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**Metacognition and Self-Awareness: Neural and
Cognitive Mechanisms in Brain Ageing and
Alzheimer's Disease**

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Statement on the use of AI-assisted language tools

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Γνῶθι σεαυτόν

« Know thyself »

*Motto inscribed on the Temple of Apollo at Delphi

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Summary

Anosognosia is well characterized in the clinical stages of Alzheimer's disease (AD), but remains comparatively under-described at the prodromal stage. This work examines how altered self-awareness may emerge along the continuum from normal ageing to AD.

We first conducted a retrospective analysis of the Wisconsin Longitudinal Study to compare participants' global memory self-ratings with spouse ratings. In individuals genetically at risk for AD, spouse assessments were the most robust predictors of subsequent cognitive decline, outperforming self-report.

These results raised a mechanistic question: which neuroanatomical systems, affected early in AD, contribute to metacognitive deficits? We therefore designed an experimental study in healthy participants to perturb Default Mode Network (DMN) connectivity by inhibiting the posterior cingulate cortex (PCC), a core DMN hub that neuroimaging studies have consistently shown to be affected early in AD. Resting-state fMRI revealed DMN desynchronization following PCC inhibition with transcranial magnetic stimulation (TMS). The same protocol was then used to assess behavioral consequences for memory performance and self-evaluation measures; stimulation spared accuracy and standard metacognitive indices but modulated confidence response times in a stimulus-dependent manner.

Overall, this thesis combines longitudinal observational evidence with a causal, network-level perturbation approach, highlighting the prognostic value of informant-based assessment and exploring how PCC/DMN mechanisms may relate to memory-related self-evaluation.

List of abbreviation

Abbreviation	Definition
A31	area 31 (posterior cingulate cortex region)
ACD	awareness of cognitive decline
AD	Alzheimer's disease
AI	Awareness Index
ANOVA	analysis of variance
APOE	apolipoprotein E
APOE4	apolipoprotein E ε4 allele
APP	amyloid precursor protein
AQ-D	Anosognosia Questionnaire for Dementia
BOLD	blood oxygenation level-dependent (BOLD) signal
CDR	Clinical Dementia Rating
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
CIBERNED	Center for Biomedical Research in Neurodegenerative Disease
CTBS	Continuous theta burst stimulation
CN	clinically normal (cognitively unimpaired)
CSF	cerebrospinal fluid
DMN	default mode network
EC	entorhinal cortex
EMG	Electromyography
FA	flip angle
FDR	false discovery rate
FDG-PET	fluorodeoxyglucose positron emission tomography
FOV	field of view
FWE	family-wise error
GLM	general linear model
HA	hyper awareness
HC	Hippocampus
JOL	judgment-of-learning
LATE	Limbic-predominant age-related TDP-43 encephalopathy
LCD	liquid crystal display
LOA	lack of awareness
MCI	mild cognitive impairment
MEPs	motor evoked potentials
MNI	Montreal Neurological Institute

Abbreviation	Definition
MP-RAGE	magnetization-prepared rapid gradient echo
NA	normal awareness
OFC	orbitofrontal cortex
PART	Primary Age-Related Tauopathy
PC/RSC	posterior cingulate / retrosplenial cortex
PCC	posterior cingulate cortex
PCN	Precuneus
PET	positron emission tomography
PI	path integration
REF	Reference
ROC	receiver operating characteristic
ROIs	regions of interest
RTs	reaction times
SAM	spouse-appraised memory
SENSE	Sensitivity Encoding (MRI acceleration technique)
SGBM	standard gradient boosting machine
SHQ	Sea Hero Quest
SMC	subjective memory complaints
SNPs	single-nucleotide polymorphisms
SNRs	signal-to-noise ratios
SS	sum of squares
TA	total acquisition time
TE	echo time
TI	inversion time
TMS	transcranial magnetic stimulation
TR	repetition time
VBM	voxel-based morphometry
WLS	Wisconsin Longitudinal Study
WM	white matter

1 Chapter 1: Introduction

1.1 Normal and pathological cognitive ageing: performance and self-evaluation

1.1.1 Deterioration or evolution of cognition across the lifespan?

The evolution of cognitive abilities across the lifespan is a major topic in neuroscience. Numerous studies have now characterized age-related changes in cognition in so-called normal ageing. The term *evolution* is used deliberately here because although ageing is indeed associated with a decline in some cognitive abilities, other domains tend to remain stable or even improve.

1.1.2 Normal cognitive ageing: expected alterations

In the scientific literature on this topic, a distinction is often drawn between so-called *crystallized* and *fluid* cognitive abilities. Crystallized abilities refer to knowledge and skills accumulated over the lifespan, such as vocabulary. Lexical knowledge typically increases over time and is considerably richer at the age of 65 than at 20 in the average healthy individual (1).

By contrast, fluid abilities, such as executive functions, reflect the capacity to process, manipulate and transform information in order to solve a given problem.

These abilities show a gradual decline across adulthood, beginning as early as 20 years of age (2).

With respect to memory, normal ageing is also associated with differential changes across memory systems. Semantic memory, that is, general knowledge about the world, tends to be maintained or even enriched with age, in line with the accumulation of experiences and learning throughout life (3). Likewise, procedural memory, which supports the acquisition and automatization of motor or cognitive skills, remains relatively preserved. In contrast, episodic memory – which underpins the encoding and retrieval of personally experienced, context-rich events – is particularly vulnerable to ageing, with increasing difficulties in forming new memories and recalling them spontaneously (4). In addition, working memory, which relies on attentional and executive resources, shows a progressive decline, reflected in a reduced capacity to maintain and simultaneously manipulate information (5). Thus, the mnemonic profile of normal ageing is not characterized by a global loss, but rather by a selective alteration of certain systems, contrasting with the relative stability, or even strengthening, of other components.

1.1.3 Age-related changes in metacognition

While the scientific literature on normal cognitive ageing contains numerous studies on quantitatively measurable cognitive abilities, such as those described above, it is

less extensive regarding metacognitive abilities, which are often more difficult to quantify.

Traditional cognitive assessment tools rely on quantitative measures such as the number of items recalled, response times, and error rates. These indicators allow performance to be standardized and objectified. However, this approach overlooks an essential aspect of cognitive functioning: the individual's capacity to judge their own performance.

Human cognition is not limited to objectively quantifiable performance. It also comprises the ability to anticipate, monitor, and evaluate one's own mental processes—that is, metacognition (6). This component enables individuals to answer questions such as: Have I understood correctly? Am I able to remember this information? Did I perform well on this task?

Metacognition plays a crucial role in cognitive self-regulation and in maintaining autonomy. In older adults, it may remain preserved, decline, or become maladaptive, sometimes independently of objective cognitive performance (7). Thus, an individual may show impaired cognitive performance while retaining good awareness of their limitations, or conversely, may markedly overestimate their abilities despite clear deficits.

1.1.4 Pathologies of cognitive ageing

Among the pathological causes of cognitive decline, neurodegenerative diseases occupy a central place. The most frequent is Alzheimer's disease, which accounts for approximately 60–70% of dementia cases (8). It initially presents with episodic memory impairment before extending to other cognitive domains. However, other neurodegenerative and neurovascular diseases can also lead to cognitive impairment.

Among the most prevalent are:

- **Vascular cognitive impairment**, secondary to multiple ischaemic or haemorrhagic lesions, which may occur abruptly, as in recurrent strokes, or more progressively in association with chronic microvascular damage (9);
- **Dementia with Lewy bodies**, combining attentional deficits, visual hallucinations and extrapyramidal signs (10);
- **Frontotemporal dementias**, characterized by prominent behavioural changes or language disorders (11);
- **Age-related cerebral degenerative processes**, including LATE (Limbic-predominant Age-related TDP-43 Encephalopathy), defined by pathological TDP-43 deposits in limbic regions (12), and PART (Primary Age-Related Tauopathy), characterized by tau protein accumulation in medial temporal structures, frequently observed in very old individuals and potentially contributing to a moderate decline in memory (13).

1.1.5 Metacognition in patients with neurodegenerative disease

In pathological contexts, metacognitive alterations have been particularly studied in patients with Alzheimer's disease. It is now well established that a certain degree of anosognosia frequently accompanies memory impairment once it is clinically manifest, reflecting a loss of awareness of cognitive deficits (14). In contrast, whether anosognosia can emerge at very early stages of the disease, when memory difficulties are still mild and sometimes difficult to objectify, remains a matter of debate and is the focus of recent investigations. The present work is situated precisely within this zone of scientific uncertainty: the study of metacognition in normal and pathological ageing, and more specifically the exploration of whether anosognosia in Alzheimer's disease is an early feature or not, with the aim of better understanding its mechanisms and clinical implications.

Investigating metacognition in normal and pathological ageing may also open up therapeutic perspectives. A more precise characterization of metacognitive dynamics can help tailor psychological support, cognitive rehabilitation interventions, and assistance for informal caregivers. The ultimate goal is to improve patients' adherence to care, promote their residual autonomy, and limit the functional and psychosocial consequences of cognitive disorders.

1.1.6 Metacognition versus anosognosia : conceptual distinction

Metacognition refers to the set of processes through which individuals monitor and control their own cognitive operations (e.g., evaluating confidence, detecting errors, predicting performance, or adjusting behaviour accordingly) (6). In experimental settings, metacognition is commonly operationalised using trial-wise confidence judgements and indices that relate subjective estimates to objective performance, including signal-detection-based approaches designed to dissociate metacognitive sensitivity from first-order performance (15,16). By contrast, anosognosia is a clinical phenomenon, classically defined as a reduced or absent awareness of one's deficits and their consequences for daily functioning, most often described in neurological and neurodegenerative conditions (14,17–19). Throughout this thesis, metacognition denotes the mechanisms supporting self-monitoring and self-regulation, typically operationalized in experimental paradigms. Anosognosia denotes the clinical expression of impaired awareness, reflecting a persistent mismatch between objective functioning, informant evidence, and self-appraisal in daily life. Conceptually, anosognosia can be framed as a disorder of self-awareness that partly reflects a breakdown of metacognitive mechanisms, particularly the ability to update self-beliefs in light of evidence and feedback, however, it is not synonymous with metacognition (20–22). Metacognitive capacities can be fractionated across domains (e.g., some monitoring may remain relatively preserved under specific task conditions, while awareness of everyday decline is

compromised), which helps explain why reduced insight may coexist with apparently intact performance monitoring in selected paradigms (16,21,23).

1.1.7 Fractionating Metacognition

Metacognition is not a unitary ability but a multidimensional set of processes that can be decomposed into:

- 1) **monitoring** (evaluating one's current or predicted performance) versus **control** (adjusting behaviour based on these evaluations).
- 2) **prospective judgements** (e.g., predicting later recall) versus **retrospective judgements** (e.g., confidence after an answer).
- 3) **local** (trial-by-trial) versus **global** (trait-like, everyday self-appraisal) levels of self-evaluation (24).

Quantitatively, experimental work further separates metacognitive sensitivity (how well confidence discriminates correct from incorrect responses) from bias (overall over-/under-confidence), with approaches such as confidence–accuracy correspondence metrics and SDT-based indices (e.g., meta-d') helping to dissociate these components from first-order task performance (15,16).

As discussed more in detail later, this fractionation is particularly useful in Alzheimer's disease: anosognosia can be conceptualised as a clinical expression of

impaired awareness that may reflect a failure to update global self-beliefs in response to evidence and feedback, even when some forms of local, task-based monitoring remain measurable under specific conditions (21,22). Mechanistically, this mismatch has been linked to disruptions in the maintenance of self-knowledge and its mnemonic foundations, supporting the idea that awareness depends on interactions between memory, self-referential processes, and metacognitive monitoring (20).

Finally, work on awareness of cognitive decline (ACD) shows that reduced awareness can emerge before the dementia stage along the AD continuum, reinforcing the notion that different metacognitive components (local confidence, global self-appraisal, belief updating, informant discrepancy) may be differentially preserved or impaired across stages and domains (25).

1.2 Alzheimer's disease

1.2.1 Epidemiology

Alzheimer's disease is a neurodegenerative disorder whose rising prevalence makes it a major public health challenge. Advances in medicine and public health have increased life expectancy and helped maintain autonomy in older adults for longer. This demographic shift contributes to a progressive inversion of the age pyramid in so-called developed countries, a trend that is also expected, in the medium term, in developing and emerging countries (26). The main risk factor for the sporadic form

of Alzheimer's disease remains ageing (27). From the age of 60, the incidence of the disease approximately doubles with each decade. Among individuals older than 85 years, around one third present with some form of dementia, in the broad sense of the term (28).

In 2018, the *World Alzheimer Report* (29) estimated that 212,000 people were living with dementia in Belgium. Based on epidemiological data indicating that Alzheimer's disease accounts for approximately 65% of dementia cases, it can be inferred that nearly 137,800 individuals were affected by this neurodegenerative disorder in Belgium in that year.

1.2.2 Neuropathology of Alzheimer's disease

Alzheimer's disease is a neurodegenerative disorder characterized by the abnormal accumulation of peptides and proteins within the central nervous system. At the brain level, these accumulations occur in both extracellular and intracellular compartments. Two major proteins have been identified as playing a central role in the neurodegenerative process: β -amyloid ($A\beta$) peptides and the tau protein (30).

The first substrate implicated in the pathophysiology of Alzheimer's disease are β -amyloid peptides, and more specifically the $A\beta_{42}$ isoform. These peptides result from the cleavage of amyloid precursor protein (APP), a transmembrane protein located in the cell membrane. Abnormal cleavage of APP, notably by γ -secretase,

leads to the production of the A β 42 isoform, whose biochemical properties promote aggregation and neurotoxicity (31).

A β peptides can be found in two main forms: a soluble form, present in the cerebrospinal fluid (CSF) and diffusely in the extracellular space, and an aggregated form organized in β -pleated sheets, a typical structure of amyloid deposits (32).

The second protein involved in the pathophysiology of Alzheimer's disease is tau. Under physiological conditions, tau plays an essential role in maintaining the neuronal cytoskeleton, particularly within axons, thereby contributing to neuronal integrity and architecture (33). In Alzheimer's disease, tau can adopt pathological, non-functional forms. At the level of post-translational modifications, so-called hyperphosphorylated forms are frequently observed. These pathological forms aggregate to form intracytoplasmic structures known as neurofibrillary tangles (34). Although the exact mechanisms by which tau aggregates induce neuronal damage are not yet fully elucidated, these tangles are thought to cause long-term neuronal injury, ultimately leading to apoptosis (35).

A causal link has long been suspected between the pathological presence of amyloid peptides forming plaques in the extracellular space of the central nervous system and the hyperphosphorylation and intracellular aggregation of tau. Although the mechanisms underlying this pathophysiological cascade are not yet completely understood, it is widely accepted that these two events represent key early steps in the development of Alzheimer's disease (36).

1.2.2.1 Sporadic and familial forms of Alzheimer's disease

The most commonly encountered form is the so-called sporadic form (26). It typically occurs in the general population from the sixth decade of life onwards. Epidemiological studies suggest that it probably results from a synergistic effect of multiple factors that may affect cognitive reserve (37). These factors are numerous and will not be reviewed exhaustively here. Among the most commonly recognized are cardiovascular risk factors, a history of repeated traumatic brain injury, educational level, diet, physical activity, and sleep quality (38).

Alzheimer's disease can also present in a so-called familial form, resulting from the transmission of genetic mutations. These forms are associated with mutations in three genes: those encoding amyloid precursor protein (APP), presenilin 1, and presenilin 2 (39). These mutations share the common consequence of promoting the formation and accumulation of β -amyloid peptides in the extracellular space. These hereditary forms follow a Mendelian mode of transmission. Clinically, carriers of these mutations are characterized by an early onset of the disease, typically around the fourth decade of life (40).

A particular form of Alzheimer's disease is observed exclusively in individuals with trisomy 21. In these patients, trisomy of chromosome 21, which contains the gene encoding amyloid precursor protein (APP), leads to increased production of A β peptides (41).

Beyond strictly hereditary forms of Alzheimer's disease, it is now well established that the sporadic form is also influenced by genetic factors. These do not take the form of classical Mendelian mutations, but rather polymorphisms with incomplete penetrance. A comprehensive description of these numerous genes lies beyond the scope of this work. However, one gene in particular stands out: the gene encoding apolipoprotein E (APOE) (42).

The importance of APOE rests on two key elements:

- First, the relatively high prevalence of its risk allele in the general population;
- Second, the fact that the presence of this allele substantially increases the risk of developing Alzheimer's disease in carriers (43).

Apolipoprotein E (APOE) exists in three main allelic forms. The $\epsilon 2$ allele is considered protective and is found in approximately 7–8% of the general population. The $\epsilon 3$ allele is neutral with respect to risk and is present in about 75–85% of individuals. Finally, the $\epsilon 4$ allele, found in roughly 15% of the population, is recognized as significantly increasing the risk of developing Alzheimer's disease (42).

Individuals who are heterozygous carriers of the $\epsilon 4$ allele of the APOE gene have a 3- to 4-fold increased risk of developing Alzheimer's disease compared with carriers of the $\epsilon 3$ allele. This risk is multiplied by approximately 12 in individuals who are

homozygous for the $\epsilon 4$ allele (43). It is also noteworthy that in patients with an early-onset form of Alzheimer's disease, the $\epsilon 4$ allele is observed up to 2.3 times more frequently than in those with late-onset forms (44).

1.2.2.2 Progression of the neurodegenerative process

Neuroimaging studies and post-mortem analyses have clearly shown that the earliest regions affected by the pathophysiological process of Alzheimer's disease are the entorhinal cortex, the hippocampus, and then midline structures such as the posterior cingulate cortex and the precuneus (45–47). Subsequently, the neurodegenerative process spreads to various associative areas, in particular parietal and frontal regions. The overall effect of these alterations is a cortico-subcortical atrophy, which is typically observed in patients with advanced stages of the disease (48).

1.2.2.3 Clinical evolution of Alzheimer's disease

Alzheimer's disease can be staged in different ways. In the following section, the terminology used is inspired by that found in the “Revised criteria for diagnosis and staging of Alzheimer's disease: Alzheimer's Association Workgroup”, published in June 2024 (49). We have chosen to adopt a staging based on the clinical evolution of the disease, as this framework will help to clarify the remainder of this work.

These stages are classically described as five in number. They will be presented chronologically: the preclinical stage, the stage of mild cognitive impairment, the early stage of major cognitive impairment, the moderate stage of major cognitive impairment, and the advanced stage of major cognitive impairment. An approximate average duration will also be discussed for each stage. It should be emphasized from the outset that these average durations are influenced by several factors, including genetic factors (e.g. APOE ϵ 4), lifestyle factors (cognitive and physical activity), and treatments. Life expectancy after diagnosis also depends on age at onset: the later the onset, the faster the progression tends to be (50).

The stages of Alzheimer's disease can be defined as follows:

- **Stage 1 - Preclinical stage**

At this stage, pathophysiological changes are already underway, but without any detectable clinical manifestation. Alterations in the profile of cerebrospinal fluid (CSF) biomarkers can already be observed, in particular an increase in tau protein concentrations (both total and hyperphosphorylated forms) and a decrease in soluble forms of β -amyloid peptides, reflecting their precipitation into amyloid plaques. Cerebral PET imaging using metabolic tracers specific for tau (THK5317, THK5351, AV-1451, PBB3) and amyloid (florbetapir [18F]AV-45, flutemetamol [18F]GE067) can already demonstrate abnormal accumulation of these

proteins in the brain at this stage. However, these pathophysiological alterations do not yet result in any detectable cognitive impairment. It is currently estimated that this stage may precede the next by 10 to 20 years (51). It is therefore conceivable that the ability to identify patients at this stage would allow early therapeutic interventions to be implemented, with the potential to modify or even prevent progression to later stages of the disease. This prospect helps explain why a substantial portion of current research focuses on this preclinical phase.

- **Stage 2 - Mild cognitive impairment**

At this stage, pathophysiological changes begin to impact the patient's cognitive abilities. Two main categories of mild cognitive impairment (MCI) are distinguished: amnesic MCI (aMCI) and non-amnesic MCI. Retrospective studies indicate that aMCI more frequently progresses to clinically overt Alzheimer's disease. Amnesic MCI is characterized by predominant impairment in the memory domain, which is affected early and severely in Alzheimer's disease. These deficits can be objectified using neuropsychological tests, but they do not yet compromise the patient's autonomy or the performance of activities of daily living. Longitudinal cohort data estimate that MCI may last on average between 2 and 5 years before conversion to dementia, with an annual progression rate between 10% and 15% (52,53). However, a non-negligible proportion of patients remain

stable over several years, or even show a reversion to normal cognitive functioning, although this phenomenon is often transient (54). These observations suggest that MCI is a heterogeneous state whose duration cannot be reduced to a single trajectory and which may be influenced by biological, environmental and behavioural factors.

- **Stage 3 – Mild dementia due to Alzheimer's disease**

The stage of mild dementia in Alzheimer's disease marks a clear break from mild cognitive impairment because of the obvious functional impact of cognitive disorders on everyday life. At this stage, cognitive deficits are no longer limited to episodic memory. They are often multidomain, also affecting language (anomia, paraphasias), executive functions (planning, cognitive flexibility), temporal orientation, and sometimes even the recognition of familiar faces (55). Behavioural disturbances may also emerge, such as apathy, irritability, anxiety symptoms or depression, although their intensity varies between individuals (56). A constant need for supervision appears for certain complex activities, notably financial management, scheduling medical appointments, or taking medications correctly (57). In contrast, simpler activities of daily living, such as personal hygiene, eating, or moving around in a familiar environment, are generally preserved, allowing some patients to continue living alone, provided they receive regular external monitoring (58). This phase is critical in the care

pathway, as it often corresponds to the moment when relatives begin to perceive the difficulties as persistent, and when support needs become structural, even if still partial. The average duration of the mild dementia stage varies across cohorts, diagnostic criteria and individual factors discussed above (age at diagnosis, comorbidities, educational level, symptomatic treatment). In longitudinal studies, this phase typically lasts between 2 and 4 years before progression to a moderate stage (59). A landmark study conducted within the « Religious Orders Study » (60) reports a mean duration of 2.9 years for the mild dementia stage, with faster trajectories in individuals who are older at the time of diagnosis.

- **Stage 4 – Moderate dementia due to Alzheimer’s disease**

At the moderate stage, patients present with marked memory disorders and multiple additional cognitive deficits, notably in language, planning abilities, and executive functions. Behavioural disturbances are also common. This multidomain impairment leads to increasing dependency, requiring supervision for most activities of daily living, including the simplest ones. Living alone is no longer possible, although living with a partner may still be feasible if behavioural disturbances are not too severe. Based on data from the Washington Heights–Inwood Columbia Aging Project (61), the average duration of the moderate phase is estimated at around 3 years before progression to severe dementia. Clinical heterogeneity

nonetheless remains substantial: some patients progress slowly, whereas others show rapid decline, in relation to age, medical comorbidities, or the lack of cognitive and social stimulation. A study by Mitchell et al. (62) confirms that functional trajectories during this phase predict progression to advanced stages, particularly when communicative and motor abilities begin to deteriorate. Thus, although the moderate stage is characterized by increasing dependency, it also represents a pivotal period during which non-pharmacological interventions (cognitive stimulation, environmental adaptations, support for caregivers) can still significantly improve quality of life and slow functional decline.

