the two were introduced in the model as fixed effects. Subject intercept and scaled age slope were included as random effects.

3.3 RESULTS

We identified 64 superagers and 55 age-matched typical older adults from the single-centre Vallecas Project longitudinal cohort (Olazarán et al., 2015) based on their free delayed recall performance on the verbal memory FCSRT (Buschke, 1984). Superagers performed at or above the mean of a person ~30 years younger with their same education level, and typical older adults performed within a normal range for their age and education. All superagers and typical older adults were 79.5 years or above when meeting the selection criteria (see full criteria in Chapter 2). For the selection of typical older adults, the original criteria proposed by Northwestern University (Gefen et al., 2015; Harrison et al., 2012) (see Methods in Chapter 2) resulted in 19 Northwestern-criteria typical older adults, producing a marked imbalance with the superager group, we therefore relaxed the criteria for control performance (see Methods in Chapter 2). All analyses were conducted between the 64 superagers and 55 typical older adults and repeated using the 19 Northwestern-criteria typical older adults.

Superagers and typical older adults showed no differences in age or sex, with a higher percentage of females in both groups. Superagers had a significantly higher number of years of education compared to typical older adults (Table S1) with a median of 16.0 years (interquartile range (IQR) [10.0-19.0]) in superagers and a median of 10.0 years (IQR [6.0-17.5]) in typical older adults.

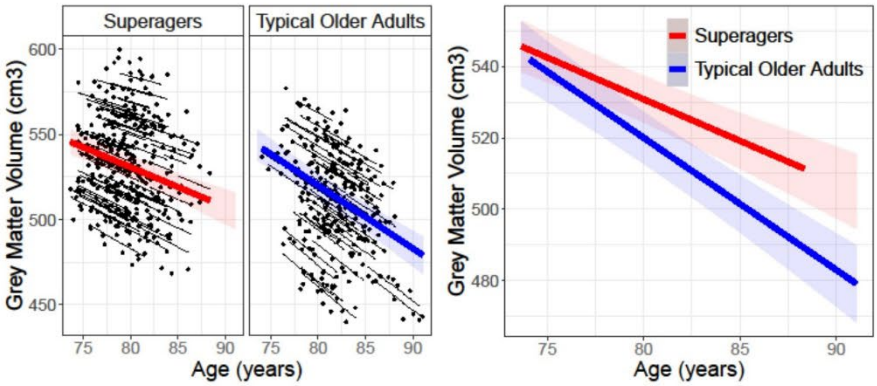
While the *APOE* ε4 allele increases the risk of non-familial Alzheimer's disease (Corder et al., 1993) relative to the ε3 allele, the ε2 allele is believed to be protective (Corder et al., 1994). Despite a previous suggestion that the ε4 allele is under-expressed in a cohort of 12 superagers (Rogalski et al., 2013), we did not find a between-group difference in the *APOE* gene allelic composition (Table S2, Table S3 and Table S4).

Levels of five blood biomarkers for neurodegeneration were assessed by Simoa. Levels of blood amyloid β (Aβ42/Aβ40 ratio), t-Tau and p-Tau181, p-Tau181/Aβ42 ratio, NfL and GFAP were equivalent between groups (Table S2, Table S4). We further explored the association between blood biomarker levels and brain grey matter volume. There was no significant association between the levels of Aβ42/Aβ40 ratio and p-Tau181 and grey matter volume in the whole cohort, nor an interaction between blood biomarker levels and group (data not shown).

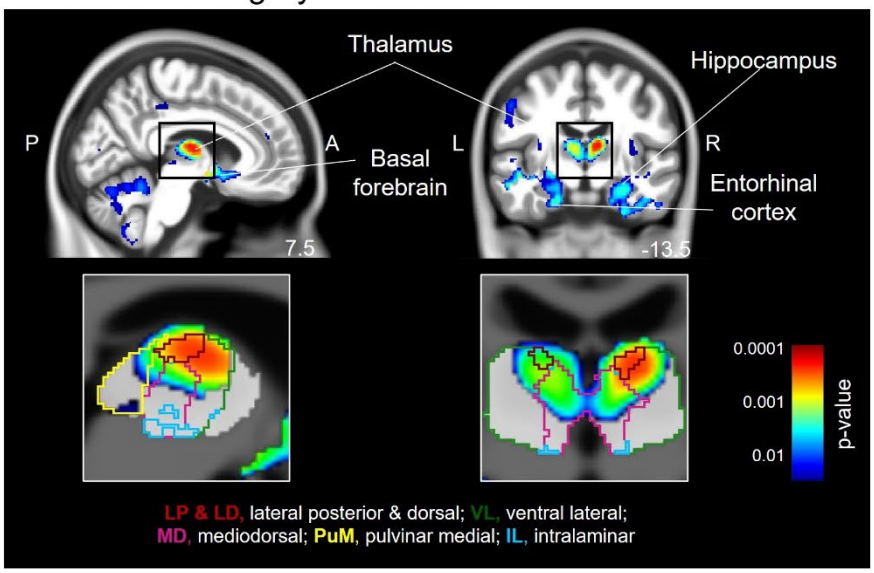
Grey matter volume was analysed cross-sectionally and longitudinally over 5 years with yearly follow-up scans, which also included scans in some superagers and typical older adults before their 80th birthday (MRI scans conducted before the visit used for cross-sectional analyses are available as the selection criteria were applied from visits 2 to 6). The median number of follow-up visits was 5.0 (IQR [5.0-6.0], range [2.0-6.0]) for superagers and 5.0 median visits (IQR [4.5-6.0], range [2.0-6.0]) for typical older adults. At cross-section, superagers showed a larger total grey matter volume compared to typical older adults (Table S1, Table S3, Table S5). Longitudinal analysis of total grey matter volume revealed a significant effect of group (β (standard error (SE)): -11.9 (4.9), P = 0.015), age (β (SE): -7.5 (1.1), P < 0.0001), and a group-by-age interaction (β (SE): -4.1 (1.7), P = 0.015). Typical older adults showed a faster decline in grey matter volume across time than superagers (slope (SE), cm^3/one SD of scaled age: superager: -7.5 (1.1); typical older adult: -11.6 (1.1)) (Figure 4A, Figure S1A, Table S6, Figure S2) indicating that superagers have a slower total grey matter atrophy rate relative to typical older adults. Inspection of linear fit of the atrophy rate of each group shows that superagers and typical older adults appear to have equivalent total grey matter volumes at the approximate age of 75 (Figure 4A).

We next explored whether the differences found in total grey matter volume are localised to specific brain areas, performing a cross-sectional MRI analysis of grey matter volume with a whole-brain approach. Superagers showed greater grey matter volume compared to typical older adults in bilateral thalamus, basal forebrain, angular gyrus and regions within the MTL including bilateral effects in hippocampus, amygdala, entorhinal cortex, parahippocampal gyrus and fusiform gyrus (P < 0.05 family-wise error (FWE)-corrected) (Figure 4B, Figure S1B). Effects were observed in multiple nuclei within the thalamus (Figure 4B), but notably not the anterior nucleus, which is the thalamic nucleus most implicated in memory function (Aggleton et al., 2010; Ghika-Schmid and Bogousslavsky, 2000). Instead, effects were greatest in ventral lateral nucleus, a major component of the motor thalamus (Bosch-Bouju et al., 2013), as well as lateral dorsal nucleus, part of the limbic thalamus (Taber et al., 2004). Given the difference in years of education between superagers and typical older adults, we repeated the model adjusting for years of education, with the main results still holding (data not shown). Cortical thickness findings consistent with previous literature were found (Figure S3).

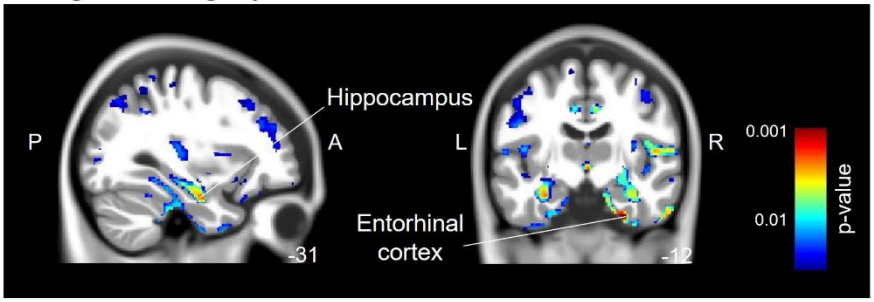
A. Grey matter atrophy



B. Cross-sectional grey matter volume



C. Longitudinal grey matter volume



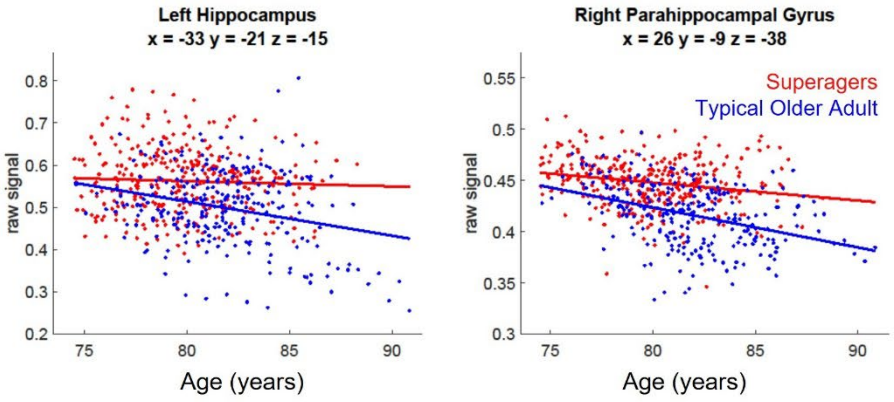


Figure 4. Neuroanatomical differences between superagers and age-matched typical older adults. A. Grey matter atrophy rates. The decline over time in total grey matter volume is slower in superagers (red) than typical older adults (blue). Individual trajectories and means for each group are shown (left) as well as means for both groups (right). Shaded error bars depict 95% confidence intervals. **B.** Cross-sectional differences in grey matter volume. Brain areas showing greater grey matter volume in superagers than typical older adults are shown as colormaps of *p*-values overlaid on sagittal and coronal sections of a canonical T1 image. Insets show the thalamic nuclei where grey matter volume is greater in superagers than typical older adults, (anatomical demarcations from the AAL3 atlas). **C.** Longitudinal differences in grey matter volume. Top: Brain areas showing reduced grey matter volume loss in superagers than typical older adults over time (significant group by time interaction, $P < 0.05$ FWE-corrected) are shown as colormaps of *p*-values overlaid on sagittal and coronal sections. Bottom: Grey matter volume trajectories over time in two representative voxels, the left hippocampus and right parahippocampal gyrus, for superagers (red) and typical older adults (blue). Superagers show a slower decline in both cases. The coordinates of the sections are given in mm. n = 119, 64 superagers and 55 typical older adults. A, anterior; L, left; P, posterior; R, right.

The slower superager atrophy in total cortical volume prompted us to examine region-specific longitudinal changes in grey matter volume over the five years follow-up period. Brain loci showing slower grey matter volume loss over time in superagers compared to typical older adults included bilateral hippocampus, entorhinal cortex, parahippocampal gyrus, left amygdala, bilateral basal forebrain, caudate, anterior insula, and right posterior cingulate ($P < 0.05$ FWE-corrected) (Figure 4C top, Figure S1C top). This between-group difference in atrophy rate is illustrated by plotting volume trajectories from left hippocampus and right parahippocampal gyrus (Figure 4C bottom, Figure S1C bottom). For this measure, it is evident that superagers and typical older adults are at the same level at around age 75 (Figure 4C bottom), however, memory superiority in superagers is evident before structural brain differences (Figure S4). Consistent neuroimaging findings were found comparing superagers with Northwestern-criteria typical older adults (Figure S1).

A Random Forest machine learning approach was employed to identify the demographic, lifestyle and clinical variables that best separated superagers from typical older adults during classification. With 89 variables, this model reached an accuracy for discriminating superagers from typical older adults of 66.4% (68.8% sensitivity, 63.6% specificity). Each variable's importance for classification and associated *p*-value is plotted in Figure 5.

The better performance of superagers in the Timed Up & Go test and the finger tapping test with dominant hand indicate that (Table S7), in addition to exceptional memory, superagers have better mobility, agility, and balance, this association between episodic memory and better mobility is supported by the significant correlation between $FCSRT_{freedelayrecall}$ and Timed Up & Go test (Pearson's r = -0.39, $P < 0.0001$). Despite these differences in motor function, we found no significant differences in the self-reported exercise frequency between groups. Superagers were also characterised by better mental health, scoring lower than typical older adults in measures of the State-Trait Anxiety Inventory and in the Geriatric Depression Scale (Table S7). On the depression scale, both superagers (1.1 (SD = 2.1)) and typical older adults (2.7 (SD = 3.1)) have average scores below 5, above which is a frequently used cut-off point for diagnosing depression (Delgado-Losada et al., 2021; Greenberg, 2012). Superagers complained less frequently about not getting enough sleep than did typical older adults (Table S7), despite no differences in self-reported sleep duration (6.8 h (SD = 1.3) for superagers, 6.6 h (SD = 1.3) and typical older adults). Superagers reported a less frequent history of glucose disorders and hypertension, a more active lifestyle during midlife, and a higher musical background —either formal or amateur— than typical older adults (Table S7). There was a higher proportion of separated/divorced members in the superager group compared to typical older adults without gender differences (Table S7). Superagers showed higher independence in their daily living and a higher score in the intelligence test (Table S7).

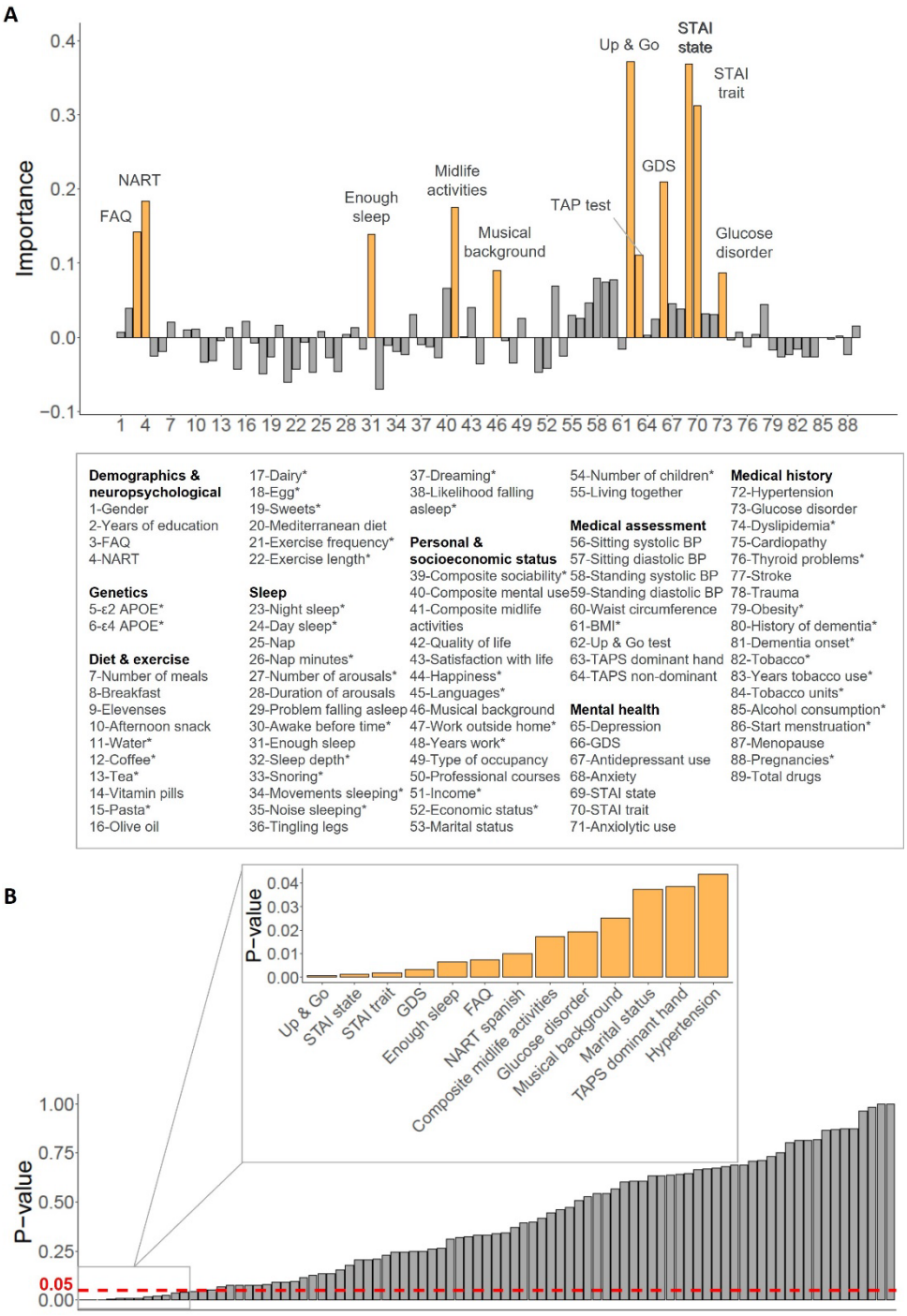


Figure 5. Classification of superagers using lifestyle and clinical variables. A Random Forest approach was used to classify superagers and age-matched typical older adults with 66.4% accuracy. **A.** The importance of the 89 variables included in the model are plotted. *Indicates variables with negative values, which have no beneficial contribution to the classification performance of the predictive model. Variables with highest importance for superager classification are highlighted (orange). **B.** Variables plotted by the *p*-value of their importance. The dashed red line indicates the threshold for significance and variables with a *p*-value < 0.05 are shown in the inset. n = 119, 64 superagers and 55 typical older adults. FAQ, Functional Activities Questionnaire; GDS, Geriatric Depression Score; NART, National Adult Reading Test; STAI, State-Trait Anxiety Inventory; TAPS, finger tapping test.

3.4 DISCUSSION

Our findings illustrate marked differences, both in brain structure and in multiple clinical and lifestyle features, between superagers and a healthy control group with normal memory for its age range. The two groups show no differences in *APOE* ε4 frequency, the major genetic risk factor for non-familial Alzheimer's disease, and equivalent levels of plasma biomarkers for dementia (levels similar to those reported for healthy ageing and distinctly lower than values observed in Alzheimer's disease patients) (Palmqvist et al., 2021) consistent with previous studies reporting no difference in amyloid burden between superagers and typical older adults (Dekhtyar et al., 2017; Gefen et al., 2021; Harrison et al., 2018). It is therefore likely that the observed between-group differences reflect a superager resistance to age-related memory decline, as opposed to the two groups being at different points of a dementia-related process.

One possible explanation for preserved brain structure in the superager phenotype is that they are simply born with larger brains, such that age-related atrophy is less evident compared to typical older adults. However, the rate of atrophy is reduced in superagers for the whole cortex and circumscribed memory-related areas comprising (anterior) (Strange et al., 2014) hippocampus and cholinergic basal forebrain. Reduced atrophy in hippocampus and basal forebrain, taken together with previous reports of lower acetylcholinesterase activity in cortical pyramidal neurons of post-mortem superager brains (Janeczek et al., 2018), could indicate an enhanced effect of acetylcholine on hippocampal and cortical neurons in superageing. A slowing of superager total grey matter atrophy relative to age-matched typical older adults accords with previous observations (Cook et al., 2017b), thus strengthening our findings, but critically, our 5 years of longitudinal study reveal that the values for total grey matter volume and focal grey matter volume appear to be equivalent in both groups at around age 75. Interestingly, memory performance is superior in superagers relative to typical older adults before this age, suggesting that there could be a lag between declining cognitive abilities and visible atrophy or that other factors beyond maintained brain structure underly superager memory prior to age 75 like functional properties (Betzel et al., 2014).

The preservation of motor thalamus grey matter volume in superageing, that is one of the thalamic groups that atrophies less in normal ageing (Choi et al., 2022) and has been also associated with faster episodic memory learning (Frank et al., 2022),

dovetails with our classification analysis highlighting finger tapping and Timed Up & Go tests as important for assigning superager status. Gait speed and balance, as well as finger tapping, are slower in patients with mild cognitive impairment and Alzheimer's disease, relative to typical older adults (Buracchio et al., 2010; Poirier et al., 2021; Roalf et al., 2018). We show here that superagers are faster than a control group with equivalent levels of blood biomarkers for neurodegenerative disease, indicating that movement speed is associated with better memory even in the absence of an evident dementia process. Although superagers self-report equivalent exercise frequencies to typical older adults, their superior movement speed could reflect their engagement in more "non-exercise" physical activity (*e.g.*, climbing stairs, gardening) (Kanning et al., 2013) than typical older adults, although further assessment of physical activity could be acquired through an objective and measurable approach to aim for greater generalisability of the findings. The potential mechanisms for physical exercise to improve cognition (or prevent dementia) include indirect effects on other modifiable risk factors (*e.g.*, cardiovascular fitness, obesity, insulin resistance, hypertension, dyslipidaemia) and direct effects on the brain, such as increased cerebral blood flow and levels of Brain Derived Neurotrophic Factor (Erickson et al., 2011), however, the direction of the association may be in the opposite and better brain health may be responsible for the faster speed of movement.

Of the 4 most important variables for classification of the superager phenotype, three were related to mental health: both questionaries for anxious state and trait and the Geriatric Depression Scale, a specific measure for depressive symptoms in the elderly. For all three scales, superagers display better mental health compared to typical older adults. An episode of depression and/or anxiety can impair performance on a memory test in both younger (Kizilbash et al., 2002) and older adults (Lohman et al., 2013), and in the long term a history of depression and anxiety is not only a risk factor for the development of dementia (Gulpers et al., 2016; Jorm, 2001) but also a symptom (McKeith and Cummings, 2005). Previous work on superageing has shown that superagers are resilient to post-operative delirium when compared to typical older adults (Katsumi et al., 2022), a condition with multifactorial aetiology in which existing depression could be a precipitating factor (Wilson et al., 2020).

The classification model highlights other variables that provide further insights into activities that may optimise memory function into the 9th decade. Superagers complain less frequently about not getting enough sleep, although self-reported sleep

duration was not considered important by our classification model. Self-reported sleep duration shows an inverted U-shaped association with global cognitive decline in ageing (Ma et al., 2020), but the average sleep duration for both superagers and typical older adults are within the non-deleterious range. The classification model highlighted musical background as a differentiating factor between groups, superagers being more likely to have some sort of musical background than typical older adults, whether it be amateur interest or formal training. This is in keeping with reports that early- to mid-life formal musical training is associated with improved late-life episodic and semantic memory (Gooding et al., 2014) and has been shown to increase grey matter volume (Gaser and Schlaug, 2003).

Previous work described an association between satisfaction in social relationships and superageing (Cook Maher et al., 2017), with superagers reporting satisfying, high-quality relationships. Our classification model did not ascribe significant importance to a composite social variable that included frequency of interactions with family/friends, feelings of solitude, and leisure activities. We did, however, observe an effect of marital status without gender differences. Being married or cohabiting with a partner is typically associated with better cognitive health later in life (Feng et al., 2014; Hakansson et al., 2009; Josefsson et al., 2012; Liu et al., 2019), but in the current sample, superagers were more likely to be separated or divorced than typical older adults. These discrepancies between our results and previous literature could be explained by the cultural dependence of social relations (Kito et al., 2017).

More years of formal education is commonly associated with the construct of "cognitive reserve", and reduced dementia risk (Josefsson et al., 2012; Meng and D'Arcy, 2012). Years of education did not, however, reach significant importance in classifying superagers. Although superagers had more years of education than the group of 55 typical older adults, comparing education level in superagers *vs*. Northwestern-criteria (Gefen et al., 2015; Harrison et al., 2012) typical older adults (n = 19), showed no difference even at trend level ($P = 0.90$). It is therefore unlikely that the superager memory phenotype is a product of more years of education, although this variable may influence performance on non-memory tasks.

As with any observational study, causality of the factors reported here and superageing cannot be inferred. This would require intervention trials involving, for example, prescribed activities to promote movement speed, tight control over

psychiatric symptoms, promoting awareness of the benefits of musical training and activities that improve perceived sleep quality (Wang and Boros, 2019). Any physical or psychiatric interventions may, however, need to be implemented in midlife, or before. Aerobic exercise interventions in healthy older adults do not appear to yield cognitive benefit even when the intervention leads to improved cardiorespiratory fitness (Young et al., 2015) and psychiatric symptoms accelerate ageing already in early mid-life (Wertz et al., 2021). We also acknowledge that, despite introducing 89 variables into our statistical model, the classification accuracy of 66.4% indicates that further variables, perhaps genetic (Nyberg and Pudas, 2019), are associated with the superageing phenotype. A potential overlap between a genetic basis for superageing and genetic association with muscle phenotypes for fast movements among the elderly (Garatachea and Lucia, 2013) could help narrow this search.

CHAPTER 4

WHITE MATTER AND STRUCTURAL CONNECTIVITY

4.1 HYPOTHESIS AND OBJECTIVES

White matter undergoes changes with ageing (Cox et al., 2016; Davis et al., 2009; Westlye et al., 2010). White matter volume describes an inverted U-shape with faster changes earlier and later in life and peaking at 5^{th}-6^{th} decade (Walhovd et al., 2011; Westlye et al., 2010), while diffusivity measures —including fractional anisotropy (FA) and mean diffusivity (MD)— peak around two decades earlier but following the same inverted U-shape (Westlye et al., 2010). These brain white matter changes both in early and late stages of life are regionally heterogeneous. The age-related white matter effects are greater in anterior than posterior regions (Davis et al., 2009; Kochunov et al., 2007; O'Sullivan et al., 2001; Pfefferbaum et al., 2005; Sullivan and Pfefferbaum, 2006). This occurs in conjunction with stronger loss of white matter microstructural properties in the thalamic radiations and association fasciculi (Cox et al., 2016; Slater et al., 2019). This white matter ageing pattern inverts the sequence of myelination early in life and supports the last-in-last-out hypothesis (Raz, 2000) since white matter tracts that first experience the effects of ageing, like the thalamic radiations and association fibres, also show protracted maturation.

White matter loss with ageing has an effect on cognitive performance affecting processing speed, primarily impairing executive functions (Kennedy and Raz, 2009; Tubi et al., 2020), but episodic memory function in cognitively healthy elderly is also negatively associated with white matter microstructural properties of the uncinate, ILF and SLF, thalamic radiations, and dorsal cingulum bundle (Lockhart et al., 2012; Sasson et al., 2013; Ziegler et al., 2010). Structural and functional connectivity has already been studied in cohorts of successful episodic memory agers, specifically in cohorts between 60-80yo, showing better white matter microstructural properties in superagers on the corpus callosum and the right SLF (Kim et al., 2020), and stronger functional connectivity within the default mode network (Zhang et al., 2020), the salience network (Zhang et al.,

2020) and the anterior, middle and posterior cingulate cortex (Lin et al., 2017a). A longitudinal functional connectivity study showed a set of regions in superagers whose oscillations were resistant to neurodegeneration including the right fusiform gyrus, right middle frontal gyrus, right anterior cingulate cortex, left middle temporal gyrus, left precentral gyrus, and left orbitofrontal cortex (Wang et al., 2019).

In this chapter we studied the brain white matter status and structural connectivity proxies in a sample of 64 superagers and 55 typical older adults that are over 80yo providing a brain characterisation of a time window in superageing that is unexplored to our knowledge in brain connectivity terms. We approached this study with a cross-sectional and longitudinal characterisation of 1) global white matter status and 2) structural connectivity derived from diffusion tensor imaging parameters. In the grey matter analysis presented in Chapter 3 (Garo-Pascual et al., 2023), we found that the prevailing ageing mechanism on superagers is resistance to age-related brain structural changes as manifested in slower atrophy and a greater grey matter volume in the MTL and motor thalamus compared to typical older adults. We hypothesise that the superager brain would show resistance to age-related white matter changes and have better global white matter status and preserved structural connectivity —higher FA and lower MD— in anterior tracts especially the ATR and association fibres in comparison to typical older adults.

4.2 MATERIALS AND METHODS

Participants. The sample used for the following analyses was described in the General Methods of Chapter 2.

MRI data acquisition. MRI images were acquired using a 3 Tesla MRI (Sigma HDxt GEHC, Waukesha, USA) with a phased array 8 channel head coil. T1-weighted images (3D fast spoiled gradient echo with inversion recovery preparation) were collected using a TR of 10ms, TE of 4.5ms, FOV of 240mm and a matrix size of 288x288 with slice thickness of 1mm, yielding a voxel size of 0.5 x 0.5 x 1 mm. Diffusion-weighted images were single-shot spin echo echo planar imaging (SE-EPI), with TR 9200ms, TE 80ms, b-value 800s/mm2 and 21 gradient directions, FOV 240mm and matrix size 128 x 128 with slice thickness of 3mm. The resting-state functional MRI acquisition without task, 5:10 s (TR 2500 ms, TE 27 ms). FOV 240 mm, matrix 240 x 240, 35 slices with a

thickness 2.6 mm. In all acquisitions, participants were instructed to stay awake with closed eyes without thinking of anything.

Brain white matter volume and white matter lesions volume. Brain white matter volume and white matter lesions volume were extracted from the segmentation of T1-weighted images using CAT12.7 toolbox (https://neuro-jena.github.io/cat/) implemented in SPM12 (version r6225; https://www.fil.ion.ucl.ac.uk/spm) (Ashburner and Friston, 2005). This pipeline was run for cross-sectional and longitudinal analyses, with the latter including scans from visit 1 to visit 6. TIV was also extracted using this protocol for analytical purposes as a covariate. White matter lesions are typically detected as hyperintense radiological observations in T2-FLAIR images. Here, however, we computed the volume of white matter lesions from T1-weighted images using the CAT12 toolbox, which provides a similar performance compared to existing methods of white matter hyperintensity detention from T2-FLAIR data (Dahnke et al., 2019).

Fazekas score. The Fazekas scale (Fazekas et al., 1987) quantifies brain white matter hyperintensities from MRI data with a scale as 0 = absence, 1 = focal lesions, 2 = start of confluent lesions and 3 = diffuse affectation in a region ± U-shaped fibres. For our cohort, the lesions were graded by a radiologist blinded to the subject's group using T2-FLAIR images.

White matter tract-based spatial statistics of diffusivity measures. For preprocessing of diffusion-weighted images, FSL was used (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki) and the pipeline included a motion and eddy current correction, the extraction of non-brain voxels and ends with the calculation of voxel-wise diffusion maps (FA and MD) for each participant. Individual diffusion maps were then used in the tract-based spatial statistics (TBSS) pipeline using the FMRIB toolbox (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki) (Smith et al., 2006). The general outline of the process is 1) FA individual maps were non-linearly registered to standard space (FMRIB58_FA template) (Andersson et al., 2007); 2) a mean FA image was created by averaging all co-registered FA maps; and 3) individually aligned images were projected onto the mean FA skeleton, representing the centres of all tracts common to the study sample (visual inspection was required to set a threshold of mean FA at 0.25 to include non-skeleton voxels) and skeletonised images were used for voxel-wise analysis. Diffusivity maps for MD were generated by applying the same steps detailed above. For cross-sectional analysis, diffusivity maps for FA and MD were entered into separate GLM

to compare differences between the superager and the control group. TIV, age, gender and years of education were used as covariates. We conducted whole-brain analyses using a TFCE approach with 5000 permutations (default parameters E = 0.5 and H = 2). Significant results are reported at a FWE-corrected level of $P < 0.05$. To visualise our results we used the multimodal analysis and visualisation tool (MMVT) (Felsenstein et al., 2019). The same preprocessing pipeline and GLM was built for additional diffusivity measures including mode of anisotropy, radial and axial diffusivity (Figure S6). FA and MD values were also explored longitudinally replicating with longitudinal scans the same preprocessing steps described above and further used for a regions of interest (ROI)-based analysis conducted by averaging the FA and MD values from 18 ROIs described in the JHU-ICBM thr25 atlas (Hua et al., 2008; Wakana et al., 2007)(Figure S7-S9, Table S10-S11). The statistical model is specified in the statistical analysis section.

Longitudinal diffusivity analysis in SPM. Whole-brain voxel-wise analyses testing longitudinal group differences in two measures derived from diffusion-weighted imaging sequences —FA and MD— were carried out using SPM12 (version r6225; https://www.fil.ion.ucl.ac.uk/spm) and FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) (Jenkinson et al., 2012). The preprocessing of diffusion-weighted images was conducted in FSL as described in the previous section. We performed eddy current correction, brain segmentation to exclude non-brain voxels and calculation of FA and MD parameters with the FMRIB toolbox (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki). The resulted FA and MD maps were normalised to standardise Montreal Neurological Institute (MNI) space using the TBSS pipeline (Smith et al., 2006), a non-linear registration to set individual's maps into the standard template FMRB58_FA. *Randomise*, the FSL function that builds GLM, does not support reliable longitudinal analysis, so the preprocessed data was further analysed in SPM similarly to previous authors (Lei et al., 2012).

The normalised FA and MD maps generated in FSL were then smoothed in SPM12 using a 6 mm FWHM Gaussian kernel. In the longitudinal toolbox in CAT12, separate GLM models were specified for FA and MD. Age at each MRI acquisition was included as a covariate interacting with the group factor. A masking threshold of 0.1 was applied to FA images to remove effects out of the brain. No masking threshold was used in MD images since the MD values have a low order of magnitude. These voxel-wise analyses were conducted using TFCE approach with 5000 permutations and default parameters (E = 0.5 and H = 2) using the TFCE tool (version r223) from CAT12 toolbox in SPM12 (https://www.neuro.uni-jena.de/tfce). Significant results are reported at FWE-

corrected level of $P < 0.05$. The neuroanatomical loci were reported according to the Mori and the JHU-ICBM thr25 atlas (Hua et al., 2008; Wakana et al., 2007) and Mango software (http://rii.uthscsa.edu/mango/) was used to produce the figure.

Statistical analysis. Cross-sectional group comparisons for white matter volume and white matter lesions volume were conducted with an analysis of covariance with TIV as covariate. Categorical data was evaluated with a Chi-squared test or Fisher exact test. Differences in the longitudinal trajectories of white matter volume, white matter lesions volume and Fazekas scores (computed as numeric due to the accumulative nature of the scale) and ROI-based FA and MD values were studied with a linear mixed-effects model built with the lme4 package in R. In these linear mixed effects models, white matter volume and white matter lesions volume were adjusted by TIV; scaled age, group and the interaction between scaled age and group were the fixed factors; and the random intercept and the random slope were included. Significant results are reported at $P < 0.05$, except for FA and MD ROI-based analysis that are reported at a FDR corrected level of $P < 0.05$ considering the 18 ROIs. All statistical analysis described were performed in R 4.0.2 (https://www.r-project.org/).

4.3 RESULTS

The general status of brain white matter in the superager brain was assessed cross-sectionally and longitudinally by three parameters: 1) total brain white matter volume, 2) volume of white matter lesions extracted automatically from T1-weighted images and 3) the Fazekas score (Fazekas et al., 1987), a radiological scale that evaluates the degree of brain white matter lesions (see Methods). Superagers and typical older adults show no cross-sectional differences in white matter volume ($F(1, 115) = 0.4$, $P = 0.54$) (Table S8) nor in the rate of atrophy over time ($t(1, 578) = 0.2$, $P = 0.81$) (Figure 6, Table S9). The volumetric segmentation of white matter lesions does not show differences at cross-section between groups ($F(1, 115) = 2.0$, $P = 0.17$) (Table S8), but the increasing load of white matter lesions volume over time is significantly slower

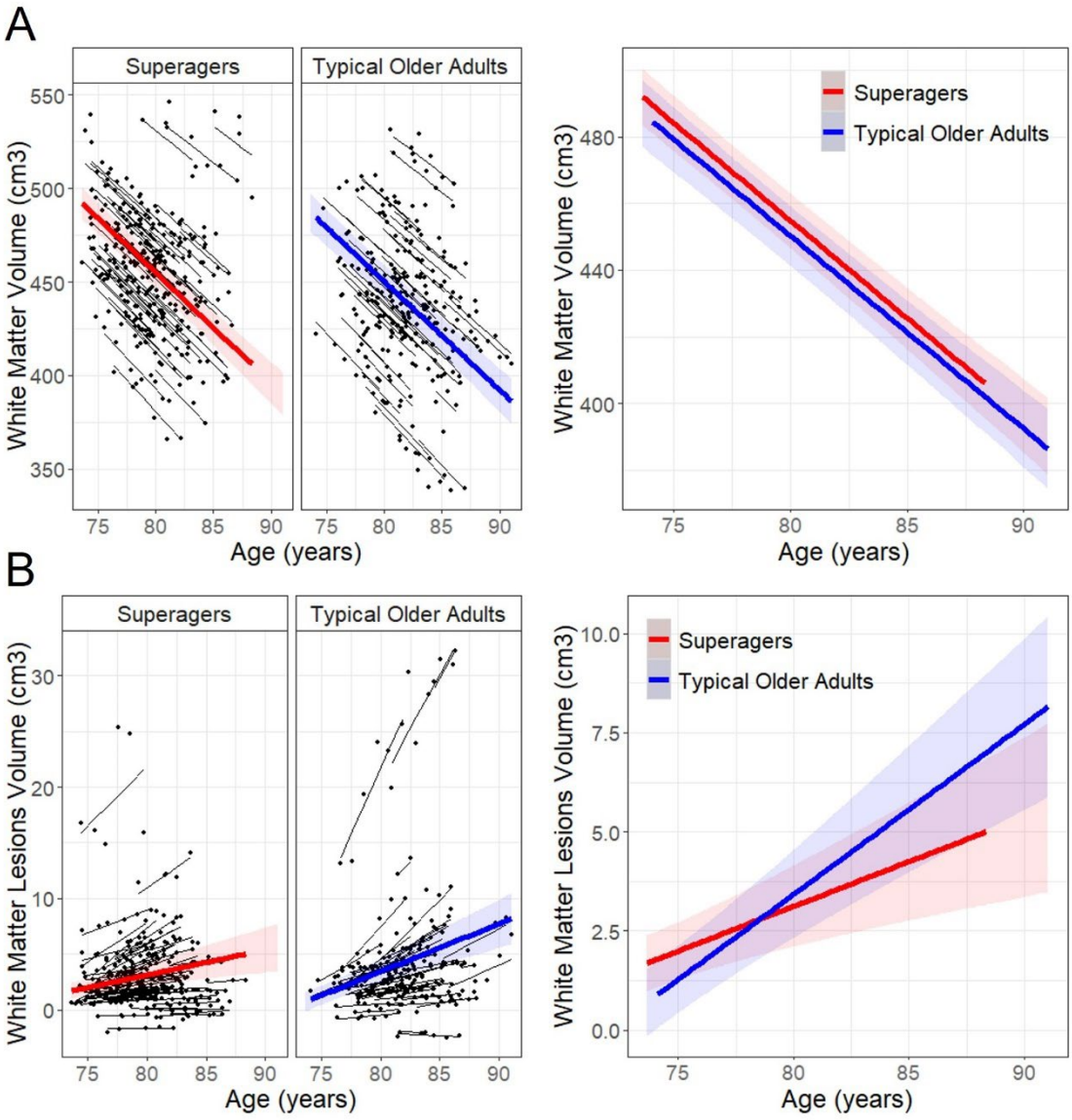


Figure 6. Longitudinal evolution of white matter volume and white matter lesions volume in superagers and typical older adults. A. Predicted trajectories of total brain white matter volume over time were plotted for superagers (red solid line) and typical older adults (blue solid line) with respective shaded areas indicating the 95% confidence interval and individual trajectories in black showing no difference at baseline or atrophy rate between groups. **B.** Accumulation over time of white matter lesions measured as white matter lesions volume. There was no baseline difference between groups, however, lesion accumulation was faster in typical older adults than superagers. White matter volumes and white matter lesions volumes have been adjusted by total intracranial volume (TIV) in the statistical model and for illustration purposes. Age was scaled in the statistical model, but raw values are shown for illustration purposes.

in superagers compared to typical older adults ($t(1, 578) = 2.4$, $P = 0.02$, β(SE) superager: 0.7 (0.18), β(SE) typical older adult: 1.36 (0.20)) (Figure 6, Table S9). The exclusion of four outliers (three typical older adults and a superager) results in the loss of significance

on the interaction between age scaled and group (t(1,558) = 1.6, *P* = 0.11). The Fazekas scores revealed that, at cross-section, a similar proportion of superagers (85.9%) and typical older adults (83.3%) have white matter lesions (χ = 0.02, *P* = 0.89) (Table S8). This high prevalence of white matter lesions is in accordance with other elderly cohorts (Association, 1994; de Leeuw et al., 2001) and there is a significant correlation between white mater lesions volume and the Fazekas score (Pearson's r = 0.73, *P* < 0.0001). There are no differences in the degree of these lesions between groups (*P* = 0.45) (Table S8) nor in the longitudinal evolution of lesions degree along the Fazekas scale (*P* = 0.80) (Table S9). Of the three global parameters assessed cross-sectionally and longitudinally, only slower white matter lesion volume loading in superagers indicates that their general white matter status could be superior of that of typical older adults.

Continuing the white matter status assessment with region-specific analysis, we explored cross-sectional voxel-wise group differences in FA and MD. A better white matter structural connectivity in terms of diffusivity translates into higher FA and lower MD values. Superagers show higher FA than typical older adults mainly in fontal regions of the inferior fronto-occipital fasciculus (IFO), ATR, right inferior longitudinal fasciculus (ILF), right forceps minor and left forceps major (*P* < 0.05 FWE-corrected) (Figure 7A). Lower MD values are found in superagers compared to typical older adults in an extensive network comprising the forceps major and minor, SLF and ILF, IFO, ATR, and cingulum bundle (*P* < 0.05 FWE-corrected) in an anteroposterior gradient showing stronger group differences in the anterior portion of the brain (Figure 7B, Figure S5). Additional diffusivity measures including axial and radial diffusivity and mode of anisotropy support the above results of superior white matter microstructural properties in the superager brain (Figure S6).

Region-specific diffusivity measures were studied longitudinally over five years with yearly follow-up scans using a voxel-wise approach and complementary ROI-based analyses were also conducted (see Appendix). We observed that FA decreases significantly slower in superagers compared to typical older adults in all white matter tracts described in the JHU-ICBM atlas (Figure 8) except for limited effects on the cingulum, hippocampal cingulum, and uncinate fasciculus with similar bilateral effects (*P* < 0.05 FWE-corrected) (Figure 8A) and consistent results are found in the ROI-based analysis (Figure S7, Table S10). The increase of MD over time is significantly slower in superagers compared to typical older adults in all tracts from the JHU-ICBM atlas with similar differences bilaterally, except for the limited effects on the cingulum (*P* < 0.05

FWE-corrected) (Figure 8B). ROI-based analysis shows different group longitudinal trajectories in all white matter tracts except for the corticospinal tract, the uncinate fasciculus and forceps minor (Figure S8, Table S11). Altogether, these results indicate a resistance to age-related changes in structural connectivity in superagers compared to typical older adults by showing a slower decrease of FA and a slower increase in MD over time.

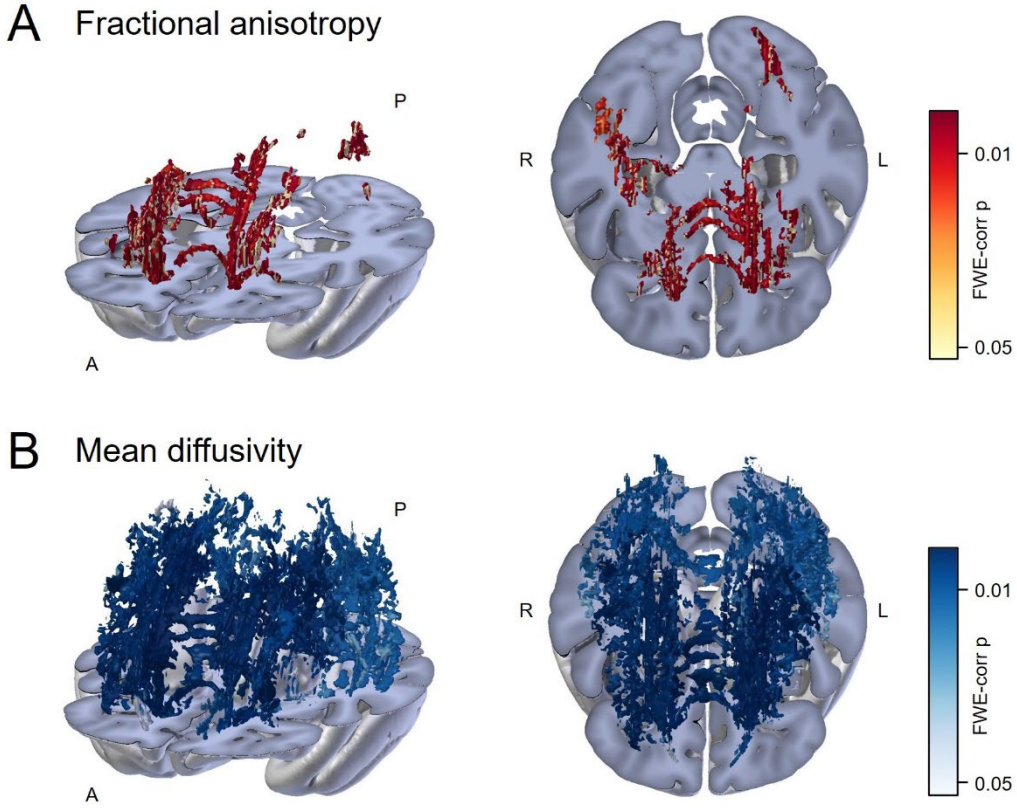


Figure 7. Better white matter structural connectivity in superagers especially in frontal white matter tracts compared to typical older adults. A. Superagers show higher fractional anisotropy (FA) than typical older adults in bilateral frontal tracts and the anterior thalamic radiation (ATR) (warm colours). **B.** Lower mean diffusivity (MD) is found in superagers compared to typical older adults in an extensive network (cold colours) showing greater differences in the anterior half of the brain (darker blue) (P < 0.05 FWE-corrected). A, anterior; FWE-corr p, family-wise corrected p-value; L, left; R, right and P, posterior.

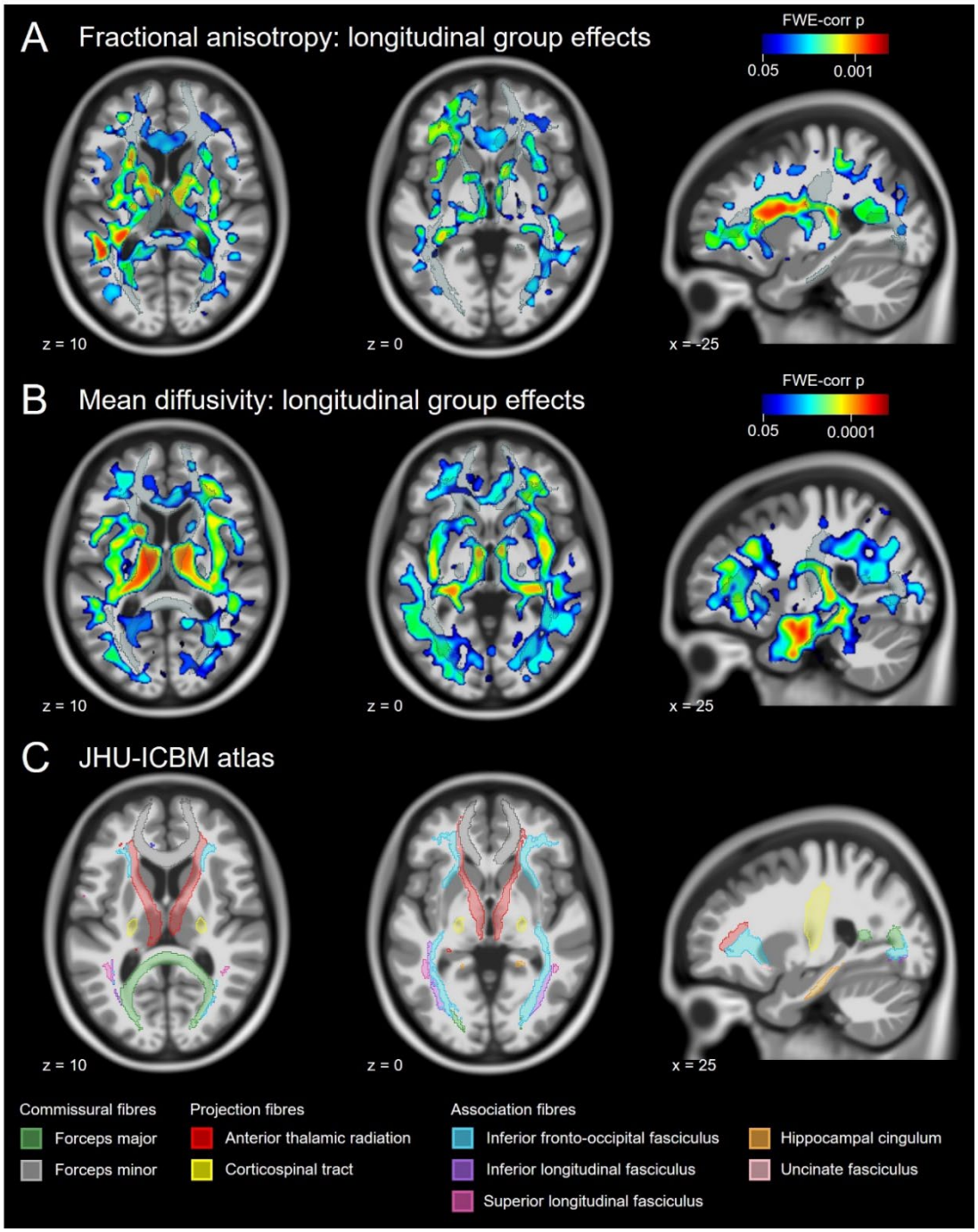


Figure 8. Longitudinal changes in structural connectivity between superagers and typical older adults. A. Longitudinal differences in fractional anisotropy (FA). Superagers show a slower decrease of FA compared to typical older adults in an extended network of white matter tracts (shaded regions) and grey matter structures like the global maximum peak in the left striatum and left thalamus (Mori atlas) ($P < 0.05$ FWE-corrected). **B.** Longitudinal differences in mean diffusivity (MD). Superagers show a slower increase of MD compared to typical older adults in an extended network of white matter tracts (shaded regions) and grey matter structures like the global maximum peak in the right hippocampus (Mori atlas) ($P < 0.05$ FWE-corrected). **C.** JHU-ICBM atlas labels of white matter tracts were used to map the significant effects shown in the rest of the panels. Note that the significant effects shown in A. and B. are not constrained to white matter since the FA and MD maps were not limited to white matter skeleton. FWE-corr p, family-wise error *p*-value.

4.4 DISCUSSION

Assessment of global cerebral white matter status indicates that superagers and typical older adults have similar white matter health at cross-section since no group differences are found in total brain white matter volume, white matter lesions volume and the Fazekas score. The longitudinal follow-up only shows a divergent pattern between groups in white matter lesions volume, where superagers have a significantly slower load of lesions. Differences in structural connectivity consistent with better white matter microstructure in superagers than typical older adults are found at cross-section and longitudinally. Cross-sectional differences show higher FA in superagers mostly in frontal fibres and lower MD in most white matter tracts following an anteroposterior gradient with greater group differences in anterior tracts. The decrease in FA over time is slower in superagers than typical older adults in all white matter tracts assessed and the increase of MD overtime is slower in superagers than typical older adults in all white matter tracts except for the corticospinal tract, the uncinate fasciculus and the forceps minor.

In global terms, the cross-sectional white matter health of our cohort does not differ between groups and the average prevalence of white matter lesions of 85%, assessed with the Fazekas scale, is consistent with other healthy older cohorts (Association, 1994; de Leeuw et al., 2001). The white matter lesion load increases with time (Ylikoski et al., 1995) and superagers show a slower longitudinal load of white matter lesions volume relative to typical older adults, suggesting better white matter status in the brain of superagers. Despite the evidence of consistency between lesion degree using the Fazekas scale and white matter lesions volume in older cohorts (Cedres et al., 2020; Valdes Hernandez Mdel et al., 2013; van Straaten et al., 2006) and the significant correlation between the two measures in our sample, we only found group differences with the volumetric approach possibly due to the lower sensitivity of the Fazekas scale (van Straaten et al., 2006), but this significant finding does not survive outliers exclusion.

White matter lesions underlie axonal and/or myelin degeneration (Haller et al., 2013), yielding negative consequences for cognitive function (de Groot et al., 2000; Prins and Scheltens, 2015). The age-related accumulation of these lesions affects processing speed, mainly impairing executive function and, to a lesser extent, the memory domain (Prins and Scheltens, 2015; Tubi et al., 2020). These lesions have been associated with

a vascular aetiology, with the prevalence of cardiovascular disease being a risk factor for their enlargement (Debette and Markus, 2010; Launer et al., 2000). Superagers showed higher prevalence of hypertension and glucose disorders than typical older adults (Garo-Pascual et al., 2023). However, they do not show differences in other cardiovascular risk factors (de Bruijn and Ikram, 2014) like high cholesterol, smoking status, obesity, diet —quantified as weekly frequency of food groups and adherence to Mediterranean diet— and physical activity (Garo-Pascual et al., 2023). Thus, the slower accumulation of white matter lesions in the brains of superagers could be explained by better cardiovascular health in this group, but not all cardiovascular risk factors support this speculation.

The regional study of structural connectivity parameters —FA and MD— confirms, firstly, better white matter microstructural properties in superagers than in typical older adults, both cross-sectionally and longitudinally, and, secondly, outlines regional brain patterns associated with ageing. Cross-sectionally, the greatest differences between groups for both FA and MD accumulate in the anterior part of the brain in line with the existing evidence that the anterior portion of the brain is more vulnerable to the effects of ageing (Davis et al., 2009; Kochunov et al., 2007; O'Sullivan et al., 2001; Pfefferbaum et al., 2005; Sullivan and Pfefferbaum, 2006). Although greater group differences in MD were found in the anterior areas, they were not constrained to the anterior portion like most FA effects. MD is a more sensitive parameter to age-related changes than FA (Cox et al., 2016), and this could explain its larger group differences.

Longitudinally, extensive group differences in white matter microstructure were found in most white mater tracts. However, ROI-based analysis revealed that MD trajectories over time were similar for both groups in the corticospinal tract, the forceps minor and the uncinate fasciculus. The absence of longitudinal differences between groups in the corticospinal tract and the forceps minor is of particular interest, as these are some of the most robust white matter tracts to the effects of ageing (Cox et al., 2016; Slater et al., 2019), supporting the first-in-last-out hypothesis (Raz, 2000). Therefore, the ageing trajectories between superagers and typical older adults mainly differ in association fibres and the ATR —which are the most vulnerable to age-related changes (Cox et al., 2016; Slater et al., 2019)— reinforcing the idea that superagers and typical older adults are both part of the healthy ageing spectrum and superagers exhibit a resistance to age-related changes (Garo-Pascual et al., 2023).

Overall, these findings support the existing idea, derived from successful ageing studies in younger cohorts, that superagers preserve robust patterns of structural and functional connectivity cross-sectionally and longitudinally (Kim et al., 2020; Lin et al., 2017a; Wang et al., 2019; Zhang et al., 2020) and extend this notion to superagers over 80 years of age. The findings presented here provide further support for our assertion that the most plausible protective mechanism in the superager brain is resistance to age-related changes, as the structural changes that will naturally arise with ageing occur at a slower rate in superagers than in typical older adults.