- **Stage 5 – Severe dementia due to Alzheimer’s disease**

At this advanced stage, cognitive disorders are generalized, with profound deficits in memory, language, executive functions, and severe spatio-temporal disorientation. Behavioural disturbances are frequent and have a major impact on the quality of life of both patients and caregivers. Individuals at this stage require constant supervision and are often institutionalized due to the severity of their cognitive deficits. The average duration of the severe dementia stage in Alzheimer’s disease is generally short, ranging from 1.5 to 3 years depending on the study, although this estimate varies according to age, sex, medical comorbidities, and care setting. This terminal phase is characterized by an almost complete loss of

autonomy, marked motor decline, and profound impairment of communication abilities. Patients often become bedridden, mute, incontinent, and totally dependent for personal care. According to Xie et al. (63), the mean life expectancy after entering the severe stage is 1.9 years, with a median of 1.3 years, reflecting high vulnerability to medical complications (respiratory infections, swallowing disorders, malnutrition). A study by Mitchell et al. (62) conducted in nursing homes indicates that 6-month survival after the onset of severe dementia associated with major functional impairment is below 50%, particularly in the presence of acute comorbidities (fever, pneumonia, rapid weight loss). Entry into this stage is therefore associated with increased morbidity and a substantial burden on caregivers, justifying the implementation of palliative care focused on the patient's comfort.

1.2.3 Cognitive domains affected in Alzheimer's disease

Depending on the stage of the disease, patients with Alzheimer's disease present with varying patterns of cognitive impairment. Memory deficits are generally the earliest symptoms and constitute the main cognitive manifestation in most cases (64).

As the disease progresses, these deficits gradually extend to other cognitive domains, eventually leading to an almost generalized impairment in advanced stages (65).

A comprehensive description of all cognitive disorders lies beyond the scope of this work. Therefore, the focus will be placed primarily on memory impairment and on anosognosia, which frequently accompanies it and represents the central axis of the present work (66).

1.2.3.1 Memory impairments

It is more appropriate to speak of multiple memory systems rather than of “memory” in the singular, as memory is classically divided into two broad categories: short-term memory and long-term memory (67). The present work focuses on long-term memory, which is commonly subdivided into two major subtypes: non-declarative and declarative memory (68). Non-declarative memory primarily encompasses procedural abilities, whereas declarative memory includes episodic (or autobiographical) memory and semantic memory. Episodic memory supports the storage of personally experienced life events, including not only the nature of the event itself but also their temporal and spatial context. Semantic memory, by contrast, contains general knowledge acquired over the lifespan, independently of the learning context—for example, knowing that Brussels is the capital of Belgium, regardless of where or when this information was acquired (28).

In Alzheimer's disease, episodic memory is the earliest and most severely affected memory system. Its impairment typically follows a gradient in which the most recent memories are lost before more remote ones, a phenomenon known as Ribot's law (69).

1.2.3.2 Other cognitive domains

As the disease progresses, other cognitive functions are also affected. The pathophysiological process extends to various associative areas of the cerebral cortex, leading to the gradual emergence of executive deficits (planning, cognitive flexibility), language disturbances (reduced vocabulary, paraphasias), and behavioural changes (abulia, depression, disinhibition, disruption of the sleep–wake cycle) (57).

The chronological sequence of involvement of these different cognitive functions reflects the anatomical progression of the lesions. Episodic memory is particularly vulnerable because it depends on the hippocampus, a structure that is initially affected by the pathological accumulation of hyperphosphorylated tau protein (70).

1.2.4 Anosognosia

1.2.4.1 Definition

Anosognosia is a symptom frequently observed in patients with Alzheimer's disease. From an etymological perspective, the term *anosognosia* is composed of three Greek elements: the privative prefix *a-*, *noso-* meaning "disease", and *-gnosis* referring to knowledge. Anosognosia can therefore be defined as an individual's inability to recognize their own illness or its consequences on autonomy and functional abilities (18).

The term *anosognosia* was first introduced by Joseph Babinski in 1914, in a communication to the *Société de Neurologie de Paris*. He described a phenomenon observed in some patients with hemiplegia who appeared unaware of their deficit despite manifest motor impairment. Babinski used the term "anosognosia" to designate this lack of awareness of the disorder, which he distinguished from a primary disturbance of perception or language (17). This seminal observation marked the beginning of the systematic study of awareness of neurological deficits and was later extended to other conditions, including dementias, in which anosognosia is particularly frequent.

It is essential to distinguish anosognosia from denial, even though these two phenomena may sometimes present similarly. Anosognosia is classically considered a neurocognitive disorder linked to brain damage. It is involuntary and unconscious: the patient does not recognize their deficits because the cerebral mechanisms

underlying self-awareness and self-evaluation (metacognition) are impaired, and the self-monitoring process remains defective.

In our view, the most decisive distinction between denial and anosognosia lies in their underlying causes. Denial is conceptualized as a subconscious psychological process and is not secondary to a structural or functional brain lesion. By contrast, anosognosia, understood as an unconscious failure of awareness, is always secondary to dysfunction in specific brain regions. Consequently, different types of anosognosia have been described, depending on the deficit involved and the brain regions affected:

- **Anosognosia in hemiplegia**, in which the patient fails to acknowledge their motor deficit. It is most often secondary to right-hemisphere lesions involving, in particular, the parietal and premotor cortices, the insula, and the cingulate cortex (19).
- **Anosognosia in cortical blindness (Anton–Babinski syndrome)**, in which the patient, despite bilateral occipital damage causing complete blindness, does not recognize their visual loss and may even produce erroneous visual descriptions. This type of anosognosia illustrates the role of primary and associative visual cortices in perceptual awareness (71,72).
- **Anosognosia for memory impairment**, frequently observed in mild forms of Alzheimer’s disease, in which the patient does not perceive their memory deficits. It is thought to be associated with alterations of the prefrontal cortex

(superior frontal gyrus), posterior cingulate cortex, temporoparietal regions, and medial temporal structures (14).

Denial, by contrast, is regarded as a psychological defense mechanism. It is a largely subconscious, active attitude whereby the individual refuses to acknowledge a reality perceived as threatening or intolerable, often in relation to emotional or existential issues. Denial may therefore be transient, fluctuating, and sensitive to the emotional context (73), whereas anosognosia is generally stable, persistent, and linked to a specific brain lesion. This distinction is crucial both diagnostically and therapeutically, as rehabilitation strategies differ according to the origin of the lack of awareness: anosognosia, arising from unconscious neurocognitive processes, calls for interventions tailored to a brain-based deficit, whereas denial, related to subconscious psychological processes, requires psychotherapeutic work aimed at facilitating acceptance of reality.

1.2.4.2 Epidemiology

Starkstein et al. (2014) describe a variability in the prevalence of anosognosia in Alzheimer's disease. It has been estimated to range between 20% and 80% across studies. This variability reported across studies may be explained by the stage at which patients are assessed, the method used to quantify it, and the clinical setting (14). More recent work, including that of Cacciamani et al. (2021) (25), suggests that

the prevalence of anosognosia in Alzheimer's disease ranges from 40% to 91%. This wide variability can be explained primarily by two factors. First, there is substantial heterogeneity in the methods used to diagnose or quantify anosognosia, such as self- and informant-report scales, discrepancy methods comparing patient and caregiver ratings, and direct clinical assessments (as will be detailed later in this thesis). Second, the characteristics of the studied samples also contribute to this variation: the frequency of anosognosia differs markedly according to the stage of Alzheimer's disease and is generally more easily detected once cognitive impairment reaches a level at which its consequences on daily functioning become clearly observable.

1.2.4.3 Functional implications and public health impact

According to data published by Sciensano in 2019, dementias (of all causes combined) shared, together with coronary heart disease, the top position as the leading cause of mortality in Belgium, each accounting for approximately 9% of all deaths recorded that year. Among neurodegenerative disorders, Alzheimer's disease remains by far the most frequent. As previously mentioned, a substantial proportion of patients will develop anosognosia, to varying degrees, over the course of the disease.

At first glance, this symptom might appear to have a protective effect for the patient. Indeed, it has been shown that individuals with anosognosia obtain lower scores on scales assessing depressive symptoms (74). However, this indifference, or even lack

of awareness of their deficits, is in fact detrimental. Anosognosia frequently contributes to delayed diagnosis: because patients are unaware of their difficulties, they do not perceive the need to seek medical attention. Responsibility for raising concern and encouraging consultation therefore often falls to relatives. In a context of increasing loneliness and social isolation, which affects a non-negligible proportion of older adults, this step may be severely compromised, further postponing the time of diagnosis.

Anosognosia may also lead patients to engage in risky behaviours, such as driving, despite unrecognized cognitive limitations. More broadly, it represents a major obstacle to the early implementation of appropriate care, as well as to the patient's adherence to treatment and therapeutic recommendations.

It is now widely acknowledged in the literature that the only genuinely promising therapeutic strategy relies on intervening as early as possible in the course of the disease. In this regard, most ongoing clinical trials, particularly those evaluating therapies targeting amyloid or tau pathology, recruit participants in the preclinical stages of Alzheimer's disease (75–77).

1.2.4.4 Diagnostic methods for anosognosia

Three main approaches are currently recognized for the assessment and detection of anosognosia in patients with Alzheimer's disease. These methods share a common

principle: the use of discrepancy indices that quantify the gap between the patient's self-evaluation of their cognitive or functional abilities and a more objective evaluation. The latter can take different forms, as detailed below.

The first method consists in computing a discrepancy index between the patient's self-evaluation and their actual performance on a standardized cognitive test, such as a memory test. This approach makes it possible to estimate the gap between the subjective perception of cognitive abilities and their objective expression (18).

The second method is based on a comparison between the patient's self-evaluation and the clinical assessment made by an experienced professional, generally through a semi-structured interview. This approach relies on the clinician's expertise to judge whether the patient's discourse is concordant or not with their actual clinical condition (78).

Finally, the third approach compares the patient's subjective ratings to those provided by an informal caregiver (spouse, child, close friend) regarding the patient's ability to carry out activities of daily living. This informant-based method is frequently used in research on anosognosia because of its ease of implementation (79).

Among these methods, the two approaches that show the strongest correlation in clinical studies are: the comparison between the patient's self-evaluation and the caregiver's evaluation, and the semi-structured interview conducted by an experienced clinician (14). These approaches, which are more contextualized and

closer to real-life situations, appear to be more sensitive to subtle manifestations of anosognosia than discrepancy indices derived from standardized cognitive tests, the interpretation of which may be biased by the patient's lack of knowledge about the expected criteria for success.

With regard to the first method (based on cognitive performance), an important limitation must be emphasized: depending on the difficulty of the cognitive task, its sensitivity may be restricted by the ceiling effect inherent to many neuropsychological tests. In other words, when cognitive impairment is very subtle, a patient may easily perform within the normal range on tests yet still present a metacognitive deficit that remains undetected. Thus, in cases of mild cognitive impairment, a patient may suffer from anosognosia without this being reflected in a marked overestimation of their abilities on objective measures. This methodological constraint limits the relevance of this approach in the early stages of the disease.

1.2.4.5 Cultural context and symptom reporting in cognitive decline

The recognition, interpretation, and reporting of cognitive symptoms are shaped by cultural norms, health literacy, stigma, language, and family roles, with downstream effects on help-seeking behaviours, access pathways, and the timing of referral to memory services (80,81). Ethno-cultural disparities in dementia reflect a complex interaction between biological risk factors and sociocultural determinants, and

highlight that cultural perceptions of what constitutes “normal aging,” limited access to care, and issues of trust between minority communities and healthcare systems can delay diagnosis and treatment. They also stress that widely used cognitive screening tools may introduce bias across groups, and discuss practical mitigation strategies such as accounting for literacy (rather than education alone) and considering longitudinal change rather than single cut-off scores (81). Importantly, the same sociocultural factors that influence patient self-report may also affect caregiver reporting; e.g., varying thresholds for noticing or disclosing change, preferences for family-centred care, or reluctance to medicalise symptoms, thereby modulating patient–informant discrepancy measures that are often used to index awareness ((80,81). In a Belgian memory-clinic context, Lebrun et al. (82) further show that CSF biomarker profiles and their age-related dynamics can differ between immigrant and native patients (including ethnic effects on T-tau and the T-tau/A β 42 ratio, and an ethnicity-by-age pattern for A β 42), underscoring that migratory/sociocultural context may intersect with diagnostic trajectories and case-mix. Taken together, these findings support interpreting discrepancy-based indices within their sociocultural context, and argue for systematic inclusion of an informant whenever possible, use of structured tools, and explicit consideration of contextual factors (language barriers, health literacy, stigma, caregiver burden, and trust) when evaluating complaint, awareness, and discordance.

1.2.5 Review of the literature

In this chapter, we discuss the evolution of theoretical and empirical models proposed to explain the emergence of anosognosia in Alzheimer's disease (AD), following a chronological logic that highlights the progressive development of scientific knowledge on this topic. This critical analysis is grounded in a selection of articles identified through a systematic literature review conducted at the beginning of the doctoral work (2017), and therefore reflects the evidence available at that time. For this review, we used the MeSH terms “anosognosia”, “neural correlate”, and “Alzheimer”. This search yielded an initial set of 11 articles, of which 3 were excluded for lack of relevance (no study of anosognosia or exclusive focus on other dementias). Thus, 8 articles were retained.

Below, we provide a critical synthesis of several of these studies, organized according to their chronological order of publication, in order to illustrate the gradual evolution of explanatory models of anosognosia and of the techniques used to investigate its underlying mechanisms.

A first fundamental theory was proposed by McGlynn and Schacter (1989) with the DICE model (Dissociable Interactions and Conscious Experience) (83). This model postulates that anosognosia results from a dysfunction of metacognitive processes of an executive nature. The authors suggest the existence of a subsystem, termed the “conscious awareness system” (CAM), responsible for comparing the individual's current abilities with a database derived from autobiographical memory, which

contains the history of past performances. This comparison would normally allow self-representations to be updated as new information is encoded. If this system is altered or disconnected from other key cognitive structures, notably autobiographical memory, its role as a central comparator would be compromised, leading to the patient's inability to recognize their deficits and, ultimately, to the emergence of anosognosia.

This theoretical foundation was subsequently elaborated in empirical work, such as that of Gil et al. (2001), who examined self-awareness from a broader perspective (84). By exploring multiple dimensions of self-awareness, from identity to morality, including introspection, the study shows that reflective self-awareness in AD is heterogeneously affected, with some components (such as anosognosia and moral judgments) being more impaired than others (such as identity or body representation). Gil et al. (2001) provide an in-depth neuropsychological analysis of self-consciousness (SC) in AD, based on a multidimensional and ecological assessment of this capacity. The originality of this study lies in its broad conception of self-awareness, considered not only as awareness of cognitive deficits (anosognosia), but also as awareness of identity, affective states, body representation, prospective memory, moral judgment, and introspective abilities. Using a 14-item structured questionnaire administered to 45 patients with mild to moderate AD, the authors demonstrate that the different dimensions of self-consciousness are heterogeneously affected: some are relatively preserved (identity,

body representation), whereas others are more severely impaired (anosognosia, moral judgments, prospective memory).

The study reveals a significant correlation between the severity of global cognitive impairment assessed by the Mini-Mental State Examination (MMSE) and self-awareness, particularly for items related to anosognosia and moral judgment. By contrast, other dimensions such as affective state, future memory, or introspective ability do not show a significant relationship with dementia severity. These findings indicate that impairment of self-consciousness cannot be reduced to a simple function of global cognitive decline, and likely relies on distinct neural networks involving multimodal and supramodal association cortices, notably dorsolateral and rostral frontal regions. The methodology, based on standardized assessment with inter-rater and test–retest reliability checks, confers good reliability to the data. The clinical sample, though modest, is relatively homogeneous, and the statistical analyses (correlations, comparisons with Bonferroni corrections) are appropriate. However, the absence of a control group and the limited discussion of the functional implications of these deficits for patients' everyday life constitute noteworthy limitations. Moreover, the relationship between self-consciousness scores and other executive functions, although suggested by the correlation with a frontal score, remains to be further explored.

Nonetheless, this study has the merit of situating anosognosia within a broader conceptual framework of reflective self-awareness, emphasizing the complexity and

fragmentation of self-referential processes in AD. In doing so, it contributes to essential reflections on the cognitive and neurobiological mechanisms of self-consciousness, and paves the way for more fine-grained clinical approaches to the assessment of awareness disturbances in neurodegenerative diseases. It can be seen as extending the DICE model by providing preliminary behavioural support for the notion of fractionation within self-awareness processes.

In this line, the study by Salmon et al. (2006) represents an important step in the objective identification of cerebral correlates of anosognosia through fluorodeoxyglucose positron emission tomography (FDG-PET) (85). Analysis of a large patient sample allowed the authors to dissociate two circuits potentially involved in anosognosia: one orbitofrontal–temporal circuit, centred on subjective evaluation of deficits, and one temporoparietal circuit, involved in adopting an external perspective.

This large-scale study, conducted in 209 patients with mild to moderate AD recruited within the European NEST-DD (Network for Efficiency and Standardization of Dementia Diagnosis) program, deepened the understanding of the neural correlates of anosognosia. Its originality lies in the cross-evaluation of awareness of deficits using a structured questionnaire completed by both the patient and an informal caregiver, covering thirteen cognitive domains (memory, attention, language, executive functions, etc.). Two distinct indices of anosognosia were derived: a patient self-rating score (reflecting impaired self-judgment of cognitive abilities) and

a discrepancy score between patient and caregiver ratings (considered an index of unawareness of others' perspectives).

Multiple regression analyses showed that patients with poorer awareness of their deficits exhibited hypometabolism in orbitofrontal regions (particularly ventromedial) and in the right parahippocampal region. These findings suggest that self-evaluation requires both updating of internal representations (orbitofrontal function) and mnemonic integration of past experiences (hippocampal function). Joint dysfunction of these regions may prevent patients from inhibiting outdated, irrelevant self-representations or adjusting their judgments to their current situation.

Furthermore, the discrepancy score, reflecting differences in perception between patients and their relatives, was associated with bilateral hypometabolism at the temporoparietal junctions and in inferior temporal cortices. These regions are known to play a role in self-referential processing and perspective taking (86,87), suggesting that anosognosia may reflect an impairment in the ability to adopt an external viewpoint on oneself, that is, to evaluate oneself as a third party would. This third-person metacognitive perspective is crucial for reflective and social self-awareness.

The strength of this study lies in its large sample size, the use of metabolic imaging, and the careful control of potential confounders (memory performance, dementia severity, apathy, etc.). However, interpretation is limited by the cross-sectional design, which does not allow inference about the temporal evolution of anosognosia.

Overall, the study supports the involvement of two partially dissociable neural circuits in anosognosia:

- a medial orbitofronto–temporal circuit associated with subjective evaluation of deficits;
- a temporoparietal circuit involved in the ability to confront one’s own point of view with that of others.

This distinction echoes earlier hypotheses suggesting that anosognosia may result both from a failure of self-evaluation processes and from a deficit in adopting a third-person perspective on oneself.

In her 2007 literature review, Souchay proposed an original, theoretically grounded approach to memory-related anosognosia in AD, drawing on the conceptual framework of metamemory (88). In contrast to most studies that address anosognosia from a purely neuropsychological or clinical angle, this review argues that the metamemory paradigm and in particular the Nelson and Narens (1994) model (6), provides a particularly suitable tool for assessing mnemonic awareness in AD. By articulating theoretical models of anosognosia (DICE, CAM) with empirical measures derived from metamemory tasks (judgments of knowledge, confidence, learning), the author highlights a fractionated profile of metacognitive abilities in patients with AD.

One major contribution of this work is the demonstration that patients can, under certain conditions, show relatively preserved monitoring of their memory

performance, especially for semantic tasks, while exhibiting marked deficits when evaluating recent episodic memory. This dissociation, which also appears in feeling-of-knowing (FOK) and judgment-of-learning (JOL) paradigms, suggests that anosognosia does not reflect a global absence of awareness, but rather a selective alteration of self-referential processes requiring autonoetic consciousness, itself compromised in AD. Detailed analysis of monitoring and control mechanisms, as well as of cognitive biases such as the “midpoint anchoring” effect, enriches our understanding of the predictive errors made by patients.

Methodologically, the article rightly emphasizes the limitations of approaches based solely on judgment calibration (overestimation/lack of awareness) and advocates the use of relative accuracy indices (such as Gamma correlations) as more fine-grained measures of mnemonic awareness. The review also discusses neural correlates of these mechanisms, drawing on neuroimaging data and frontotemporal disconnection models, thereby contributing to a closer link between the functional disintegration of memory/consciousness networks and the metacognitive deficits observed in AD.

However, some limitations must be acknowledged. The approach remains primarily theoretical, and the empirical data are drawn from heterogeneous studies with sometimes small samples. Moreover, although the article proposes an innovative reading of anosognosia, the clinical and rehabilitative implications of this conceptualization are only briefly outlined.

In sum, this review enriches the debate by offering a nuanced perspective on patients' predictive errors, while emphasizing that certain forms of metacognition, such as the feeling of knowing, may remain partially intact.

These theoretical reflections paved the way for more integrative models, such as the “petrified self” hypothesis proposed by Mograbi et al. (2009) (89). The work of McGlynn and Schacter probably laid the theoretical groundwork that later led Mograbi and colleagues to formulate their “petrified self” theory in 2009. In our view, this is one of the most influential models for explaining the neuropsychological mechanisms underlying the emergence of anosognosia in patients with AD (89) . According to this hypothesis, the sense of self is closely dependent on autobiographical memory. Since autobiographical memory is affected early in AD, patients become unable to update their self-image in light of recent events. As a result, their self-representation remains “frozen” at a premorbid level and no longer reflects their actual cognitive status, leading to a lack of awareness of accumulated deficits.

In their literature review, Mograbi, Brown and Morris integrate data from neuroscience and neuropsychology (including theories of the self) to propose an original model of anosognosia. They build in particular on the *autobiographical self model* (90) and the *Self Memory System* (91), to emphasize the importance of personal memories in constructing a sense of continuity of one's identity. Patients with AD, due to their memory deficits, cannot integrate new information about their

current state, whereas according to Ribot's law (69), older, especially semantic, memories tend to remain relatively preserved. Self-evaluation in this context therefore relies on an outdated personal database, leading to underestimation of current cognitive difficulties.

The *petrified self* hypothesis is also supported by experimental and clinical data, notably several case studies (92,93). In these reports, patients evaluate their personality in a way that remains consistent with their premorbid past, without taking recent changes into account. This pattern led the authors to suggest, in their own words, the existence of a subsystem within semantic memory specifically devoted to “*self-image*”.

Thus, the *petrified self* model offers a neurocognitive account of anosognosia in Alzheimer's disease, centered on the interaction between autobiographical memory, self-awareness, and the capacity to update the self. It provides an integrative framework for understanding how the progressive deterioration of memory systems leads to a distorted self-evaluation, despite the apparent preservation of a personal identity frozen at its premorbid state. This model is grounded in case studies and empirical data, and articulates concepts from cognitive neuroscience with models such as the *Self-Memory System*.