CHAPTER 5

TELOMERE LENGTH

5.1 HYPOTHESIS AND OBJECTIVES

Telomeres are the ends of linear chromosomes and their repetitive sequence of nucleotide tandems associated with protein complexes play a protective role for genetic material (Blackburn, 1991; de Lange, 2005). In mammals, telomeres shorten each replication (Olovnikov, 1972; Watson, 1972) and telomerase, the cellular machinery responsible for telomere elongation, is repressed in most somatic cells (Hiyama and Hiyama, 2007). Telomere shortening accounts for part of the functional decline that an organism experiences with ageing (Blasco, 2007) and, for this reason, telomere attrition is considered a hallmark of ageing (Lopez-Otin et al., 2013). In addition to ageing, there are other factors that determine telomere length at the end of an individual's life. Lifestyle influences telomere length in both directions: smoking and high body weight have deleterious effects, while following a Mediterranean diet and exercising can slow the rate of shortening (Canudas et al., 2020; Shammas, 2011; Song et al., 2010; Valdes et al., 2005). However, the greatest inter-individual variability in telomere length in adulthood is established at birth (Benetos et al., 2013) and the estimated heritability of telomere length is 70% (Broer et al., 2013). In sum, telomere length aggregates into a single value the burden of an individual's ageing, lifestyle and genetics, the same factors that contribute to complex degenerative diseases such as Alzheimer's disease.

Telomere length has been previously studied in the context of cognitive ageing and Alzheimer's disease. A recent meta-analysis shows an association between better global cognition and higher brain volume with longer leukocyte telomere length in non-demented subjects (Gampawar et al., 2022). Greater hippocampal volume is also associated with longer leukocyte telomeres in a sample of *APOE* ε4 negative healthy adults (Jacobs et al., 2014). In Alzheimer's disease patients, shorter telomeres are found relative to healthy peers (Forero et al., 2016), although there is increasing evidence pointing to a U-shaped relationship between Alzheimer's disease and telomere length, as both short and long telomeres have been associated with a higher risk of Alzheimer's

disease (Fani et al., 2020) and amnestic mild cognitive impairment, the prodromal state of Alzheimer's disease (Roberts et al., 2014).

In this chapter, we investigated telomere length in the context of successful ageing of episodic memory and we have measured telomere length of blood cells in a population of superagers that, to our best knowledge, has not been explored before. The closest approximation would be studies in the oldest-olds, elderly people who outlive their life expectancy without a diagnosis of cancer, cardiovascular and major pulmonary disease, diabetes, and Alzheimer's disease. While most studies report longer or more stable telomeres in the oldest-old for their age (Franzke et al., 2015), other oldest-old cohorts samples show no difference in telomere length compared to younger groups (Halaschek-Wiener et al., 2008; Tedone et al., 2014), although the variability of telomere length is lower in the oldest-old than in young adults (Halaschek-Wiener et al., 2008). Here we aim to characterise telomere length in superagers and typical older adults by studying median telomere length, proportion of telomeres below 6 kilobase pairs (Kbp) and 3 Kbp and the 20^{th} percentile. We measured telomere length in blood cells in a sample of 57 superagers and 48 age-matched typical older adults using a high-throughput quantitative in-situ hybridisation (HT Q-FISH) approach. We hypothesise that superagers have longer median telomere length and a smaller proportion of short telomeres relative to typical older adults.

5.2 MATERIALS AND METHODS

Participants. The selection criteria of superagers and typical older adults was described in Chapter 2. Telomere length was measured in 57 superagers and 48 typical older adults (out of the 64 superagers and 55 typical older adults from the analyses presented in Chapter 3 and 4) from the visit used for cross-sectional analyses.

Neuropsychological tests. Neuropsychological tests different form the ones used as selection criteria are shown in this study for descriptive purposes. As estimators for general cognitive performance, we used the total score of the Mini Mental State Examination and Functional Activities Questionnaire, the delayed recall score of the Rey-Osterreith Complex Figure test as a visuospatial memory measure, the Lexical Fluency test with letter P and the Spanish version of the National Adult Reading Test as vocabulary and intelligence estimators respectively.

High-throughput Q-FISH technique for telomere length determination. Telomere length in our sample was measured with a high-throughput (HT) Q-FISH technique (Life length, Spain) (Canela et al., 2007). This method is a quantitative fluorescence in-situ hybridisation approach for cells in interphase where telomeres are hybridised with a fluorescent peptide nucleic acid probe that recognises three telomere repeats (sequence: Alexa488-OO-CCCTAACCCTAACCCTAA, Panagene). The intensity of the fluorescent signal of the probes is proportional to telomere length and is translated into base pairs by a standard regression curve generated using control cell lines with known telomere length.

Preparation of the blood samples including extraction, processing, and storage before the telomere determination procedure was done at CIEN Foundation (Madrid, Spain) following the Vienna Protocol (Institute of Neurology, University of Vienna, May 2001. Modified proposal blood collection WHO 080501) as previously described (Kenny et al., 2019; Olazarán et al., 2015). Blood samples were drawn using BD Vacutainer CPT tubes with 0.1M sodium citrate and mononuclear cells were isolated following manufacturer's instructions, after centrifugation and two washes with PBS they were stored frozen at -80ºC until use, including transportation to Life Length facilities. On processing day at the Life Length laboratory, the samples and control cell lines frozen in liquid nitrogen were thawed at 37°C. Cells were seeded in clear bottom black-walled 384-well plates at the density of 15,000 cells per well with 5 replicates of each sample and 8 replicates of each control cell line. Cells were fixed with methanol/acetic acid (3/1, vol/vol) and treated with pepsin to digest the cytoplasm and the nuclei and then processed for in-situ hybridisation. After several washing steps following standard DAPI incubation for DNA staining, the wells were filled up with mounting medium and the plate was stored overnight at 4°C. Samples were processed in 2 batches.

Quantification of telomere length started with image acquisition of the blood cells nuclei on a High Content Screening Opera Phenix System (Perkin Elmer), using the Columbus software, Version 2.9 (Perkin Elmer). Images were captured using a 40 x 0.95 NA water immersion objective. UV and 488 nm excitation wavelengths were used to detect the DAPI and A488 signals respectively. With constant exposure settings, 15 independent images were captured at different positions for each well. Next, the nuclei images were used to define the region of interest for each cell, measuring telomere fluorescence intensity of the A488 image in all of them. The results of intensity for each foci were exported to the Columbus 2.4 software (Perkin Elmer). The quality of the

samples was ensured by performing the measurement on a minimum of 10,000 telomeres per sample in 5 replicates with a coefficient of variation of less than 10% (samples with less than 3 valid replicates were discarded). Telomere length distribution and median telomere length were calculated with Life Length's proprietary algorithms and the four telomere variables analysed in this chapter are median telomere length, percentage of telomeres bellow 6 Kbp and 3 Kbp and the 20th percentile.

***APOE* genotyping.** Genotyping of *APOE* gene polymorphisms was determined by a genome wide association analysis (GWAS) using the Axiom 815K Spanish biobank array (Thermo Fisher) as described elsewhere (de Rojas et al., 2021).

Potential confounding variables. Age, sex, years of education, BMI, *APOE* ε4 carrier, smoking, hypertension, cell viability, storage time and batch were considered as potential confounding variables following previous literature (Canela et al., 2007; Fani et al., 2020). BMI was estimated as weight in kilograms per height in meters squared, smoking was categorised as ex-smoker or never smoker (none of the subjects was a smoker at the time of blood extraction) and hypertension was categorised as subjects with or without history of hypertension by self-report. The values for all these variables were recorded at the same visit when the blood used for telomere length determination was drawn. Cell viability was estimated after thawing the blood samples by the Tripan-Blue method and the storage time was calculated from the date of collection to the date of analysis of the blood samples.

Statistical analysis. The four variables of study are median telomere length, percentage of telomeres below 6 Kbp and 3 Kbp and the 20th percentile of telomere length. The normality of these variables was assessed by visual inspection with Q-Q plots and by the Shapiro-Wilk normality test. We tested with individual regression models the relationship between telomere variables and each potential confounding variable (age sex, years of education, BMI, *APOE* ε4 carrier, smoking, hypertension, cell viability, storage time and batch), and only cell viability, storage time and batch showed a significant association with telomere variables. We fitted telomere variables using all confounding variables and compared this full model with a simplified model adjusted only by cell viability, storage time and batch, given that both models have a similar fit to the data, in this chapter we report group comparisons for telomere variables adjusted by cell viability, storage time and batch. To test group differences, chi-squared tests are used for categorical variables and two-sample t-tests (two-tailed) were used for

numerical variables with significance level set at 0.05. The association of telomere variables with age was assessed with regression models where telomere variables were the dependent variable individually and age, group and the interaction between the two were included as independent variables. All analyses were performed at R version 4.1.2 (https://www.r-project.org/).

5.3 RESULTS

The demographic characteristics of the subset of superagers and typical older adults used for telomere length analyses follows the same pattern as the complete dataset shown in Chapter 3. There is no age difference between superagers and typical older adults, the sex distribution is similar between groups, with slightly higher female representation in both groups, and superagers have a significantly higher number of years of education compared to typical older adults (Table S12). Superagers perform significantly better in the cognitive tests that are part of the selection criteria (delayed free-recall score of the Free and Cued Selective Reminding Test, the Animal Fluency test, Digit Symbol Substitution Test, 15-item Boston Naming Test) but also in other cognitive tests including the Mini-Mental State Examination, the Functional Activities Questionnaire, the delayed recall score of the Rey-Osterreith Complex Figure test, a lexical language test and an estimator of intelligence.

Group differences in telomere length distributions (Figure 9A, B) show a significantly shorter median telomere length in superagers compared to typical older adults (t = -2.3, *P* = 0.02) (Figure 9C, Table S12). Measures of centrality such as median telomere length are relevant, however, the proportion of short telomeres may be even more informative (Hemann et al., 2001). When telomeres have shortened too much, chromosomal integrity is compromised, inducing cell cycle arrest or apoptosis. By analysing the percentage of short (<6 Kbp) and extremely short (<3 Kbp) telomeres, we obtain an approximation of how close the cells of the blood samples are to the limit of telomere protection over genetic material and thus cellular survival. Superagers show a significantly higher percentage of short (t = 2.1, *P* = 0.04) (Figure 9D, Table S12) and extremely short telomeres (t = 2.2, *P* = 0.03) (Figure 9E, Table S12) compared to typical older adults. Consistent with these results, the 20^{th} percentile of the telomere length distribution show significantly shorter telomere length in superagers compared to typical older adults (t = -2.3, *P* = 0.02) (Figure 9F, Table S12). Taken together, superagers have

shorter median telomere length and a higher proportion of short telomeres than age-matched typical older adults.

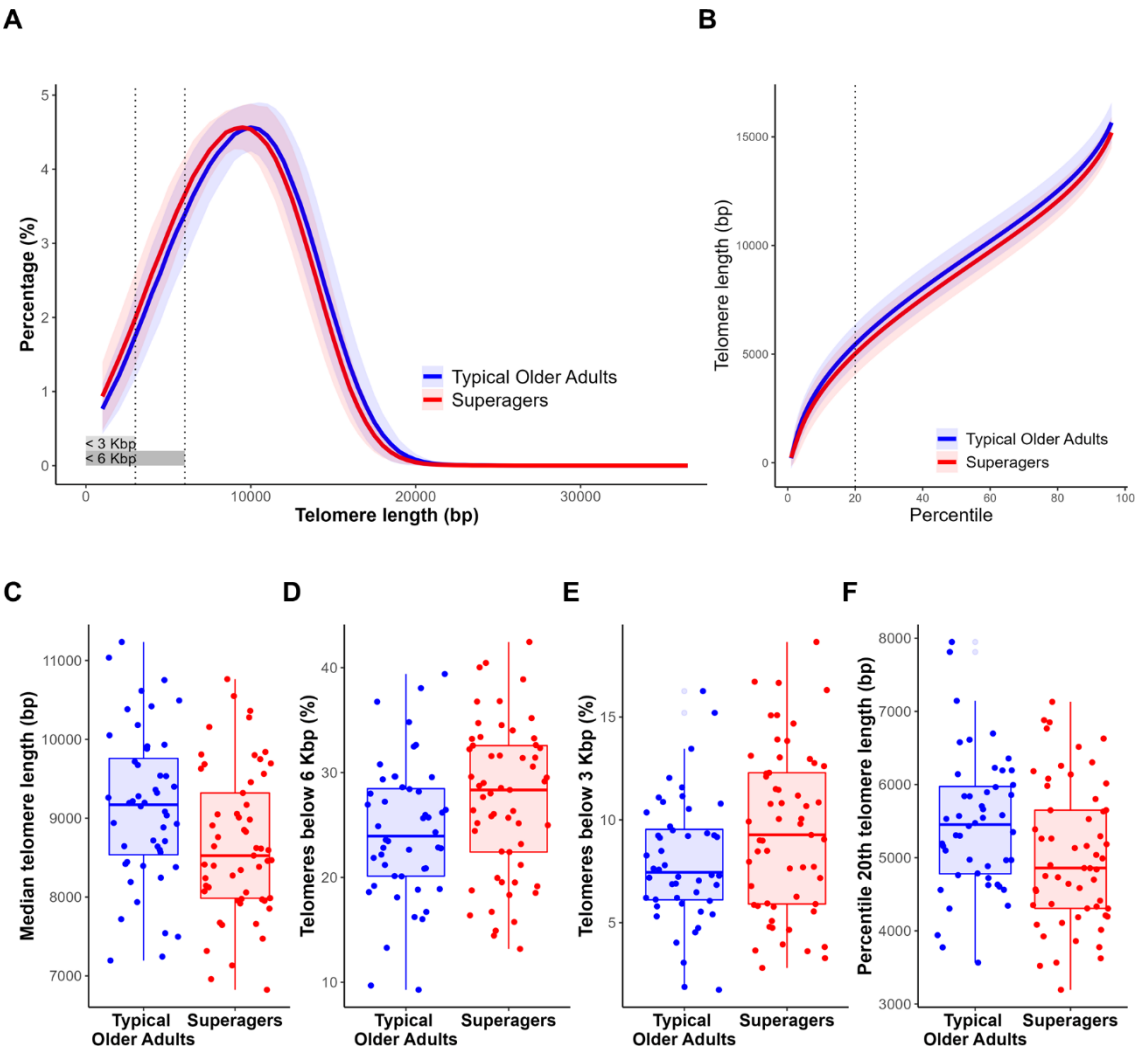


Figure 9. Telomere length distribution in a population of superagers and age-matched typical older adults. A. Average group distributions of telomere length (distributions build in 500 bp intervals) illustrate group differences in centrality measures of telomere length and percentage of short telomeres (dotted lines indicate the 6 Kbp and 3 Kbp mark). **B.** Percentiles of telomeres ranked by their length. Dotted line refers to the 20th percentile. **C.** Median telomere length is significantly higher in typical older adults than in superagers (t = -2.3, *P* = 0.02). **D.** The percentage of telomeres below 6 Kbp is significantly higher in superagers than in typical older adults (t = 2.1, *P* = 0.04). **E.** Superagers also have a significantly higher percentage of telomeres below 3 Kbp (t = 2.2, *P* = 0.03). **F.** Superagers' 20th percentile is on average significantly shorter than in typical older adults (t = -2.3, *P* = 0.02). Raw data is plotted, and the statistical contrasts referred in C-F were conducted with telomere variables adjusted by cell viability, storage time and batch. Kbp, kilobase pairs; bp, base pairs.

Finally, as telomere attrition is a hallmark of ageing (Lopez-Otin et al., 2013), we explored the association between age and telomere variables in our cross-sectional dataset with an age range of 79.5-87.9 years. There is a significant main effect of age over median telomere length in the whole sample (t = -2.2, *P* = 0.03) (Figure 10C) but there is not a significant interaction between age and group (t = 1.5, *P* = 0.14) (Figure 10D), thus median telomere length has the same ageing trajectory in both groups. Same findings were replicated in the rest of the telomere variables (data not shown).

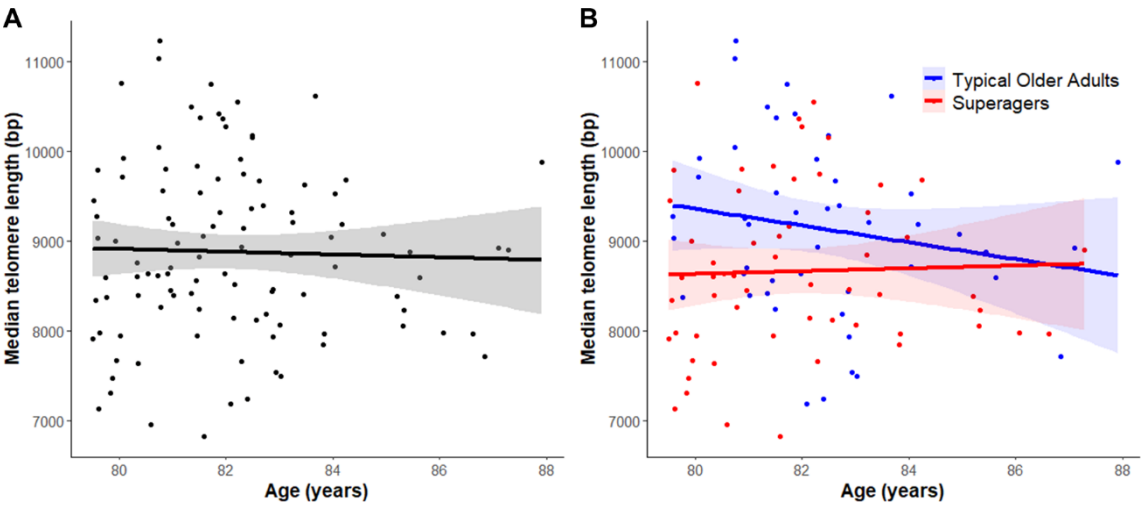


Figure 10. Association between median telomere length and age in a population of superagers and age-matched typical older adults. A. There is a main effect of age on median telomere length in the whole sample (t = -2.2, *P* = 0.03). **B.** Ageing trajectories on telomere length are similar between groups (t = 1.5, *P* = 0.14). Raw data is plotted, and the statistical contrasts referred were conducted with median telomere length adjusted by cell viability, storage time and batch. Bp, base pairs.

5.4 DISCUSSION

This chapter investigated the cross-sectional distribution of telomere length in a sample of elderly subjects with successful episodic memory —superagers— compared to an age-matched control group with normal episodic memory for their age. Significantly shorter median telomere length and higher proportion of short telomeres was observed in superagers compared to typical older adults together with a negative association between telomeric variables and age in the whole sample with no

differentiated ageing trajectories between groups. We hypothesised that superagers had a longer median telomere length and fewer short telomeres than typical older adults, however, significant differences were found in the opposite direction. Our original hypothesis was founded on the solid consideration of telomere attrition as a hallmark of ageing (Lopez-Otin et al., 2013) and on our previous findings showing that superagers are resistant to age-related brain atrophy (Garo-Pascual et al., 2023). Most of the literature on telomere length and brain ageing reinforced our hypothesis, as in the systematic review and meta-analysis by Gampawar and colleges where the majority of studies pointed to a positive association between telomere length and cognitive performance and brain volume in healthy elderly people (Gampawar et al., 2022). Nonetheless, our paradoxical results are not unique in the context of telomeres and cognitive ageing. While meta-analyses conclude that telomeres are shorter among Alzheimer's disease patients (Forero et al., 2016) and longer and more stable in oldest-old (Franzke et al., 2015), long telomeres do not always reflect a benefit for cognition. There is evidence that both short and long telomeres are associated with increased risk of Alzheimer's disease (Fani et al., 2020) and amnestic mild cognitive impairment (Roberts et al., 2014), some cohorts of oldest-old have a shorter telomere length than younger controls (Halaschek-Wiener et al., 2008; Tedone et al., 2014) and in other cognitively healthy cohorts, the genetic predisposition for long telomeres is not associated with cognitive performance including episodic memory (Demanelis et al., 2021; Rodriguez-Fernandez et al., 2022).

An analysis of the three factors that contribute to determining an adult's telomere length —ageing, lifestyle and genetics— is of relevance in interpreting our results. In terms of the ageing mechanism, superagers are resistant to structural brain age-related changes, as their atrophy rate is slower than typical older adults (Cook et al., 2017b; Garo-Pascual et al., 2023). In our previous findings, the same superager cohort assessed in this chapter show better mental health, better mobility, less complaints for not having enough sleep and lower incidence of glucose disorders and hypertension than typical older adults (Garo-Pascual et al., 2023). These are not the same factors associated with long telomere length (following a Mediterranean diet, exercising, not smoking and a low body weight) (Canudas et al., 2020; Shammas, 2011; Song et al., 2010; Valdes et al., 2005), although they also suggest better overall health status. Current understanding of the mechanisms of ageing in the superagers and the factors associated with this phenotype lead us to hypothesise that their telomeres must be longer than those of typical older adults, however, genetic information is missing and the heritability of

telomere length is 70% (Broer et al., 2013) so the greatest inter-individual variability in telomere length in adulthood is determined early in life (Benetos et al., 2013). We can only speculate that the longer telomeres of typical older adults could have been established early in life or already at birth by genetic variants. Considering that the absolute difference in median telomere length between groups is 474 bp and the attrition rate of telomeres in humans is ~70 bp per year (Canela et al., 2007), superagers have 6.8 more "years" in telomere length terms than typical older adults. Thus, it is likely that the successful episodic memory performance of superagers is not determined by optimised telomere function.

This study has certain limitations. Telomere length was measured in leukocytes, and although recent work reports correlations between telomere length in blood and other human tissues, including the brain (Demanelis et al., 2020), it is uncertain whether brain tissue telomere variables would be better indicators of cognitive ageing than blood cell measurements. The blood samples used have an unknown proportion of granulocytes and lymphocytes which is sensitive to ageing (Valiathan et al., 2016) and dementia (van der Willik et al., 2019), and lymphocytes have higher levels of telomerase activity than granulocytes (Weng et al., 1997) potently resulting in telomere length differences between the two cell types. An important advantage of the HT Q-FISH technology used for telomere length determination is the low detection limit of < 100 bp (Canela et al., 2007) that allows short telomeres to be studied and this parameter better reflects telomere dysfunction compared to measures of telomere length centrality (Hemann et al., 2001).

Future studies should aim to assess telomere length longitudinally to calculate the rate of telomere attrition, as it is a better estimate of life spam than telomere length per se (Whittemore et al., 2019) and will clarify what the contribution of the ageing process on telomere length is, as genetic make-up and lifestyle factors will remain fixed intrasubject. In addition, the genetic component related to telomere length in the superager population is still unknown and could be explored with the genetic variants associated with long telomeres (Li et al., 2020; Machiela et al., 2017; Prescott et al., 2011).

CHAPTER 6

EPISODIC LEARNING RATE

A version of the following chapter is published in Neuroimage:

Frank D*, **Garo-Pascual M***, Reyes Velasquez PA, Frades B, Peled N, Zhang L, Strange BA. Brain structure and episodic learning rate in cognitively healthy ageing. Neuroimage. 2022 Sep 13;263:119630.

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6.1 HYPOTHESIS AND OBJECTIVES

Ageing is accompanied by a decline in cognition, most characteristically in episodic memory performance (Glisky, 2007; Tromp et al., 2015), the ability to remember personal experiences. Episodic memory impairments in ageing can manifest in different ways depending on the studied phase (*i.e.,* encoding, consolidation, or retrieval process). It is difficult to study these phases independently behaviourally, although previous work has reported that distinct processes may be affected unequally during ageing. For example, more prominent deficits have been found for encoding relative to retrieval in older adults (Friedman et al., 2007; Morcom et al., 2003). Furthermore, exploring the neural underpinnings of these various manifestations (*e.g.*, learning versus retention) could inform dissociations between normal age-related decline and decline driven by neurodegenerative diseases such as dementia. Memory decline in ageing is often

measured in terms of retention. However, impairments could also be driven by a diminished ability to learn information over a period of time, rather than to retrieve it. We therefore aimed to elucidate the structural brain properties underlying episodic learning rate in ageing.

Ageing has been associated with a global reduction in grey matter volume (Farokhian et al., 2017; Grieve et al., 2007), although to different extents across brain regions (Cox et al., 2018; Resnick et al., 2003). Numerous studies have found a specific grey matter volume loss in prefrontal, temporal and parietal cortices (Cox et al., 2021; Elliott, 2020), associated with general cognitive and memory-specific decline (Cox et al., 2021; Fjell and Walhovd, 2010; Gorbach et al., 2017). White matter age-related differences in FA and MD have also been reported (Bennett et al., 2010; Fjell and Walhovd, 2010; Madden and Parks, 2017). FA and MD are negatively correlated such that loss of white matter microstructure is indexed by a decrease in FA and an increase in MD. Like grey matter, age-related white matter effects are apparent throughout the brain (Farokhian et al., 2017; Grieve et al., 2007), although with greater effects in anterior than posterior tracts (Bennett et al., 2010). Whilst these structural differences contribute to our understanding of brain ageing, it is vital to also consider their cognitive manifestations.

A commonly observed form of age-related cognitive decline is impaired memory, which has been associated with reduced hippocampal volume (Gorbach et al., 2017; Hedden et al., 2016; Persson et al., 2012), as well as with damage to the microstructure of frontal and temporal white matter tracts (de Mooij et al., 2018; Kennedy and Raz, 2009; Rizvi et al., 2020b), and specifically limbic tracts (Bennett et al., 2015). Furthermore, recognition performance on neuropsychological episodic memory tests has been shown to correlate with FA and MD measures in the fornix, cingulum, and superior and inferior longitudinal fasciculi (Sasson et al., 2013). However, other studies have not found correlations between white matter microstructure and episodic retrieval in ageing (Gorbach et al., 2017; Laukka et al., 2013; Salami et al., 2012).

Memory performance is usually quantified by the ability to recognise or recall information correctly, a retrieval impairment could be caused by a reduced ability to encode or learn information (Boujut and Clarys, 2016; Cadar et al., 2018). Encoding, which is potentially dissociable from retrieval processes (Bennett et al., 2015; Kwok and Buckley, 2010), has been shown to underlie several memory deficits observed in ageing

(Grady, 2012). Whilst learning rate is part of the encoding process, in the current context it specifically refers to an improvement in learning over time (or repetitions). Indeed, there is evidence for reduced error-driven (Nassar et al., 2016) and probabilistic learning rates (Herff et al., 2019; Samanez-Larkin et al., 2012) in older adults, but evidence for similar deficits in episodic learning rate is lacking.

A potential way to probe episodic learning rate is through the FCSRT. The FCSRT is one of the most commonly used free-recall paradigms for episodic memory assessment, including immediate and delayed free- and cued-recall (Buschke, 1984). Worse recall performance of cognitively normal older adults on the FCSRT has been associated with reduced hippocampal grey matter volume (Zammit et al., 2017), reduced fornix FA (Hartopp et al., 2019; Metzler-Baddeley et al., 2011) and increased frontal MD (Nicolas et al., 2020). In addition to such retrieval effects, using the immediate free recall components across three consecutive trials, the FCSRT enables investigating episodic learning by examining how many additional words are successfully recalled on each trial.

In a large cross-sectional cohort of healthy older adults from the Vallecas Project, we first calculated the learning rate across the three consecutive FCSRT immediate recall trials and investigated 1) the association with demographic variables —sex, age and level of education—, 2) its neural manifestation in grey matter volume using a voxel-based morphometry approach, and 3) in white matter microstructure using TBSS on FA and MD measures and 4) differences in the learning rates in two subgroups of the Vallecas Project cohort —superagers and typical older adults. We hypothesised that 1) learning rate is independent of sex, is negatively associated with age and positively associated with level of education; 2) hippocampal grey matter volume and 3) white matter microstructure of limbic tracts correlate with learning rate given the critical role of the hippocampus in episodic memory and its correlation with structural changes in ageing; and 4) superagers have a faster learning rate than typical older adults.