This model finds a direct echo in the multimodal study by Perrotin et al. (2015), which provides a neurofunctional demonstration of a disconnection syndrome between self-referential and memory networks (94). Combining fluorodeoxyglucose

positron emission tomography (FDG-PET) and resting-state functional MRI, this study aimed to characterize the cerebral alterations underlying unawareness of memory deficits in patients with Alzheimer's disease. To this end, the authors computed an anosognosia index based on the discrepancy between objective memory performance and subjective self-evaluation in 23 patients with probable Alzheimer's disease and 30 healthy controls.

The results showed that patients significantly underestimated their memory deficits. At the neurofunctional level, anosognosia was associated with hypometabolism in the medial orbitofrontal cortex (OFC) and the posterior cingulate cortex (PCC), two regions of the cortical midline network thought to be critically involved in self-referential processing and self-awareness. More innovatively, the study showed that anosognosia was also related to reduced intrinsic functional connectivity between these regions and medial temporal lobe structures, particularly the hippocampus.

These findings suggest that loss of awareness of memory impairment in Alzheimer's disease is not only linked to local alterations, but also to disrupted functional communication between networks involved in memory and those involved in self-processing. The authors therefore proposed a disconnection syndrome between the self-referential network (notably OFC and PCC) and the memory network (including the hippocampus), compromising comparison processes between current performance and internal representations of cognitive functioning. This disconnection is thought to impair monitoring of ongoing mnemonic information

and to prevent its integration into the personal knowledge base, ultimately leading to reduced awareness of cognitive deficits.

The major strength of this study lies in its multimodal methodology, combining metabolic data and functional connectivity measures, which allows anosognosia to be conceptualized as a dynamic phenomenon affecting large, interconnected circuits. Nonetheless, several limitations must be noted. First, connectivity alterations were assessed only at the functional level, not at the structural level: for example, white-matter tract integrity was not examined using structural imaging techniques. Second, a potential weakness of the work lies in the anosognosia index itself, which is based on standardized scores that may lack ecological validity. Despite these limitations, the findings strengthen the view that anosognosia in Alzheimer's disease can be understood as the consequence of an integrated dysfunction of neural networks supporting episodic memory and self-awareness.

Overall, the authors show that anosognosia is related not only to hypometabolism in medial regions (OFC, PCC), but also to altered functional connectivity between these regions and the hippocampus. This dissociation between mnemonic content and self-processing prevents effective integration of current performance into the personal knowledge base.

In line with this approach, the study by Vannini et al. (95) examined the same processes in a prodromal population with amnesic mild cognitive impairment (aMCI). Using a multimodal design combining molecular imaging (FDG-PET) and

resting-state functional MRI, the authors investigated metabolic alterations and connectivity dysfunctions associated with reduced awareness of memory impairment. The methodology, which included a large reference cohort (251 cognitively normal controls) and a quantitative anosognosia index based on the discrepancy between objective performance and subjective judgments, provides substantial robustness to the study.

The results revealed a significant reduction in cerebral metabolism in the precuneus and right hippocampus among patients with more pronounced anosognosia. In addition, reduced functional connectivity was observed between the precuneus and key regions of the self-referential network, including the orbitofrontal cortex, posterior cingulate cortex, and inferior parietal lobes. These data support the hypothesis that anosognosia results from a joint alteration of self-referential processing and episodic memory. This perspective is consistent with previous work on the role of the default mode network (DMN) in self-awareness and metamemory processes.

However, several limitations must be emphasized. First, the cross-sectional nature of the study precludes any firm conclusions about the predictive value of the observed alterations. Second, the aMCI group is relatively small ($n = 31$), which may limit the generalizability of the findings, particularly for voxel-wise analyses. The absence of a comparison group of patients with Alzheimer's dementia also restricts the evolutionary scope of the conclusions.

Despite these limitations, the study offers interesting avenues for the identification of functional biomarkers of cognitive awareness and opens the way to longitudinal research aimed at clarifying the mechanisms underlying the progression of anosognosia in Alzheimer's disease. The results tend to confirm that anosognosia is accompanied by metabolic alterations and functional disconnection between the precuneus and frontal and parietal regions of the DMN. This work reinforces the hypothesis of a coordinated dysfunction between episodic memory and self-awareness and suggests that these mechanisms are already at play in the earliest stages of neurodegeneration.

Finally, Fujimoto et al. (2017) provide complementary insights by focusing on the structural aspects of anosognosia using voxel-based morphometry (VBM) in patients with mild Alzheimer's disease (96). This study examined the structural neural bases of anosognosia for memory impairment in mild AD using MRI and voxel-wise morphometric analysis. In contrast to many previous studies that relied mainly on functional or metabolic approaches, this work is distinguished by its exclusive focus on the anatomical correlates of unawareness of memory deficits. The results indicate a significant correlation between anosognosia and reduced grey matter volume in the left superior frontal gyrus, a region not traditionally considered among the earliest affected in Alzheimer's disease, such as the hippocampus or medial temporal cortex.

Methodologically, the study used a homogeneous sample of 49 patients who were not receiving any medication likely to affect cognitive functioning. Anosognosia was

quantified by the discrepancy between patients' and caregivers' ratings on a validated questionnaire. Statistical analyses took into account several relevant covariates, including age, educational level, and total intracranial volume. The inclusion of depression as an additional covariate in complementary analyses further strengthens the robustness of the conclusions.

However, several limitations must be considered. The cross-sectional and retrospective design does not allow causal or longitudinal relationships between brain atrophy and anosognosia to be inferred. Moreover, the assessment is restricted to anosognosia for memory impairment and does not address other domains such as behaviour or activities of daily living, which limits the functional implications of the proposed model. In addition, although the superior frontal gyrus is identified as a key region, the discussion remains cautious regarding its exact role in self-evaluation processes, referring in particular to its possible involvement in the default mode network and introspective functions.

This study nonetheless has the merit of drawing attention to a brain region that has been relatively neglected in the context of memory-related anosognosia and supports the hypothesis that awareness of deficits in Alzheimer's disease involves neural networks that are at least partly distinct from those underlying the cognitive deficits themselves. It thus opens up interesting perspectives for the identification of structural biomarkers of anosognosia, particularly at early stages of the disease.

Chapter 1

Reference	Year	Model / Theory	Study population	Methodology	Main findings	Limitations
McGlynn & Schacter	1989	DICE model (Conscious Awareness System)	N/A (theoretical)	Conceptual, neuropsychological analysis	Anosognosia suspected to result from dysfunction of a central comparator linking autobiographical memory with current abilities.	Theoretical, no empirical validation.
Gil et al.	2001	Reflective self / self-consciousness	45 patients with mild to moderate AD	14-item structured questionnaire	Anosognosia and moral judgments more impaired than identity and body representation; correlation with MMSE.	Small sample, no control group.
Salmon et al.	2006	Neural correlates of anosognosia	209 patients with mild to moderate AD	FDG-PET, patient/caregiver questionnaires	Orbitofrontal and parahippocampal hypometabolism associated with impaired self-evaluation; temporoparietal circuit involved in adopting an external perspective.	Cross-sectional study, causality not established.
Souchay	2007	Metamemory	Literature review, AD	Analysis of FOK and JOL paradigms	Selective deficit of episodic monitoring despite relatively preserved semantic monitoring.	Heterogeneous data, clinical implications only briefly outlined.
Mograbi et al.	2009	“Petrified self” model	N/A (theoretical)	Synthesis of neuropsychology and neuroscience	Impaired autobiographical memory prevents updating of the self, generating anosognosia.	Theoretical, need for empirical validation.
Perrotin et al.	2015	Disconnection between memory and self networks	23 AD patients, 30 healthy controls	FDG-PET + resting-state fMRI	Hypometabolism in OFC/PCC and reduced connectivity with the hippocampus; indicators of functional disconnection.	Limited sample size, standardized anosognosia score.
Vannini et al.	2017	Prodromal anosognosia (aMCI)	31 aMCI patients, 251 controls	FDG-PET + resting-state fMRI	Metabolic alterations and DMN connectivity changes associated with memory-related anosognosia.	Cross-sectional; small aMCI cohort; no direct comparison with AD.
Fujimoto et al.	2017	Structural correlates	49 patients with mild AD	MRI voxel-based morphometry (VBM)	Reduced grey matter volume in the left superior frontal gyrus correlated with memory-related anosognosia.	Cross-sectional study; assessment limited to memory impairment.

1.2.6 Default mode network, precuneus, posterior cingulate cortex, and the self

The default mode network (DMN) comprises several brain regions that serve as key nodes within the network, including the medial prefrontal cortex (mPFC) and the posterior medial cortex, particularly the posterior cingulate cortex (PCC) and precuneus, as well as the inferior parietal lobule/angular gyrus and lateral temporal cortex (97). The importance of this network in self-representation is supported by neurodevelopmental studies showing a correlation between the maturation of these regions (in particular the PCC and PCN) and the development of the narrative self (98).

The PCN and PCC are implicated in multiple cognitive functions. Both structures are activated within the DMN when individuals are not engaged in tasks requiring externally directed attention (99), and when they allow their thoughts to wander freely. The DMN is also recruited when individuals are explicitly asked to think about themselves or to recall autobiographical memories (100).

These observations suggest that the PCC and PCN subserve at least two major functions: first, the ability to orient attention towards the internal rather than the external world; second, the construction of a self-image through autobiographical memory, as captured by the concept of the narrative self.

The PCC can be divided into two subregions: dorsal PCC (dPCC) and ventral PCC (vPCC). The vPCC appears to be involved in introspective reflective processes, whereas the dPCC may play a regulatory role, facilitating transitions between neural networks involved in external attention and those supporting internal attention (101).

Similarly, the PCN can be subdivided into dorsal (dPCN) and ventral (vPCN) precuneus. It has been shown that the vPCN exhibits strong functional connectivity with the DMN, unlike the dPCN, which is not considered part of this network. Indeed, fMRI studies have demonstrated a distinct pattern of functional connectivity with the DMN for the vPCN, but not for the dPCN (102).

Several studies have highlighted the involvement of the PCN in self-consciousness, the processing of self-relevant autobiographical information, and reflective self-awareness. The PCN thus emerges as a central region in the construction of self-image. This role may be explained by its function as a hub within the DMN, supporting both mental imagery strategies and the retrieval of self-related episodic memories (103).

If the PCC/PCN complex and the DMN play a central role in self-representation, it can be hypothesized that, in Alzheimer's disease, dysfunction of this network may compromise patients' ability to evaluate their own cognitive and functional performance and thus contribute to the emergence of Alzheimer-specific anosognosia.

1.2.6.1 Anosognosia, self-awareness and autobiographical memory

As discussed above, anosognosia leads patients to hold a distorted perception of their functional abilities. More generally, this phenomenon reflects an alteration in the way patients represent themselves, a notion commonly referred to in the English-language literature as the *self*.

Within this conceptual framework, a distinction is often drawn between two forms of self: the *minimal self* and the *narrative* or *extended self*. The minimal self corresponds to the immediate awareness of ongoing sensory experiences, independent of any reflective or memory-based elaboration (104). For example, a newborn exposed to sensory stimuli is able to perceive and react to them, without yet possessing a consolidated representation of themselves. This phenomenon is particularly evident in the mirror self-recognition paradigm, where infants do not recognize their own reflection as a representation of themselves (105).

By contrast, the narrative self is constructed on the basis of autobiographical memories and enables the formation of a coherent self-image over time. This form of self thus depends directly on the integrity of autobiographical memory, which is possibly compromised at an early stage in Alzheimer's disease.

A close theoretical link can therefore be envisaged between impairment of the narrative self and anosognosia in Alzheimer's disease. As suggested by several neurocognitive hypotheses (20), anosognosia may result from a failure of the

mechanisms responsible for updating autobiographical memory, in relation to the encoding and consolidation deficits that are characteristic of the disease.

1.2.6.2 Default mode network and Alzheimer's disease

Dysfunction of the default mode network (DMN) has been consistently demonstrated in patients with Alzheimer's disease (AD). Functional MRI studies have shown that the activity pattern of the DMN in these patients differs significantly from that observed in healthy older adults (106). In addition, early involvement of the posterior cingulate cortex (PCC) and the precuneus (PCN), two key DMN nodes, has been documented in the course of Alzheimer's disease (107). Correlational studies suggest that lesions affecting these regions contribute to the clinical expression of anosognosia in patients with AD (95).

After the hippocampal regions, the PCC and adjacent PCN are among the areas secondarily affected by the pathophysiological process of Alzheimer's disease (27). Indeed, the earliest abnormal deposits of tau protein are located in the hippocampal and peri-hippocampal regions, before spreading to the PCC, probably because of the dense axonal connections linking these structures (108,109). This pathophysiological process leads to a reduction in functional connectivity within the DMN, altering interactions between its various nodes, as demonstrated by several functional MRI studies (110,111)

1.2.6.3 Discrepancy between scientific literature and clinical experience

As discussed above, anosognosia was considered a symptom that predominantly characterizes the dementia stages of Alzheimer's disease, an assumption based on numerous studies conducted in the early 1990s (Feher et al., 1991 (112); Migliorelli et al., 1995a; Migliorelli et al., 1995b; Reed et al., 1993 (113); Sevush & Leve, 1993 (114). However, day-to-day clinical practice suggests that impaired awareness of deficits can also occur at much earlier stages of the disease. It is not uncommon, for example, to encounter patients who, at their first consultation and despite only mild cognitive impairment, display a marked indifference to the concerns expressed by their relatives regarding emerging cognitive difficulties, or attempt to minimize them.

This discrepancy between clinical experience and earlier conceptualizations has led to the hypothesis of *early* anosognosia, manifesting as early as the mild or even prodromal stages of Alzheimer's disease. This clinical intuition was the starting point for a series of studies aiming to characterize more precisely the trajectory of anosognosia over the course of the disease. These investigations have relied both on retrospective clinical studies and on the development of experimental paradigms in healthy participants, with the goal of better understanding the neurocognitive mechanisms underlying awareness of cognitive deficits.

2 Chapter 2: Experimental section

This second chapter presents the experimental component of the present work, structured around two main questions arising from the literature review:

- (1) At what point does anosognosia become detectable over the course of Alzheimer's disease, particularly at preclinical and prodromal stages?
- (2) What are the anatomo-clinical and neurofunctional correlates of this disturbance, and to what extent can dysfunction of brain systems involved in self-awareness be linked, at least partially causally, to the emergence of a deficit in metacognitive self-evaluation?

The first question is addressed through a retrospective observational study based on the Wisconsin Longitudinal Study (WLS) (115), a large community cohort followed since late adolescence and reassessed several times in mid- and late adulthood. This database provides a unique framework for examining, in older adults who are still clinically free of dementia, the relationships between objective memory performance, subjective memory complaints (SMC), and spouse-appraised memory (SAM). By comparing these different indices over a period of approximately six years, and by taking into account genetic status (APOE ϵ 4 carrier vs. non-carrier), the study aims to determine to what extent self-evaluation and spouse evaluation

predict subsequent memory decline, and whether patient–spouse disagreement can reveal an early self-evaluation deficit consistent with emerging anosognosia.

The second question concerns the neurofunctional and anatomo-clinical correlates of these self-evaluation disturbances. Neuroimaging data suggest the involvement of specific brain networks in self-awareness but remain predominantly correlational. To move beyond this limitation, we developed an experimental neuromodulation model in healthy participants, combining deep transcranial magnetic stimulation with measures of functional connectivity and metacognition. This model allows a more direct test of whether transient disruption of regions implicated in self-referential processes can modify the dynamics of confidence judgments and the relationship between performance accuracy and self-evaluation.

2.1 First objective: At what point does anosognosia become detectable?

To investigate anosognosia in the context of early memory impairment, we required a database including middle-aged to older adults, and more specifically individuals in their sixties, an age range in which the incidence of Alzheimer’s disease increases significantly. This database needed to contain both neuropsychological test data assessing memory performance and subjective measures in the form of self-evaluations of memory.

The simultaneous availability of these two types of data would allow us to analyze the relationship between the objective evolution of memory abilities and individuals' subjective perception of them. Moreover, in order to compute a discrepancy index between patients' self-evaluations and a more objective assessment, it was also necessary that the database include informant-based evaluations, ideally provided by relatives or healthcare professionals.

These methodological requirements were met by the Wisconsin Longitudinal Study (WLS) (115), a large American longitudinal study that has followed, for several decades, a cohort of participants who initially graduated from high school in 1957. This database includes neuropsychological information, self-ratings, and informant-based cognitive evaluations, making it particularly suitable for exploring metacognitive processes and anosognosia in older adults.

We therefore conducted a retrospective observational study based on WLS data, with the aim of examining the relationships between memory performance, subjective perception of memory, and the potential presence of an early metacognitive deficit.

2.1.1 Spouse-Appraised Memory Functioning Predicts Memory Decline Better Than Subjective Memory Complaints in Community Dwelling Older Adults at Genetic Risk for Alzheimer's Disease

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Objective:

Alzheimer's disease (AD) begins with subtle memory decline, years before dementia onset. The presence of subjective memory complaints (SMC) has been proposed as a marker of preclinical AD. However, recent evidence has demonstrated early and progressive loss of awareness of memory difficulties in non-demented older adults harboring AD pathology. We investigated the respective contributions of SMC and spouse-appraised memory functioning (SAM) to predict memory decline in a large cohort of community dwelling older adults.

Methods:

The Wisconsin Longitudinal Study collected cognitive data from a community-based cohort of 3,583 participants in both 2005 and 2011. The participant and the participant's spouse were each asked to rate the participant's memory functioning using a Likert scale. We predicted change in objective episodic memory with models including baseline SMC, baseline SAM, or both SMC and SAM. We also evaluated an awareness index (SMC minus SAM). We then tested the interaction between Apolipoprotein E (APOE ϵ 4) carrier status and SMC/SAM to evaluate whether the effects were driven by individuals at-risk for AD pathology. Results: In separate models, SMC (-0.081 ± 0.036 , $p = 0.025$) and SAM (-0.084 ± 0.278 , $p = 0.003$) were both associated with memory decline over ~6 years. However, the AI was not significantly associated with memory decline (0.031 ± 0.024 , $p = 0.19$). When both predictors were included in the same model, SAM (-0.074 ± 0.03 , $p = 0.0092$) was associated with memory decline, while SMC was not significant (-0.061 ± 0.04 , $p = 0.99$). The association between SAM and memory decline was stronger in the APOE ϵ 4 carriers than in the non-carriers (APOE-by-SAM interaction: $F = 6.07$; $p = 0.002$), and follow-up analyses revealed that SAM was particularly predictive of decline only for APOE ϵ 4 carriers. The association between SMC and memory decline was independent of APOE ϵ 4 carrier status (APOE-by-SMC interaction: $F = 2.29$; $p = 0.13$)

Conclusions:

Spouse-appraised memory functioning was more predictive of memory decline than SMC or an awareness index, particularly in APOE ϵ 4 carriers, who are at increased risk for AD pathology.

INTRODUCTION:

Longitudinal studies of clinically unimpaired older individuals suggest subtle memory change is evident as early as 12 years before the diagnosis of Alzheimer's disease (AD) dementia (116). Because cross-sectional neuropsychological evaluation often remains within the normal range for a long time, subjective memory complaints (SMC) have been suggested as a possible harbinger of preclinical AD (117–119). However, anosognosia –a symptom defined as the lack of awareness (LOA) of one's own cognitive deficits– is well documented in AD dementia (120,121), and it may already be observed in the preclinical and prodromal stages of AD (122,123). Awareness is often operationalized by subtracting the degree or level of complaints expressed by a relative, most often the spouse, (i.e., SAM for spouse-appraised memory functioning) from the participant's degree or level of complaints (SMC). An awareness index of zero, therefore, suggests that the participant's insight into his/her level of memory functioning matches the spouse's observations. When

the index is positive, the participant may be reporting more memory concerns (or may be more hyperaware) than the spouse's observations would suggest. Finally, when it is negative, the participant may be underreporting memory difficulties relative to the spouse's report, suggesting the possibility of at least partial unawareness of his/her memory abilities. Previous studies have shown that AD biomarker-positive patients with mild cognitive impairment (MCI) already show LOA (14,124,125), whereas pre-symptomatic amyloid-positive individuals may initially be hyperaware and may express SMC (14,122,126,127). However, it is not clear whether some degree of LOA may already be present during the preclinical, asymptomatic stage of AD, thereby decreasing the value of SMC for predicting subsequent memory decline. To our knowledge, a study directly comparing SAM and SMC in clinically unimpaired older individuals at risk of developing AD has yet to be performed. To this end, we investigated a unique community-based cohort of clinically unimpaired older adults from the Wisconsin Longitudinal Study (WLS) (128). We used SAM, SMC, and an awareness index to predict changes in memory performance over the next 6 years. We hypothesized that because of incipient LOA, SAM would be more predictive than SMC of memory decline.

METHODS

Participants

The Wisconsin Longitudinal Study (WLS) includes more than 10,000 high school graduates from the State of Wisconsin. Most participants have been followed longitudinally since their graduation in 1957 (14). The data were collected via various media (interview, telephone and written questionnaire) at several time points, most recently in 2005 and 2011. The WLS provides neuropsychological and functional assessment data in addition to genetic data, including Apolipoprotein E (APOE), whose $\epsilon 4$ allele is a well-known genetic risk factor for developing sporadic AD (128). From this sample, 3,583 participants were selected based on the availability of the needed data. Genetic data were available in a subgroup of 2,605 subjects. A data sharing agreement and ethical approval were obtained for data analysis at UCLouvain (2018/07JUI/245).

Subjective Memory Complaint, Spouse Appraisal of Memory and Awareness

In 2005, participants and their spouses each completed surveys rating the level of the participant's memory functioning. Participants were asked, "During the past 4 weeks, how would you describe your ability to remember things?" The possible responses were: 1-remember most things, 2-somewhat forgetful, 3-very forgetful, and 4-unable to remember anything, which collectively reflected the severity of the participants' SMC. For the spouse appraisal of memory, spouses were asked, "How would you rate your spouse's memory at the present time?" The possible responses were: 1-Poor, 2-Fair, 3-Good and 4-Very Good. Because the two surveys were on

different scales and in reverse order, we recoded the spouse's report (4 became 1, etc.). We then created an awareness index (AI) by computing the difference between each participant's self-evaluation and the assessment made by the participant's spouse, which ranged from -3 to $+3$. Increasingly negative scores reflect more underestimation of memory difficulty, while increasingly positive scores suggest overestimation of memory difficulties, relative to what the spouse reported. This method of assessing awareness using a caregiver appraisal of the participant's memory functioning and the participant's self-evaluation of his/her own memory functioning was inspired by the Starkstein Anosognosia Questionnaire for Dementia (AQ-D) (129).

Objective Memory Performance

Objective memory performance was measured both in 2005 and 2011 using a composite score based on the single-trial immediate and delayed recall of a 10-word list (c-memory). Raw scores for both the 2005 and 2011 evaluations were standardized to obtain z-scores using the mean and standard deviation of the whole group within each time point. Finally, we calculated an arithmetic mean of the immediate and delayed z-scores to obtain the following scores: c-memory 2005 (baseline) and c-memory 2011 (follow-up).