6.2 MATERIALS AND METHODS

Participants. All participants in this study were part of the Vallecas Project, a single-centre longitudinal study of community-dwelling volunteers aged 69-86 without any cognitive or psychiatric disorder that compromised their daily functioning at the time of recruitment. Inclusion and exclusion criteria have been further described elsewhere

(Olazarán et al., 2015) and in the General Methods chapter. Data from the baseline visit of 982 cognitively normal participants (mean age = 74.8, SD = 3.9, 637 (64.9%) women) were included in the current study. Any subject with a diagnosis of mild cognitive impairment or Alzheimer's disease at this first visit was excluded. Additional analyses were performed with two subgroups of this cohort, the 64 superagers and 55 typical older adults previously described in the General Methods chapter, data from the same cross-sectional visit used in previous analyses (Chapter 3-5) in used in these analyses.

Neuropsychological assessment. Participants completed a battery of neuropsychological assessments as part of the Vallecas Project protocol. In this study, we report the total score of the Mini Mental State Examination (Folstein et al., 1975) and we mainly focus on the FCSRT (Buschke, 1984), assessing learning and retention of verbal memory, with immediate and delayed recall components. The test was administered using standard procedures (Peña-Casanova et al., 2009a). Participants were presented with cards containing four words and asked to identify the word corresponding to a specific semantic category, going through all four words, on four different cards (16 words in total). The words presented are not the most obvious member of each semantic category. Following the presentation phase, participants were asked to recall as many words as possible in three consecutive recall trials each one followed by 20 s of interference counting backwards (Figure 11A). For each trial, participants were asked to freely recall as many words as possible with a time limit of 90 s, then examiners provided the semantic category clue for the forgotten items. These three free and cued recalls constitute the three immediate recall trials of the task. This immediate recall phase is followed by a 30 min delay, after which the delayed phase of the test starts. Participants were asked on a single trial to freely recall as many words as possible otherwise cues were provided (Figure 11A). To assess the learning rate across trials, we fit a linear mixed-effects model of the number of items freely recalled in each immediate trial, as a function of the recall trial (first, second, and third) using the lme4 package in R 4.0.2 (https://www.r-project.org/). The model also included a random slope of the recall trial, and a random intercept per participant, capturing inter-individual variability in learning rate (across the three trials). The learning rate coefficient for each participant was extracted using the *coef* function for subsequent analyses. Next, we built a multiple regression model where the learning rate was the dependent variable, sex, age and level of education were the predictors and the delayed free recall score of the FCSRT was included as a covariate to rule out the retrieval phase of the memory process.

Extraction and plotting of the effects reported below were conducted using the effects (Fox, 2003) and ggplot2 (Wickham, 2009) packages in R.

MRI data acquisition. Images were acquired using a 3T MRI (Signa HDxt GE) with a phased array eight-channel head coil. T1-weighted images (3D fast spoiled gradient echo with inversion recovery preparation) were collected using a TR of 10ms, TE of 4.5ms, FOV of 240mm and a matrix size of 288 × 288 with slice thickness of 1mm, yielding a voxel size of 0.5 × 0.5 × 1 mm3. Diffusion-weighted images were single-shot SE-EPI, with TR 9200ms, TE 80ms, b-value 800s/mm2 and 21 gradient directions, FOV 240mm and matrix size 128 × 128 with slice thickness of 3mm.

Grey matter volume. The analysis was carried out in SPM12 (version r6225; https://www.fil.ion.ucl.ac.uk/spm). T1-weighted images were segmented into grey matter, white matter and cerebrospinal fluid and then aligned and normalised to MNI space using the DARTEL algorithm (Ashburner, 2007). Prior to statistical modelling, the normalised images were smoothed using a 6mm FWHM Gaussian kernel. The pre-processed grey matter maps were entered into a GLM with learning rate from the memory task as the predictor of interest, and TIV, sex, and the delayed free recall score of the FCSRT as covariates. Age and education were not used in the model as additional covariates since FCSRT delayed free recall is sensitive to the effects of age and level of education. Nonetheless, to ensure the model is capturing variance associated with these variables we devised a second model without FCSRT delayed free recall and including TIV, sex, age and education as covariates and the same results were obtained (see Appendix). We conducted whole-brain analyses using a TFCE approach with 5000 permutations and default parameters (E = 0.5 and H = 2) using the TFCE tool (version r223) for CAT12 toolbox in SPM (https://www.neuro.uni-jena.de/tfce) (Gaser et al., 2022). Therefore, our analyses fully correct for mass-univariate testing (and associated multiple-comparisons problem) by employing a whole-brain FWE-correction. Furthermore, we used the TFCE approach to overcome cluster-based inference issues. The AAL3 atlas neuroanatomical labels were used to describe neuroanatomical loci (Rolls et al., 2020) and Mango software was used to produce the figure (http://rii.uthscsa.edu/mango/). These analyses assessed which regions were positively associated with the episodic learning rate. Significant results are reported at a FWE corrected level of $P < 0.05$.

White matter microstructure. Of the 982 participants, seven were excluded as they did not have diffusion data. For preprocessing these images, the FSL toolbox (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki) was used for motion and eddy current correction, the extraction of non-brain voxels and, lastly, the calculation of voxel-wise diffusion maps (FA and MD) for each participant. Individual FA and MD maps were then used in the FSL TBSS pipeline (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/TBSS/UserGuider) (Smith et al., 2006). The general outline of the process is: 1) FA individual maps were non-linearly registered to standard space (FMRIB58_FA template); 2) a mean FA image was created by averaging all co-registered FA maps and 3) individually aligned images were projected onto the mean FA skeleton —representing the centers of all tracts common to the study sample— and skeletonised images were used for voxel-wise analysis. Diffusivity maps for MD were generated by applying the same steps detailed above. The same GLM design matrix as the grey matter analysis was used along with the TFCE approach with 5000 permutations (default parameters E = 0.5 and H = 2). Significant results are reported at a FWE-corrected level of $P < 0.05$. To visualise our TBSS results we used the MMVT (Felsenstein et al., 2019). The pipeline follows these steps: 1) binary masking: all the voxels in the TBSS volume below the threshold (0.95) were set to zero; 2) outlier voxels removal using the Open3D python package (Zhou et al., 2018); 3) smoothing the volumetric data using a 3D Gaussian filter (Virtanen et al., 2020); 4) surface creation from the volume's TBSS surfaces using the marching cubes algorithm (Lorensen and Cline, 1987), for that, we re-calculate the threshold to give us the same number of voxels after the smoothing step; 5) translation for the surfaces' vertices coordinates; and 6) projection of the volumetric data on the surfaces.

Statistical analysis. Differences in the learning rate between groups were studied with a linear mixed-effects model built with the lme4 package in R. In this linear mixed-effects model, trial number, group and the interaction between trial number and group were fixed factors. Age, sex, level of education and the FCSRT delayed free recall score were considered as covariates of no interest. The random intercept and the random slope were also included in the model. All statistical analysis described in this section were performed in R 4.0.2 (https://www.r-project.org/).

6.3 RESULTS

Performance across the three immediate recall trials of the FCSRT improved, reflecting a positive episodic learning rate that on average was 1.4 (SD = 0.3) (*i.e.*, on average participants recall 1.4 more items each trial) (Figure 11B, Table S13). Our linear model predicting the learning rate as a function of age, sex, and level of education revealed significant effects of the three predictors after correcting for FCSRT delayed free recall score. Learning rate and FCSRT delayed free recall were positively correlated (Pearson's r = 0.7; $P < 0.0001$). Age had a negative effect on learning rate ($F(1,965) = 10.5$, $P = 0.001$) (Figure 11C), sex also had an effect ($F(1,965) = 4.7$, $P = 0.031$) and being a woman was positively associated with learning rate (Figure 11D). Finally, having a higher level of education was positively associated with learning rate ($F(3,965) = 5.2$, $P < 0.0001$) (Figure 11E). There was no significant interaction between the three predictors (age, sex and level of education).

We found a positive correlation between episodic learning rate and grey matter volume in the bilateral hippocampus, with more pronounced effects on the left side and the left superior temporal gyrus (Figure 12A, Figure S9), and the right anterior thalamic nucleus with some extension to adjacent nuclei (right ventroanterior and ventrolateral thalamic nuclei) (Figure 12B, Table S14, Figure S9).

We first examined FA as a proxi of white matter microstructure and found a bilateral network of temporal, parietal and occipital tracts showing a positive association with episodic learning rate. Among tracts showing significant positive correlations were the bilateral ATR, fornix, IFO, ILF, SLF, and uncinate fasciculus (Figure 13A-B, Table S15, Figure S10). Next, we examined MD and found a negative association between a similar network of bilateral tracts and episodic learning rate, including bilateral ATR, corticospinal tract, forceps major and minor, cingulum, IFO, ILF, SLF, uncinate and fornix (Figure 13C-D, Figure S10, Table S15).

Whilst the classification of superagers and typical older adults relies on the delayed free recall component of the FCSRT, the learning rate derived from the three immediate free recall trials has not been explored. We tested for group differences in learning rate from the FCSRT test administered at the same visit used for previous cross-sectional analyses presented in Chapter 3-5 (all different from the initial visit) (see General Methods Chapter 2) by fitting a linear mixed-effects model adjusted for sex, age, level of education and FCSRT delayed free recall score. Of special interest is the

correction for the FCSRT delayed free recall score, as it helped us to dissect learning rates independently of the score that classified participants into groups. There was a significant and positive effect of trial number ($\chi(343) = 37.7$, $P < 0.0001$) (Figure 14) showing that participants in both groups recalled more words across trials and no group

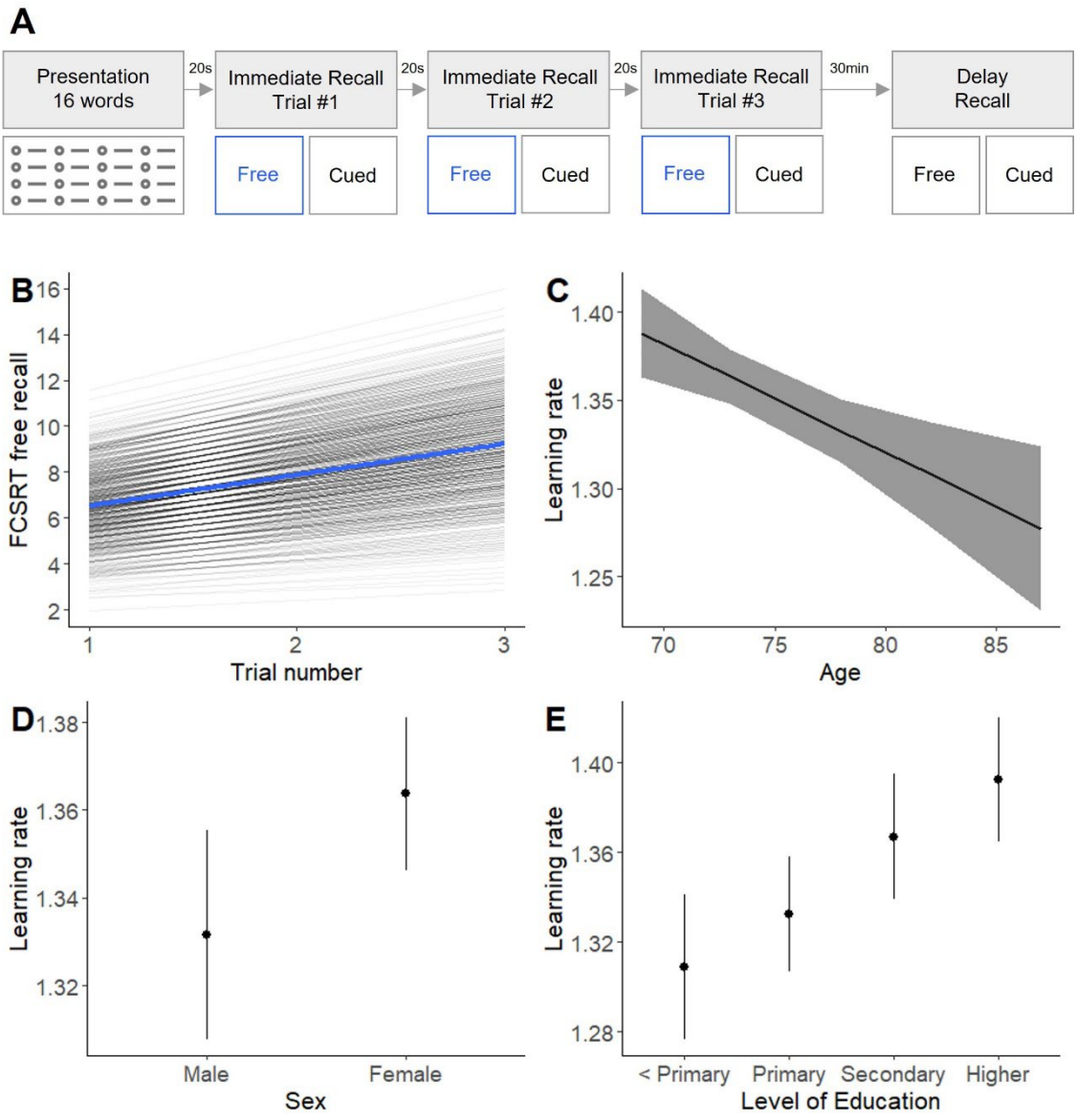


Figure 11. Learning rate across FCSRT trials is related to age, sex, and level of education. A. Diagram of the FCSRT protocol used to assess memory in this study in which the learning rate is calculated from the free recall of the three immediate trials (blue). **B.** Mean and individual learning rates across the three free immediate recall FCSRT trials. After controlling for the rest of the model predictors, **C.** learning rate decreases with age, **D.** females learn faster than males and **E.** level of education is positively associated with learning rate. Error bars represent 95% confidence intervals. FCSRT, Free and Cued Selective Reminding Test.

effect was found after adjustment ($\chi(343) = 0.08$, $P = 0.78$) (Figure 14). The interaction between trial number and group showed significant differences ($\chi(343) = 5.51$, $P = 0.02$) (Figure 14) where superagers had a faster learning rate (β (SE) = 1.20 (0.12)) than typical older adults (β (SE) = 0.79 (0.13)). These results suggest that superagers learnt on average 1.20 more items per trial, while typical older adults learnt on average 0.79 more items per trial. We did not find a differential association for grey matter volume and learning rate between the groups (data not shown).

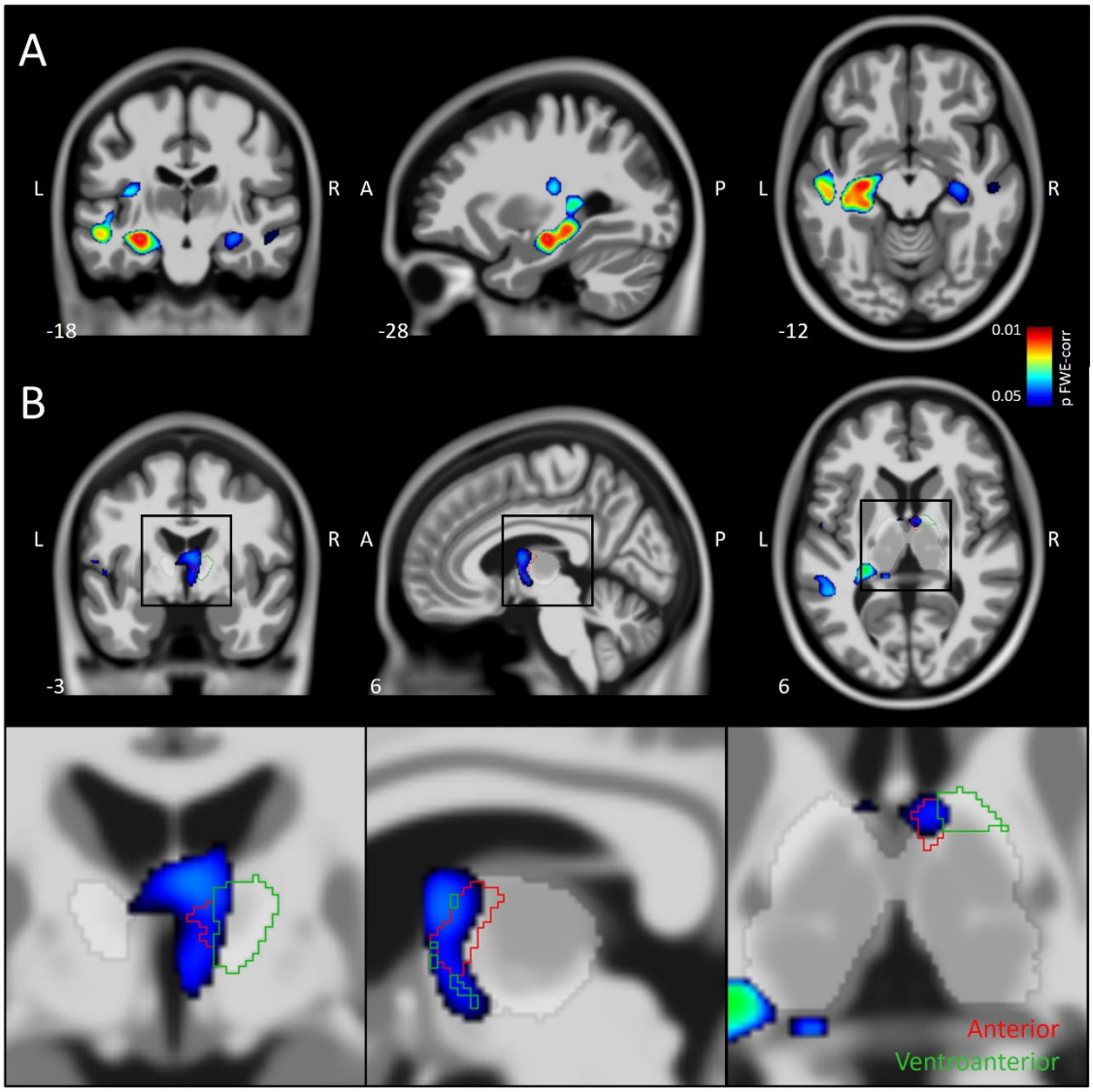


Figure 12. Grey matter volume correlates with episodic learning rate in older adults. The positive correlation has been overlaid on a canonical T1 image (thresholded at $P < 0.05$ FWE-corr) to show a significant effect in **A.** hippocampus bilaterally and left superior temporal gyrus and **B.** right anterior (red) and ventroanterior (green) thalamic nuclei. Thalamic ROIs in the inset come from the AAL3 atlas (Rolls et al., 2020). The coordinates of the sections are given in mm. A, anterior; L, left; P, posterior; R, right.

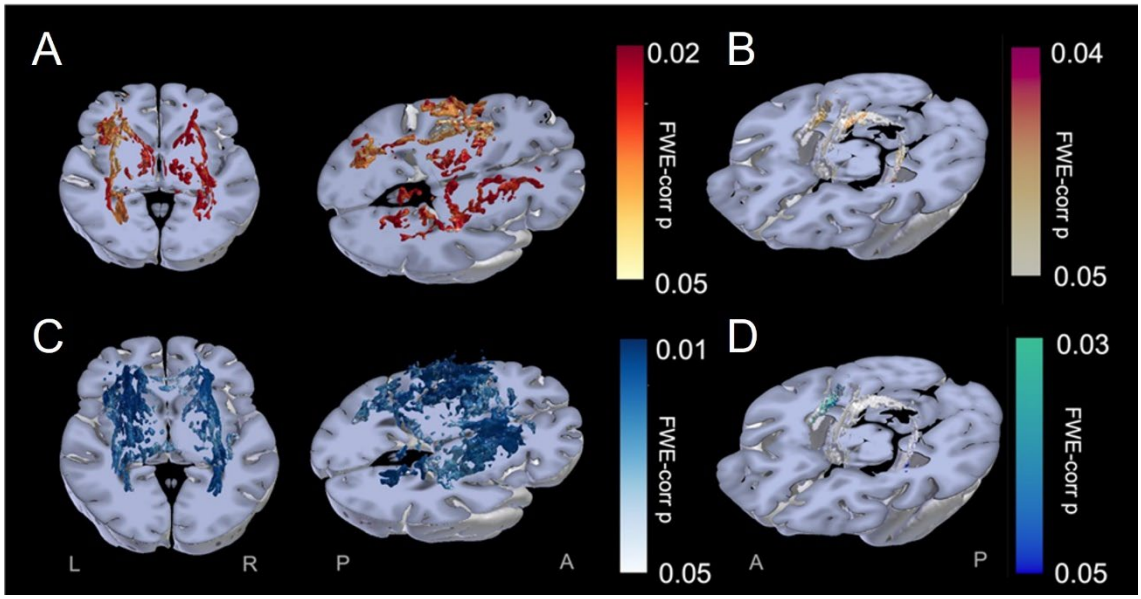


Figure 13. The microstructure of an extensive network of white matter tracts is related to episodic learning rate in older adults. A. Positive correlation between fractional anisotropy (FA) and episodic learning rate (warm colours; $P < 0.05$ FWE-corr). **B.** FA effects overlaid on the fornix. **C.** Negative correlation between mean diffusivity (MD) and episodic learning rate (cold colours; $P < 0.05$ FWE-corr). **D.** MD effect overlaid on the fornix. A, anterior; L, left; P, posterior; R, right.

As the average learning rate reported for the whole sample at baseline visit (1.4 (SD = 0.3)) was unadjusted for sex, age and FCSRT delayed free recall it is difficult to compare it to the adjusted average learning rate reported above for superagers and typical older adults. Therefore, we computed the unadjusted learning rates for each group using the FCSRT administered at baseline visit. The unadjusted learning rate of superagers at baseline was 1.59 (SD = 0.25), higher than the average for the whole sample, and 1.10 (SD = 0.25) for typical older adults. We also tested the significant interaction between trial number and group with the memory performance at baseline and replicated the significant effects found with the memory scores of the selected visits for cross-sectional analyses (data not shown).

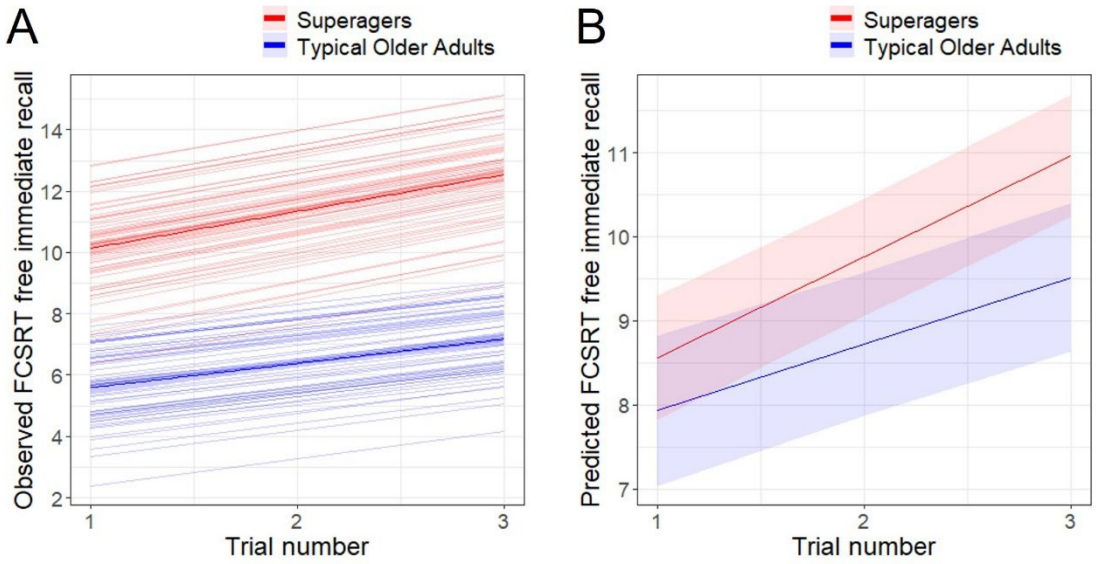


Figure 14. Differences in episodic learning rate across FCSRT immediate free recall trials between superagers and typical older adults. A. Individual learning rates of the observed values are plotted for superagers (red) and typical older adults (blue) together with the group averages represented by thicker lines (respective shaded areas indicating the 95% confidence interval) suggesting offset differences between groups. **B.** These offset differences are not significant after adjusting by sex, age, level of education and FCSRT delayed free recall score. The predicted learning rates showed, however, a significantly faster learning in superagers (red solid line) than typical older adults (blue solid line), shaded areas indicate the 95% confidence interval. FCSRT, Free and Cued Selective Reminding Test.

6.4 DISCUSSION

Our results show that within a cross-sectional cohort of cognitively healthy older adults (69-86yo), women and individuals with more years of formal education had a faster episodic learning rate across the three consecutive FCSRT immediate free recall trials. This learning rate declined with age in the whole healthy cohort and was associated with neuroanatomical structural properties. We found a positive correlation between grey matter volume and episodic learning rate, where participants with greater volume in hippocampus, anterior thalamic nucleus and left superior temporal gyrus learned at a faster rate than those with lower volume. Furthermore, we found that FA was positively associated with episodic learning rate in an extended network including limbic tracts, indicating that the structural integrity of these tracts indexed learning ability. A complementary negative association was observed for MD, in similar tracts, such that decreased MD was associated with a faster episodic learning rate. The converging grey and white matter findings suggest that structural properties of the hippocampal-anterior thalamic circuit contribute to learning ability in ageing and may potentially inform age-related decline in encoding (Friedman et al., 2007; Morcom et al., 2003). In addition, a faster rate of episodic learning was observed in the superager subgroup —defined by youthful performance in the FCSRT delayed free recall score— compared to typical older adults.

Previous research on neural substrates of cognitive decline in ageing has shown a hippocampal volume decline with age that correlated with memory performance and with FCSRT recall specifically (Zammit et al., 2017). The presence of hippocampal volume findings with relation to both episodic learning rate and recall components of the FCSRT task suggests the hippocampus may be involved in these two separate processes, both of which are impaired in ageing. The more pronounced effect we observed in the left hippocampus is in accordance with previous findings of verbal memory tasks (Ezzati et al., 2016), and the general lateralisation of verbal functions. Our results, therefore, extend previous research on the relationship between hippocampal volume and memory decline in ageing, showing episodic learning rate is also indexed by hippocampal volume. Note that it is unlikely that these effects reflect memory function in general, given that delayed free recall was included as a covariate in our model.

Structural properties of extra-hippocampal limbic regions were also associated with learning ability. Our grey matter thalamic findings indicate a correlation between

episodic learning rate and the right anterior thalamic nucleus, extending to right ventroanterior and right ventrolateral nuclei. The anterior thalamic nuclei have been suggested to play an important role in learning and memory (Aggleton et al., 2010; Sweeney-Reed et al., 2021; Winocur, 1985), that extend beyond its established role in spatial processing (Nelson, 2021; Wolff and Vann, 2019). For example, fMRI studies in younger adults suggest that the activation of the anterior thalamic nuclei supports recognition memory performance (Pergola et al., 2013) and evidence from intracranial electroencephalogram studies indicates theta-synchronisation between anterior thalamus and frontal and parietal regions supporting successful memory formation (Sweeney-Reed et al., 2014). Furthermore, and in line with our results, Leszczyński and Staudigl posited that the anterior thalamus might modulate information flow, via attention allocation, to support learning (Leszczyński and Staudigl, 2016). Taken together with the increased hippocampal volume, which was related to a better learning rate, our results indicate that the limbic system may play an important role in learning ability in ageing and might explain some of the impairments in navigating in a novel environment (Grzeschik et al., 2021), and impaired learning strategies observed in mild cognitive impairment (Ribeiro et al., 2007).