Statistical Analysis

We performed a Spearman correlation between SAM and SMC baseline data (2005), to evaluate the association between the spouse's assessment of the participant's level of memory functioning and the participant's own subjective self-evaluation of his or her memory functioning. In order to assess the association between the subjects' and spouses' complaints and the objective memory scores at baseline we performed a multiple regression analysis using c-memory 2005 as the dependent variable and SAM, SMC, and AI as predictors by taking into account the effects of age, sex and education. To evaluate the prediction of memory decline from the subjective complaints, we used the c-memory scores to assess memory decline from 2005 to 2011. We predicted the 2011 scores from the 2005 scores using bivariate regression, and we saved the standardized residual for each participant as their residualized change scores. Multiple regressions evaluated the association between memory decline (residualized change scores) and SMC, SAM and AI by taking into account the effects of age, sex and education. We performed a t-test to assess differences between APOE ϵ 4 carriers and non-carriers for baseline memory performance and memory decline. Finally, we conducted two-way ANOVAs to test for an interaction effect between APOE ϵ 4 carrier status and the three rating scores (i.e., SMC, SAM, and AI) when predicting memory decline. The analyses were performed using MedCalc for Windows V.19.0.5 (MedCalc Software, Ostend, Belgium) (130).

RESULTS

Preliminary Analysis of Baseline 2005 Demographics, APOE Status and the Awareness Measures (SMC, SAM, and AI)

As shown in Table 1, a majority of participants (76.7 %) declared they “remember most things” (level 1), but an important minority (23.2%) reported some forgetfulness, most often a mild degree (level 2; 22.6%). Overall, spouses were more critical of participants’ memory functioning than the participants themselves. For example, 29.5% of the spouses reported the participant’s memory function to be at level 2 or higher (suggesting greater forgetfulness) compared to only 23.2 % of the participants. The correlation between participants and spouses was statistically significant (Spearman rho = 0.209; $p < 0.0001$), although the magnitude of this relationship was relatively modest, as the participants’ self-reports explained only 4.3% of the variance in the spouse report. Because no participants and very few spouses endorsed deficient functioning at level 4 (Table 1), ratings at level 3 and 4 were pooled for further analysis (SMC score: 1-remember most things, 2-somewhat forgetful, 3-very forgetful; SAM score: 1-very good, 2-good, 3-poor). It is important to note that there was considerable variability in SMC ratings for the “very forgetful” level (see also Figures 1, 2, 4 below), presumably due to relatively few participants who considered their own memory functioning to be problematic. However, spouse ratings at all three levels were much less variable, suggesting the possibility of greater reliability of spouse-appraised memory relative to the participant’s own

SMCs. The calculated AI showed: -3 (0.3%); -2 (3%); -1 (17.9%); 0 (66%); 1 (12.7%) 2 (0.1%) and 3 (0%). Subsequently, we pooled the scores into three groups: negative scores as “Lack of awareness” (LOA), the null score as “Normal awareness” (NA) and the positive scores as “Hyper awareness” (HA). The demographics and APOE status for the whole sample and all the subgroups (SMC, SAM, and AI) are shown in Table 2. For the whole sample, 684 participants were $\epsilon 4$ carriers, 1,921 were $\epsilon 4$ non-carriers, and 978 had unknown genotype (subpopulation with available APOE genotyping = 2605). In the $\epsilon 4$ carrier group, 46 participants were homozygous for the $\epsilon 4$ allele while 638 were heterozygous.

Objective Memory Performance According to SMC, SAM, and AI

Baseline 2005

As shown in Figure 1, decreased objective memory performance was modestly associated with increasing SMC in a multiple regression model including age, sex and education (adjusted $R^2 = 0.10$; unstandardized SMC coefficient: -0.118 , $SE = 0.032$, $p < 0.0001$) and with increasing SAM (adjusted- $R^2 = 0.11$; unstandardized SAM coefficient: -0.165 , $SE = 0.025$, $p < 0.0001$). The AI highlighted that participants who overestimated their memory had lower objective memory performance in a multiple regression model including age, sex and education (adjusted- $R^2 = 0.104$; unstandardized AI coefficient = 0.077 , $SE = 0.021$; $p < 0.0001$). Both SMC and SAM independently predicted the objective c-memory

composite score when both were included in a multiple regression model including sex, age and education: (adjusted- $R^2 = 0.11$; unstandardized SMC coefficient: -0.077 , $SE = 0.033$, $p = 0.019$ and unstandardized SAM coefficient: -0.153 , $SE = 0.025$, $p < 0.0001$), indicating that SAM was associated more strongly with objective memory than was SMC at baseline. Because AI is a composite index based on the discrepancy between SAM and SMC, a multiple regression model including these three predictors was not performed as it would have created a perfect multicollinearity between AI and the SMC and SAM predictors.

Memory Decline From 2005 to 2011

As shown in Figure 2, in separate models, including age, sex and education, both SAM (adjusted- $R^2 = 0.068$; unstandardized coefficient: -0.084 , $SE = 0.278$, $p = 0.003$) and SMC (adjusted $R^2 = 0.067$; unstandardized coefficient: -0.081 , $SE = 0.036$, $p = 0.025$) were associated with memory decline over ~6 years. However, the AI was not significantly associated with memory decline (adjusted- $R^2 = 0.066$; unstandardized AI coefficient: 0.031 , $SE = 0.024$, $p = 0.195$). When both SAM and SMC were included in the same multiple regression model, including age, sex and education, only SAM was associated with memory decline (adjusted $R^2 = 0.069$; unstandardized SAM coefficient = -0.074 , $SE = 0.03$, $p < 0.0092$) while SMC was not (unstandardized SMC coefficient = -0.061 , $SE = 0.04$, $p = 0.991$). Adding the objective memory score from 2005 to the model did not modify this result. That is,

SAM predicted subsequent memory decline above and beyond baseline objective memory performance and SMC. Because of the perfect multicollinearity between AI and the SAM and SMC predictors, a model including all three predictors was not performed.

Objective Baseline Memory Performance and Memory Decline According to the APOE Carrier Status

Baseline memory performance in 2005 was not statistically different between $\epsilon 4$ carriers and non-carriers. T-test [$t(2603) = -0.43, p = 0.08$] (see also Figure 3). However, for memory decline $\epsilon 4$ carrier status was shown to be significant (Figure 3), T-test: [$t(2603) = -3.99, p < 0.001$].

Interaction Effect Between SMC, SAM, AI, and APOE Status on Memory Decline

As shown in Figure 4, the relationship between SAM and the memory decline appeared to be mediated by the APOE status, whereas this is not the case for SMC and AI. We tested for the presence of an interaction effect between SMC, SAM, AI, and APOE status on memory decline using three separate factorial ANOVAs, each with two factors. When SMC was considered, the analysis showed a trend for an $\epsilon 4$ carrier status main effect [$F(1, 2599) = 3.52; p = 0.061$], a significant main effect for

SMC [$F(2, 2599) = 7.09$; $p = 0.001$], and no significant interaction effect [$F(2, 2599) = 1.33$; $p = 0.265$]. Because there were very few participants with ratings of level 3 in both APOE subgroups (eight participants in each group), we pooled levels 2 and 3. After doing so, the $\epsilon 4$ carrier status main effect was statistically significant [$F(1, 2601) = 16.9$; $p < 0.001$], as was the SMC main effect [$F(1, 2601) = 14.2$; $p < 0.001$]. However, there still was no significant interaction effect [$F(1, 2601) = 2.289$; $p = 0.130$]. The same analysis performed with the spouses' ratings (SAM) showed a main effect of $\epsilon 4$ carrier status [$F(1, 2599) = 18.85$; $p < 0.001$], a main effect for SAM [$F(2, 2599) = 12.6$; $p < 0.001$], and a significant interaction effect [$F(2, 2599) = 6.07$; $p = 0.002$, (see Figure 4). When level 2 and 3 were pooled, the main effect for $\epsilon 4$ carrier status remained statistically significant [$F(1, 2601) = 23.8$; $p < 0.001$], as did the SAM main effect [$F(1, 2601) = 19.3$; $p < 0.001$] and the interaction effect between $\epsilon 4$ carrier status and SAM [$F(1, 2601) = 11.6$; $p = 0.001$]. Decomposition of this significant interaction revealed that for non-carriers, there were no statistically significant differences in memory decline between the two SAM rating categories [$F(1, 1919) = 0.9$, $p = 0.3$]. In contrast, for carriers, memory decline was greater for persons with larger SAM ratings [$F(1, 682) = 18.6$, $p < 0.0001$]. When AI was used as independent variable with $\epsilon 4$ carrier status, the analysis showed only a non-significant trend for the interaction [$F(2, 2599) = 2.74$; $p = 0.065$], while main effects of both $\epsilon 4$ carrier status [$F(1, 2599) = 15.19$; $p < 0.001$] and AI [$F(2, 2599) = 7.96$; $p < 0.001$] remained significant.

DISCUSSION

Our results demonstrate that SAM is a particularly strong nonbiological predictive index of cognitive decline in a preclinical population, compared to other markers such as SMC or AI that are commonly used with patients with neurodegenerative disease, and it is particularly predictive of decline for APOE ϵ 4 carriers. APOE ϵ 4, a well-known surrogate marker of the presence of amyloid deposits in the brain, was the most powerful predictor of memory decline in our sample.

This finding concurs with the vast literature supporting a specific relationship between this genetic polymorphism and AD risk (112,131), where memory impairment is the earliest and most salient cognitive deficit (132). Interestingly, APOE ϵ 4 carriers and non-carriers were similar on baseline memory scores, suggesting the absence of a relationship between cognitive performance and the presence of the APOE ϵ 4 allele at the sample's mean age of 64 in 2005. Nearly one quarter (21%) of community dwelling older adults in our sample rated their memory functioning as being better than their spouse considered it to be. In contrast, 13% showed an opposite pattern of increased SMC relative to spousal ratings (i.e., the participant reported more memory difficulty than the spouse reported).

Our analyses also suggested APOE carrier status was independent of SMC severity ratings when predicting cognitive decline. In contrast, when the SAM index was considered with carrier status, greater SAM severity ratings were associated with cognitive decline only in participants carrying the APOE ϵ 4 risk allele. These results

suggest that SAM predicts cognitive evolution in a population at risk of developing AD at a preclinical stage. These two preclinical predictors, APOE and SAM, are easily obtained, inexpensive, and ultimately not invasive. Moreover, the synergistic use of these two risk markers makes it possible to identify a group at the greatest risk for memory decline, several years in advance.

For almost three decades, it has been very clearly demonstrated in the literature that a large proportion of patients with AD dementia experience anosognosia. The percentage of anosognosic AD patients increases throughout the disease course. At the severe stage, up to 80% of patients show some degree of anosognosia (14). Indeed, across all stages of AD severity, all past studies demonstrate a clear loss of awareness in a substantial proportion of AD patients (112,132,133). The presence of unawareness of deficits in MCI and during the preclinical stage has been less consistently demonstrated. Several studies have highlighted that among persons with MCI, anosognosia may be present in a less obvious fashion than in AD, but it is nevertheless already well-established in high amyloid MCI (122,123). On the other hand, it has also been demonstrated that hyperawareness of deficits manifested by SMCs can predict subsequent cognitive decline during the preclinical stage and in persons with MCI (117–119,134,135).

Looking at the extant body of literature, a way of reconciling these apparently diverse results is to conceptualize an evolution of awareness to unawareness during the pathophysiological progression of AD. During preclinical stages, patients may

show increased awareness of cognitive changes. Subsequently, as the disease course progresses to MCI, anosognosia becomes increasingly prevalent until it reaches clinically obvious levels in AD.

Unexpectedly, our study suggests that persons at the pre-dementia stage are, in fact, not hyper-vigilant. Our results revealed that while each participant's own self-rating only weakly predicted his or her cognitive decline, the spouse's assessment did so more effectively. Therefore, our findings are at odds with those of several past studies that have reported the preclinical presence of SMC and implicating an early hypervigilance toward cognitive changes.

Given the discrepancies in results between our study and the conclusions of others, it is important to point out possible study differences that could account for this discrepancy. First, these previous studies in SMC did not take into account SAM. Consistent with these studies, we observed a weak association between SMC and subsequent decline.

Moreover, the study population in the WLS is younger (average age of participants 64 years) than other studies showing a relationship between SMC and cognitive decline [mean age 72 years (20) and 69 years (21) respectively]. As the prevalence of AD increases exponentially with age, it is probable that the memory deficit would have been greater in the previously studied cohorts. This difference could have played out both ways on SMC, making the participants more willing to report memory concerns because of a greater memory deficit (increasing SMC), but at the

same time, the number of participants showing an onset of anosognosia due to incipient AD (decreasing SMC) may also have increased.

This combination could result in a complex relationship that is not directly comparable to our cohort. Most importantly, the absence of SAM evaluation in previous studies does not allow a meaningful comparison. However, despite having a younger cohort with, arguably, better memory functioning than in previous studies, SAM was strongly associated with cognitive decline, whereas SMC was clearly less so.

It is well-known that the prevalence of AD increases with age. However, despite having a younger cohort characterized only for $\epsilon 4$ and not for amyloid, SAM was strongly associated with cognitive decline, whereas SMC was clearly less so taking into account SAM in future studies may help identify earlier clinically normal older adults who may be at risk of cognitive decline in the near future.

Major strengths of our study include the very large sample size and relative homogeneity of participant ages at both measurement periods. These factors enabled us to demonstrate the power of SAM for predicting cognitive decline that was independent of SMC. Indeed, given the very modest correlation between SAM and SMC the two indexes appear to be evaluating different dimensions of awareness.

The major limitation of our study lies in its retrospective nature, not permitting us to identify prospective risk factors that would be sensitive to cognitive decline. In addition, only one measure of each participant's memory self-evaluation and of the

spouse's evaluation of each participant's memory in 2005 was available. This measure was not obtained in 2011, making it difficult to determine whether either evaluation may have changed over time due to disease progression or other factors. The data from this study were also obtained from participants with spouses, and it is uncertain whether participants without spouses may have responded in the same manner.

Finally, participants who responded to the cognitive survey were included in our study. The participants who did not respond were excluded, leading to possible selection bias in our analysis because of potential differences between the group who endorsed the cognitive survey and the persons who did not respond. To our knowledge, this study is the first to show the value of SAM as an early marker of cognitive decline in community dwelling older adults, in combination with genetic risk for AD.

This study suggests that evaluation of memory functioning by close informants, such as family members or regular caregivers, may be more effective than an awareness index or SMC for identifying at an early stage individuals at risk for cognitive decline, particularly among carriers of the APOE ϵ 4 allele.

Figures and tables

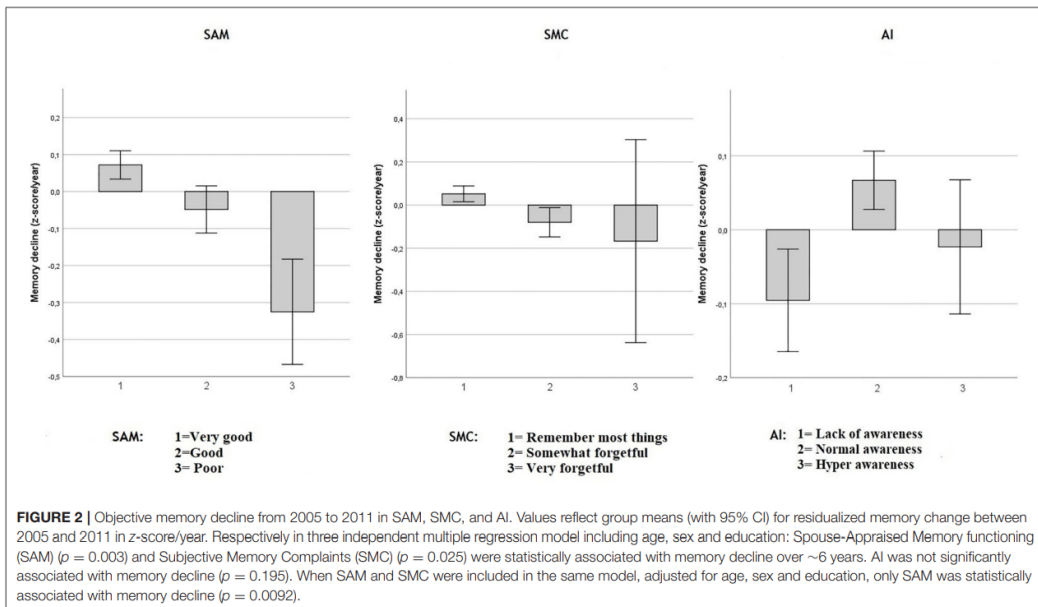
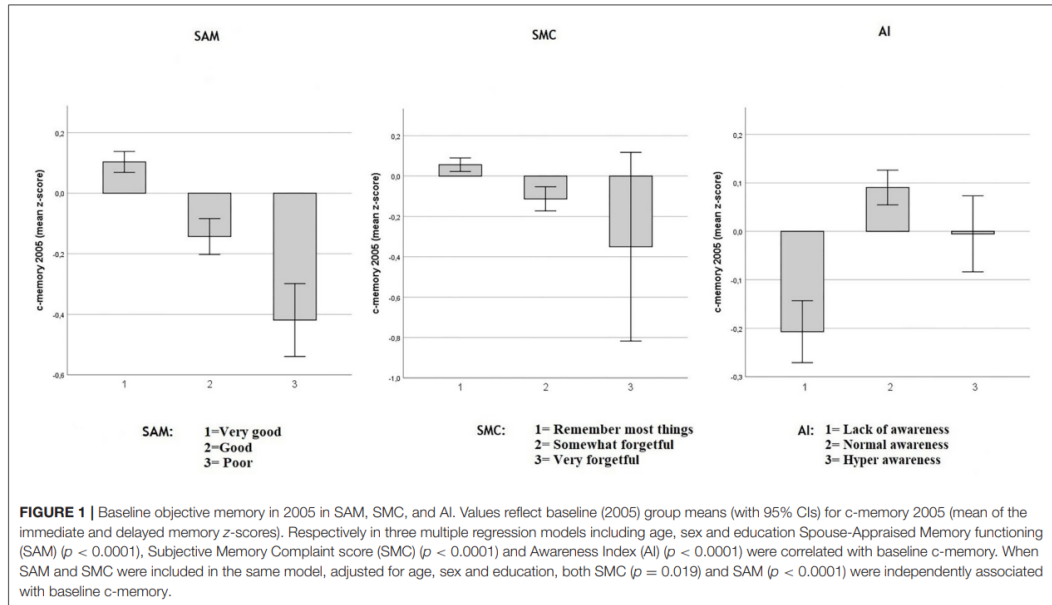
TABLE 1 | Self and spouse memory evaluation in 2005.

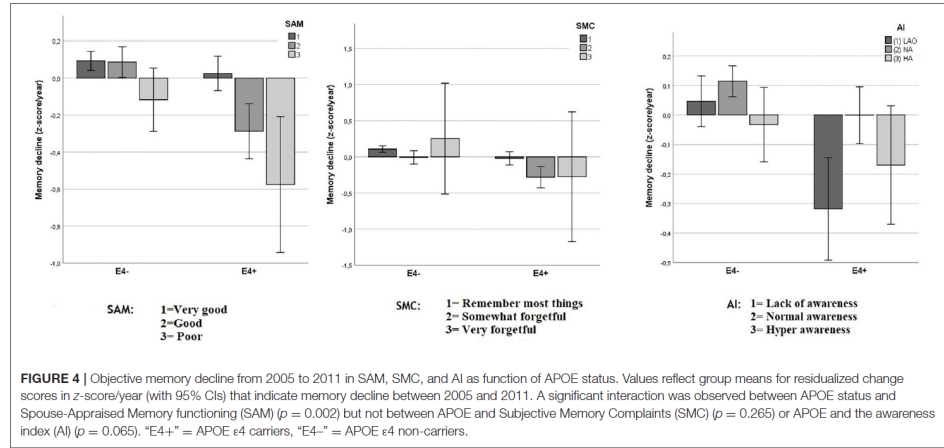
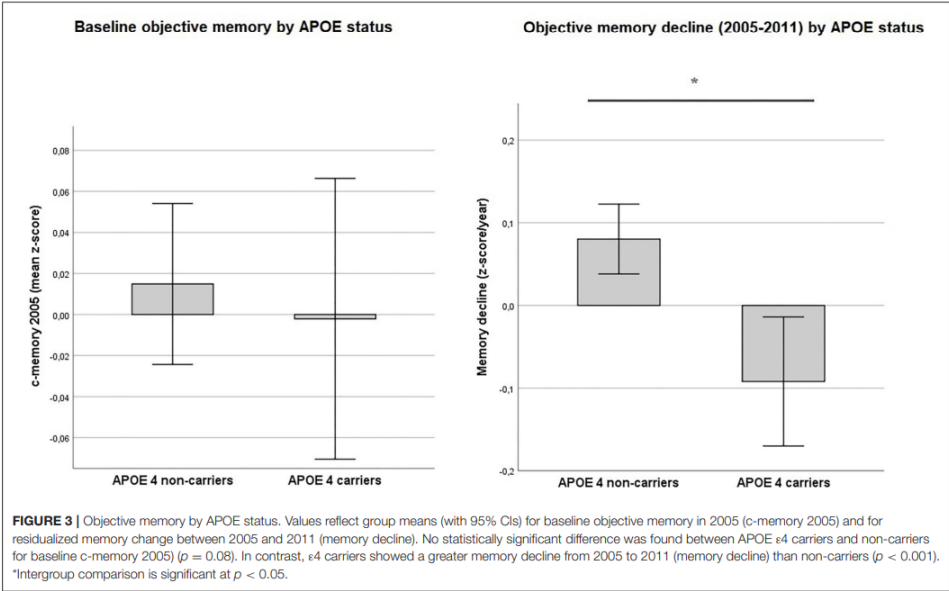
		Spouse evaluation				Total
		Very Good	Good	Fair	Poor	
Self-evaluation	Remember most things	2,078	565	96	11	2,750 (76.7%)
	Somewhat forgetful	447	281	72	11	811 (22.6%)
	Very forgetful	4	7	7	3	21 (0.6%)
	Unable to remember anything	0	0	1	0	1 (<0.1%)
Total		2,529 (70.6%)	853 (23.8%)	176 (4.9%)	25 (0.7%)	3,583

TABLE 2 | Baseline sample demographics.

	All (3,583)	SMC = 1 (2,750)	SMC = 2 (811)	SMC = 3 (22)
Mean age in 2005 (years)	63.8	63.8	63.9	64.7
Sex (% female)	45.8%	46.4%	44.5%	27.3%
Education (years)	14.2	14.4	13.8	12.9
APOE4 carriers (%) (subpopulation n = 2,605)	26.1%	25.3%	28.7%	50%
	All (3,583)	SAM = 1 (2,529)	SAM = 2 (853)	SAM = 3 (201)
Mean age in 2005 (years)	63.8	63.7	64.0	64.3
Sex (% female)	45.8%	48.8%	40.4%	31.3%
Education (years)	14.2	14.5	13.6	13.3
APOE4 carriers (%) (subpopulation n = 2,605)	26.1%	25.4%	28.8%	26.2%
	All (3,583)	AI = LOA = -3,-2,-1 (758)	AI = NA = 0 (2,366)	AI = HA = +1,+2,+3 (459)
Mean age in 2005 (years)	63.8	64.1	63.7	63.8
Sex (% female)	45.8%	39.4%	47%	50.3%
Education (years)	14.2	13.6	14.4	14.2
APOE4 carriers (%) (subpopulation n = 2,605)	26.1%	26.0 %	26.2%	27.1%

SMC 1, remember most things; SMC 2, somewhat forgetful; SMC3, very forgetful. SAM 1, Very good; SAM 2, Good; SAM 3, Poor. For AI, LOA, lack of awareness; NA, normal awareness; HA, Hyper awareness.





2.1.2 From behavioural index to neural correlates of anosognosia

In summary, the WLS study shows that spouse-rated memory functioning is not only a simple cross-sectional indicator of cognitive vulnerability, but also a robust longitudinal predictor of objective memory decline over a period of up to six years in individuals who are still clinically unimpaired, particularly among APOE $\epsilon 4$ carriers. Lower spouse evaluations of the subject's memory at baseline are associated with greater subsequent decline, even when standard neuropsychological performance remains within the normal range. Crucially, baseline memory scores in 2005 did not differ between APOE $\epsilon 4$ carriers and non-carriers, suggesting that, at the time of initial assessment, participants were most likely at a very early, prodromal stage, in which emerging pathological processes are not yet detectable on conventional memory tests.

Although this measure cannot, on its own, be considered a neuropsychological biomarker of Alzheimer's disease, this patient-spouse discrepancy suggests that subtle alterations in awareness of deficits can already be detected at a preclinical stage. In this sense, the WLS findings provide an initial, indirect answer to our first research question: signs of emerging anosognosia, or at least of a fragile metacognitive self-evaluation, may become detectable well before the onset of clinically overt dementia.

This observation raises a direct question: which brain networks underlie this vulnerability of self-evaluation, and how might their dysfunction contribute to the

later development of full-blown anosognosia? If subtle unawareness emerges at such an early stage, it is plausible that the brain regions supporting self-referential processing and metacognition are themselves affected early in the neurodegenerative process, in parallel with medial temporal and associative cortical changes. The second objective of this thesis builds on this question, aiming to characterise the anatomo-functional correlates of anosognosia, with a particular focus on the posterior cingulate cortex and the default mode network.

2.2 Second objective of the thesis: What are the neuroanatomical correlates associated with the emergence of anosognosia in Alzheimer's disease?

2.2.1 Experimental approach :

In 2016, Massimini's team (136) developed an objective and quantitative method to assess the level of consciousness in patients. The study initially focused on patients in coma, compared with fully conscious individuals. In a second step, they extended their investigation to other profiles along the spectrum of impaired consciousness, namely patients in a vegetative state, under general anesthesia, or in a minimally conscious state.

The methodology relied on analyzing EEG variations induced by transcranial magnetic stimulation. On this basis, they created an index quantifying the perturbation of cerebral complexity. Massimini's group hypothesized that the electrical activity recorded by EEG in a conscious patient contains more information than that recorded in an unconscious patient.

This complexity, or degree of “information”, was measured using an algorithm derived from the Lempel–Ziv–Welch method (137). This algorithm compresses information in a manner analogous to the compression of files into a zip format: the more repetitive and less complex the signal, the more easily it can be compressed. Conversely, a complex response to stimulation will be less compressible by the algorithm.

Using this approach, Massimini's team demonstrated a clear difference between patients with a normal level of vigilance and those with disorders of consciousness. Their method showed perfect specificity and sensitivity (100%) for distinguishing comatose patients from healthy controls, as well as high sensitivity (94.7%) for detecting patients in a minimally conscious state.

2.2.1.1 Adaptation of the “Zap and Zip” approach to the study of anosognosia

Strongly inspired by this work, we designed a project aiming to demonstrate a difference in the perturbational complexity index between patients with anosognosia

and patients without anosognosia during self-evaluation tasks. This hypothesis was based on the idea that metacognitive processes rely on brain mechanisms characterized by a high degree of complexity. Consequently, an alteration of metacognitive abilities should be reflected in reduced activity within the relevant brain areas.

To test this hypothesis, we initially planned to administer transcranial magnetic stimulation targeting the posterior cingulate cortex, and to measure the cortical response in these regions using EEG. This method would have allowed us to quantify a complexity index whose reduction could have reflected impaired metacognitive processes, particularly those related to anosognosia. We then intended to compare this index with classical measures of anosognosia, such as discrepancy indices, in order to explore potential correlations between these parameters.

However, the onset of the COVID 19 pandemic prevented the implementation of this project, notably due to constraints on recruiting patients with neurodegenerative diseases, who were considered too vulnerable to participate in experimental protocols. In response to this situation, we reoriented our study population towards young, healthy participants.

2.2.1.2 Development of an experimental model to study metacognitive deficits in healthy participants

Within this new framework, our interest focused on the possibility of demonstrating, in a causal manner, the involvement of specific brain regions in the clinical expression of reduced self-evaluation abilities. This approach, however, raised several challenges that we sought to address.

The first step was to verify that deep TMS (deep transcranial magnetic stimulation) could inhibit or disrupt the connectivity of the posterior cingulate cortex with the rest of the neural network, in particular the default mode network (DMN). This hypothesis was based on previous work reporting such effects in the context of Alzheimer's disease (110,138,139).

The second step was to test whether this disruption of DMN connectivity would be accompanied by alterations in metacognitive performance, as measured by recognition accuracy and confidence-based behavioural indices. The results obtained in these two phases led to the preparation of two manuscripts: the first is currently under revision, and the second is in preparation.

2.2.2 First phase

In this first phase, we sought to establish a model of controlled perturbation of the default mode network (DMN) in healthy participants by specifically targeting the right posterior cingulate cortex (PCC). Neuroimaging studies have shown stronger right-hemispheric PCC activation during reflective self-awareness tasks(140), and converging evidence suggests that the right PCC plays a leading role in the representation of the mental self (141). Moreover, right PCC hypometabolism has been associated with impaired self-awareness in AD and mild cognitive impairment (95,141–143). The aim was to determine, in an experimental and causal manner, whether a transient inhibition of this region, known to be critically involved in self-referential processes and self-awareness, is sufficient to desynchronize functional connectivity within the DMN.

In other words, the goal was to move beyond the essentially correlational data derived from Alzheimer's disease, where alterations of the PCC and DMN are associated with anosognosia, towards a neuromodulation paradigm in young healthy adults, allowing a direct test of the impact of deep TMS on the network-centered integrity of the PCC.

The study presented in this section therefore constitutes a proof of concept: it aims to demonstrate the feasibility and effects of a continuous deep stimulation protocol (cTBS) applied to the right PCC, and to document, using resting-state functional MRI, how this manipulation reshapes DMN synchronisation in anticipation of the metacognitive investigations detailed in the subsequent phase. More specifically,

this first step was designed to verify that deep TMS effectively targets the intended PCC node within the DMN, and that the induced changes in connectivity primarily reflect modulation of this self-referential network rather than unspecific disruption of unrelated large-scale systems.

2.2.3 Modulating the default mode network with deep TMS: a preliminary step toward understanding self-awareness.

Abstract

Introduction:

Self-referential and metacognitive processes rely on the default mode network (DMN), particularly the posterior cingulate cortex (PCC). The right PCC, in particular, has been implicated in self-awareness and insight, with neuroimaging studies showing its involvement in metacognition and anosognosia in aging and Alzheimer's disease. However, causal evidence linking right PCC activity to DMN synchrony in humans remains scarce. This study examined whether transient modulation of the right PCC using continuous theta-burst deep transcranial magnetic stimulation (deep TMS) alters DMN connectivity in healthy adults.

Aim:

To assess whether transient inhibition of the right PCC using continuous theta-burst deep transcranial magnetic stimulation (deep TMS) modulates DMN functional connectivity in healthy adults.

Methods:

Twelve right-handed healthy participants (aged 26–30 years) underwent two randomized sessions: deep TMS targeting the right PCC and a sham condition. Resting-state fMRI was acquired 15 minutes ($\pm$ 3.4 min) post-stimulation. Connectivity changes were assessed through (1) a correlation-matrix analysis between the right PCC and 79 regions of the Brainnetome atlas, and (2) a whole-brain seed-based paired t-test. Statistical significance was evaluated using cluster-level Monte-Carlo correction ($p < 0.05$).

Results

The region-based correlation matrix showed reduced connectivity between the PCC and three DMN-related regions (right precuneus, right inferior parietal gyrus, left superior temporal gyrus), although these did not survive multiple-comparison correction. The whole-brain analysis revealed eight clusters with significantly decreased connectivity after stimulation compared to sham, including six key DMN hubs (bilateral precuneus, bilateral supramarginal gyri, right angular gyrus, left posterior superior temporal gyrus).

Deep TMS of the right PCC induced a transient desynchronization of DMN connectivity, supporting a causal contribution of the right PCC to large-scale self-

referential network integrity. While no behavioral assessments were yet performed, this protocol provides a mechanistic foundation for future studies investigating the neural basis of self-awareness and its disruption in aging and neurodegenerative disease.

Conclusion:

Deep TMS of the right PCC induced a transient desynchronization of DMN connectivity, supporting a causal contribution of the right PCC to large-scale self-referential network integrity. While no behavioral evaluation was performed at this stage, these results provide a proof-of-principle that transient modulation of the right PCC can alter DMN synchrony, opening the way for future studies combining deep TMS with metacognitive and self-awareness assessments to directly investigate awareness mechanisms in healthy aging and neurodegenerative disease.

Introduction

The default mode network (DMN) is a large-scale brain network involved in internally directed cognition, including autobiographical memory, internal mentation, and self-referential thought (144,145). Among its core hubs, the posterior

cingulate cortex (PCC) plays a pivotal role in self-focused attention and the maintenance of self-related information during rest and introspection (140,146,147). Neurodevelopmental data support the involvement of DMN nodes, including the PCC, in the emergence of narrative self-representation (144). During rest or self-reflection, the PCC consistently shows increased activation, highlighting its role in internal awareness and cognitive self-processing (145,146).

Converging evidence suggests that the PCC contributes to self-awareness processes and introspective evaluation. PET and fMRI studies have demonstrated PCC engagement during self-referential judgments and reflective self-awareness (140,146,147). Its central anatomical and functional position in the DMN enables it to integrate self-relevant information with autobiographical memory systems (103). Consequently, disruption of PCC function is hypothesized to impair insight into one's own cognitive abilities and internal states.

This hypothesis is supported by research in Alzheimer's disease (AD), where anosognosia, i.e., reduced awareness of cognitive, emotional, and behavioral deficits, is frequently observed (148). Functional MRI studies show abnormal DMN connectivity in AD compared to healthy aging (106,149), and early involvement of DMN hubs, including the PCC, has been demonstrated across modalities (95,141,150,151). Functional connectivity and metabolic studies suggest that PCC dysfunction is associated with anosognosia in prodromal and established AD (95,122,141,142,152). After the hippocampus, the PCC is among the earliest regions

affected by AD pathology, displaying hypometabolism (150,151), amyloid deposition (109), and structural degeneration (95,153). Reduced metabolism in the PCC predicts progression to dementia (154) and correlates with anosognosia severity (122,141,143).

Notably, evidence also comes from preclinical populations. Young carriers of the apolipoprotein-E $\epsilon 4$ (APOE4) allele exhibit DMN hyperconnectivity relative to non-carriers, interpreted as a compensatory mechanism (155). Taken together, these findings suggest that the PCC may play an important role in supporting awareness of cognitive abilities, thereby motivating efforts to experimentally modulate PCC function to further explore its potential contribution to awareness-related processes.

Importantly, several studies emphasize a preferential involvement of the right PCC in self-referential processing and anosognosia (89,95,106,153). Neuroimaging experiments have shown stronger right-hemispheric PCC activation during reflective self-awareness tasks (140), and evidence suggests the right PCC plays a leading role in the representation of the mental self (146). Moreover, right PCC hypometabolism has been associated with impaired self-awareness in AD and mild cognitive impairment (95,141–143). These findings provide a rationale for selectively targeting the right PCC when experimentally probing the neural basis of self-awareness.

Despite this accumulated evidence, current knowledge remains largely correlational. A causal demonstration linking PCC dysfunction to self-awareness is lacking,

primarily because clinical lesions in AD are multifocal and confounded by widespread pathology (14,20,89,156). Direct, reversible disruption of neuronal activity in the PCC could therefore constitute a critical mechanistic test. Transcranial magnetic stimulation (TMS) offers a means to transiently perturb cortical function (157), and continuous theta-burst stimulation (cTBS) provides a reliable method to induce inhibitory neuromodulatory after-effects (158). However, because of its deep anatomical location, the PCC cannot typically be reached by classical TMS coils. Advances in deep TMS technology now allow stimulation of deeper neural structures, providing a unique opportunity to transiently modulate PCC activity in healthy subjects.

The aim of the present study was to develop an experimental model of DMN disruption by targeting the right PCC using deep TMS in healthy young adults. Demonstrating that right PCC inhibition alters DMN connectivity would represent an essential first step toward establishing the causal contribution of this region to self-awareness mechanisms in aging and neurodegenerative disease.

Methods

Participants:

Twelve young healthy volunteers (6 females, 6 males; age range 26–30 years, mean age 28) took part in the study. All participants were right-handed, as confirmed using

the Edinburgh Handedness Inventory, and had no contraindication to transcranial magnetic stimulation (TMS) or MRI. Exclusion criteria included, history of any brain lesion, a personal or family history of epilepsy and the use of chronic medication.

A narrow young-adult age range was selected to ensure greater homogeneity in brain functional connectivity and to minimize age-related confounding factors, such as variability in neurovascular coupling, early microvascular lesions, or preclinical neurodegenerative changes, which are more common in older individuals.

All participants provided written informed consent in accordance with the Declaration of Helsinki, and the study protocol was approved by the Ethical Committee of UCLouvain (Ref: 2019/19SEP/410, B403201941608).

Design

Participants completed three study visits, each scheduled one week apart. The first session consisted of acquiring a high-resolution structural MRI used to generate an individual 3D brain model for neuronavigation. The structural MRI was inspected to exclude the presence of any brain lesion that would contraindicate continued participation in the experiment.

During the two subsequent visits, participants received either real deep TMS targeting the right posterior cingulate cortex (rPCC) or sham stimulation, in a randomized and counterbalanced order. Thus, half of the participants received real

stimulation during the second visit and sham during the third, whereas the remaining half received sham first followed by real stimulation. In both sessions, stimulation was followed by an fMRI examination.

Resting-state fMRI was acquired 15 minutes later (± 3.4 min) after the end of stimulation. During this interval, participants remained seated quietly and were instructed to stay relaxed. MRI acquisition began with a T1-weighted scan, followed by resting-state fMRI. During the resting-state acquisition, participants lay with their eyes closed and were instructed to let their thoughts wander naturally without focusing on anything in particular.

Deep TMS protocol:

Continuous theta-burst stimulation (cTBS) was used as the neuromodulation protocol. This patterned stimulation approach is known to induce inhibitory after-effects on cortical excitability lasting up to approximately one hour after stimulation (158). The cTBS train consisted of bursts of three pulses at 50 Hz, repeated every 200 ms (5 Hz) for 40 s, yielding a total of 600 pulses.

Stimulation intensity was set at 80% of the motor threshold, which was determined individually for each participant. The motor threshold was established by delivering single-pulse TMS over the lower-limb motor cortex, located at a cortical depth comparable to the rPCC, while recording surface electromyography (EMG) responses from the left tibialis anterior muscle. Surface EMG electrodes were positioned over the tibialis anterior muscle belly with an inter-electrode distance of

2 cm, and a ground electrode was placed ipsilaterally over the ankle. Electrode placement followed the European recommendations for surface EMG (159,160). The motor threshold was defined as the lowest stimulation intensity producing motor evoked potentials (MEPs) exceeding 50 μ V in $\geq 50\%$ of 10 consecutive trials (161).

Deep magnetic stimulation was delivered using a double-cone coil (MagVenture DB80), suitable for targeting deeper cortical structures. The right posterior cingulate cortex (rPCC) was targeted using MNI coordinates (4, -53, 42). Individual structural MRI scans were used to reconstruct a 3D anatomical model for precise target localization, and neuronavigation software (Visor 2, ANT Neuro) ensured coil positioning accuracy during stimulation.

To preserve participant blinding, sham stimulation was delivered by positioning the coil at a 90° angle over the vertex, reproducing the acoustic click and scalp sensation without effective cortical stimulation. During the active stimulation condition, the coil was also placed over the vertex to match sensory perception; at this location, stimulation is far from the motor cortex and does not evoke peripheral muscle responses. Therefore, participants were blind to stimulation condition, while the experimenter administering stimulation was not.

MRI scanning:

All participants underwent three MRI sessions consisting of one high-resolution three-dimensional (3D) T1-weighted structural scan and two resting-state functional

MRI (rs-fMRI) runs. Data were acquired on a 3 T MRI system (SIGNA Premier, GE Healthcare, USA) equipped with a 48-channel head coil at the Cliniques universitaires Saint-Luc (UCLouvain, Brussels, Belgium).

Structural imaging. A whole-brain 3D T1-weighted MPRAGE sequence (1 mm³ isotropic) was acquired with inversion time (TI) = 900 ms, repetition time (TR) = 2188.16 ms, echo time (TE) = 2.96 ms, flip angle (FA) = 8°, field of view (FOV) = 256 × 256 mm², matrix = 256 × 256, 156 slices, slice thickness = 1 mm, no gap; total scan time = 5 min 36 s. Structural images were inspected to exclude brain lesions that would contraindicate continued participation.

Functional imaging. Resting-state fMRI data were collected with multiband echo-planar imaging (multiband factor = 3; ARC = 2 parallel imaging), FOV = 220 × 220 mm², matrix = 110 × 110, TE = 30 ms, FA = 90°, 64 ascending, interleaved slices, slice thickness = 2 mm; TR = 1500 ms. Each run comprised 250 whole-brain volumes (duration = 6 min 15 s).

MRI pre-processing and Data Analysis:

The MRI data were analyzed using BrainVoyager [Version 2.8, Brain Innovation, Maastricht, The Netherlands]. Preprocessing of the resting-state data consisted of a linear trend removal to exclude scanner-related signal drift, temporal high-pass filter to remove frequencies lower than 0.005 Hz and correction for head movements using a rigid body algorithm for rotating and translating each functional volume in 3D

space. Data were also corrected for time differences in the acquisition of the different slices. Data were co-registered with their 3D T1-weighted scans and normalized in the MNI space. All co-registrations were verified and movement corrections were optimized, using a sinc interpolation. Because spontaneous low-frequency fluctuations are not exclusively BOLD-related fluctuations, but are also contaminated by non-neural signals (i.e., artifacts), several additional pre-processing steps were added to remove these undesirable sources of variance. Regression analyses were performed to remove artifacts due to residual motion (the six movement regressors were obtained via rigid body correction of head motion as implemented in BrainVoyager) and changes in ventricles (the signal from a ventricular region of interest defined in each subject). The final data were smoothed in the spatial domain (Gaussian filter: Full Width at Half Maximum = 5mm). We used BrainVoyager and a customized MATLAB code (The Mathworks) to calculate cross-correlations between the average time-course signals, extracted from 79 regions of interest (ROIs) intersected with the individual brain mask of each subject. These regions were derived from the Brainnetome atlas [23]. This resulted in 30 135 pairs of functional connectivity per subject. In a second time, we generated first-level connectivity maps for each participant, between the seed region and all voxels in the whole brain, and entered for the second-level analysis, these connectivity maps in a whole-brain paired t-test to investigate the effect between the two conditions.

Result

We computed, for each participant and for each condition (real rPCC stimulation vs. sham), the Pearson correlation between the BOLD time course in the right posterior cingulate cortex (rPCC; Brainnetome A31) and the time courses of the 79 default mode network (DMN) subregions defined in the Brainnetome atlas. Condition-wise correlation coefficients were compared with paired *t*-tests.

Three DMN parcels exhibited significant condition differences (sham > stimulation), indicating reduced connectivity following stimulation: Left superior temporal gyrus (A22C; $p = 0.039$), Right inferior parietal gyrus (A40C; $p = 0.007$), and Right precuneus (A7M; $p = 0.031$). In all three parcels, the BOLD time course of the rPCC (A31) was more strongly correlated under sham than under stimulation, consistent with a decrease in functional connectivity induced by the cTBS protocol over rPCC. Given the number of ROI-wise tests (79), these effects did not survive familywise control using a Bonferroni correction procedure across ROIs.

Secondly, a voxelwise paired *t*-test contrasted sham vs. rPCC-stimulation seed-based connectivity maps, using cluster-extent Monte Carlo correction for multiple comparisons ($p < .05$; cluster-size threshold = 187 mm³). Eight clusters survived correction, all showing lower correlation with the rPCC seed (A31) during stimulation relative to sham. Six of the significant clusters were located within canonical DMN territories—namely the right angular gyrus, right supramarginal gyrus, left supramarginal gyrus, right precuneus, left precuneus, and left posterior

superior temporal gyrus—and two additional clusters outside the core DMN (see Table 1 for peak coordinates and anatomical labels). All significant effects were in the same direction (stimulation < sham), and the cluster-level results survived the Monte Carlo multiple-comparison correction.

Discussion

Studying the neuroanatomical substrates of self-awareness in humans remains challenging. Correlational evidence links default mode network (DMN) dysfunction with anosognosia in Alzheimer's disease (AD), with particular emphasis on the posterior cingulate (PCC) complex as an early and vulnerable hub. Within contemporary models, the PCC is a highly connected, metabolically active node supporting internally directed cognition and self-referential processing, embedded in partially dissociable DMN subsystems (162,163). Yet, despite these associations, causal tests of whether perturbing this network can transiently alter connectivity, and thereby offer a tractable human model for probing self-awareness, are scarce.

Our goal was to provide such a proof of concept by disrupting the DMN via focal stimulation of the right PCC in healthy young adults. Several practical obstacles had to be addressed. First, the right PCC lies deep on the medial wall, rendering it largely inaccessible to conventional figure-of-eight coils. We therefore used deep TMS with neuronavigation to focus energy at the required depth, a strategy supported by

bended double-cone coil, design studies demonstrating increased penetration compared with standard coils (164,165). Second, to calibrate stimulation intensity at depth, we used lower-limb motor-evoked potentials (MEPs) as an individualized proxy because the leg area of M1 lies at a comparable depth to the PCC. Third, because many DMN hubs are deep, scalp EEG is ill-suited to detect network-wide repercussions; we thus combined stimulation with resting-state fMRI and adopted a protocol with sustained after-effects. Continuous theta-burst stimulation (cTBS) was selected given its capacity to induce minutes-to-hours changes in cortical excitability in humans (166).

The results indicate that cTBS over the right PCC reduces resting-state functional connectivity between the seed region and multiple DMN clusters, including the right angular gyrus, bilateral supramarginal gyri, bilateral precuneus, and the left posterior superior temporal gyrus. These network-level decreases are consistent with prior demonstrations that TMS can modulate intrinsic connectivity within and between large-scale systems, including the DMN(167,168). Importantly, effects were measured acquired on average 15.2 min ($\pm$ 3.4 min), after stimulation, well within a window compatible with behavioral testing; this suggests that online assessments of self-monitoring could be interleaved with, or immediately follow, stimulation blocks to probe causality at the level of metacognition.

A potential concern is that the precuneus (PCN), located between the coil and the PCC, may have been partially stimulated. Two points mitigate this. First, coil

geometry and neuronavigation were used specifically to focus induced fields at the targeted depth, reducing spread. Second, the additional clusters showing decreased coupling to PCC were topographically distant from the stimulation site, making a direct local-activation account unlikely. We therefore interpret the observed reductions in synchrony as network-level reconfiguration initiated by perturbation of a key hub, rather than as the by-product of unintended stimulation.