The fornix is a major hippocampal input/output pathway and has been associated with visuo-spatial learning across species (Buckley et al., 2008; Hodgetts et al., 2020; Hofstetter et al., 2013). The fornix links the hippocampus with the anterior thalamic nuclei directly and via the mammillary bodies (Aggleton et al., 1986; Aggleton et al., 2010), with both the hippocampus and the anterior thalamic nuclei showing grey matter volume relationships with episodic learning rate. Furthermore, we found that fornix integrity, as captured by bilateral FA and MD, correlated with episodic learning rate in older adults. Together with previous findings linking fornix integrity to recall performance on the FCSRT task (Hartopp et al., 2019; Metzler-Baddeley et al., 2011), our results extend its role in memory processes, indicating that the fornix also supports verbal episodic learning. We also found that the white matter integrity of the ATR was correlated with learning rate. The ATR connects the anterior and dorsomedial thalamus with the prefrontal cortex (Grodd et al., 2020), which has been suggested to play a role in learning rate (McGuire et al., 2014).

In addition to changes in limbic grey matter volume and white matter microstructure, we found episodic learning rate was associated with broader changes within bilateral white matter tracts connecting occipital-temporal-frontal regions (ILF,

SLF, IFO). This result might point toward an overall white matter microstructure effect, as previously noted in age-related cognitive decline (de Mooij et al., 2018; Farokhian et al., 2017; Grieve et al., 2007; Molloy et al., 2021; Rizvi et al., 2020a). With respect to specific cognitive functions, ILF and SLF have been shown to relate to memory performance in normal ageing (Sasson et al., 2013). ILF and IFO facilitate the flow of visual information up the visual stream (Rokem et al., 2017), and IFO has been associated with semantic processing (Duffau, 2008), potentially supporting learning performance in our task. Therefore, the observed relationship between microstructure of these tracts and learning ability might reflect a more general aspect of cognitive ability.

Superagers showed a faster learning rate than typical age-matched older adults and their cross-sectional structural grey matter signature described in Chapter 3 (Garo-Pascual et al., 2023) and white matter signature described in Chapter 4 has a partial overlap with the structural correlates of episodic learning rate in the cross-sectional cohort of healthy older adults reported in this chapter. The overlap in grey matter volume includes hippocampal effects and a partial overlap in the ventrolateral thalamic nuclei; FA effects overlap in the ATR, IFO and ILF; and MD effects overlap in the ATR, forceps minor and major, IFO, ILF and SLF. The frontal predominance of the white matter microstructure in superagers was not observed in the correlation map of episodic learning rate and the signature of superageing did not extend to the fornix, which was described as a key element among the structural correlates of episodic learning rate.

Finally, it is important to note some limitations of the current study; we analysed data from a cross-sectional cohort of healthy older adults. As grey and white matter properties and memory function both deteriorate with age, future longitudinal studies would be needed to better understand the relationship between learning ability and structural changes as ageing progresses and eliminate age-related confounds in cross-sectional studies (Elliott, 2020). We used the learning rate across trials in an established neuropsychological memory task (FCSRT) as a measure for episodic learning; it would be interesting to examine neural correlates of learning rates in tasks such as error-driven and statistical learning (Herff et al., 2019; Nassar et al., 2016; Samanez-Larkin et al., 2012), as well as consider learning ability as a potential cognitive phenotype in pathological ageing. Finally, future research with hippocampal subfield resolution could examine their differential contribution to episodic learning rate. It would be interesting to explore whether volumetric effects are more pronounced in the subiculum, the principal

source of hippocampal projections to the anterior thalamus and mammillary bodies (Hartopp et al., 2019).

In conclusion, in a cross-sectional cohort of healthy older adults, we found learning rate on the FCSRT task was positively associated with extensive grey and white matter structural effects including the hippocampus, fornix and anterior thalamic nucleus, structures part of the limbic system. Furthermore, there was a positive correlation between episodic learning rate and white matter microstructure of long-range white matter tracts (ILF, SLF, IFO). Superagers, in addition to their superiority in FCSRT delayed free recall, also showed a faster episodic learning rate relative to typical older adults. Our findings indicate that episodic learning rate is associated with key anatomical structures implicated in memory function, and therefore may inform further exploration of the relationship between episodic learning rate and retrieval in ageing.

CHAPTER 7

GENERAL DISCUSSION

The challenge posed by the pathological loss of episodic memory at both the individual and collective level is the motivation for this thesis. Alzheimer's disease is characterised by a primary impairment of episodic memory and is the most prevalent type of dementia, with a current burden of 32 million people diagnosed worldwide and many millions more in a prodromal state (Gustavsson et al., 2023). The severe loss of episodic memory suffered by Alzheimer's patients compromises their identity as individuals, since this function allows us to remember our personal past events and to learn through experience about the circumstances around us. At the collective level, the pathological loss of episodic memory affects different parties, with the most affected being the patients' families and the healthcare system. The alarming estimations coexist with the incipient approval of lecanemab, an anti-amyloid antibody that has shown to slow cognitive decline in Alzheimer's disease (van Dyck et al., 2023), in addition to numerous alternative strategies to prevent or slow the progressive cognitive decline. This PhD thesis does not focus on the pathological mechanisms of Alzheimer's disease, but rather investigates an elderly population that naturally ages with successful episodic memory function. To this end, I identified a large group of elderly subjects with exceptional episodic memory for their age in the longitudinal cohort of the Vallecas Project adapting a previous definition of superaeging (Gefen et al., 2015; Harrison et al., 2012). Along with a sample of 64 superagers, whose episodic memory was comparable to that of a healthy person 30 years younger, I selected as a control group a sample of 55 typical age-matched older adults with an episodic memory performance considered normal for their age and educational level. The aim of this thesis is to advance conceptually the field of successful episodic memory ageing through an in-depth characterisation of the superager phenotype and, ultimately, to open new avenues for the prevention and/or treatment of pathological loss of episodic memory.

Recapitulating the results of this PhD thesis, the first study reveals a significantly slower rate of grey matter atrophy in superagers compared to typical older adults in total

grey matter volume, but specifically in the MTL, and cross-sectional differences showing a greater grey matter volume in the motor thalamus, MTL, and cholinergic forebrain of the superager brain (Garo-Pascual et al., 2023). Faster movement speed and better mental health are the factors that best differentiate superagers from typical older adults, along with less prevalence of glucose disorders and hypertension problems, fewer complaints of not getting enough sleep, a more active lifestyle during midlife, higher musical background, higher proportion of separated participants, higher independence in daily living, and higher score in an intelligence test in superagers relative to typical older adults. Similar frequency in superagers and typical older adults is found for the *APOE* ε4 allele, the major genetic risk factor for non-familiar Alzheimer's disease, and similar group levels of blood biomarkers of dementia in line with those reported for healthy ageing (Palmqvist et al., 2021). The second study shows group differences in structural connectivity consistent with a better white matter microstructure in superagers than in typical older adults, both cross-sectionally and longitudinally. The cross-sectional differences show higher FA in superagers in frontal tracts, and lower MD in most white matter tracts, following an anteroposterior gradient with greater group differences in anterior regions. The third study shows significantly shorter median telomere length and a higher proportion of short telomeres in the blood cells of superagers compared to typical older adults. The final study shows a significant correlation between brain structure and learning rate across the three consecutive immediate free recall trails of the FCSRT episodic memory task in the large (n = 982) cross-sectional cohort of cognitively healthy ageing from the Vallecas Project (Frank et al., 2022) in the hippocampus, anterior thalamic nucleus and left superior temporal gyrus and in an extended network of limbic white matter tracts including the fornix. These results suggest the contribution of the hippocampal-anterior thalamic circuit to learning ability in ageing and, ultimately, a faster learning rate is observed in the superager group —group defined by a superior performance on the FCSRT delayed free recall score— compared to typical older adults.

The conceptual advance that this thesis aims to bring to the field of successful episodic memory ageing involves identifying the dominant ageing mechanism in the brain of superagers and the demographic, clinical and lifestyle factors associated with this phenotype. Longitudinal neuroimaging data may help to identify this mechanism of brain ageing, as superageing may reflect resistance or resilience to dementia or age-related processes. In the context of dementia, resistance refers to the avoidance of disease and resilience is understood as the successful coping with the disease

(Arenaza-Urquijo and Vemuri, 2018). However, as both superagers and typical older adults are negative for neurodegenerative biomarkers and their levels are consistent with healthy agers (Palmqvist et al., 2021), in line with previous studies of superageing (Baran et al., 2018; Dang et al., 2019; Dekhtyar et al., 2017; Harrison et al., 2018), we have extended the same framework to describe ageing processes in the absence of neuropathology. Thus, in a healthy ageing context, resistance refers to avoidance of typical age-related memory decline and translates, in brain structural terms, into brain preservation in superagers, while resilience refers to successfully coping with effects of ageing and implies a similar brain structure between superagers and normal ageing peers. Longitudinal findings on grey and white matter show slower age-related brain changes in superagers compared to typical older adults, suggesting that the predominant ageing mechanism in the brain of superagers is resistance to age-related structural changes. This resistance is consistent with previous longitudinal studies of successful episodic memory ageing (Cook et al., 2017b; Wang et al., 2019) and discrepancies are found in younger cohorts (Dang et al., 2019) that may be capturing a different phase of the non-linear ageing process (Nyberg et al., 2012; Ronnlund et al., 2005).

There are regional differences in the pattern of brain ageing between grey and white matter. Grey matter atrophy is especially slow in the MTL when comparing superagers to typical older adults, whereas the pattern of changes in white matter microstructure over time is more widespread and affects almost all white matter tracts, except tracts reported to show high resistance to age-related changes (*i.e.*, corticospinal tract and the forceps minor) (Cox et al., 2016; Slater et al., 2019). Accordingly, resistance to age-related grey matter atrophy in superagers may have a more specific role in the success of episodic memory function, compared to the general ageing pattern observed in white matter microstructure (Giorgio et al., 2010; Pfefferbaum et al., 2013; Raz et al., 2005). Despite these regional differences in the longitudinal trajectories of grey matter volume and white matter microstructure, they all converge in both groups at age 75, where there is already a group difference in episodic memory function. Before the age of 75, the difference in episodic memory performance between superagers and typical older adults may not be explained by brain structural differences, but may depend on different brain properties such as functional properties (Betzel et al., 2014). Therefore, despite the structural brain signature of superageing, potential markers of biological brain age based on structural features might not be the best retrospective predictors of superageing.

In the attempt to predict the phenotype of superagers, the machine learning model was used to identify which factors differentiate superagers from typical older adults. Due to the observational nature of this study, a causal relationship between the factors obtained from the machine learning model and the superager phenotype cannot be established. Unravelling the directionality of this association will require randomised trials or a Mendelian randomisation approach. The model achieves an accuracy of 66.4%, which is far from being able to predict superageing. Accuracy could be improved if the many self-reported variables were supplemented with objective measures that would allow for greater generalisability of the results, however, the accuracy of the model could be given a quantitative leap by introducing genetic information beyond the *APOE* genotype already present. Given the omission of the genetic component in the machine learning model, its current accuracy may reflect the existence of a genetic predisposition to the superageing phenotype that remains to be explored. Therefore, it is likely that the superager phenotype is the result of an interaction between a genetic predisposition and certain clinical and lifestyle factors. Previous studies suggest an association of the superageing phenotype with genetic variants (Huentelman et al., 2018) but large sample sizes are needed to draw robust conclusions on genetic variants and this will require pooling different cohorts of superagers.

The identification of a marker or set of markers that can inform successful episodic memory ageing is a future direction that could be useful in both research and clinical settings. Such a marker could potentially replace complex neuropsychological screening criteria for this population, which in some cases are not accessible or are subject to high variability among cohorts. To that end, telomere length was explored in our sample of superagers and typical older adults as a known marker of biological age that is sensitive to genetics, lifestyle and the ageing process (Lopez-Otin et al., 2013), the same three factors that are possibly contributing to the superageing phenotype. The interpretation of short telomere length in our sample of superagers may be misleading, as this is a highly heritable trait (Benetos et al., 2013; Broer et al., 2013) and the genetic variants associated with long telomere length have not been studied. Similarly, a longitudinal analysis of telomere length will reveal the rate of telomere length attrition, which may be more informative of lifespan than a cross-sectional measure (Whittemore et al., 2019). Other biological age markers whose sensitivity to episodic memory ageing is worth exploring are epigenetic, transcriptomic or metabolic profiles (Jylhava et al., 2017).

Nonetheless, the factors associated with the superageing phenotype might already be pointing to a possible explanatory mechanism for the excellent episodic memory of superagers that could involve enhanced positive neuromodulation and a more strategic activation of the default mode network. The better speed of mobility of superagers, derived from their better performance on the Timed Up & Go test, could have a positive impact on memory function through different neuromodulation pathways. On one hand, there is evidence that action, *i.e.* being physically active during memory formation, enhances episodic memory function through noradrenergic modulation from the locus coeruleus (Yebra et al., 2019). On the other hand, the dopaminergic system via the ventral tegmental area and substantia nigra signals rewarding and novel stimuli boosting their memory (Lisman and Grace, 2005; Shohamy and Adcock, 2010). Then, the physical mobility component of exploring a new environment with potentially rewarding cues could be related to improved memory function (Duzel et al., 2010; Kakade and Dayan, 2002). A spatial navigation memory task that requires exploration related with functional MRI can reveal whether the activation maps include the ventral tegmental area and substantia nigra in the superager brain. The possible relationship between the faster speed of mobility and the larger volume of the motor thalamus in superagers is still unknown. The ventrolateral thalamic nuclei, where the largest volumetric differences between superagers and typical older adults are found, receive inputs from the cerebellum and project to motor cortices especially the primary motor cortex and this pathway is involved in the performance of movements in response to sensory inputs (Sommer, 2003). The larger volume in superagers could be attributed to the greater number of motor inputs processed as a result of a more active lifestyle. The association between motor thalamus volume and speed of mobility could be tested in both young and old adults to determine whether there is a relationship between this subcortical structure, speed of mobility and episodic memory function.

Additionally, the superager phenotype is associated with better mental health, as reported by depression and anxiety scales. There is correlation between depression and anxiety and impaired episodic memory (Kizilbash et al., 2002; Lohman et al., 2013). Mental health problems are both risk factors (Gulpers et al., 2016; Jorm, 2001) and symptoms for Alzheimer's disease (McKeith and Cummings, 2005). One element that can be present in both depression and anxiety is rumination (Bar, 2009; McLaughlin and Nolen-Hoeksema, 2011; Mor and Winquist, 2002; Nolen-Hoeksema, 2000) –repetitive and passive thoughts focused on autobiographical memories, current distress symptoms and their possible negative consequences (Nolen-Hoeksema et al., 2008).

Ruminations are associated with an increased activation of the default mode network (Burkhouse et al., 2017; Cooney et al., 2010; Zhou et al., 2020) that is the mental state when no other perceptual, attentional or language task is being performed, and this network processes internal information, such as episodic memory (Buckner et al., 2008; Raichle et al., 2001). Rumination is detrimental to attention, memory processing and decision-making and this detrimental effect could be due to increased recruitment of the default mode network (Disner et al., 2011; Forner-Phillips et al., 2020; Nolen-Hoeksema, 2000). If the default mode network of superagers is less saturated by rumination, it can potentially perform episodic memory tasks more successfully. This hypothesis could be assessed with a test of episodic memory after a distraction condition or a rumination induction condition to activate the default mode network. Faster speed of mobility and better mental health may positively impact episodic memory function in superagers via different pathways that may act in parallel but also intertwined, as depression and mobility are mutually associated (Lampinen and Heikkinen, 2003; Speiser et al., 2022).

Despite the limitations detailed throughout this general discussion, to my best knowledge, this thesis has identified one of the largest samples of superagers over 80 years of age with annual longitudinal MRI scans for up 5 years with a comprehensive battery of demographic, clinical and lifestyle variables. The findings derived from this thesis involve the identification of resistance to age-related brain changes as the dominant mechanism of ageing in superagers, together with the regional mapping of grey and white matter superageing signature and the identification of factors associated with this phenotype in a compendium of variables that extends beyond studies to date. This field of research still has much to reveal about how to age with healthy episodic memory function, and this work also serves to generate new hypotheses regarding the mechanisms underlying successful episodic memory ageing that, if tested, could broaden the preventive and therapeutic outlook for Alzheimer's disease.

CONCLUSIONS

Episodic memory is the function that allows us to travel through time and imagine ourselves in the past, present and future. Paradoxically, this cognitive function is one of the most vulnerable to the passage of time. A slight decline in episodic memory is part of natural ageing, but its pathological loss is a primary feature of Alzheimer's disease, the most common form of dementia. Throughout this thesis I have extensively explored a large population of superagers, who are older people with episodic memory comparable to that of a healthy person 30 years younger, to understand how episodic memory can naturally age successfully. Longitudinal analyses of brain structure over a 5-year period have shown that atrophy of grey matter volume and white matter microstructure is slower in superagers than in typical older adults, probably reflecting superagers' resistance to age-related brain changes. Cross-sectional differences in the brains of superagers compared to typical older adults include regions with a well-established role in episodic memory function, such as the medial temporal lobe, and potentially novel ones, such as the motor thalamus. This latter observation may be related to findings from a machine learning model that identified faster movement speed as one of the factors that best differentiates superagers from typical older adults. This model also identified better mental health in superagers, suggesting an overlap between factors associated with superageing and factors protective of dementia. To elucidate possible mechanisms underlying superageing, we measured telomere length in blood cells and analysed the rate of episodic learning; superagers have a significantly shorter telomere length compared to typical older adults of the same age, and a faster rate of learning. In summary, this work identifies resistance to age-related structural changes as the most likely mechanism of brain ageing in superagers, provides unexplored neural substrates for episodic memory function, and identifies clinical and lifestyle factors associated with the superager phenotype that may motivate future interventions to promote healthy episodic memory ageing.

CONCLUSIONES

La memoria episódica es la función que nos permite viajar en el tiempo e imaginarnos a nosotros mismos en el pasado, el presente y el futuro. Paradójicamente, esta función cognitiva es de las más vulnerables al paso del tiempo. Un ligero deterioro de la memoria episódica es parte del envejecimiento natural, pero su pérdida patológica es una característica primordial de la enfermedad de Alzheimer, la forma más común de demencia. A lo largo de esta tesis he explorado exhaustivamente una amplia población de superancianos, que son personas mayores con una memoria episódica comparable a la de una persona sana 30 años más joven, para comprender cómo, de forma natural, la memoria episódica puede envejecer satisfactoriamente. Los análisis longitudinales de la estructura cerebral durante un periodo de 5 años han demostrado que la atrofia del volumen de sustancia gris y de la microestructura de la sustancia blanca es más lenta en los superancianos que en los adultos mayores típicos, lo que probablemente refleja la resistencia de los superancianos a los cambios cerebrales relacionados con la edad. Las diferencias transversales en el cerebro de los superancianos respecto a los adultos mayores típicos incluyen regiones con un papel bien establecido en la función de la memoria episódica, como el lóbulo temporal medial, y otras potencialmente nuevas, como el tálamo motor. Esta última observación podría estar relacionada con los hallazgos del modelo de aprendizaje automático que identificó una mayor velocidad de movimiento como uno de los factores que mejor diferencian a los superancianos de los adultos mayores típicos. Este modelo también identificó una mejor salud mental en los superancianos, lo que sugiere un solapamiento entre los factores asociados a los superancianos y los factores protectores de la demencia. Para dilucidar los posibles mecanismos subyacentes a los superancianos, se midió la longitud de los telómeros en células sanguíneas y también se analizó la tasa de aprendizaje episódico; los superancianos tienen una longitud telomérica significativamente más corta en comparación con los adultos mayores típicos de su misma edad, y una tasa de aprendizaje más rápida. En resumen, este trabajo identifica la resistencia a los cambios estructurales asociados a la edad como el mecanismo más probable de envejecimiento cerebral en los superancianos, proporciona sustratos neurales inexplorados en relación

con la función de la memoria episódica e identifica factores clínicos y de estilo de vida asociados con el fenotipo del superanciano que pueden motivar futuras intervenciones para promover el envejecimiento saludable de la memoria episódica.

APPENDIX

A.1 SUPPLEMENTARY FILES

A.1.1 Supplementary information Chapter 3

Table S1. Demographic, neuropsychological, and brain volumetric differences between superagers and typical older adults. Comparison between superagers and typical older adults at the visit selected for cross-sectional analysis for numerical variables was performed using a t-test, for categorical data a Chi-Square test was performed, and an analysis of covariance was run for brain volumetric variables with total intracranial volume (TIV) as covariate. False discovery rate (FDR) correction performed over the 15 variables. 15-BNT, 15-items Boston Naming Test; [a], used to define group membership; CI, confidence interval; Corr. *P*, FDR-corrected *p*-value; FAQ, Functional Activities Questionnaire total score; $FCSRT_{freedelrecall}$, Free and Cued Selective Reminding Test free delayed recall; $FLU_{lexicaltotal}$, Lexical Fluency with letter "P" total score; $FLU_{semantictotal}$, animal fluency test total score; IQR, interquartile range; MMSE, Mini Mental State Examination total score; NART, Spanish version of the National Adult Reading Test total score; *P*, *p*-value; $REY_{delayrecall}$, Rey-Osterreith Complex Figure delayed recall score; SD, standard deviation.

	Superagers (n = 64)	**Typical older adults** (n = 55)	**95% CI of mean difference**	**Statistic**	***P***	**Corr. *P***
Demographics						
Age, median (IQR), years	81.6 (80.4 - 83.1)	82.1 (81.3 - 83.0)	-1.22, 0.16	t = -1.5	0.13	0.16
Women, No. (%)	38 (59%)	35 (64%)	-	χ = -0.1	0.77	0.83
Men, No (%)	26 (41%)	20 (36%)	-	χ = -0.1	0.77	0.83
Education, median (IQR), years	16.0 (10.0 - 19.0)	10.0 (6.0 - 17.5)	0.52, 5.36	t = 2.4	0.019	0.026
Neuropsychology – selection criteria variables						
$FCSRT_{freedelayrecall}$[a], median (IQR)	13.0 (12.0 - 14.25)	7.0 (5.0 - 8.0)	6.32, 7.41	t = 25.0	<0.0001	<0.0001
$FLU_{semantictotal}$, mean (SD)	21.2 (4.8)	15.9 (4.1)	3.65, 6.87	t = 6.5	<0.0001	<0.0001
Digit Symbol Substitution Test, mean (SD)	21.2 (6.1)	15.3 (5.8)	3.75, 8.07	t = 5.4	<0.0001	<0.0001
15-BNT, median (IQR)	14.0 (13.0 - 15.0)	12.0 (9.0 - 13.5)	1.55, 3.06	t = 6.1	<0.0001	<0.0001
Neuropsychology – other variables						
MMSE, median (IQR)	29.0 (28.8 - 30.0)	28.0 (27.0 - 29.0)	0.73, 1.80	t = 4.7	<0.0001	<0.0001
FAQ, median (IQR)	0.0 (0.0 - 0.0)	0.0 (0.0 - 1.0)	-0.65, -0.13	t = 3.0	0.0033	0.0050
$REY_{delayrecall}$, mean (SD)	15.5 (4.3)	10.4 (5.2)	2.24, 7.85	t = 3.6	<0.0001	<0.0001
$FLU_{lexicaltotal}$, mean (SD)	17.1 (4.4)	12.8 (4.3)	2.69, 5.86	t = 5.3	<0.0001	<0.0001
NART, median (IQR)	56.0 (48.0 - 58.3)	50.0 (40.0 - 56.0)	2.36, 10.48	t = 3.2	0.0023	0.0038
Brain volumetry						
Total intracranial volume, mean (SD), cm^3	1400.06 (156.42)	1404.99 (155.51)	-61.07, 52.94	t = -0.1	0.89	0.89
Grey matter volume, mean (SD), cm^3	523.09 (42.05)	511.64 (43.35)	$6.03x10^{-3}$, 1.00	F = 6.3	0.013	0.020

Table S2. *APOE* genotype and neurodegenerative blood biomarkers differences between superagers and typical older adults. Aβ42, Aβ40, t-Tau and p-Tau181 were measured in plasma while GFAP and NfL were measured in serum. Group comparisons were performed using the Fisher's test for the categorical variable. Group comparisons for blood biomarkers were performed with a t-test for those biomarkers that followed a normal distribution after log-transformation (GFAP, NfL, Aβ42/40 ratio and t-Tau); otherwise, the Mann-Whitney U-test was used on original values (p-Tau181 and p-Tau181/Aβ42). Log transformed data is shown for GFAP, NfL, Aβ42/40 ratio and t-Tau. False discovery rate (FDR) correction performed over the 7 variables. Aβ, amyloid beta; *APOE*, apolipoprotein E gene; CI, confidence interval; Corr. *P*, FDR-corrected *p*-value; GFAP, Glial Fibrillary Acidic Protein; IQR, interquartile range; NfL, Neurofilament Light polypeptide; *P*, *p*-value; p-Tau181, tau phosphorylated at threonine 181; t-Tau, total tau.

		Superagers (n = 64)	**Typical older adults** (n = 55)	**95% CI of mean difference**	**Statistic**	***P***	**Corr. *P***
Genetic risk factor for late onset Alzheimer's Disease							
APOE, No (%), alleles	ε2/ε3	7 (11%)	10 (18%)	-	Fisher's Test	0.47	0.86
	ε3/ε3	47 (73%)	37 (67%)				
	ε3/ε4	10 (16%)	7 (13%)				
	ε4/ε4	0 (0%)	1 (2%)				
Blood biomarkers for neurodegeneration							
Aβ42/40 ratio, mean (SD), pg/ml		-2.7 (0.26)	-2.8 (0.24)	-0.04, 0.14	t = -1.1	0.28	0.86
t-Tau, mean (SD), pg/ml		0.909 (0.407)	0.911 (0.242)	-0.12, 0.12	t = -0.04	0.97	0.97
p-Tau181, median (IQR), pg/ml		1.41 (0.96 - 1.93)	1.37 (0.95 - 2.06)	-	Z = -0.2	0.84	0.97
p-Tau181/Aβ42, median (IQR), pg/ml		0.12 (0.08 -0.18)	0.12 (0.09 - 0.20)	-	Z = -0.8	0.45	0.86
GFAP, mean (SD), pg/ml		5.08 (0.64)	5.16 (0.69)	-0.33, 0.16	t = -0.7	0.49	0.86
NfL, mean (SD), pg/ml		2.78 (0.49)	2.74 (0.69)	-0.25, 0.19	t = 0.3	0.76	0.97

Table S3. Demographic, neuropsychological, and brain volumetric differences between superagers and Northwestern-criteria typical older adults. Comparison between superagers and Northwestern-criteria typical older adults for numerical variables was performed using a t-test, for categorical data a Chi-Square test was performed, and analysis of covariance was run for brain volumetric variables to correct for total intracranial volume (TIV). 15-BNT, 15-items Boston Naming Test; $FCSRT_{freedelrecall}$, Free and Cued Selective Reminding Test free delayed recall; FAQ, Functional Activities Questionnaire total score; $FLU_{semantictotal}$, animal fluency test total score; $FLU_{lexicaltotal}$, Lexical Fluency with P total score; IQR, interquartile range; MMSE, Mini Mental State Examination total score; NART, Spanish version of the National Adult Reading Test total score; *P*, *p*-value; $REY_{delayrecall}$, delayed recall score; SD, Standard Deviation.

	Superagers (n = 64)	**Northwestern-criteria typical older adults** (n = 19)	**Statistic**	***P***
Demographics				
Age, median (IQR), years	81.6 (80.4-83.1)	81.9 (80.5-84.3)	t = 1.1	0.30
Female, No. (%)	38 (59)	11 (58)	$\chi = 1x10^{-31}$	1.00
Education, median (IQR), years	16.0 (10.0-19.0)	16.0 (9.0-19.0)	t = -0.07	0.95
Neuropsychology – selection criteria variables				
$FCSRT_{freedelayrecall}$, median (IQR)	13.0 (12.0-14.25)	6.0 (5.0-8.0)	t = 15.7	<0.0001
$FLU_{semantictotal}$, median (IQR)	21.0 (18.0-24.0)	17.0 (15.0-19.0)	t = 5.2	<0.0001
Digit Symbol Substitution Test, median (IQR)	21.0 (17.0-26.0)	15.0 (13.0-20.0)	t = 3.7	0.0006
15-BNT, median (IQR)	14.0 (13.0-15.0)	13.0 (11.5-14.0)	t = 3.5	0.0016
Neuropsychology – other variables				
MMSE, mean (SD)	29.1 (1.1)	28.4 (1.5)	t = 1.7	0.10
FAQ, mean (SD)	0.2 (0.5)	0.5 (0.8)	t = -1.6	0.12
$REY_{delayrecall}$, mean (SD)	15.5 (4.3)	8.9 (6.1)	t = 2.7	0.029
$FLU_{lexicaltotal}$, median (IQR)	17.5 (14.0-19.3)	14.0 (13.0-16.5)	t = 2.0	0.056
NART, median (IQR)	56.0 (48.0-58.3)	53.5 (44.0-56.8)	t = 1.0	0.31
Brain volumetry				
Total intracranial volume, mean (SD), cm^3	1399.99 (156.42)	1373.77 (161.30)	t = 0.6	0.54
Grey matter volume, mean (SD), cm^3	523.10 (42.05)	507.19 (42.20)	F = 2.7	0.10

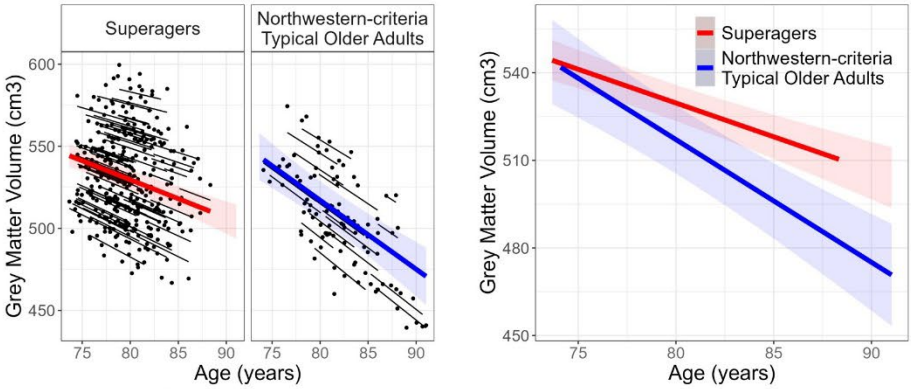
Table S4. *APOE* genotype and blood neurodegeneration biomarkers for the superagers and Northwestern-criteria typical older adults. Aβ42, Aβ40 and t-Tau were measured in plasma while p-Tau181, GFAP and NfL were measured in serum. Group comparisons for blood biomarkers were performed with a t-test for those biomarkers that followed a normal distribution after log-transformation (Aβ42/40 ratio and t-Tau); otherwise, the Mann-Whitney U-test was used on original values (p-Tau181, p-Tau181/Aβ42, GFAP and NfL). Log transformed data is shown for Aβ42/40 ratio and t-Tau. Aβ, amyloid beta; *APOE*, apolipoprotein E gene; GFAP, Glial Fibrillary Acidic Protein; IQR, interquartile range; NfL, Neurofilament Light polypeptide; *P*, *p*-value; p-Tau181, tau phosphorylated at threonine 181; t-Tau, total tau.

		Superagers (n = 64)	**Northwestern-criteria typical older adults** (n = 19)	**Statistic**	***P***
Genetic risk factor for late onset Alzheimer's Disease					
APOE, No (%), alleles	ε2/ε3	7 (11)	2 (11)	Fisher's Test	0.45
	ε3/ε3	47 (73)	14 (74)		
	ε3/ε4	10 (16)	2 (11)		
	ε4/ε4	0 (0)	1 (5)		
Blood biomarkers for neurodegeneration					
Aβ42/40 ratio, mean (SD), pg/ml		-2.74 (0.26)	-2.75 (0.20)	t = 0.3	0.77
t-Tau, mean (SD), pg/ml		0.91 (0.41)	0.96 (0.26)	t = -0.7	0.51
p-Tau181, median (IQR), pg/ml		1.41 (0.96 - 1.93)	1.24 (0.92 - 2.20)	Z = 0.1	0.94
p-Tau181/Aβ42, median (IQR), pg/ml		0.12 (0.08 - 0.18)	0.13 (0.08 - 0.20)	Z = -0.5	0.61
GFAP, median (IQR), pg/ml		180.76 (118.14 - 258.94)	200.43 (102.09 - 299.79)	Z = -0.8	0.44
NfL, median (IQR), pg/ml		16.91 (11.86 - 22.81)	16.73 (12.78 - 25.85)	Z = -0.7	0.50

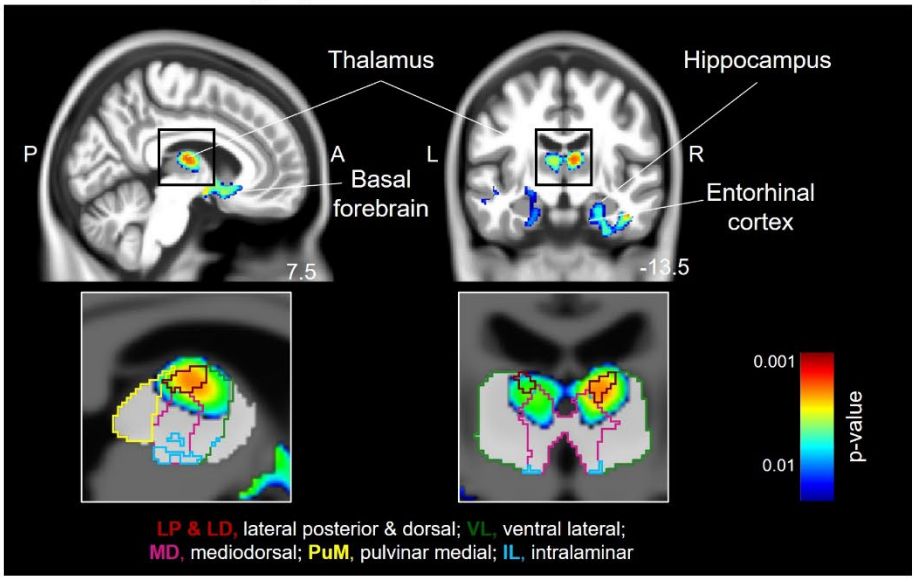
Table S5. Cross-sectional differences in whole-brain volumetric measures across superagers and typical older adults. Volume differences between superagers and typical older adults (n = 55) and Northwestern-criteria typical older adults (n = 19) were analysed with an analysis of covariance using total intracranial volume (TIV) as a covariate. Data extracted from FreeSurfer pipeline. A multiple comparison correction based on false discovery rate (FDR) was displayed over the 25 variables analysed ($P < 0.05$). CC, cingulate gyrus; Corr. *P*, false discovery rate corrected *p*-value; SD, standard deviation; *P*, *p*-value.

Areas	**Superagers** (n = 64)	**Typical older adults** (n = 55)	***P*** (Superager *vs.* Typical older adults)	**Corr. *P*** (Superager *vs.* Typical older adults)	**Northwestern-criteria typical older adults** (n = 19)	***P*** (Superager *vs.* Northwestern-criteria typical older adults)	**Corr. *P*** (Superager *vs.* Northwestern-criteria typical older adults)
Total Intracranial Volume, mean (SD), cm^3	1451.44 (132.65)	1476.86 (165.54)	0.37	0.67	1466.81 (159.11)	0.70	0.94
Cortical Volume, mean (SD), cm^3	418.86 (36.11)	412.61 (31.99)	0.0017	0.028	405.92 (30.63)	0.0015	0.038
Cerebral White Matter, mean (SD), cm^3	371.80 (44.59)	367.85 (48.10)	0.047	0.20	364.22 (43.99)	0.10	0.28
Sub. Cortical Grey Matter, mean (SD), cm^3	47.97 (4.23)	48.09 (4.96)	0.49	0.77	48.45 (6.19)	0.90	0.94
Total Grey Matter, mean (SD), cm^3	563.82 (41.99)	557.68 (45.57)	0.0022	0.028	552.47 (42.71)	0.0066	0.075
Cerebellum White Matter, mean (SD), cm^3	26.13 (3.23)	26.12 (3.08)	0.70	0.96	26.40 (3.05)	0.90	0.94
Cerebellum Cortex, mean (SD), cm^3	95.47 (10.18)	95.35 (8.59)	0.38	0.67	96.47 (10.68)	0.92	0.94
Lateral Ventricle, mean (SD), cm^3	29.56 (11.25)	37.49 (20.75)	0.012	0.088	35.39 (19.20)	0.09	0.28
Inf. Lateral Ventricle, mean (SD), cm^3	1.17 (0.52)	1.54 (1.04)	0.022	0.11	1.60 (1.08)	0.012	0.075
Third Ventricle, mean (SD), cm^3	1.64 (0.55)	1.88 (0.75)	0.070	0.25	1.86 (0.89)	0.19	0.37
Fourth Ventricle, mean (SD), cm^3	1.55 (0.39)	1.58 (0.48)	0.93	0.96	1.49 (0.45)	0.45	0.70
CSF, mean (SD), cm^3	1.17 (0.23)	1.20 (0.27)	0.81	0.96	1.18 (0.27)	0.94	0.94
Thalamus, mean (SD), cm^3	11.38 (1.11)	11.45 (1.12)	0.84	0.96	11.53 (1.29)	0.75	0.94
Caudate, mean (SD), cm^3	6.51 (1.00)	6.63 (1.22)	0.95	0.96	6.82 (1.54)	0.35	0.59
Putamen, mean (SD), cm^3	7.71 (1.01)	7.75 (1.34)	0.77	0.96	7.86 (1.78)	0.76	0.94
Pallidum, mean (SD), cm^3	3.22 (0.49)	3.24 (0.47)	0.80	0.96	3.36 (0.60)	0.35	0.59
Hippocampus, mean (SD), cm^3	6.85 (0.73)	6.74 (0.78)	0.16	0.50	6.62 (0.88)	0.15	0.28
Amygdala, mean (SD), cm^3	2.51 (0.32)	2.47 (0.48)	0.22	0.61	2.39 (0.56)	0.11	0.28
Accumbens, mean (SD), cm^3	0.92 (0.16)	0.86 (0.17)	0.014	0.088	0.82 (0.18)	0.0093	0.075
Ventral Diencephalon, mean (SD), cm^3	7.25 (0.65)	7.23 (0.69)	0.33	0.67	7.28 (0.84)	0.89	0.94
CC. Posterior, mean (SD), cm^3	0.95 (0.13)	0.94 (0.20)	0.40	0.67	0.88 (0.18)	0.039	0.19
CC. Mid. Posterior, mean (SD), cm^3	0.46 (0.09)	0.49 (0.15)	0.28	0.67	0.48 (0.12)	0.70	0.94
CC. Central, mean (SD), cm^3	0.47 (0.10)	0.47 (0.12)	0.96	0.96	0.43 (0.08)	0.13	0.28
CC. Mid. Anterior, mean (SD), cm^3	0.43 (0.10)	0.42 (0.11)	0.39	0.67	0.39 (0.09)	0.064	0.27
CC. Anterior, mean (SD), cm^3	0.80 (0.12)	0.82 (0.15)	0.60	0.89	0.76 (0.09)	0.11	0.28

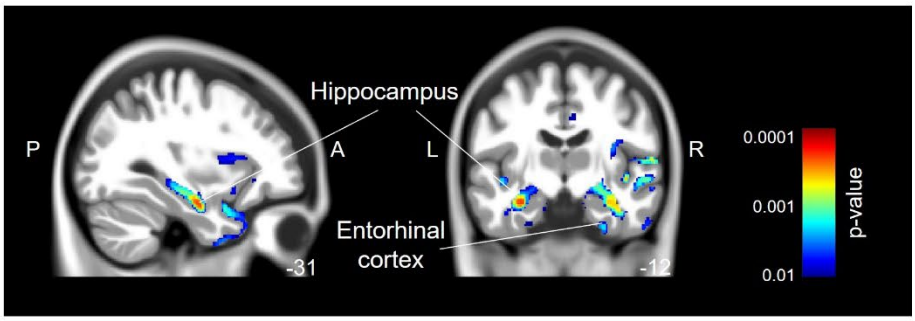
A. Grey matter atrophy



B. Cross-sectional grey matter volume



C. Longitudinal grey matter volume



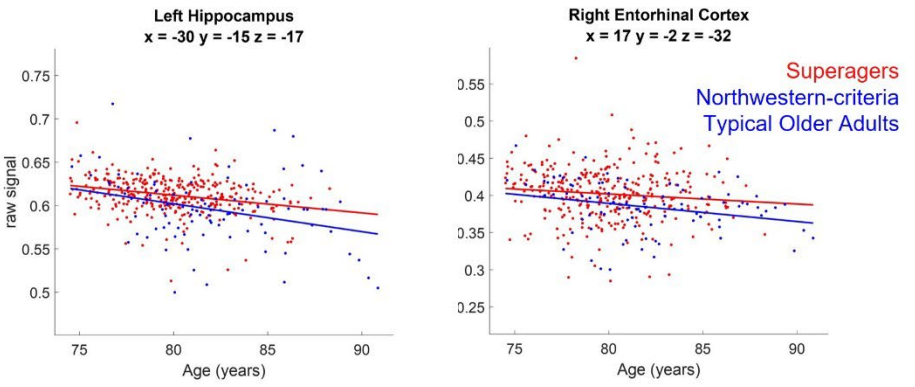


Figure S1. Neuroanatomical differences between superagers and Northwestern-criteria typical older adults. A. Grey matter atrophy. The decline over time in grey matter volume is slower in superagers (red) than Northwestern-criteria typical older adults (blue). Individual trajectories and means and 95% confidence interval for each group are shown (left) as well as means and 95% confidence interval for both groups (right). **B.** Cross-sectional differences in grey matter volume. Brain areas showing greater grey matter volume in superagers than Northwestern-criteria typical older adults are shown as colormaps of *p*-values overlaid on sagittal and coronal sections of a canonical T1 image. Insets show the thalamic nuclei where grey matter volume is greater in superagers than typical older adults, anatomical demarcations from the AAL3 atlas. **C.** Longitudinal differences in grey matter volume. Top: Brain areas showing reduced grey matter volume loss in superagers than Northwestern-criteria typical older adults over time (significant group by time interaction, $P < 0.05$ FWE corrected) are shown as colormaps of *p*-values overlaid on sagittal and coronal sections. Bottom: Grey matter volume trajectories over time in two representative voxels, the left hippocampus and right entorhinal cortex, for superagers (red) and Northwestern-criteria typical older adults (blue). Superagers show a slower decline in both cases. The coordinates of the sections are given in mm. n = 83, 64 superagers and 19 Northwestern-criteria typical older adults. A, anterior; L, left; P, posterior; R, right.

Table S6. Model of longitudinal trajectories of total grey matter volume in superagers and typical older adults. Linear mixed model coefficients (β), standard errors (SE) and *p*-values (*P*). Total grey matter volume is the dependent variable and was adjusted by total intracranial volume (TIV), both measures extracted from CAT12 pipeline. Group, scaled age and the interaction between the two were introduced in the model as predictor variables (fixed effects). Random effects included subject intercept and scaled age slope. SD, standard deviation.

	Model superagers and typical older adults		**Model superagers and Northwestern-criteria typical older adults**	
	β (SE)	***P***	**β (SE)**	***P***
Group	-11.9 (4.9)	0.015	-13.1 (6.6)	0.048
Age (scaled)	-7.5 (1.1)	< 0.0001	-7.7 (1.2)	< 0.0001
Interaction Group × Age (scaled)	-4.1 (1.7)	0.015	-6.0 (2.4)	0.014
Superager slope, cm^3/one SD of scaled age	-7.5 (1.1)	-	-7.7 (1.2)	-
Typical older adult slope, cm^3/one SD of scaled age	-11.6 (1.1)	-	-13.7 (2.1)	-

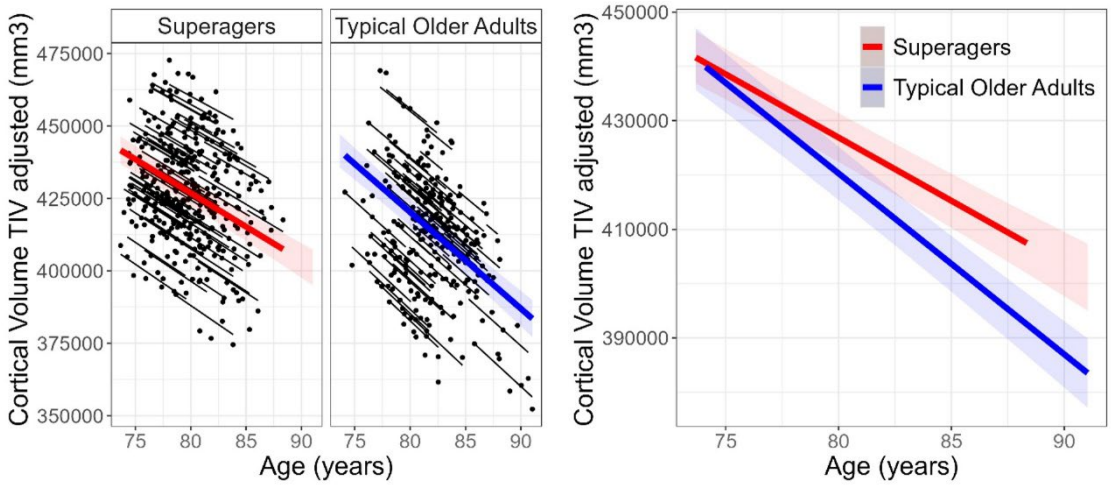


Figure S2. Cortical atrophy rate differences in superagers and typical older adults. The decline over time in cortical volume is slower in superagers (red) than Northwestern-criteria typical older adults (blue). Individual trajectories and means and 95% confidence interval for each group are shown (left) as well as means and 95% confidence interval for both groups (right). Total cortical volume was extracted with FreeSurfer and this replicates previous analysis on superagers (Cook et al., 2017b). Longitudinal analysis of total cortical volume revealed a significant effect of group (β (SE): -7658.5 (3365.8), P = 0.023), age (β (SE): -7312.9 (645.0), P < 0.0001), and a group-by-age interaction (β (SE): -3151.1 (995.7), P = 0.0016). Typical older adults showed a faster decline in cortical volume across time than superagers (slope (SE), mm^3/one SD of scaled age: superager: -7346.9 (645.0); typical older adults: -10498.0 (758.6)). SD, standard deviation; SE, standard error; TIV, total intracranial volume.

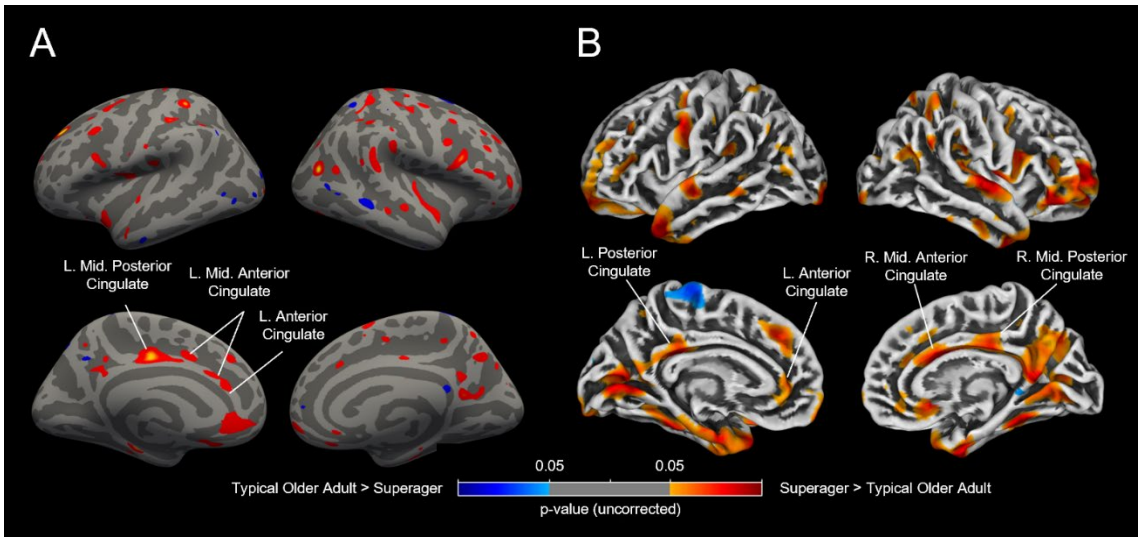


Figure S3. Regional differences in cortical thickness between superagers and typical older adults with two approaches. A. FreeSurfer approach. Red clusters indicate the regions where cortical thickness is greater in superagers than typical older adults (P < 0.05 uncorrected). These clusters are overlaid on the default inflated brain cortical template (FreeSurfer version 6.0). **B.** CAT12 approach. Red clusters indicate the regions where cortical thickness is greater in superagers than typical older adults (P < 0.05 uncorrected). These clusters are overlaid on the default brain cortical template from CAT12. Both approaches show uncorrected effects in areas within the cingulate gyrus, consistent with previous findings in superageing (Gefen et al., 2015; Harrison et al., 2018; Harrison et al., 2012; Sun et al., 2016).

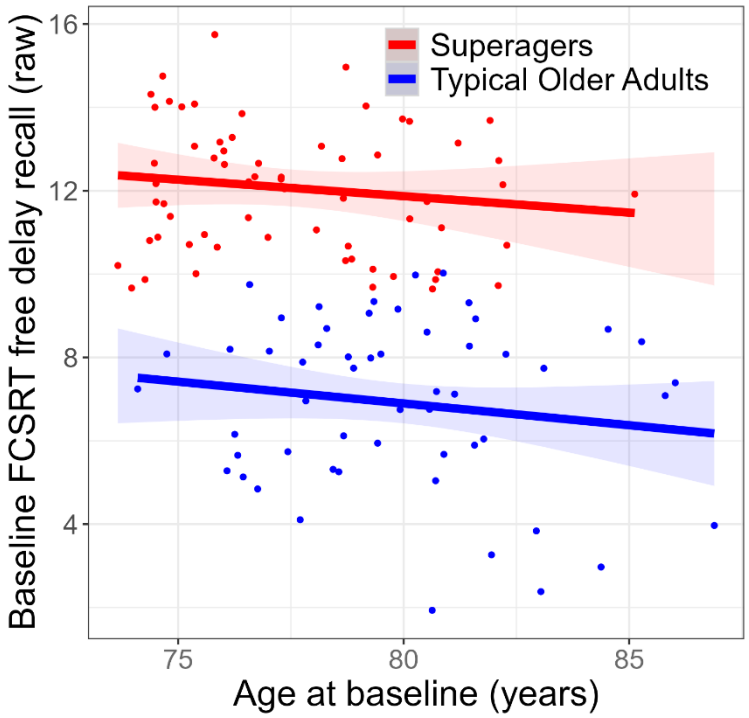


Figure S4. Memory superiority in superagers is evident prior to meeting the superager or control age criterion. The longitudinal nature of the Vallecas project from which superagers and typical older adults were selected enabled a retrograde examination of neuropsychological and neuroimaging features for up to 5 years. We therefore examined memory performance retrospectively in superagers and typical older adults. Superager performance on the FCSRT free delay recall score at baseline visit are significantly different ($P < 0.0001$, $t(31) = 5.9$) when comparing the average score of 75-78yo superagers (12.5 (SD = 1.4)) and typical older adults (6.6 (SD = 1.8)) after controlling for age. Raw FCSRT scores are plotted, rather than the age- and education-corrected values that were used in the selection criteria, in order to represent memory performance as a function of age. FCSRT, Free and Cued Selective Reminding Test; SD, standard deviation.

Table S7. Factors differentiating superagers from typical older adults as identified by the Random Forest model. Univariate comparisons between superagers and typical older adults were performed using a t-test or a Mann-Whitney U-test for numerical variables and a Chi-Square test or a Fisher's exact test for categorical data. This univariate approach was only applied to determine the direction of group differences for variables previously identified as significant by the multivariate approach performed in the Random Forest model. FAQ, Functional Activities Questionnaire total score; GDS, Geriatric Depression Score; NART, Spanish version of the National Adult Reading Test total score; P, p-value; SD, Standard Deviation; STAI, State Trait Anxiety Inventory.

		Superagers (n = 64)	**Typical older adults** (n = 55)	**Statistic**	***P***
Timed Up & Go test, mean (SD), sec		6.6 (1.4)	8.3 (2.4)	t = -4.4	<0.0001
STAI state, mean (SD)		6.6 (8.4)	14.3 (10.2)	Z = -4.2	<0.0001
STAI trait, mean (SD)		8.0 (9.2)	17.4 (12.2)	Z = -4.5	<0.0001
GDS test, mean (SD)		1.1 (2.1)	2.7 (3.1)	Z = -3.7	<0.0001
Enough sleep, No (%)		6 (9.8)	14 (26.9)	χ = -4.5	0.03
FAQ test, mean (SD)		0.2 (0.5)	0.6 (0.8)	Z = -3.1	0.002
NART$_{Spanish}$, mean (SD)		52.4 (8.4)	46.0 (12.0)	Z = 3.3	<0.0001
Composite midlife activities, mean (SD)		58.9 (15.9)	47.7 (14.1)	t = 3.6	<0.0001
Glucose disorder history, No (%)		12 (18.8)	18 (32.7)	χ = -2.4	0.12
Musical background, No (%)	No background	20 (35.1)	32 (62.7)	χ = 8.7	0.01
	Amateur	28 (49.1)	16 (31.4)		
	Formal training	9 (15.8)	3 (5.9)		
Marital status, No (%)	Single	5 (7.9)	4 (7.5)	Fisher's Test	0.03
	Married	34 (54.0)	25 (47.2)		
	Widowed	17 (27.0)	24 (45.3)		
	Separated/Divorced	7 (11.1)	0 (0)		
TAPS dominant hand, mean (SD), count		64.4 (7.6)	60.2 (8.6)	t = 2.7	0.008
Hypertension history, No (%)		36 (56.3)	42 (76.4)	χ = -4.4	0.04

A.1.2 Supplementary information Chapter 4

Table S8. Cross-sectional group differences of white matter volume and white matter lesions. Volumetric group differences were calculated with an analysis of covariance and total intracranial volume (TIV) was regress out. The mean and standard deviation (SD) reported in the table correspond to the raw data. Group differences in the Fazekas scale were assessed with a Chi-square test and Fisher's exact test. *P*, *p*-value.

		Superagers	Typical older adults	Statistic	*P*
White matter volume, mean (SD), cm^3		441.80 (54.99)	439.08 (55.46)	F = 0.4	0.54
White matter lesions volume, mean (SD), cm^3		3.28 (3.32)	4.52 (6.35)	F = 1.9	0.17
White matter lesion presence, No. (%), Fazekas score		55 (85.9)	45 (83.3)	χ = 0.02	0.89
Fazekas score, No. (%)	1	35 (63.6)	24 (53.3)	Fisher's Test	0.45
	2	17 (30.9)	16 (35.6)		
	3	3 (5.5)	5 (11.1)		

Table S9. Longitudinal evolution of white matter volume, white matter lesions volume and Fazekas score. Coefficients (β) correspond to the three linear mixed-effects model predicting the longitudinal evolution of total brain white matter volume, brain white matter lesions volume and Fazekas score progression respectively. In the three independent models, age, group and the interaction between age and group were fixed effects (scaled age was introduced in the model) and the random intercept and slope were also considered in the model. White matter volume and white matter lesions volume were adjusted by total intracranial volume (TIV). *P*, *p*-value; SD, standard deviation; SE, standard error.