This model has potential implications for the study of anosognosia. Pathological case series in AD implicate PCC dysfunction in impaired awareness (169), but such data are inevitably confounded by diffuse pathology and compensatory reorganization (170). A causal perturbation approach in healthy brains avoids these confounds and can be paired with sensitive metacognitive measures.

Because young healthy adults are not anosognosic, self-awareness should be operationalized with trial-by-trial confidence and accuracy during brief tasks administered within the post-stimulation window. Signal-detection-based metrics (e.g., meta- d' , metacognitive efficiency) provide performance-independent indices of self-monitoring suitable for this purpose (15,16). If right-PCC cTBS reliably reduces metacognitive efficiency while leaving first-order accuracy intact, this would constitute a mechanistic demonstration linking PCC-centered DMN function to self-evaluation.

Limitations warrant caution. First, the sample size was modest; That said, the primary contrast still yielded a large within-subject effect (Cohen's $d_h = 0.933$) and

a paired $t(11) = 3.233$. With $n = 12$ paired observations, this corresponds to an approximate two-tailed achieved power of ~ 0.85 at $\alpha = .05$, and the 95% CI for the mean difference (-0.09396 to -0.01784) excludes zero. In a within-subject design that attenuates inter-individual variance, these indices support that the present sample size is sufficient to draw robust inferences about the direction and presence of the network-level effect, while acknowledging that larger cohorts will refine its precision and generalizability.

Second, although our ROI-wise effects did not survive stringent familywise corrections across all 79 parcels, convergent, cluster-corrected seed-based findings suggest a coherent pattern; nevertheless, the broader debate on cluster-extent inference in fMRI underscores the value of transparent thresholds, nonparametric validation, and replication (171).

Third, our experiment did not incorporate a behavioral assessment aligned with the post-stimulation window; consequently, we cannot directly determine whether transient disruption of the DMN via right-PCC stimulation affected participants' self-evaluation (i.e., metacognitive awareness). The current findings therefore speak to network-level connectivity changes only. Without concurrent behavioral probes, such as trial-by-trial confidence ratings, calibration indices of over-/underconfidence, and signal-detection-based measures (e.g., meta- d' and metacognitive efficiency) collected during the ~ 10 – 20 -min after-effect window, any inference about a reduction in insight remains provisional. Future iterations should

embed brief, time-locked tasks to jointly quantify first-order performance and second-order awareness, enabling a direct brain–behavior link for right-PCC-centered DMN perturbation.

Finally, the mechanisms underlying loss of insight in aging and AD are likely multifactorial, with contributions from executive control and salience/attention systems in addition to PCC-centered DMN processes (172); refined task batteries and multimodal approaches will be necessary to map these interactions.

In conclusion, by combining deep-TMS continuous theta-burst stimulation over the right posterior cingulate cortex with resting-state fMRI, we show that a brief, focal perturbation is sufficient to down-regulate connectivity within the default mode network (DMN) in healthy young adults. Seed-based analyses identified eight clusters, predominantly within canonical DMN territories, that were less coupled with the rPCC under stimulation than under sham, and ROI-wise results converged in the same direction even when conservative familywise control across parcels was applied. Despite a modest sample ($n = 12$), the within-subject contrast yielded a large effect (Cohen's $d^z = 0.933$; $t(11) = -3.233$; 95% CI $[-0.09396, -0.01784]$), indicating reliable network-level change within the post-stimulation window. Together, these findings establish a practical human model for transient, PCC-centered DMN disruption and provide a causal entry point for probing self-referential processing and metacognition.

Key limitations include the absence of concurrent behavioral measures and the restriction to young, healthy participants. Future studies should pair stimulation with brief, time-locked metacognitive tasks, refine target specificity with individualized electric-field modeling and neuronavigation, and extend the paradigm to older adults and clinical cohorts. More broadly, demonstrating brain–behavior links under controlled perturbation may help bridge systems-level accounts of the DMN with symptom mechanisms such as anosognosia in Alzheimer’s disease.

Figures and tables

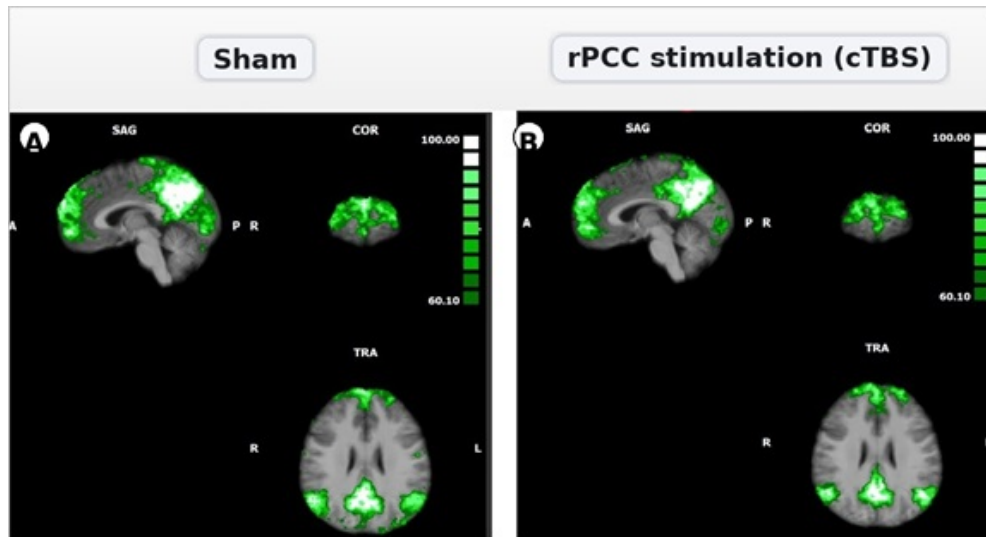


Figure 1. Seed-based connectivity maps under **sham** (panel A, left) and **right-PCC stimulation** (panel B, right) following deep TMS with a continuous theta-burst stimulation (**cTBS**) protocol. Three orthogonal views are shown (SAG, COR, TRA; orientation labels A/P and R/L). Overlays (green scale; 60.10–100.00) depict the thresholded group maps of coupling with the rPCC seed; brighter shades indicate stronger map intensity. Visual inspection highlights a reduction of DMN-consistent coupling in the stimulation condition. Resting-state fMRI was acquired 10–20 minutes after the end of stimulation (mean 15.2 ± 3.4 min); in each session, the T1-weighted structural scan preceded rs-fMRI. Each rs-fMRI run comprised 250 volumes (TR = 1500 ms; TE = 30 ms; 64 slices; multiband factor = 3; 2-mm isotropic voxels; duration ≈ 6 min 15 s).

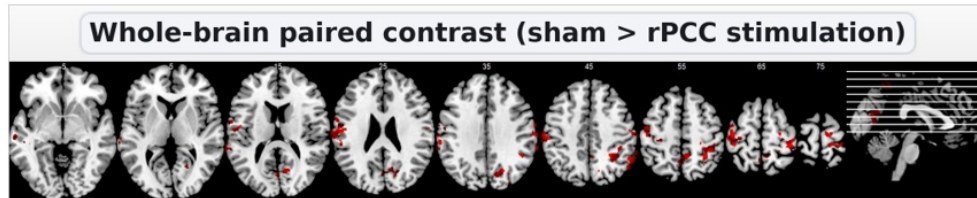


Figure 2. Whole-brain paired contrast (**sham > rPCC stimulation**) of seed-based connectivity. Red clusters mark regions showing lower coupling with the rPCC during stimulation relative to sham. Axial slices are displayed in MNI space at $z = 5, 15, 25, 35, 45, 55, 65,$ and 75 mm; the sagittal panel (right) indicates slice positions. Eight clusters survived cluster-extent Monte-Carlo correction ($p < .05$; cluster-size threshold = **187 mm³**) and correspond primarily to DMN territories (right angular gyrus, bilateral supramarginal gyri, bilateral precuneus, left posterior superior temporal gyrus; see **Table 1** for peak coordinates).

Table 1.

Brain region*	Cluster size			
	x	y	z	(mm ³)
Right angular gyrus*	59	-50	44	2535
Right supramarginal gyrus*	62	-14	40	1886
Right superior parietal gyrus	32	-38	59	5992
Right precuneus*	12	-69	23	3995
Left precuneus*	-2	-50	56	1984
Left postcentral gyrus	-39	-19	62	3922
Left posterior superior temporal gyrus*	-64	-28	4	1753
Left supramarginal gyrus*	-61	-18	31	5279

Comment. Comparing condition-wise BOLD time courses (sham vs. rPCC stimulation with deep cTBS) revealed **eight clusters** with significantly **lower** coupling to the rPCC under stimulation in a whole-brain paired *t*-test corrected for multiple comparisons ($p < .05$; cluster-extent threshold = 187 mm³). Six clusters (*)

lie within DMN regions.** Seed-based voxelwise contrast (**sham > rPCC stimulation**) following deep TMS with a continuous theta-burst stimulation (**cTBS**) protocol. **Eight clusters** exhibited reduced functional connectivity with the rPCC under stimulation. Significant clusters were predominantly within the DMN: right angular gyrus, right and left supramarginal gyri, right and left precuneus, and left posterior superior temporal gyrus. Results are cluster-extent corrected by Monte-Carlo simulation ($p < .05$; cluster-size threshold = **187 mm³**).

2.2.3.1 Summary of main findings and implications of the first phase

Taken together, this first experimental phase fulfils its primary objective: it demonstrates that transient inhibition of the right posterior cingulate cortex by deep TMS is sufficient to disrupt functional connectivity within the default mode network in young healthy adults. The decreases in connectivity observed between the stimulation site and several parietal and temporal DMN nodes provide an additional causal argument to complement the predominantly correlational data from the Alzheimer's disease literature. They suggest that the PCC is not merely a passive marker of neurodegeneration, but a strategic hub whose perturbation is enough to induce a reconfiguration of the self-referential network.

Beyond this proof-of-concept result, the study also validates a neuromodulation protocol that combines neuronavigation, a deep TMS coil, and resting-state functional MRI under conditions compatible with subsequent behavioural

assessment. It thus paves the way for an original experimental approach: to perturb, in a controlled manner, a key DMN hub and then examine, within the same temporal window, the consequences of this perturbation on fine-grained measures of self-evaluation and metacognition. This transition, from the network level to the behavioural level, lies at the core of the second phase of our work.

2.2.4 Second phase

Building on the first phase, this second study aimed to test whether the transient desynchronisation of the default mode network (DMN), demonstrated at the level of functional connectivity, would translate into measurable changes in cognitive self-evaluation. In other words, the goal was to move from a purely neurofunctional model, in which stimulation of the PCC alters coupling between network nodes, to a model that explicitly integrates the behavioural dimension of metacognition, through confidence ratings and discrepancy indices between objective performance and subjective judgment.

As previously mentioned, three main discrepancy-based methods are classically used to assess anosognosia or self-evaluation deficits, but only the approach comparing objective cognitive performance with subjective prediction of that performance could be applied in a healthy population. We therefore reused the deep TMS protocol targeting the right PCC, applying the same theta-burst stimulation pattern, but

replacing resting-state fMRI with a visual memory task followed by trial-by-trial self-evaluations.

This second phase is a direct extension of the first: it is grounded in the same theoretical framework (the role of the PCC and DMN in self-awareness) and translates the question of anosognosia into an experimental model in healthy participants, by examining whether an acute perturbation of the self-referential network is sufficient to alter the calibration between memory accuracy and confidence.

2.2.5 Modulating confidence timing without altering accuracy: effects of deep TMS over the default mode network in a color versus grayscale memory task.

Abstract

Background:

Self-evaluation of cognitive performance is thought to rely on distributed brain networks that overlap with the default mode network (DMN), and in particular the posterior cingulate cortex (PCC). Deep transcranial magnetic stimulation (deepTMS) targeting the PCC provides a means to test its causal contribution to metacognitive processes such as confidence and metacognitive bias (discrepancy between confidence and accuracy).

Methods:

Eleven healthy adults completed a randomized, sham-controlled, within-subject crossover protocol with continuous theta-burst deep transcranial magnetic stimulation (deep TMS) over the right PCC. In two sessions (active vs. sham, separated by one week), they performed a 2AFC colour vs. black-and-white

recognition task with 4-point confidence ratings, beginning 15 minutes after each stimulation session. We analysed recognition accuracy, confidence, and response times, and quantified metacognitive sensitivity using the area under the type 2 ROC curve (type 2 AUC).

Results

Active deepTMS over the right PCC did not significantly change overall recognition accuracy, mean confidence, mean RTs, or global discrepancy relative to sham. Participants showed a mild underconfidence in both conditions, with no reliable TMS effect on metacognitive bias or its evolution over time. Across sessions, RTs for memory and confidence decreased markedly with trial order, indicating strong practice effects, but the corresponding slopes did not differ between conditions. Exploratorily, a significant Condition $\times$ Stimulus Format interaction emerged for confidence RTs: in the sham session, confidence judgments were faster for black-and-white than for color stimuli, whereas after TMS this pattern was attenuated or reversed, with relatively faster confidence for color items.

Conclusions:

Right-PCC deepTMS in healthy adults does not disrupt recognition performance, average confidence, or metacognitive bias, but may subtly modulate the temporal dynamics of confidence formation in a stimulus-dependent manner. These preliminary findings support a nuanced role of PCC–DMN circuitry in the timing

and contextualization of metacognitive judgments rather than in first-order memory accuracy or gross confidence calibration.

Introduction

Awareness of one's own cognitive abilities is a central feature of human cognition. In everyday life, we continually monitor how well we remember, perceive, and make decisions, and we adapt our behaviour accordingly. These metacognitive processes can be probed experimentally using confidence judgments and indices that relate subjective estimates to objective performance. In neurological conditions, such monitoring can be markedly altered, leading to anosognosia, i.e. a reduced or absent awareness of deficits. In Alzheimer's disease (AD), impaired insight into memory decline is frequent and clinically important, as it has been associated with worse functional outcomes and faster progression to dementia (152).

A converging body of imaging work implicates the default mode network (DMN) and particularly the right posterior cingulate cortex (PCC) in self-referential processing and awareness of cognitive abilities (173). The DMN is a large-scale network preferentially engaged during internally oriented cognition, including autobiographical memory (174), future thinking and self-related thought (173,175). Within this system, the PCC occupies a central hub position, with dense structural and functional connectivity to medial prefrontal and lateral parietal regions, and is

thought to integrate self-relevant information with episodic memory and other associative networks (176). In the AD continuum, functional and metabolic abnormalities of the PCC complex are among the earliest identifiable signatures, and several studies have linked hypometabolism or disconnection within PCC-centred DMN subnetworks to reduced awareness of memory deficits and to clinical progression(177,178). These observations have motivated our hypothesis that anosognosia for memory impairment may, at least in part, reflect a disruption of PCC-centred self-referential networks.

In parallel, the broader metacognition literature has highlighted a distributed set of brain regions that contribute to confidence and self-evaluation, including prefrontal, parietal and midline structures(23). Trial-by-trial confidence ratings, analysed using signal-detection–based measures or calibration metrics, have become a standard tool to quantify metacognitive sensitivity and efficiency in healthy and clinical populations (179,180). Functional neuroimaging studies generally suggest that metacognitive judgments recruit both task-relevant frontoparietal control regions and DMN hubs such as the PCC and angular gyrus, with DMN activity often associated with internally focused evaluation and self-related processing(181). Alterations in DMN connectivity have been reported in association with changes in metacognitive accuracy or self-awareness, however, most of these links remain correlational, and the precise contribution of PCC-centred networks to different aspects of metacognition is still being clarified.

One way to move beyond correlational evidence is to transiently perturb specific brain regions and assess the impact on confidence and performance. Non-invasive neuromodulation techniques such as transcranial magnetic stimulation (TMS) has been widely used to probe the neural basis of metacognition(182,183). Conversely, stimulation of sensory cortices can modify perceptual confidence by adding noise to sensory evidence, again without consistently changing objective performance(184). Together, these findings indicate that neuromodulation can influence metacognitive judgments through several cortical entry points; however, they have so far provided relatively little direct evidence about the specific role of midline DMN hubs such as the PCC.

A major methodological challenge is that the PCC is deep and medially located, placing it near the limits of conventional figure-of-eight coils. Deep TMS with specialized bend coil geometries offers a partial solution by increasing the penetration of induced electric fields into medial and subcortical regions. In our own previous study, continuous theta-burst deep TMS applied to the right PCC in healthy young adults transiently reduced resting-state DMN connectivity, with decreased coupling between the stimulated site and several canonical DMN hubs, thereby providing a proof-of-principle that PCC activity and its network embedding can be causally manipulated. While such network-level findings are promising, it is still unclear whether comparable perturbations of PCC-centred circuits are sufficient to produce measurable changes in metacognitive behaviour.

If PCC-centred DMN integrity contributes to monitoring one's own cognitive performance, then transient inhibition of the right PCC might be expected to alter the relationship between objective task accuracy and subjective confidence.

In dementia research, this relationship is often operationalised as a discrepancy between objective performance and self-rated abilities, with larger discrepancies interpreted as reduced insight or emerging anosognosia (14). Translating this logic to experimental neuromodulation, one can ask whether perturbing PCC-centred DMN activity in healthy participants modifies the calibration between what they actually remember and how confident they feel about their memory decisions, even if first-order performance remains largely preserved.

In the present study, we combined right-PCC deep TMS with a forced-choice visual memory task and trial-by-trial confidence judgments. Healthy participants completed two sessions, one with real continuous theta-burst deep TMS over the right PCC and one with sham stimulation, in a randomized cross-over design. Exactly 15 minutes after stimulation, within the time window in which we previously observed connectivity changes in our previous fMRI study. We asked participants to perform a two-alternative forced-choice (2AFC) recognition task in which they judged, for each item, whether it had previously been presented in colour or in black-and-white, and then rated their confidence on a four-point scale. This paradigm allowed us to quantify (I) first-order memory performance, (II) confidence distributions and response times, and (III) the alignment between confidence and

accuracy across conditions. By manipulating the visual format (colour vs. grayscale) and analysing trial order, we could also explore, whether PCC neuromodulation differentially affects specific stimulus categories or the temporal dynamics of metacognitive judgments.

Our primary aim was to test whether transient inhibition of the right PCC via deep TMS modulates metacognitive behaviour in healthy young adults, over and above any change in objective memory performance. Based on prior evidence implicating DMN and PCC dysfunction in anosognosia, we anticipated that PCC stimulation was more likely to produce subtle changes in confidence calibration, discrepancy indices, or reaction times than a marked drop in accuracy. At the same time, given the modest sample size and the relatively simple nature of the memory task, we considered it equally plausible that behavioural effects would be small or restricted to specific dimensions (such as the timing of confidence formation). By directly linking a PCC-centred neuromodulation protocol to behavioural readouts of confidence, this study seeks to provide an experimental bridge between systems-level models of DMN dysfunction and mechanistic accounts of self-awareness that are relevant for ageing and neurodegenerative disease.

Methods

Participants

Twelve healthy young adults (6 women, 6 men; age range 26–30 years, mean age 28 years) took part in the study. All participants were right-handed according to the Edinburgh Handedness Inventory and reported no history of neurological or psychiatric disease, no personal or family history of epilepsy, and no other contraindication to transcranial magnetic stimulation (TMS) or MRI. Exclusion criteria included the use of chronic medication and any structural brain abnormality visible on MRI. A relatively narrow age range was chosen to minimise inter-individual variability in brain functional connectivity and to reduce the risk of age-related confounds such as microvascular lesions or preclinical neurodegenerative changes.

All participants gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee of UCLouvain (Ref: 2019/19SEP/410, B403201941608). Twelve participants were enrolled; one was excluded from the present behavioural analyses because of incomplete data in one stimulation session, leaving 11 participants for all reported analyses.

Experimental design and procedure

The experiment followed a randomised, sham-controlled, within-subject cross-over design embedded in a broader protocol that also included an fMRI component reported elsewhere. The stimulation and imaging aspects of this protocol have been described in detail in our previous report on deep TMS modulation of the default mode network (Bellaali et al. 2025 under review). Each participant attended three visits scheduled one week apart. During the first visit, a high-resolution structural MRI scan was acquired and used to build an individual 3D brain model for neuronavigation and to exclude incidental brain lesions.

During the second and third visits, participants received either real deep TMS targeting the right posterior cingulate cortex (rPCC) or sham stimulation, in a counterbalanced order across participants. Half of the sample received real stimulation during the second visit and sham during the third; the remaining half received sham first followed by real stimulation. In the original imaging protocol, stimulation was followed by resting-state fMRI acquisition. In the present study, we used the same stimulation sessions but replaced the fMRI acquisition with a visual memory and confidence task.

In each of the two stimulation sessions (real vs. sham), the procedure was as follows: (I) deep TMS or sham stimulation over the rPCC; (II) the visual memory task, comprising an encoding phase a waiting period of 15 minutes, during which participants remained seated quietly eyes closed; (III) followed by a recognition-

plus-confidence phase. The 15-minute delay was chosen to coincide with the mean post-stimulation interval in our previous study (Bellaali et al. 2025, under review) during which PCC desynchronization from the default mode network (DMN) was observed following the same continuous theta-burst protocol.

Deep TMS protocol

Continuous theta-burst stimulation (cTBS) was used as the neuromodulation protocol. This patterned stimulation is known to induce inhibitory after-effects on cortical excitability that can last up to approximately one hour after stimulation (185,186). The cTBS train consisted of bursts of three pulses at 50 Hz, repeated every 200 ms (5 Hz) for 40 s, yielding a total of 600 pulses.

Stimulation intensity was set at 80% of each participant's individual motor threshold. The motor threshold was determined using single-pulse TMS over the lower-limb primary motor cortex, chosen because its cortical depth is comparable to that of the rPCC. Surface electromyography (EMG) was recorded from the left tibialis anterior muscle using standard electrode placement and ground referencing according to European recommendations for surface EMG (160). The motor threshold was defined as the lowest stimulation intensity producing motor-evoked potentials greater than 50 μ V in at least 50% of 10 consecutive trials.

Deep magnetic stimulation was delivered with a bended double-cone coil (MagVenture DB80) connected to a MagPro X100 stimulator (MagVenture, Farum, Denmark), which is suitable for targeting deeper cortical structures. The right PCC

target was defined using MNI coordinates (4, -53, 42). Individual structural MRI scans were used to reconstruct 3D anatomical models, and a neuronavigation system (Visor 2, ANT Neuro, Enschede, Netherlands) ensured accurate coil positioning throughout stimulation.

In the real stimulation condition, the coil was positioned tangentially to the scalp over the rPCC target, with the handle oriented to maximise the induced electric field at the target according to the neuronavigation guidance. In the sham condition, the same coil was held at a 90° angle over the same scalp location, producing a comparable auditory click and scalp sensation while markedly reducing the effective intracranial electric field. Participants were blind to stimulation condition; the experimenter delivering stimulation was not.

Visual memory and confidence task

Encoding phase

We used a two-alternative forced-choice (2AFC) visual recognition task to probe mnemonic performance and metacognitive evaluation after stimulation.

During the encoding phase of each session (real TMS and sham), participants viewed 130 visual items presented sequentially at the centre of the screen. For each session, 65 items were shown in grayscale (black-and-white) and 65 in colour. Stimulus format (colour vs. black-and-white) was randomised across trials. Images were

drawn from a the standardized Snodgrass and Vanderwart object set, using the colourized versions by Rossion and Pourtois with validated low-level perceptual properties, ensuring that colour and grayscale versions were comparable in visual salience and complexity (187,188). Each image was paired with a unique verbal label (noun) presented below the picture.

Participants were instructed to pay attention to both the item and its visual format (colour vs. black-and-white), as they would later be asked to recall the format associated with each item. No overt response was required during encoding.

Recognition and confidence phase

Following encoding, participants performed the recognition and confidence task on the same set of items. On each trial, only the verbal label was presented on the screen, without the corresponding image. Participants were asked to indicate whether the originally presented image associated with that label had been in colour or in black-and-white. The two response alternatives (“colour” vs. “black-and-white”) were presented on the screen. The order of trials in the recognition phase was randomised, independently of the encoding sequence.

Immediately after each recognition response, participants provided a trial-wise confidence judgement regarding the accuracy of their decision. Confidence was rated on a four-point scale with the following anchors: 1) totally at random, 2) almost random, 3) almost certain, 4) certain. Participants were instructed to use the full

range of the scale and to base their rating solely on how sure they felt that their response was correct. Both recognition responses and confidence ratings were self-paced; reaction times (RTs) were recorded from stimulus onset to keypress for each decision.

Behavioural measures

For each participant, session (Sham, TMS), and trial, we recorded: (I) recognition accuracy (correct vs. incorrect format discrimination), (II) confidence rating (1–4), (III) RT for the memory decision, and (IV) RT for the confidence judgement.

Objective memory performance was quantified as the proportion of correct responses in the 2AFC task. Mean confidence level was computed as the average rating across trials. RTs were averaged separately for memory decisions and confidence judgements.

Metacognitive sensitivity (type 2 AUC)

To quantify metacognitive sensitivity, we used a type 2 receiver operating characteristic (ROC) approach based on trial-wise confidence ratings, inspired by signal detection theoretic treatments of metacognition (189). For each participant and condition (Sham, TMS), objective accuracy was coded as 1 for correct format judgements and 0 for incorrect judgements, and confidence ratings (1–4) were

treated as an ordinal decision variable indicating the perceived likelihood of being correct. Type 2 ROC curves were constructed by progressively collapsing confidence levels into “high” versus “low” confidence criteria and computing, for each criterion, the hit rate (proportion of correct trials rated with high confidence) and false-alarm rate (proportion of incorrect trials rated with high confidence). Metacognitive sensitivity was then summarised as the area under the type 2 ROC curve (type 2 AUC), which can be interpreted as the probability that a randomly chosen correct trial receives a higher confidence rating than a randomly chosen incorrect trial. A value of 0.5 corresponds to chance-level metacognitive discrimination, whereas values approaching 1 indicate near-perfect discrimination between correct and incorrect responses on the basis of confidence. Type 2 AUC was computed separately for each participant and condition and served as our primary index of metacognitive sensitivity. In exploratory analyses, we also examined potential time-on-task effects by splitting each 130-trial session (sham and TMS, administered on separate days) into an early block (trials 1–65) and a late block (trials 66–130) and recomputing type 2 AUC for each block..

Statistical analysis

Data analysis was performed using R (version 4.3.2) (190). All analyses were conducted on participant-level summary measures using two-sided tests with an alpha level of 0.05. For the main outcomes, we first computed, for each participant

and condition, the mean recognition accuracy, mean confidence rating, and mean RTs for memory decisions and confidence judgements. These participant-level averages were compared between Sham and TMS using paired-sample t-tests.

To assess the effects of stimulus format, we performed 2×2 repeated-measures ANOVAs with within-subject factors Condition (Sham vs. TMS) and Stimulus format (Colour vs. Black-and-white). These ANOVAs were run separately for recognition accuracy, mean confidence ratings, memory RTs, and confidence RTs. Partial eta-squared (η^2) was reported as an effect-size index for ANOVA effects. To characterize time-on-task effects, we modelled the evolution of performance and RTs across the sequence of trials (order 1–130). For each participant and condition, we fitted simple linear regressions relating trial order to (i) accuracy, (ii) confidence level, (iii) memory RT, and (iv) confidence RT, and compared the resulting slopes between Sham and TMS with paired t-tests. In a complementary analysis, we divided each session into an early block (trials 1–65) and a late block (trials 66–130) and entered the corresponding confidence RT means into a 2 (Condition) $\times$ 2 (Format) $\times$ 2 (Block) repeated-measures ANOVA to test for practice-related speeding and its interaction with TMS and stimulus format.

For metacognitive sensitivity, we computed type 2 area under the ROC curve (type 2 AUC) from trial-wise confidence ratings, separately for each participant and condition. Type 2 AUC was defined as the probability that a randomly chosen correct trial received a higher confidence rating than a randomly chosen incorrect trial, with

0.5 indicating chance-level discrimination and values approaching 1 indicating near-perfect metacognitive sensitivity. Participant-level type 2 AUC values were compared between Sham and TMS using paired-sample t-tests. In exploratory analyses, we also examined time-on-task effects on metacognitive sensitivity by computing type 2 AUC within successive trial blocks and submitting these block-wise indices to within-subject comparisons between conditions.

Because metacognitive and RT measures may deviate from normality and can be sensitive to outliers, we complemented all key parametric comparisons with non-parametric tests. Specifically, we repeated the main Sham–TMS comparisons on median accuracy, confidence, and RT using Wilcoxon signed-rank tests, and we compared the distribution of Spearman correlation coefficients (e.g., discrepancy vs. order) between conditions using Wilcoxon tests. Where appropriate, we also examined non-parametric indices of Condition $\times$ Format interactions (e.g., median colour–minus–black-and-white differences) to check the robustness of the ANOVA findings.

Results

Overall memory performance and confidence

For each participant and condition, we computed mean recognition accuracy (proportion correct in the 2AFC task), mean confidence rating, and mean response times (RTs) for the memory decision and the subsequent confidence judgement. Paired-sample t-tests on these participant-level averages revealed no main effect of stimulation on any of these global measures.

Mean recognition accuracy was similar in the Sham ($M = 0.77$, $SD = 0.11$) and TMS sessions ($M = 0.78$, $SD = 0.10$), $t(10) = 0.81$, $p = 0.44$. Mean confidence ratings (on the 1–4 scale) did not differ between conditions (Sham: $M = 2.88$, $SD = 0.56$; TMS: $M = 2.91$, $SD = 0.56$), $t(10) = 0.42$, $p = 0.68$. Likewise, there was no evidence for a stimulation effect on mean RTs for the memory decision (Sham: $M = 2.44$ s, $SD = 0.66$; TMS: $M = 2.44$ s, $SD = 0.56$; $t(10) = 0.02$, $p = 0.99$) or for the confidence judgement (Sham: $M = 0.72$ s, $SD = 0.26$; TMS: $M = 0.72$ s, $SD = 0.16$; $t(10) \approx 0.01$, $p \approx 0.99$). These analyses indicate that, at a global level, deep TMS over the right PCC did not reliably alter recognition performance, subjective confidence, or overall response speed.

Metacognitive sensitivity (type 2 AUC)

Beyond mean confidence levels, we quantified metacognitive sensitivity by computing type 2 receiver operating characteristic (ROC) curves from trial-by-trial confidence ratings and deriving the corresponding area under the curve (type 2 AUC) for each participant and condition. This index reflects the probability that confidence ratings are higher for correct than for incorrect responses, and thus provides a distribution-free measure of how well participants can discriminate between their own correct and incorrect decisions. At the whole-experiment level, type 2 AUC was clearly above chance in both conditions and did not differ between real and sham stimulation (sham: $M = 0.74$, $SD = 0.07$; TMS: $M = 0.75$, $SD = 0.09$; paired t-test: $t(10) = 0.60$, $p = 0.56$, $d_z = 0.18$), despite comparable objective memory performance (Figure 1A). In exploratory analyses, we further examined potential time-on-task effects within each session, taking into account that sham and TMS were administered on separate days in a counterbalanced order. For each 130-trial session (sham and TMS), trials were ordered according to their temporal position and divided into an early block (trials 1–65) and a late block (trials 66–130), and type 2 AUC was recomputed separately for each block and condition. Descriptively, type 2 AUC values remained in a similar range across early and late blocks in both conditions (sham: early $M = 0.77$, $SD = 0.08$; late $M = 0.72$, $SD = 0.10$; TMS: early $M = 0.76$, $SD = 0.11$; late $M = 0.75$, $SD = 0.09$). A 2 (Condition: sham, TMS) $\times$ 2 (Block: early, late) repeated-measures ANOVA on block-wise AUCs revealed

neither a main effect of Condition ($F(1,10) = 0.17, p = 0.69$) nor of Block ($F(1,10) = 1.06, p = 0.33$), and the Condition $\times$ Block interaction was likewise non-significant ($F(1,10) = 1.33, p = 0.28$; Figure 1B). To provide a more fine-grained view of potential temporal dynamics, we also computed trial-wise estimates of metacognitive sensitivity using a moving-average smoothing procedure. As illustrated in Figure 2, both sham and TMS sessions remained consistently above chance and showed no systematic divergence over the course of the task. Overall, these converging analyses suggest that deep TMS over the right PCC did not measurably affect participants' ability to monitor the correctness of their recognition judgements, even though it produced subtle modulations of the temporal dynamics of confidence responses (see below; Figures 1–2).

Effects of stimulus format on accuracy and confidence

To test whether performance depended on stimulus format and whether any such effects were modulated by stimulation, we ran 2×2 repeated-measures ANOVAs with within-subject factors Condition (Sham vs. TMS) and Stimulus format (colour vs. black-and-white). For recognition accuracy (Table 1A), there was no main effect of Condition, $F(1,10) = 0.66, p = 0.44$, partial $\eta^2 = 0.06$, but a significant main effect of Stimulus format, $F(1,10) = 6.31, p = 0.031$, $\eta^2 = 0.39$, reflecting slightly better performance for black-and-white images than for colour images across both

conditions (e.g., Sham: $M = 0.81$ vs. 0.72 ; TMS: $M = 0.84$ vs. 0.72). The Condition $\times$ Format interaction on accuracy was not significant, $F(1,10) = 0.38$, $p = 0.55$, $\eta^2 = 0.04$.

Analogous ANOVAs for mean confidence ratings and for memory-decision RTs did not reveal any significant main effects or interactions (all p -values ≥ 0.30), consistent with the paired t -tests on global averages. Thus, the intrinsic visual format of the stimuli influenced recognition accuracy, with a modest advantage for black-and-white images, but this effect was not detectably modulated by PCC stimulation.

Time-on-task effects

We next examined within-session dynamics as a function of trial order (1–130). For each participant and condition, we fitted simple linear regressions relating trial order to accuracy, confidence level, memory RT, and confidence RT, and compared the resulting slopes between Sham and TMS sessions. Across participants, slopes for accuracy and confidence were close to zero and did not differ between conditions (all $|t(10)| \leq 1.40$, all p s ≥ 0.19), indicating relatively stable performance and confidence over time. Slopes for RTs tended to be negative in both conditions, consistent with practice-related speeding, but again did not differ reliably between Sham and TMS.

To provide a more interpretable summary of time-on-task effects on confidence RTs, we divided each session into an early block (trials 1–65) and a late block (trials 66–130) and collapsed across condition and format. Confidence RTs decreased robustly from the first to the second half of the task (Early: $M = 0.82$ s, $SD = 0.19$; Late: $M = 0.62$ s, $SD = 0.14$; $t(10) = -7.21$, $p < 0.001$; Table 2), indicating a strong practice or habituation effect.

A complementary 2 (Condition) $\times 2$ (Format) $\times 2$ (Block) repeated-measures ANOVA on confidence RTs confirmed a strong main effect of Block, $F(1,10) = 45.43$, $p < 0.001$, $\eta^2 = 0.82$ (participants became faster over time), and a significant Condition $\times$ Format interaction, $F(1,10) = 6.48$, $p = 0.029$, $\eta^2 = 0.39$, similar to the simpler 2×2 model. No three-way interaction Condition $\times$ Format $\times$ Block was observed, $F(1,10) = 0.10$, $p = 0.76$, indicating that practice-related speeding was broadly similar in Sham and TMS sessions and for both stimulus formats, and that the TMS $\times$ Format interaction on confidence RTs did not depend strongly on task period.

TMS $\times$ stimulus format interaction on confidence response times

In contrast to the null effects on accuracy and mean confidence level, a more subtle effect emerged for the RTs of the confidence judgements. A 2×2 ANOVA on confidence RTs with factors Condition and Stimulus format (Table 1B) showed no

main effect of Condition, $F(1,10) = 0.00$, $p = 0.99$, $\eta^2 < 0.001$, and no main effect of Format, $F(1,10) = 0.01$, $p = 0.93$, $\eta^2 \approx 0.001$. However, the Condition $\times$ Format interaction was significant, $F(1,10) = 5.31$, $p = 0.044$, $\eta^2 = 0.35$.

Descriptively, in the Sham condition confidence judgements tended to be slightly faster for black-and-white than for colour stimuli ($M = 0.71$ s vs. 0.74 s), whereas in the TMS condition this pattern was reversed, with slightly faster confidence judgements for colour than for black-and-white stimuli ($M = 0.70$ s vs. 0.74 s) (Figure 2). Although the absolute differences were small (on the order of 30–40 ms), the crossover pattern suggests that deep TMS over PCC may selectively influence the temporal dynamics of confidence formation as a function of stimulus format, without producing large changes in accuracy or confidence magnitude.

Discussion

In this experiment, we examined whether deep transcranial magnetic stimulation (deep TMS) applied over the right posterior cingulate cortex (rPCC) modulates memory performance and metacognitive judgements in healthy adults. Participants performed a two-alternative forced-choice (2AFC) recognition task (colour vs.

black-and-white) followed by trial-by-trial confidence ratings, after either real or sham stimulation.

Overall, deep TMS did not produce robust changes in objective memory accuracy, mean confidence level, global response times, or metacognitive sensitivity as indexed by type 2 area under the ROC curve (type 2 AUC). The main effects that emerged concerned (i) a reliable practice-related acceleration of reaction times across trials, (ii) stable above-chance metacognitive sensitivity over time, accompanied by a gradual, stimulation-independent decrease in mean confidence ratings across blocks and (iii) a modest but consistent interaction between stimulation condition and stimulus format on confidence reaction times. Below, we first situate these findings in the context of PCC/DMN function, then consider the specific metacognitive effects, practice-related dynamics and methodological constraints, before discussing implications for anosognosia and Alzheimer's disease and outlining directions for future work.

PCC, DMN, and metacognitive monitoring

The PCC is a core hub of the default mode network (DMN), with dense connectivity to medial prefrontal, lateral parietal, and medial temporal structures(191). This network is engaged by internally oriented cognition, autobiographical memory, and self-referential processing, and it has often been implicated in monitoring one's own mental states(192). Building on our previous work showing that the same deep TMS

protocol can transiently down-regulate PCC-centred DMN connectivity at rest, we reasoned that applying cTBS over the right PCC and probing participants ~15 minutes later might alter how they monitor and evaluate their own performance on a memory task.

The present behavioural data provide little evidence for a large, global impact of this PCC protocol on either first-order performance or metacognitive monitoring. Both parametric and non-parametric analyses converged on null or clearly non-significant effects when comparing Sham and TMS sessions for mean accuracy, mean confidence ratings, and mean reaction times for memory and confidence decisions. Critically, type 2 AUC, our primary index of metacognitive sensitivity based on trial-wise confidence ratings, was clearly above chance in both conditions and did not differ between Sham and TMS sessions, nor did it show any consistent divergence across blocks. Within the limits of our sample size and task sensitivity, these findings suggest that transient PCC perturbation alone is not sufficient to substantially disrupt performance monitoring, at least as captured by standard behavioural and metacognitive indices in a relatively simple perceptual memory task in healthy young adults. Against this background of largely preserved performance and metacognitive sensitivity, the more subtle effects on confidence dynamics become particularly informative.

Subtle stimulation × format effects on confidence dynamics

The most consistent TMS-related effect concerned confidence reaction times. In the Sham condition, confidence judgements tended to be slightly faster for black-and-white stimuli than for coloured stimuli, whereas in the TMS condition this pattern was reduced or reversed, with marginally faster confidence judgements for colour images. This Condition × Format interaction was statistically significant in our ANOVA models and showed a similar trend in the non-parametric analyses, although the absolute magnitude of the effect remained small.

One tentative interpretation is that PCC modulation may alter how low-level visual properties (such as the presence or absence of colour) are integrated into the temporal dynamics of confidence formation. Confidence judgements are known to be shaped not only by sensory evidence but also by top-down expectations, task structure, and heuristic cues (193). If the PCC acts as a hub integrating internal context with perceptual evidence within the DMN, a mild perturbation could subtly re-weight the contribution of stimulus format to the timing of metacognitive evaluation, without necessarily changing overall accuracy, the mean level of confidence, or the ability of confidence to discriminate between correct and incorrect responses (as indexed by type 2 AUC). The fact that the interaction is restricted to RTs, rather than to confidence magnitude or metacognitive sensitivity, is compatible with models in which confidence evolves as a post-decisional process that can be sped up or slowed down without large shifts in decision criteria (194).

Given the small number of participants and the exploratory nature of this contrast, these observations should be interpreted cautiously. At the same time, they are broadly consistent with our initial hypothesis that PCC–DMN perturbation might influence metacognitive processes more through fine-grained adjustments of internal monitoring dynamics than through marked behavioural deficits. This view is reinforced when one considers the joint evolution of metacognitive sensitivity and confidence over time.

Metacognitive sensitivity and confidence over time

Our type 2 AUC analyses provide a complementary view on metacognition that focuses on sensitivity rather than bias. Across both sessions, metacognitive sensitivity was robust: type 2 AUC values were substantially above chance (~ 0.75 on average), indicating that participants' confidence ratings reliably distinguished correct from incorrect trials. Importantly, we found no evidence that PCC stimulation altered this sensitivity. Participant-level type 2 AUC values were highly similar in Sham and TMS conditions, and block-wise analyses showed no systematic drift or interaction with stimulation, suggesting that the ability to monitor performance remained stable over the course of the task.

In contrast, mean confidence ratings showed a clear temporal trend: confidence declined gradually from early to late trials in both Sham and TMS sessions. This pattern suggests that participants became more conservative in their explicit

confidence judgements over time, even though their objective accuracy and metacognitive sensitivity remained relatively stable. One possibility is that, as the task progressed, participants incorporated accumulated uncertainty or subjective fatigue into their confidence reports, without losing access to trial-wise information about correctness. From a metacognitive standpoint, this dissociation between stable sensitivity (type 2 AUC) and decreasing confidence levels is informative: it suggests that PCC-targeted cTBS did not disrupt the mapping between evidence and confidence, but that global confidence calibration can drift for reasons unrelated to stimulation.

We also observed stimulus-format effects on behaviour that were not mirrored by strong metacognitive changes. Black-and-white images tended to be recognised slightly better than colour images overall, yet our metacognitive sensitivity analyses did not reveal a clear format $\times$ stimulation interaction. Participants thus maintained relatively well-calibrated confidence discrimination across formats, even when accuracy and confidence RTs varied subtly with stimulus properties. Together, these results point to a metacognitive system that is resilient to acute PCC perturbation in healthy adults, at least under the present task demands.

Practice effects, temporal dynamics and methodological considerations

Across both stimulation conditions, we observed robust decreases in reaction times for memory and confidence decisions as a function of trial order and block (early vs.

late), consistent with practice-related speeding. Order-related regressions and block-wise analyses revealed large early-to-late effects, and non-parametric tests confirmed that these temporal dynamics were comparable in Sham and TMS sessions. Thus, deep TMS did not produce a nonspecific acceleration or deceleration of processing, but rather seemed to be superimposed on a general trend toward faster responses and slightly declining confidence over time.

These findings must, however, be interpreted in light of several methodological constraints. First, the modest sample size ($N = 11$) limits statistical power, especially for relatively noisy measures such as metacognitive indices, so the present results should be viewed as preliminary effect-size estimates rather than definitive evidence for or against PCC involvement in specific metacognitive operations. Second, overall recognition performance was relatively high (~75–80% correct), which may have reduced the sensitivity of our behavioural and metacognitive measures to subtle changes in error monitoring. Third, the memory judgement was perceptual and low-level (colour vs. black-and-white), whereas the PCC and DMN are thought to play a more prominent role in self-referential and autobiographical processes; stronger effects of PCC modulation might emerge with tasks that tap these domains more directly.

An additional temporal limitation concerns the latency between stimulation and task performance: the metacognitive task was administered 15 minutes after rPCC cTBS. Although this delay was chosen based on prior evidence that DMN modulation peaks

in this window, it is possible that the behavioural impact of stimulation had already begun to diminish by the time participants began the task. Notably, in our previous study using the same protocol, robust PCC-centred DMN down-regulation was detected 15 minutes after stimulation using resting-state fMRI, a measure considerably more sensitive than behavioural indices. In contrast, metacognitive performance, which inherently carries more noise, may require either a shorter post-stimulation delay or concurrent assessment to capture subtle network perturbations. Thus, the absence of clear TMS effects in the present dataset may partly reflect a temporal mismatch between peak neurophysiological modulation and the behavioural window during which metacognition was probed.

Finally, we used a single stimulation site (right PCC), one continuous theta-burst protocol. DMN modulation is likely to depend on stimulation parameters and individual network architecture, suggesting that a broader exploration of protocols, ideally combined with concurrent neuroimaging, will be necessary to fully characterise how PCC perturbation influences metacognitive behaviour.

Implications for anosognosia and Alzheimer's disease

A central motivation for targeting the PCC is its early and prominent involvement in the Alzheimer's disease continuum, where anosognosia for memory deficits has been repeatedly linked to DMN hypometabolism and dysconnectivity (195). Network-based accounts propose that anosognosia may arise from disrupted error-

monitoring and self-evaluation mechanisms anchored in PCC and related DMN hubs, in interaction with medial prefrontal, hippocampal, and frontoparietal control regions (196).

Our findings in healthy individuals suggest that an acute, focal perturbation of the PCC is not by itself sufficient to generate a marked breakdown of metacognitive monitoring, at least in a simple perceptual memory task. Even when using a relatively sensitive ROC-based index of metacognitive sensitivity, we observed no reliable stimulation-induced degradation of the ability to distinguish correct from incorrect responses via confidence ratings. Instead, clinical anosognosia in Alzheimer's disease likely reflects a chronic, progressive, and distributed network disruption affecting multiple DMN and control nodes simultaneously. From this perspective, the present results are not inconsistent with DMN-based models of anosognosia; rather, they could indicate that PCC dysfunction must reach a certain threshold, and interact with broader network changes, before large behavioural manifestations of unawareness emerge.

Future directions

Within the broader framework of our work on PCC-centred DMN modulation, this experiment provides a behavioural complement to previous imaging findings. It shows that deep TMS over PCC can be applied safely in healthy adults without degrading memory performance, average confidence, or metacognitive sensitivity as

indexed by type 2 AUC, while possibly inducing fine-grained, stimulus-dependent changes in the temporal dynamics of confidence judgements and a global, stimulation-independent adjustment of confidence levels over time.