	White matter volume (TIV-adjusted)		**White matter lesions volume** (TIV-adjusted)		**Fazekas score**	
	β (SE)	***P***	**β (SE)**	***P***	**β (SE)**	***P***
Group	-4.62 (6.11)	0.45	0.45 (0.80)	0.57	-0.07 (0.14)	0.63
Age (scaled)	-18.58 (1.13)	< 0.0001	0.71 (0.18)	< 0.0001	0.10 (0.04)	0.007
Group x Age	0.40 (1.73)	0.81	0.64 (0.27)	0.02	0.01 (0.05)	0.80
Superager slope, cm^3/one SD of scaled age	-18.58 (1.13)	-	0.71 (0.18)	-	0.11 (0.03)	-
Typical older adult slope, cm^3/one SD of scaled age	-18.18 (1.30)	-	1.36 (0.20)	-	0.10 (0.04)	-

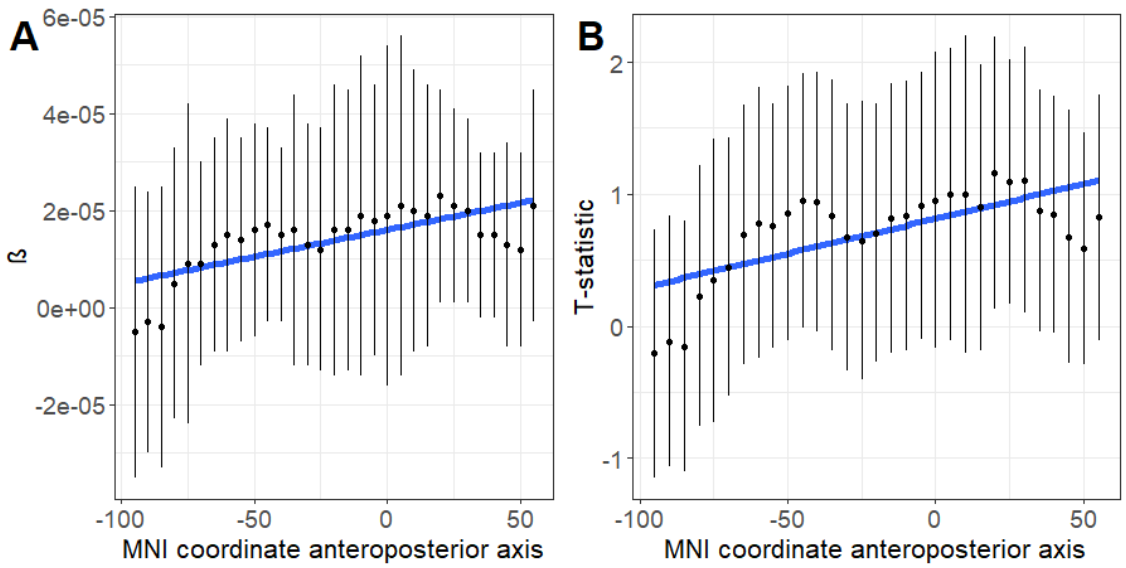


Figure S5. Greater differences in mean diffusivity (MD) between superagers and typical older adults in the anterior portion of the brain. A. The parameter estimates (β) and **B.** t-statistics from the contrast of higher MD in typical older adults than in superagers are plotted along the anteroposterior axis (positive Montreal Neurological Institute (MNI) coordinates for the anterior portion of the brain). The unthresholded β and t-statistic maps were sliced in the anteroposterior axis every five 5 mm and values in each slide were averaged and represented in these plots together with ± standard error. To assess the anteroposterior gradient on the MD group effects, we fitted a linear regression model of the two parameters separately as a function of the MNI coordinates and a significant effect of MNI coordinate on β ($t(29) = 5.31$, $P < 0.0001$) and the t-statistic ($t(29) = 4.94$, $P < 0.0001$) is found. The fitted trend of both linear regression models is shown in blue.

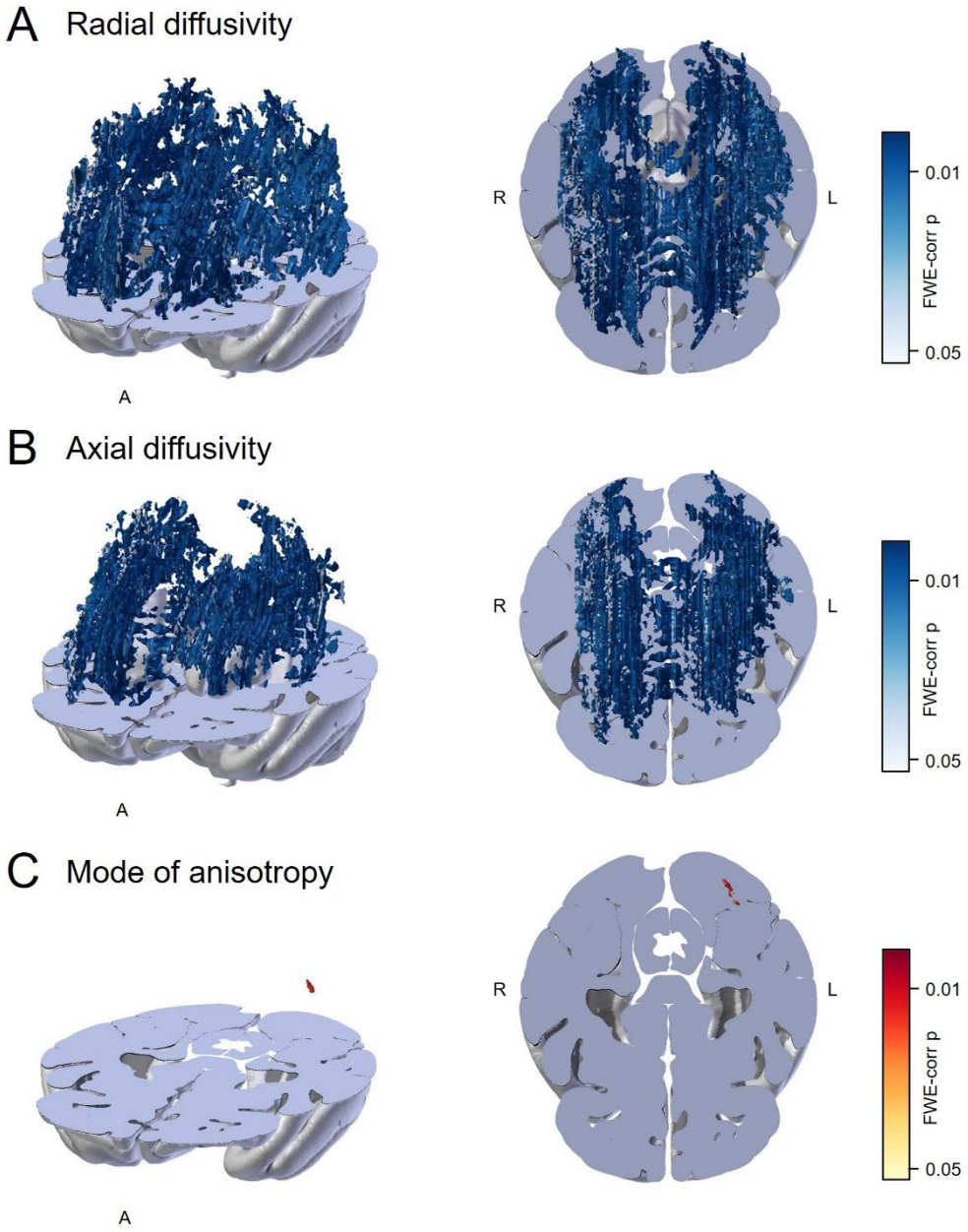


Figure S6. Radial and axial diffusivity and mode of anisotropy cross-sectional differences between superagers and typical older adults. A. Lower radial diffusivity and **B.** lower axial diffusivity is found in superagers compared to typical older adults in an extensive network (cold colours) comprising all the tracts described in JHU-ICBM atlas. **C.** Higher mode of anisotropy is found in superagers than typical older adults in a small part of the left inferior longitudinal fasciculus (ILF) and forceps major (warm colours) (*P* < 0.05 FWE-corrected). A, anterior; FWE-corr p, family-wise error *p*-value; L, left; R, right and P, posterior.

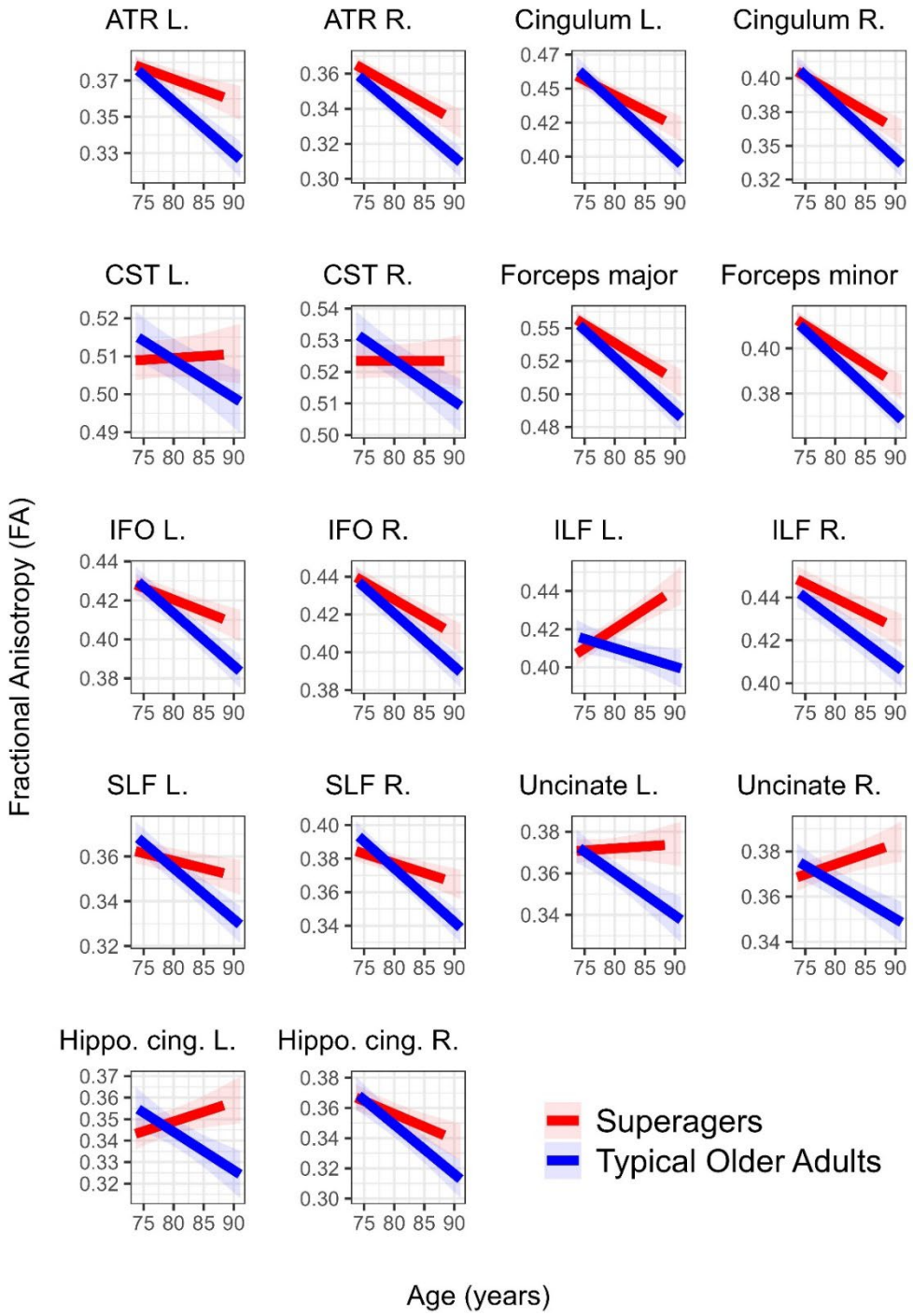


Figure S7. ROI-based longitudinal trajectories of white matter fractional anisotropy (FA). Longitudinal group differences were studied in 18 regions of interest (ROIs) from the JHU-ICBM atlas. The group average predicted trajectory is represented with the solid line and shaded areas indicate the 95% confidence interval. A linear mixed-effects model was used to predict average FA in each of the ROIs with group, scaled age and the interaction between the two as fixed factors, the random intercept and slope were included in the model. Age was scaled in the statistical model, but raw values are shown for illustration purposes. Details of the statistical tests are shown in Table S10. ATR, anterior thalamic radiation; CST, corticospinal tract; Hippo. cing., hippocampal cingulum; IFO, inferior fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus; L, left; R, right; SLF, superior longitudinal fasciculus.

Table S10. ROI-based longitudinal analysis of white matter fractional anisotropy (FA). Longitudinal group differences were studied in 18 white matter tracts or regions of interest (ROIs) from the JHU-ICBM atlas with a linear mixed-effects model to predict average FA in each of the ROIs with group, scaled age and the interaction between the two as fixed factors, the random intercept and slope were included in the model. ATR, anterior thalamic radiation; β, coefficients; Corr. *P*, false-discovery-rate corrected *p*-value; CST, corticospinal tract; Hippo. cing., hippocampal cingulum; IFO, inferior fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus; L, left; *P*, *p*-value; R, right; SE, standard error; SLF, superior longitudinal fasciculus.

	Group			**Age (scaled)**			**Group x age (scaled)**			**Slope superagers**	**Slope typical older adults**
	β (SE)	*P*	Corr. *P*	β (SE)	*P*	Corr. *P*	β (SE)	*P*	Corr. *P*	estimate (SE)	estimate (SE)
ATR L.	-0.013 (0.004)	0.002	0.01	-0.004 (0.001)	$3.3x10^{-5}$	$5.9x10^{-5}$	-0.005 (0.001)	$6.5x10^{-5}$	0.0003	-0.0037 (0.0009)	-0.0091 (0.001)
ATR R.	-0.012 (0.004)	0.006	0.03	-0.006 (0.001)	$8.3x10^{-16}$	$3.7x10^{-15}$	-0.003 (0.001)	0.004	0.004	-0.0060 (0.0007)	-0.0092 (0.0008)
Cingulum L.	-0.007 (0.005)	0.17	0.21	-0.007 (0.001)	$3.2x10^{-12}$	$9.6x10^{-12}$	-0.006 (0.002)	0.0001	0.0003	-0.0070 (0.001)	-0.0129 (0.0012)
Cingulum R.	-0.008 (0.005)	0.11	0.19	-0.008 (0.001)	$<2.2x10^{-16}$	$1.3x10^{-15}$	-0.005 (0.001)	0.0005	0.001	-0.0082 (0.0009)	-0.0129 (0.001)
CST L.	-0.001 (0.004)	0.75	0.79	$3.5x10^{-4}$ ($7.3x10^{-4}$)	0.64	0.67	-0.003 (0.001)	0.002	0.003	0.0003 (0.0007)	-0.0031 (0.0009)
CST R.	$-6.4x10^{-4}$ ($4.0x10^{-3}$)	0.87	0.87	$-1.2x10^{-5}$ ($7.9x10^{-4}$)	0.99	0.99	$-4.1x10^{-3}$ ($1.2x10^{-3}$)	0.0007	0.001	-0.0001 (0.0008)	-0.0041 (0.0009)
Forceps major	-0.012 (0.005)	0.02	0.06	-0.009 ($9.7x10^{-4}$)	$<2.2x10^{-16}$	$1.3x10^{-15}$	-0.004 (0.001)	0.003	0.004	-0.0087 (0.001)	-0.0130 (0.0011)
Forceps minor	-0.008 (0.004)	0.03	0.07	-0.007 ($6.2x10^{-4}$)	$<2.2x10^{-16}$	$1.3x10^{-15}$	-0.003 ($9.6x10^{-4}$)	0.001	0.002	-0.0066 (0.0006)	-0.0097 (0.0007)
IFO L.	-0.008 (0.004)	0.06	0.11	-0.003 (0.001)	0.0004	0.0007	-0.005 (0.001)	0.002	0.003	-0.0035 (0.001)	-0.0081 (0.0011)
IFO R.	-0.008 (0.004)	0.06	0.11	-0.006 ($7.5x10^{-4}$)	$1.2x10^{-14}$	$4.3x10^{-14}$	-0.003 (0.001)	0.007	0.008	-0.0058 (0.0007)	-0.0089 (0.0009)
ILF L.	-0.012 (0.004)	0.002	0.01	0.007 (0.001)	$3.7x10^{-9}$	$9.5x10^{-9}$	-0.010 (0.002)	$2.3x10^{-8}$	$4.1x10^{-7}$	0.0064 (0.0011)	-0.0030 (0.0013)
ILF R.	-0.011 (0.004)	0.01	0.04	-0.004 ($7.4x10^{-4}$)	$5.1x10^{-9}$	$1.2x10^{-8}$	-0.002 (0.001)	0.05	0.05	-0.0044 (0.0007)	-0.0066 (0.0009)
SLF L.	-0.004 (0.004)	0.35	0.39	-0.002 (0.001)	0.03	0.03	-0.005 (0.001)	$9.6x10^{-5}$	0.0003	-0.0020 (0.0009)	-0.0073 (0.001)
SLF R.	-0.004 (0.004)	0.29	0.35	-0.004 (0.001)	0.006	0.008	-0.007 (0.002)	0.0006	0.002	-0.0037 (0.0013)	-0.0104 (0.0015)
Uncinate L.	-0.013 (0.004)	0.0008	0.01	0.001 (0.002)	0.42	0.47	-0.007 (0.002)	0.003	0.004	0.0012 (0.0016)	-0.0058 (0.0018)
Uncinate R.	-0.010 (0.004)	0.01	0.04	0.003 ($9.4x10^{-4}$)	0.002	0.003	-0.008 (0.001)	$1.1x10^{-7}$	$9.9x10^{-7}$	0.0029 (0.0009)	-0.0048 (0.0011)
Hippo. cing. L.	-0.007 (0.005)	0.13	0.19	0.003 (0.001)	0.02	0.02	-0.008 (0.002)	$6.4x10^{-6}$	$3.8x10^{-5}$	0.0028 (0.0012)	-0.0056 (0.0014)
Hippo. cing. R.	-0.008 (0.006)	0.14	0.20	-0.005 (0.001)	$2.6x10^{-5}$	$5.2x10^{-5}$	-0.005 (0.002)	0.01	0.01	-0.0054 (0.0013)	-0.0103 (0.0015)

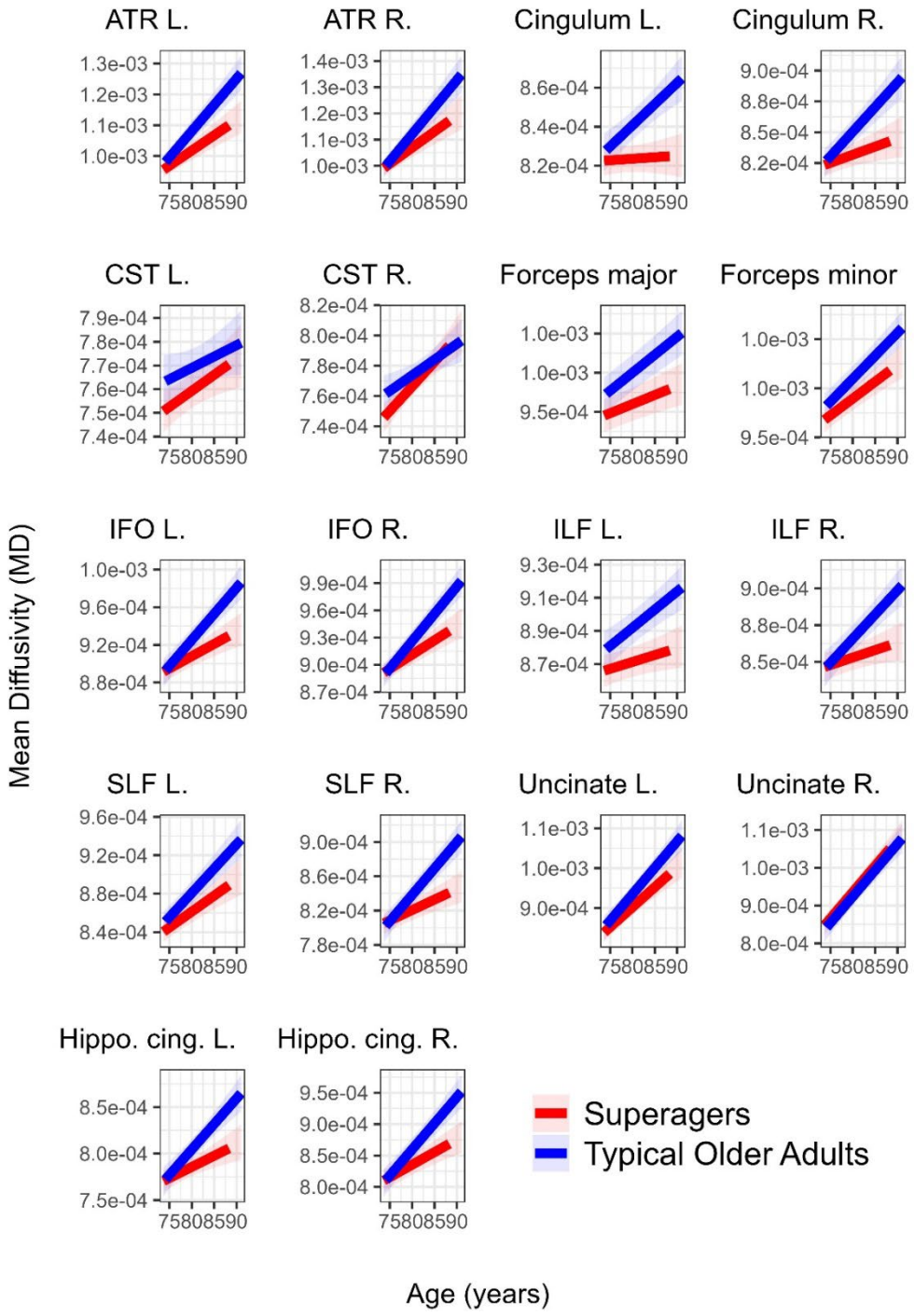


Figure S8. ROI-based longitudinal trajectories of white matter mean diffusivity (MD). Longitudinal group differences were studied bilaterally in 18 regions of interest (ROIs) from the JHU-ICBM atlas. The group average predicted trajectory is represented with the solid line and shaded areas indicate the 95% confidence interval. A linear mixed-effects model was used to predict average MD in each of the ROIs with group, scaled age and the interaction between the two as fixed factors, the random intercept and slope were included in the model. Age was scaled in the statistical model, but raw values are shown for illustration purposes. Details of the statistical tests are shown in Table S11. ATR, anterior thalamic radiation; CST, corticospinal tract; Hippo. cing., hippocampal cingulum; IFO, inferior fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus; L, left; R, right; SLF, superior longitudinal fasciculus.

Table S11. ROI-based longitudinal analysis of white matter mean diffusivity (MD). Longitudinal group differences were studied in 18 white matter tracts or regions of interest (ROIs) from the JHU-ICBM atlas with a linear mixed-effects model to predict average MD in each of the ROIs with group, scaled age and the interaction between the two as fixed factors, the random intercept and slope were included in the model. ATR, anterior thalamic radiation; β, coefficients; Corr. *P*, false-discovery-rate corrected *p*-value; CST, corticospinal tract; Hippo. cing., hippocampal cingulum; IFO, inferior fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus; L, left; *P*, *p*-value; R, right; SE, standard error; SLF, superior longitudinal fasciculus.

	Group			**Age (scaled)**			**Group x age (scaled)**			**Slope superagers**	**Slope typical older adults**
	β (SE)	*P*	Corr. *P*	β (SE)	*P*	Corr. *P*	β (SE)	*P*	Corr. *P*	estimate (SE)	estimate (SE)
ATR L.	$6.7x10^{-5}$ ($2.5x10^{-5}$)	0.007	0.02	$3.1x10^{-5}$ ($4.9x10^{-6}$)	$5.1x10^{-10}$	$2.9x10^{-9}$	$2.3x10^{-5}$ ($7.4x10^{-6}$)	0.002	0.001	$3.1x10^{-10}$ ($4.9x10^{-11}$)	$5.3x10^{-10}$ ($5.5x10^{-11}$)
ATR R.	$5.7x10^{-5}$ ($3.0x10^{-5}$)	0.06	0.07	$3.8x10^{-5}$ ($6.1x10^{-6}$)	$4.3x10^{-10}$	$2.9x10^{-9}$	$2.6x10^{-5}$ ($9.2x10^{-6}$)	0.004	0.01	$3.9x10^{-10}$ ($6.2x10^{-11}$)	$6.5x10^{-10}$ ($6.8x10^{-11}$)
Cingulum L.	$1.9x10^{-5}$ ($5.1x10^{-6}$)	0.0002	0.004	$8.2x10^{-8}$ ($1.4x10^{-6}$)	0.95	0.95	$6.7x10^{-6}$ ($2.2x10^{-6}$)	0.002	0.001	$2.0x10^{-12}$ ($1.4x10^{-11}$)	$6.7x10^{-11}$ ($1.6x10^{-11}$)
Cingulum R.	$2.1x10^{-5}$ ($7.0x10^{-6}$)	0.003	0.02	$3.9x10^{-6}$ ($1.8x10^{-6}$)	0.03	0.04	$8.7x10^{-6}$ ($2.8x10^{-6}$)	0.002	0.01	$4.0x10^{-11}$ ($1.8x10^{-11}$)	$1.3x10^{-10}$ ($2.1x10^{-11}$)
CST L.	$9.0x10^{-6}$ ($5.5x10^{-6}$)	0.10	0.11	$4.3x10^{-6}$ ($1.5x10^{-6}$)	0.005	0.007	$-1.0x10^{-6}$ ($2.4x10^{-6}$)	0.66	0.70	$4.3x10^{-11}$ ($1.5x10^{-11}$)	$3.2x10^{-11}$ ($1.8x10^{-11}$)
CST R.	$6.0x10^{-6}$ ($4.8x10^{-6}$)	0.21	0.22	$1.0x10^{-5}$ ($1.8x10^{-6}$)	$1.3x10^{-8}$	$3.9x10^{-8}$	$-3.5x10^{-6}$ ($2.8x10^{-6}$)	0.21	0.25	$1.0x10^{-10}$ ($1.8x10^{-11}$)	$6.4x10^{-11}$ ($2.1x10^{-11}$)
Forceps major	$4.1x10^{-5}$ ($1.6x10^{-5}$)	0.01	0.03	$6.7x10^{-6}$ ($2.1x10^{-6}$)	0.002	0.003	$8.0x10^{-6}$ ($3.2x10^{-6}$)	0.01	0.02	$6.7x10^{-11}$ ($2.1x10^{-11}$)	$1.5x10^{-10}$ ($2.5x10^{-11}$)
Forceps minor	$2.1x10^{-5}$ ($8.9x10^{-6}$)	0.02	0.03	$1.1x10^{-5}$ ($1.7x10^{-6}$)	$8.0x10^{-10}$	$2.9x10^{-9}$	$4.1x10^{-6}$ ($2.7x10^{-6}$)	0.12	0.16	$1.1x10^{-10}$ ($1.7x10^{-11}$)	$1.5x10^{-10}$ ($2.0x10^{-11}$)
IFO L.	$1.9x10^{-5}$ ($7.9x10^{-6}$)	0.02	0.03	$7.6x10^{-6}$ ($2.9x10^{-6}$)	0.009	0.01	$9.0x10^{-6}$ ($4.4x10^{-6}$)	0.04	0.06	$7.7x10^{-11}$ ($2.9x10^{-11}$)	$1.7x10^{-10}$ ($3.2x10^{-11}$)
IFO R.	$1.7x10^{-5}$ ($8.2x10^{-6}$)	0.04	0.05	$9.6x10^{-6}$ ($1.6x10^{-6}$)	$7.0x10^{-10}$	$2.9x10^{-9}$	$9.1x10^{-6}$ ($2.4x10^{-6}$)	0.0001	0.002	$9.7x10^{-11}$ ($1.5x10^{-11}$)	$1.9x10^{-10}$ ($1.8x10^{-11}$)
ILF L.	$2.2x10^{-5}$ ($6.7x10^{-6}$)	0.0009	0.008	$2.2x10^{-6}$ ($1.5x10^{-6}$)	0.13	0.14	$4.8x10^{-6}$ ($2.3x10^{-6}$)	0.03	0.05	$2.3x10^{-11}$ ($1.5x10^{-11}$)	$6.9x10^{-11}$ ($1.7x10^{-11}$)
ILF R.	$1.5x10^{-5}$ ($6.2x10^{-6}$)	0.02	0.03	$3.2x10^{-6}$ ($1.6x10^{-6}$)	0.05	0.06	$7.0x10^{-6}$ ($2.5x10^{-6}$)	0.005	0.01	$3.2x10^{-11}$ ($1.6x10^{-11}$)	$1.0x10^{-10}$ ($1.9x10^{-11}$)
SLF L.	$2.1x10^{-5}$ ($8.7x10^{-6}$)	0.02	0.03	1.0e-05 (1.9e-06)	$6.8x10^{-8}$	$1.8x10^{-7}$	$5.6x10^{-6}$ ($2.9x10^{-6}$)	0.05	0.07	$1.0x10^{-10}$ ($1.9x10^{-11}$)	$1.6x10^{-11}$ ($2.2x10^{-11}$)
SLF R.	$2.1x10^{-5}$ ($8.5x10^{-6}$)	0.02	0.03	$7.0x10^{-6}$ ($3.0x10^{-6}$)	0.02	0.03	$1.2x10^{-5}$ ($4.5x10^{-6}$)	0.006	0.01	$7.2x10^{-11}$ ($3.0x10^{-11}$)	$1.9x10^{-10}$ ($3.3x10^{-11}$)
Uncinate L.	$3.7x10^{-5}$ ($1.5x10^{-5}$)	0.02	0.03	$3.2x10^{-5}$ ($6.2x10^{-6}$)	$2.1x10^{-7}$	$4.7x10^{-7}$	$1.0x10^{-5}$ ($9.3x10^{-6}$)	0.26	0.29	$3.2x10^{-10}$ ($6.2x10^{-11}$)	$4.2x10^{-10}$ ($6.9x10^{-11}$)
Uncinate R.	$-1.1x10^{-5}$ ($1.8x10^{-5}$)	0.55	0.55	$4.3x10^{-5}$ ($4.0x10^{-6}$)	$<2.0x10^{-16}$	$3.6x10^{-15}$	$9.6x10^{-7}$ ($6.1x10^{-6}$)	0.88	0.88	$4.3x10^{-10}$ ($4.0x10^{-11}$)	$4.4x10^{-10}$ ($4.6x10^{-11}$)
Hippo. cing. L.	$2.1x10^{-5}$ ($7.5x10^{-6}$)	0.005	0.02	$7.6x10^{-6}$ ($2.3x10^{-6}$)	0.001	0.002	$9.8x10^{-6}$ ($3.6x10^{-6}$)	0.007	0.01	$7.9x10^{-11}$ ($2.3x10^{-11}$)	$1.7x10^{-10}$ ($2.7x10^{-11}$)
Hippo. cing. R.	$2.7x10^{-5}$ ($1.2x10^{-5}$)	0.03	0.04	$1.3x10^{-5}$ ($3.1x10^{-6}$)	$5.0x10^{-5}$	$1.0x10^{-4}$	$1.3x10^{-5}$ ($4.8x10^{-6}$)	0.005	0.01	$1.3x10^{-10}$ ($3.1x10^{-11}$)	$2.6x10^{-10}$ ($3.6x10^{-11}$)

A.1.3 Supplementary information Chapter 5

Table S12. Characteristics of the population of superagers and typical older adults. Raw data is shown and the statistical contrast for telomere variables were conducted with variables adjusted by cell viability, storage time and batch. 15-BNT, 15-item Boston Naming Test; *APOE*, apolipoprotein E; BMI, body mass index; bp, base pairs; FAQ, Functional Activities Questionnaire; $FCSRT_{freedelayrecall}$, delayed free-recall score of the Free and Cued Selective Reminding Test; $FLU_{lexicaltotal}$, Lexical Fluency test with letter P; $FLU_{semantictotal}$, the Animal Fluency test; Kbp, kilobase pairs; MMSE, Mini-Mental State Examination; NART, National Adult Reading Test Spanish version; *P*, *p*-value; $REY_{delayrecall}$, delayed recall score of the Rey-Osterreith Complex Figure test; SD, standard deviation.

	Superagers (n = 57)	**Typical older adults** (n = 48)	**Statistic**	***P***
Demographics				
Age, mean (SD), years	81.87 (1.95)	82.40 (1.90)	Z = -1.7	0.10
Female, No. (%)	35 (61.4)	31 (64.6)	χ = 0.1	0.74
Education, mean (SD), years	14.63 (5.77)	11.37 (7.34)	Z = 2.5	0.01
Neuropsychology – selection criteria variables				
$FCSRT_{freedelayrecall}$, mean[a] (SD)	16.77 (1.27)	10.67 (1.88)	Z = 8.8	<0.0001
$FLU_{semantictotal}$, mean (SD)	12.95 (3.20)	9.88 (3.19)	t = 4.9	<0.0001
Digit Symbol Substitution Test, mean (SD)	21.39 (6.09)	14.85 (5.19)	t = 5.9	<0.0001
15-BNT, mean (SD)	13.79 (1.36)	11.48 (2.59)	Z = 4.9	<0.0001
Neuropsychology – other variables				
MMSE, mean (SD)	29.05 (1.09)	27.96 (2.16)	Z = 3.9	0.0001
FAQ, mean (SD)	0.19 (0.44)	0.60 (0.80)	Z = -3.1	0.0020
$REY_{delayrecall}$, mean (SD)	15.10 (4.64)	10.43 (5.02)	t = 3.1	0.0036
$FLU_{lexicaltotal}$, mean (SD)	17.04 (4.54)	12.73 (4.43)	t = 4.9	<0.0001
NART, mean (SD)	52.58 (8.27)	44.95 (12.48)	Z = 3.5	0.0004
Confounding variables				
BMI, mean (SD), kg/m2	26.96 (3.63)	26.54 (3.05)	t = 0.6	0.52
***APOE* ε4 carrier**, No (%)	7 (12.3)	7 (14.6)	χ = 0.1	0.73
Smoking, No (%)	22 (38.6)	18 (37.5)	χ = 0.01	0.91
Hypertension, No (%)	32 (56.1)	38 (79.2)	χ = 6.2	0.01
Cell viability, mean (SD), %	43.25 (38.60)	64.81 (28.93)	Z = 2.2	0.03
Storage time, mean (SD), days	2070.30 (462.02)	2716.48 (548.29)	t = 6.5	<0.0001
Telomere variables – raw data is shown; the statistical contrasts correspond to the variables adjusted by cell viability and batch				
Median Telomere Length, mean (SD), bp	8665.40 (922.64)	9139.33 (969.24)	t = -2.3	0.02
Telomere <6 Kbp, mean (SD), %	27.42 (7.19)	24.37 (6.62)	t = 2.1	0.04
Telomere <3 Kbp, mean (SD), %	9.45 (3.97)	7.99 (3.03)	t = 2.2	0.03
20^{th} Percentile Length, mean (SD), bp	5035.17 (970.22)	5462.04 (932.79)	t = -2.3	0.02

A.1.4 Supplementary information Chapter 6

Table S13. Demographic and neuropsychological profile of the total sample and the superagers and typical older adults group. In the analysis of the total sample, the baseline visit of the Vallecas project was used whereas the data of the superagers and the typical older adults correspond to the visits selected for previous cross-sectional analysis to matched in age groups and they are all different from the baseline visit (see more details in Chapter 2 of General Methods). Raw learning rates are reported in this table, whereas group rates adjusted by sex, age, education and delayed free recall FCSRT are reported in Results. FCSRT, Free and Cued Selective Reminding Test; MMSE, Mini Mental State Examination total score; *P*, *p*-value; SD, standard deviation.

		Total sample (n = 982)	**Superagers** (n = 64)	**Typical older adults** (n = 55)	**Statistic** (Superagers *vs.* Typical older adults)	***P*** (Superagers *vs.* Typical older adults)
Demographics						
Age, years, mean (SD)		74.8 (3.9)	81.9 (1.9)	82.4 (1.9)	Z = 1.8	0.08
Sex, female (%)		637 (64.9)	38 (59.4)	35 (63.6)	χ = 0.1	0.77
Levels of education, count (%)	Less than primary	185 (18.8)	8 (12.5)	11 (20.0)	χ = 6.7	0.08
	Primary	295 (30.0)	10 (15.6)	17 (30.9)		
	Secondary	245 (24.9)	24 (37.5)	13 (23.6)		
	Higher education	257 (26.2)	22 (34.4)	14 (25.5)		
Neuropsychological performance						
MMSE, total score, mean (SD)		28.6 (1.6)	29.1 (1.1)	27.8 (1.8)	Z = 4.5	<0.0001
Trial 1 immediate free recall FCSRT, items recalled, mean (SD)		6.5 (2.1)	10.00 (2.38)	5.71 (1.65)	Z = -8.2	<0.0001
Trial 2 immediate free recall FCSRT, items recalled, mean (SD)		7.9 (2.4)	11.61 (2.00)	6.15 (1.94)	Z = -8.8	<0.0001
Trial 3 immediate free recall FCSRT, items recalled, mean (SD)		9.2 (2.5)	12.41 (1.92)	7.29 (1.71)	Z = -9.0	<0.0001
Learning rate immediate free recall FCSRT, items recalled/trial, mean (SD)		1.4 (0.3)	1.11 (0.07)	0.90 (0.06)	Z = -9.1	<0.0001
Delayed free recall FCSRT, items recalled, mean (SD)		9.4 (2.6)	13.36 (1.43)	6.49 (1.55)	Z = -9.4	<0.0001

Table S14. Coordinates of grey matter volume effects in the VBM analysis. The Montreal Neurological Initiative (MNI) coordinates for the global maximum and local maxima of each cluster are indicated in mm for the three sections in space (x, y and z). Neuroanatomical labels from the AAL3 atlas are indicated. Cerebelum 3, lobule III of cerebellar hemisphere; Corr. *P*, Family Wise Error corrected *p*-value; L, left; OFCpost, posterior orbital gyrus; *P*, *p*-value; R, right; Temporal Mid, middle temporal cortex; TFCE, Threshold Free Cluster Enhancement local spatial support; Thal VL, ventrolateral thalamic nuclei; Thal VA, ventroanterior thalamic nuclei.

Number of voxels in cluster	Corr. *P*	TFCE	*P*	x	y	z	AAL3 labels
2287	0.007	1337.90	0	-28	-18	-12	Hippocampus L (Extended to the left superior temporal gyrus)
	0.008	1318.49	0	-40	-30	-8	Hippocampus L
	0.008	1308.50	0	-26	-31	-8	Hippocampus L
184	0.032	999.29	0.002	4	-3	12	Thal VL R
	0.038	959.07	0.001	4	-5	-2	Thal VA R
168	0.033	994.55	0.001	30	-19	-12	Hippocampus R
	0.045	922.07	0.002	20	-23	-16	Parahippocampal R
46	0.044	928.22	0.001	20	-67	22	Cuneus R
	0.049	904.13	0.001	18	-59	16	Calcarine R
64	0.046	915.85	0.002	52	-19	-12	Temporal Mid R
	0.047	911.21	0.002	44	-11	-18	Hippocampus R
22	0.048	909.89	0.002	-24	18	-18	OFCpost L
7	0.049	903.11	0.001	14	-28	-24	Cerebellum 3 R

Table S15. Brain clusters for correlation between episodic learning rate and fractional anisotropy (FA) and mean diffusivity (MD) values in the main analysis. Neuroanatomical labels from the JHU-ICBM white matter atlas are indicated. ATR, anterior thalamic radiation; Corr. *P*, Family Wise Error corrected *p*-value; IFO, inferior fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus; L, left; R, right; SLF, superior longitudinal fasciculus.

Number of voxels in cluster	Corr. *P*	Main tracts
Positive correlation with FA values		
4917	0.025	IFO, SLF, fornix, ATR R
3123	0.036	IFO, ILF, SLF, fornix, ATR L
1310	0.036	IFO, ILF, forceps major R
359	0.047	ILF, forceps major L
239	0.044	SLF L
128	0.047	Corpus callosum
61	0.049	Forceps minor
8	0.050	Corpus callosum
Negative correlation with MD values		
9921	0.013	IFO, ILF, SLF, ATR R, forceps minor, corpus callosum
7467	0.015	IFO, ILF, SLF, fornix, L, corpus callosum
3	0.050	Corticospinal tract
1	0.050	Corticospinal tract

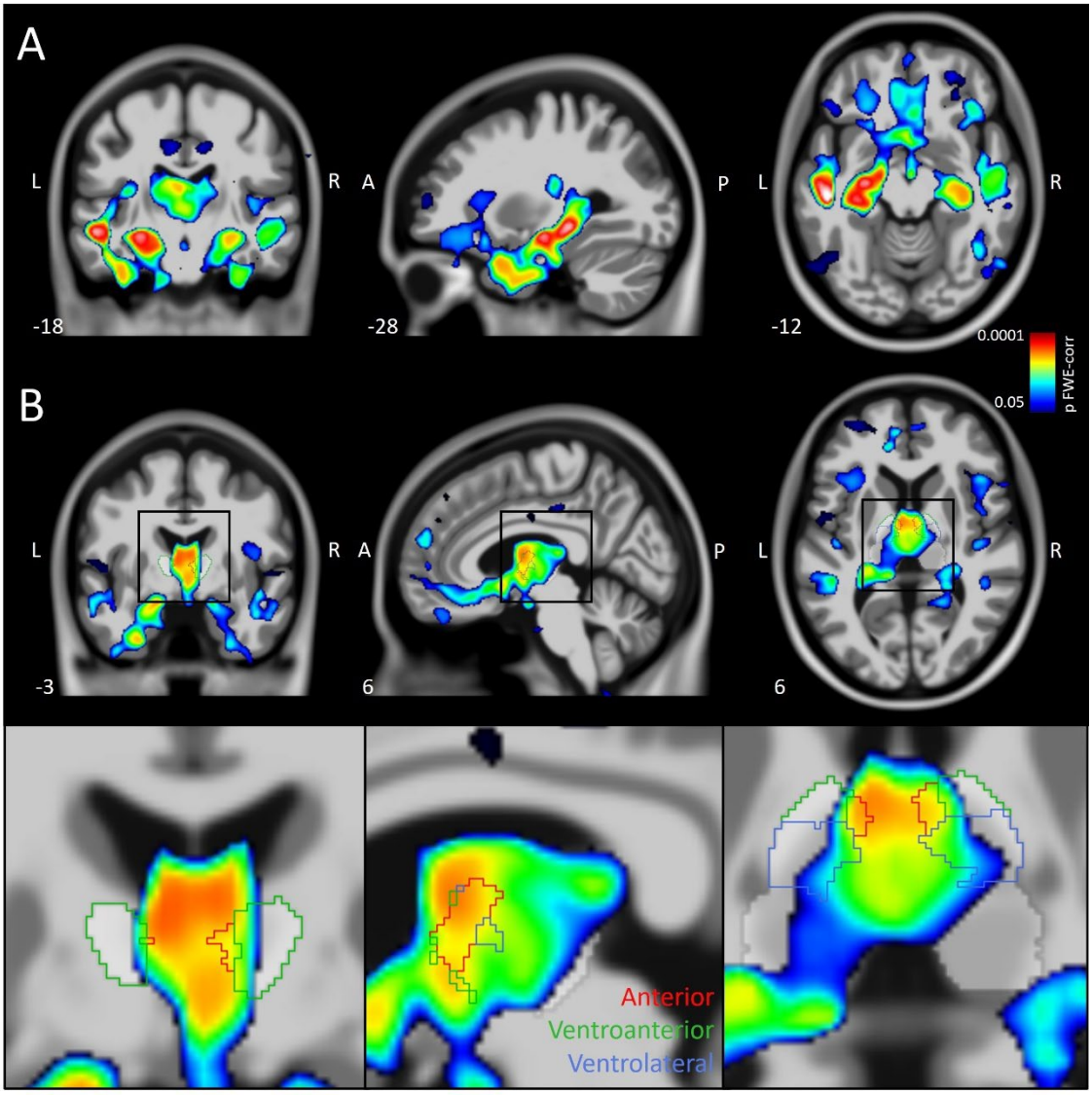


Figure S9. Grey matter volume correlates with episodic learning rate in older adults with an alternative statistical model. For this model, age and education were introduced in the model whereas the delayed free Free and Cued Selective Reminding Test (FCSRT) score was removed. The positive correlation has been overlaid on a canonical T1 image (thresholded at $P < 0.05$ FWE-corr) to show a significant effect in **A**. hippocampus bilaterally and in the **B**. thalamus, specifically the right anterior (red), ventroanterior (green) and ventrolateral thalamic nuclei (blue) (thalamic regions of interest in the inset come from the AAL3 atlas (Rolls et al., 2020). The coordinates of the sections are given in mm. A, anterior; L, left; P, posterior; p FWE-corr, Family Wise Error corrected *p*-value; R, right.

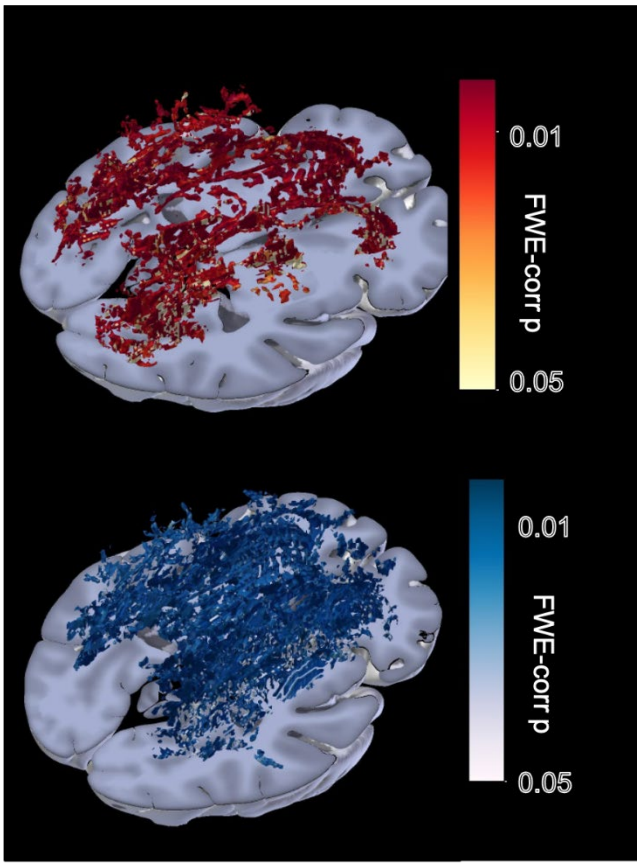


Figure S10. Extensive network of white matter microstructure is related to episodic learning rate in older adults with an alternative statistical model. For this model, age and education were introduced in the model whereas the delayed free Free and Cued Selective Reminding Test (FCSRT) score was removed. Positive correlation between FA and learning rate (warm colours; $P < 0.05$ FWE-corr) and negative correlation between MD and learning rate (cold colours; $P < 0.05$ FWE-corr) are shown. FWE-corr p, Family Wise Error corrected *p*-value.

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Frank D*, **Garo-Pascual M***, Reyes Velasquez PA, Frades B, Peled N, Zhang L, Strange BA. Brain structure and episodic learning rate in cognitively healthy ageing. Neuroimage. 2022 Sep 13;263:119630.

*These authors contributed equally to this work.

Garo-Pascual M*, Gaser C*, Zhang L, Tohka J, Medina M, Strange BA. Brain structure and phenotypic profile of superagers compared with age-matched older adults: a longitudinal analysis from the Vallecas Project. The Lancet Healthy Longevity. 2023 Jul 12.

*These authors contributed equally to this work.

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ACKNOWLEDGEMENTS

Bryan Strange, thank you for giving me the opportunity to be part of this team and thank you for believing, and making me believe, that I could build this project from scratch. Thank you for your implication in my training and for the opportunities to travel to congresses, courses, and stays that you have given me.

Javier de Felipe, thank you for trusting in this project and for contributing the idea of telomeres, the most risky and novel one. I feel fortunate that you were able to supervise my work and grateful for the speed with which you handled the bureaucracy related to my PhD school.

Christian Gaser, thank you for welcoming me with open arms in Jena, you were tremendously generous with your time, and I thank you for everything you taught me during my stay with you.

Linda Zhang, I would not even know where to begin to thank you. Thank you for walking with me every day on this journey, from the beginning to the end of my PhD. Thank you for the time you have dedicated to me and for all that I have learned from you, which is a big part of what I know.

Miguel Medina, thank you for your unconditional support. Your values and sense of responsibility have been an example.

Darya Frank, you have been a turning point for me both personally and professionally. Thank you for not judging but supporting my feelings. I feel fortunate to have shared this little common project that has given me so much joy.

Jussi Tohka and **Özgür Onur**, thank you both for your support in respective collaborations and thank you for the time you spent in writing the report for my PhD school.

To the current and past members of **The Strange Lab**, thank you for the supportive environment that has reigned over the years. **Kimberly Bress**, thank you for accompanying me during the first months of my PhD. **Svenja Treu**, thank you for your

support, especially in this final phase when I was sending you daily messages. **Diana Ortega Cruz** and **Alba Peris Yagüe**, thank you for crossing my path. Being able to share this time with you has made it more fun and you have been oxygen for me on more occasions than you can imagine. **Manuela Costa**, your attitude is inspiration and having you around recharges my batteries. **Marta Casales**, thank you for being so generous with your work. If there is someone with special merit in maintaining the harmony of the group, it is you. **Marta García Huéscar**, the superagers project could not be in better hands. Thanks also to the rest of the team members because, although we have not shared so many moments, you have been part of the retreats in Cercedilla and the dinners in Malasaña.

Thanks to the management and coordination staff of the **UAM Doctoral School** and the **UAM Doctoral Programme in Neuroscience**, it is a pleasure when you find people who facilitate the bureaucratic processes.

Vallecas Project participants, infinite thanks for your willingness that has been and will be the beginning of many research studies. **CIEN Foundation staff**, thank you for having made this wonderful data collection possible. Thanks to the people who devised this project, to those who have taken care of its logistics, to the clinical team, to the laboratory team, to the neuropathology team and, especially, to the neuroimaging team.

Beyond the professional aspect, there are many people I must thank for the refuge they have given me during this time. To my friends, my brother, my family, thank you for listening to me, for trying to understand me, for celebrating with me the good times and for being with me in the bad times. These words are not enough to thank you for your support.

Before concluding this thesis, I would like to acknowledge how privileged I have been to be able to dedicate years to study and research. This privilege is the fruit of the effort that my grandparents initiated, and, above all, my parents have made. You cannot change the cards that life deals you, but if you play them well, you can bring better luck to the next generation. Thank you.

AGRADECIMIENTOS

Bryan Strange, gracias por darme la oportunidad de formar parte de este equipo y gracias por creer, y hacerme creer, que era capaz de levantar este proyecto desde cero. Gracias por tu implicación en mi formación y por las oportunidades para viajar a congresos, cursos y estancias que me has brindado.

Javier de Felipe, gracias por creer en este proyecto y por aportar la idea de los telómeros, la más arriesgada y novedosa. Me siento afortunada de que hayas podido supervisar mi trabajo y agradecida por la rapidez con la que has gestionado la burocracia relacionada con mi escuela de doctorado.

Christian Gaser, gracias por acogerme con los brazos abiertos en Jena, fuiste tremendamente generoso con tu tiempo y estoy agradecida por todo lo que me enseñaste durante mi estancia contigo.

Linda Zhang, no sabría ni por dónde empezar a agradecer. Gracias por acompañarme cada día en este camino, desde el principio hasta el final de mi doctorado. Gracias por el tiempo que me has dedicado y por todo lo que he aprendido de ti, que es gran parte de lo que sé.

Miguel Medina, gracias por tu apoyo incondicional. Tus valores y tu sentido de la responsabilidad son un ejemplo.

Darya Frank, has sido un punto de inflexión para mí tanto a nivel personal como profesional. Gracias por no juzgar, sino acompañar mis sentimientos. Me siento afortunada de haber compartido ese pequeño proyecto en común que tantas alegrías me ha dado.

Jussi Tohka y **Özgür Onur,** gracias a ambos por vuestro apoyo en sendas colaboraciones y gracias por el tiempo que dedicasteis en escribir el informe para mi escuela de doctorado.

A los miembros presentes y pasados de **The Strange Lab**, gracias por el compañerismo que ha reinado estos años. **Kimberly Bress**, gracias por acompañarme

durante los primeros meses de mi doctorado. **Svenja Treu**, gracias por tu apoyo, sobre todo en la fase final cuando te mandaba mensajes diarios. **Diana Ortega Cruz** y **Alba Peris Yagüe**, gracias por haber aparecido en mi camino. Poder compartir esta etapa con vosotras lo ha hecho más divertido y habéis sido oxígeno para mí en más ocasiones de las que podáis imaginar. **Manuela Costa**, tu actitud es inspiración y tenerte cerca recarga las pilas. **Marta Casales**, gracias por ser tan generosa con tu trabajo. Si hay alguien con especial mérito en mantener la convivencia del grupo, esa eres tú. **Marta García Huéscar**, el relevo del proyecto de los superagers es tuyo y sé que está en buenas manos. Gracias también al resto de miembros del equipo porque, aunque no hayamos compartido tantos momentos, sí que habéis sido parte de los retiros en Cercedilla y las cenas por Malasaña.

Gracias al personal de gestión y coordinación de la **Escuela de Doctorado de la UAM** y del **Programa de Doctorado de Neurociencia de la UAM**, es un gusto cuando encuentras personas que te facilitan los procesos burocráticos.

Participantes del Proyecto Vallecas, gracias infinitas por vuestro carácter voluntarioso que ha sido y será el inicio de muchos estudios de investigación. **Trabajadores de la Fundación CIEN**, gracias por haber hecho posible esta colección de datos tan maravillosa. Gracias a las personas que idearon este proyecto, a quienes se han ocupado de su logística, al equipo clínico, al equipo de laboratorio, al equipo de neuropatología y, en especial, al equipo de neuroimagen.

Más allá de lo profesional, hay muchas personas a las que debo agradecer el refugio que me han dado en esta etapa. A mis amigos, a mi hermano, a mi familia, gracias por escucharme, por tratar de entenderme, por alegraros conmigo en los buenos momentos y por estar en los malos momentos. Estas palabras se quedan cortas para agradeceros vuestro apoyo.

Antes de poner el punto final a esta tesis, quiero reconocer lo privilegiada que he sido al poder dedicarme durante años a estudiar e investigar. Este privilegio es fruto del esfuerzo que empezaron mis abuelos y que, sobre todo, han hecho mis padres. Las cartas que te da la vida no las puedes cambiar, pero si las juegas bien, puedes brindarle mejor suerte a la siguiente generación. Gracias.