Future studies could build on these preliminary findings by (i) increasing sample size and statistical power; (ii) using tasks more directly tied to self-referential and autobiographical processes and (iii) extending the protocol to populations along the Alzheimer's continuum, where PCC–DMN dysfunction and anosognosia are central clinical issues. By systematically varying stimulation parameters, task demands, and metacognitive measures (e.g., combining ROC-based indices with model-based approaches), such work may help delineate the conditions under which perturbing a key DMN hub is sufficient or insufficient to alter metacognitive performance, and may ultimately contribute to the development of network-informed interventions targeting self-awareness in neurodegenerative disease.

Figures and tables

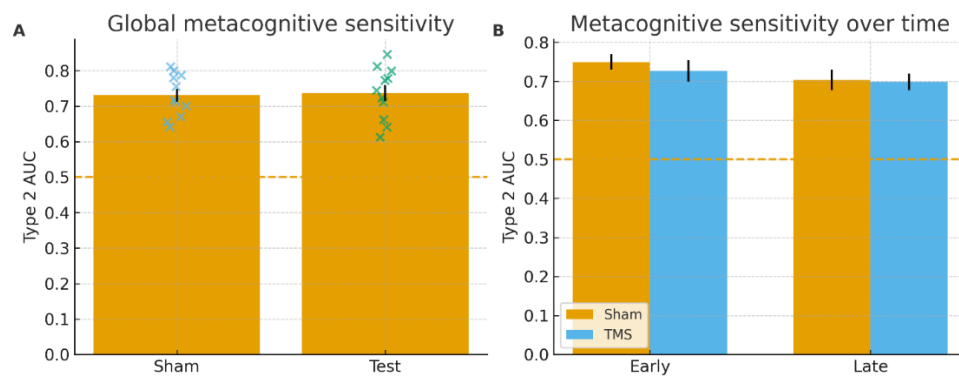


Figure 1. Metacognitive sensitivity (type 2 AUC) following deep TMS over the right PCC.

(A) Global type 2 AUC for sham and TMS sessions ($n = 11$). Bars show the mean across participants and error bars indicate ± 1 SEM; individual dots represent single participants. Metacognitive sensitivity is clearly above chance (0.5) in both conditions and does not differ between sham and TMS. (B) Block-wise type 2 AUC for early (trials 1–65) and late (trials 66–130) periods within each 130-trial session. A 2 (Condition: sham, TMS) $\times$ 2 (Block: early, late) repeated-measures ANOVA revealed no significant main effects or Condition $\times$ Block interaction, indicating that metacognitive sensitivity remained stable over time in both sessions. The horizontal dashed lines indicate chance-level performance (type 2 AUC = 0.5).

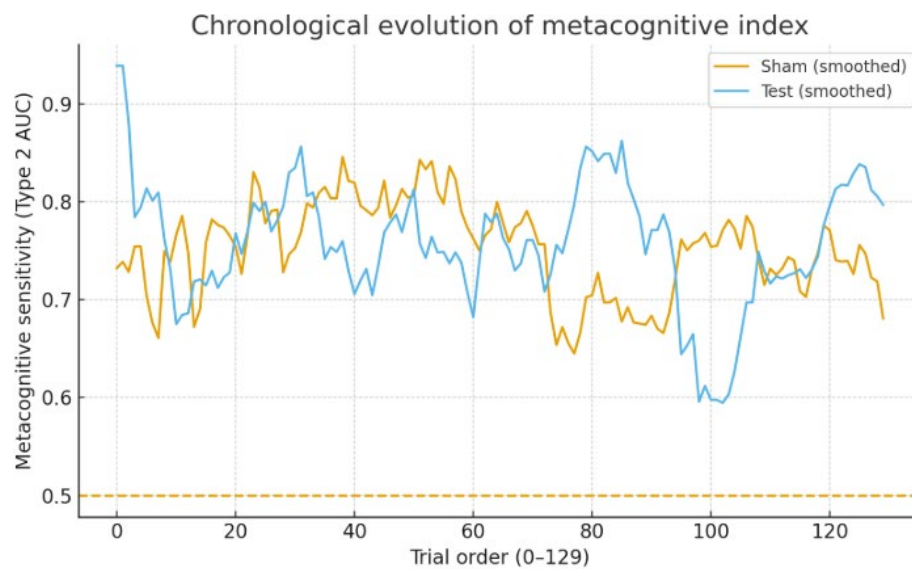


Figure 2. Chronological evolution of metacognitive sensitivity across trials.

Trial-wise metacognitive sensitivity (Type 2 AUC) is shown for Sham and TMS conditions after applying a moving-average smoothing procedure. Both conditions remained consistently above chance level (dashed line at 0.5), indicating preserved metacognitive discrimination throughout the task. No systematic divergence between Sham and TMS sessions was observed over the course of the 130 trials, suggesting that PCC stimulation did not alter the temporal dynamics of metacognitive sensitivity.

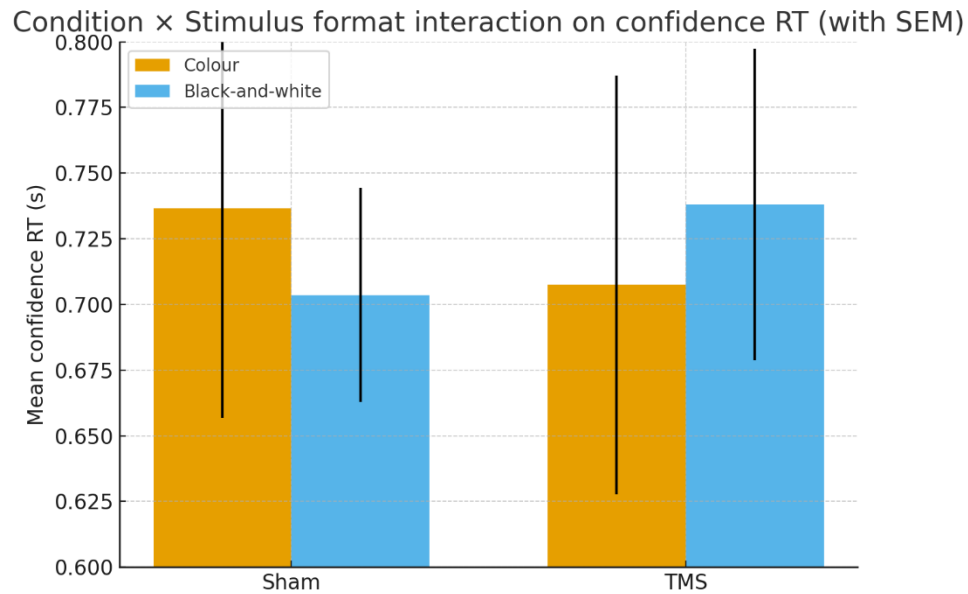


Figure 3. Condition $\times$ Stimulus format interaction on confidence response times.

Mean confidence RTs (ms) for colour and black-and-white stimuli in the Sham and TMS sessions. Error bars represent ± 1 SEM. While no main effects of Condition or Format were observed, a significant Condition $\times$ Format interaction emerged ($p = .044$), reflecting a subtle crossover pattern whereby black-and-white stimuli elicited faster confidence judgements than colour stimuli in the Sham condition, whereas the opposite trend was observed in the TMS condition.

Table 1A. Recognition accuracy (Condition $\times$ Format)

Effect	F-value	p-value	Effect size
Main effect: Condition	F(1,10)=0.66	p=.44	$\eta p^2=.06$
Main effect: Format	F(1,10)=6.31	p=.031	$\eta p^2=.39$
Interaction: Condition $\times$ Format	F(1,10)=0.38	p=.55	$\eta p^2=.04$

Descriptive accuracy values:

Condition	Colour (M)	B&W (M)
Sham	0.72	0.81
TMS	0.72	0.84

Table 1A. Recognition accuracy by stimulation condition and stimulus format.

A significant main effect of Format indicated slightly better recognition for black-and-white than for colour stimuli, independent of stimulation. No effects of Condition or Condition $\times$ Format interaction were observed.

Table 1B. Confidence RT (Condition × Format)

Effect	F-value	p-value	Effect size
Main effect: Condition	F(1,10)=0.00	p=.99	$\eta p^2 < .001$
Main effect: Format	F(1,10)=0.01	p=.93	$\eta p^2 \approx .001$
Interaction: Condition×Format	F(1,10)=5.31	p=.044	$\eta p^2 = .35$

Descriptive RT values (sec):

Condition	Colour (M)	B&W (M)
Sham	0.74	0.71
TMS	0.70	0.74

Table 1B. Confidence response times as a function of Condition and Stimulus format.

A significant Condition × Format interaction reflected a crossover pattern: confidence judgments were faster for black-and-white stimuli in the Sham session, but faster for colour stimuli in the TMS session. No main effects were detected

Table 2. Time-on-task effect on confidence RTs

Mean confidence RTs per block:

Block	Mean (s)	SD
Early (Trials 1–65)	0.82	0.19
Late (Trials 66–130)	0.62	0.14

Paired comparison:

Contrast	Δ Mean (ms)	t(10)	p
Late – Early	-200	-7.21	< .001

Table 2. Confidence response times in early vs. late task blocks

Participants showed markedly faster confidence judgments in the second half of the task. The Late-Early paired comparison revealed a strong practice effect ($t(10) = -7.21, p < .001$).

2.2.5.1 Summary of main findings and implications of the second phase

Taken as a whole, the results of this second phase suggest that acute perturbation of the rPCC by deep TMS is not, under the experimental conditions tested, sufficient to induce a clear-cut self-evaluation deficit analogous to anosognosia. Neither objective performance on the memory task, nor mean confidence level, nor the main index of metacognitive sensitivity (type 2 AUC) was significantly altered by stimulation. The observed effects remain subtle and concern primarily the temporal dynamics of confidence judgments (modulation of reaction times in interaction with stimulus format) and the gradual evolution of performance across trials. In other words, the targeted network appears to be modulated without provoking an abrupt “breakdown” in the subjects’ ability to discriminate between their correct and incorrect responses.

These apparently negative results must, however, be interpreted with caution. On the one hand, the modest sample size and the relatively simple nature of the memory task limit both the statistical power and the sensitivity of the metacognitive indices used. On the other hand, clinical anosognosia as observed in Alzheimer’s disease most likely reflects a chronic, progressive and diffuse alteration of several DMN nodes and control networks, rather than the isolated and transient perturbation of a single hub. From this perspective, our protocol can be viewed as an exploratory model: it shows that one can selectively modulate the activity of a central node within the self-awareness network without an immediate collapse of metacognition,

which argues in favour of a distributed and resilient architecture of self-evaluation mechanisms.

Finally, comparing this second phase with the first provides an important methodological lesson for the remainder of this work: combining targeted neuromodulation approaches with fine-grained behavioural measures is feasible and well tolerated in healthy participants, but demonstrating robust effects on self-awareness will probably require more ecologically valid tasks, larger samples, and a more systematic consideration of global self-perception dimensions. These aspects will be revisited and discussed in greater detail in the general discussion.

3 Chapter 3 : General discussion

3.1 Objectives and summary of main findings

The aim of this thesis was to explore the cognitive and neurobiological mechanisms that may account for the alteration of metacognition in the context of brain ageing, with a particular focus on anosognosia in Alzheimer's disease. Two main questions structured the work as a whole. The first concerned the temporal onset of impaired awareness of deficits: is anosognosia essentially a late phenomenon, primarily associated with fully developed dementia stages, or can it already be detected during the preclinical or prodromal phases of the disease? The second question addressed the anatomo-functional bases of this deficit: to what extent does dysfunction of brain networks involved in self-awareness, particularly the posterior cingulate cortex (PCC) and the default mode network (DMN), contribute causally to the emergence of a metacognitive self-evaluation deficit?

To address these questions, we adopted a two-pronged, complementary approach. The first component relied on a longitudinal observational study conducted within the Wisconsin Longitudinal Study (WLS), a large community-based cohort of older adults initially recruited in adolescence and followed over several decades. In this context, we examined, over a six-year period, the predictive value of different indices of awareness of memory problems: subjective memory complaints reported by the

participants themselves (subjective memory complaints, SMC), spouse-appraised memory (SAM), and an awareness index derived from the discrepancy between these two measures. By integrating genetic status (APOE ϵ 4 carrier vs. non-carrier) and the evolution of objective memory performance, we tested the hypothesis that a patient–spouse discrepancy might constitute an early behavioral marker of increased risk for cognitive decline.

The second component was based on the development of an experimental neuromodulation model in healthy participants. Building on numerous correlational studies implicating the DMN and the PCC in self-referential processes and anosognosia, we combined deep transcranial magnetic stimulation (deep TMS) with functional neuroimaging and metacognitive measures. A first proof-of-concept study showed that inhibitory stimulation applied to the right PCC in young healthy adults induced a transient decrease in functional connectivity within the DMN. A second study, directly extending the first, sought to determine whether this acute perturbation of the self-referential network translated into a measurable alteration of self-evaluation in a visual memory task requiring trial-by-trial confidence judgments.

Taken together, these findings do not provide direct evidence for a causal link between transient perturbation of the PCC/DMN and a measurable disruption of metacognitive performance in healthy individuals. Although our stimulation protocol reliably induced network-level changes in core DMN regions, global

recognition accuracy and standard indices of metacognitive efficiency remained largely preserved. Rather than demonstrating a central and isolated role of the PCC in generating metacognitive failure, our results therefore suggest a relative resilience of metacognition to acute, focal modulation of this network in young adults. Several non-mutually exclusive explanations for this discrepancy between physiological and behavioural effects will be examined in more detail below, including limited sample size, potential ceiling effects in the metacognitive task, the choice and timing of the stimulation protocol, and the possibility that the contribution of the PCC to self-evaluation becomes more apparent in populations with greater cognitive vulnerability.

At the same time, exploratory analyses revealed a subtle but consistent effect of stimulation on confidence response times that depended on stimulus quality (colour vs. black-and-white). Under active TMS, the temporal dynamics of confidence judgements appeared more sensitive to the difficulty of the perceptual evidence, which may indicate that we are targeting a functionally relevant node for self-referential processing, even if classical accuracy- and calibration-based measures failed to capture large behavioural changes. This discrete effect, although preliminary, supports the idea that more sensitive experimental settings, including larger cohorts, refined metacognitive paradigms designed to avoid ceiling effects, and optimized stimulation parameters and timing relative to task administration, could yet reveal a clearer causal impact of PCC/DMN perturbation on metacognition.

The general discussion that follows aims to situate these mixed results within the context of recent developments in the literature on anosognosia, to specify the specific contributions of this work, to examine its limitations, and to outline its main clinical and theoretical implications.

3.2 Confrontation with the literature and evolution of models of anosognosia (2017–2025)

When this thesis was initiated in 2017, the literature on anosognosia in Alzheimer's disease was already substantial, but several grey areas remained. Clinically, most studies converged in highlighting the high frequency of anosognosia at dementia stages, with prevalence rates reaching 40–80% depending on the sample and method (14,148). Conceptually, dominant models such as those proposed by Clare et al. (18), Morris et al. (20) (2013), or Mograbi et al. (89) emphasized the multidimensional nature of anosognosia, arising from the interaction between metacognitive, mnemonic, executive, and emotional processes. However, the possibility of genuinely *early* anosognosia, present from prodromal stages onward, remained debated (152,197).

At the neurobiological level, structural and metabolic imaging studies had repeatedly implicated frontal regions (in particular orbitofrontal and anterior cingulate cortices), medial temporal structures, the insula, and posterior parietal areas, including the PCC, as correlates of anosognosia (14,85). These findings fitted within a broader

body of work describing the role of the DMN in self-referential processes, autobiographical memory, and self-awareness (103,106,109). Nevertheless, all of these studies remained correlational, and the substantial heterogeneity of anosognosia measures, imaging paradigms, and diagnostic criteria made it difficult to derive a unified model (198,199).

Between 2017 and 2025, several major syntheses have refined and nuanced this picture. The 2019 systematic review by Mondragón et al. (198) showed that, despite methodological diversity, studies converge on the involvement of an extended network including the PCC, precuneus, temporoparietal junctions, medial frontal cortex, and medial temporal structures. This supports the view that anosognosia results from perturbations within distributed systems rather than strictly focal lesions. Hallam et al. (2020) emphasized the links between metacognition and anosognosia, showing that the neuroanatomical correlates of performance self-evaluation and awareness of deficits partially overlap but do not fully coincide, suggesting the existence of distinct components within the cognitive “self” (199).

In parallel, Cacciamani et al. (2021), through a systematic review and meta-analysis, proposed the concept of *awareness of cognitive decline* as a unifying framework to capture variations in awareness of deficits across the course of the disease(25). Their work shows that alterations in awareness of cognitive difficulties can be detected as early as predementia stages, and that these alterations are heterogeneous: they vary according to cognitive domain, assessment method, and severity level. More

recently, work from Salmon's group (200) has attempted to link cognitive models of anosognosia, such as the Cognitive Awareness Model, more directly with functional connectivity data, showing that altered interactions between the DMN, frontoparietal control networks, and medial temporal regions may account for differentiated anosognosia profiles.

In addition, several studies focusing on very early stages, such as mild cognitive impairment or subjective memory complaints, have strengthened the idea that awareness of deficits follows a non-linear trajectory in brain ageing. In 2021, Bastin et al. (152) showed that anosognosia can already be present at a prodromal stage, particularly in patients with biomarkers compatible with Alzheimer pathology, while other work has suggested that forms of "hyper-awareness" may precede the establishment of stable anosognosia (201). These findings invite us to consider anosognosia not as a binary state (present/absent), but as a dynamic process modulated by neuropathological progression, compensatory strategies, and the psychosocial context.

Against this rapidly evolving background, the results presented in this thesis add several complementary elements. The WLS study first shows, at a population level, that spouse-rated memory functioning is a more robust predictor of subsequent decline than the subject's own subjective complaint, and this is particularly marked in APOE $\epsilon 4$ carriers. This observation is consistent with the work of Cacciamani et al. (25) and other studies underlining the role of informants in early detection of

cognitive change, but it extends these findings to an initially non-clinical cohort, suggesting that behavioural signs of anosognosia can be detected well before the emergence of a clinically overt dementia syndrome.

The neuromodulation studies complement this picture by providing a first experimental test of the causal contribution of the PCC and DMN to self-awareness. The demonstration that inhibitory stimulation of the right PCC reversibly modifies functional connectivity within the DMN, particularly at posterior temporoparietal nodes, is in line with the correlational data summarized by Mondragón et al. (2019) and Hallam et al. (2020) (198,199). While showing that these links can be manipulated experimentally in humans, the fact that this acute perturbation does not, under our experimental conditions, induce a detectable metacognitive self-evaluation deficit suggests, that anosognosia in Alzheimer's disease cannot be reduced to a transient dysfunction of a single DMN node. Rather, it likely results from the combination of progressive alterations within distributed networks and the failure of the metacognitive system to update self-representations accordingly.

3.3 Specific contributions of the thesis to the field of anosognosia

Beyond its confrontation with recent literature, the work presented in this thesis makes several original contributions at the conceptual, methodological, and clinical levels.

3.3.1 Early anosognosia in at-risk older adults: insights from the WLS cohort

At the conceptual level, the first study clarifies how early anosognosia should be operationalised in community-dwelling older adults at genetic risk for Alzheimer's disease. Rather than contrasting individuals with versus without subjective memory complaint (SMC), our data emphasise the structure of the discrepancy between self- and informant-based ratings.

In the WLS cohort, SMC showed a robust association with baseline episodic memory performance and a more modest association with subsequent decline, indicating that subjective concerns do capture part of the underlying cognitive signal. Crucially, however, baseline (2005) memory performance did not differ between APOE $\epsilon 4$ carriers and non-carriers, suggesting that, at study entry, both groups were still at a very early, preclinical stage in which standard neuropsychological tests remain within the normal range. The divergence in objective performance between $\epsilon 4$ carriers and non-carriers only became apparent six years later, in 2011, consistent with the expected temporal lag between underlying pathological processes and their clinical expression.

Within this longitudinal framework, informant-based evaluations proved particularly informative. When considered separately, both SMC and spouse-appraised memory (SAM) were associated with subsequent memory decline, whereas a simple

awareness index (SMC – SAM) was not. However, when SMC and SAM were entered together into multivariable models that also included APOE ε4 carrier status, SAM systematically emerged as the stronger and more specific predictor of longitudinal decline, while SMC lost its independent predictive value. Notably, the prognostic contribution of SAM was largely concentrated in APOE ε4 carriers, for whom lower SAM scores identified individuals with the steepest subsequent decline, whereas the association between SMC and decline remained weak and independent of APOE status. These results indicate that, years before group differences become detectable on standard cognitive tests, close informants already capture subtle changes that signal increased vulnerability in genetically at-risk individuals.

These results invite caution in the clinical interpretation of subjective complaint. Our findings suggest that SMC alone provides limited prognostic specificity once informant-based appraisal is considered, and should therefore not be treated as a gatekeeping criterion for further assessment. This cautious stance is consistent with evidence that SMC/SCD show substantial heterogeneity and are influenced by non-neurodegenerative factors (e.g., mood symptoms, stress, sleep difficulties, health concerns), which can inflate complaint rates independently of objective decline (62,119). Conversely, along a trajectory of emerging unawareness, subjective complaint may become attenuated despite increasing impairment, further reducing its value as a standalone marker. In our data, patient self-appraisal may even contribute noise to discrepancy-based indices: the awareness score (SMC–SAM) did not capture longitudinal risk as robustly as spouse-appraised memory alone,

suggesting that the prognostic signal is primarily carried by the informant's report rather than by the discrepancy metric itself. This interpretation should nevertheless remain cautious, as both SMC and SAM were derived from a highly parsimonious, single-item questionnaire in the present study. Practically, this supports prioritising informant input and discrepancy-based profiles whenever possible, and interpreting subjective complaint within a broader context (objective testing, functional change, and affective/sleep status) to improve clinical decision-making (119).

This pattern converges with recent work on awareness of cognitive decline (ACD) along the Alzheimer's disease continuum. Cacciamani et al (2021) showed, in a large systematic review and meta-analysis, that poorer ACD distinguishes patients with amnesic MCI and mild AD from healthy controls more clearly than subjective cognitive decline alone, supporting the view that reduced awareness can serve as an early, clinically meaningful marker of the disease (25).

Longitudinal population-based studies have further highlighted the specific added value of the informant's perspective: Numbers et al (2023) reported that initial informant-reported complaints and their increase over time are more strongly associated with incident dementia than the participant's own reports, even when based on a single binary question (202).

Complementing this, Aaronson et al. (2025) recently demonstrated that quantitative measures of discordance between self- and study partner-reported cognitive decline on the Everyday Cognition Scale reliably distinguish cognitively unimpaired

individuals from those with MCI, with high diagnostic specificity (203). Our findings extend these observations to a genetically stratified, community-based cohort, emphasizing that the prognostic signal in early anosognosia is carried less by the presence of complaint per se than by the way self-appraisal diverges from informant-based evaluations in the context of emerging decline.

Taken together, these findings support a shift from a “complaint/no complaint” view of prodromal anosognosia towards a relational framework in which the key signal lies in the progressive decoupling between a relatively preserved or slowly changing self-evaluation and increasingly negative informant reports in the context of emerging objective decline. Conceptually, this pattern corresponds to a form of “petrified self” (89), in which autobiographical representations of one’s abilities are insufficiently updated by everyday experiences and by feedback from others. By grounding this notion in a large, population-based cohort with genetic stratification and longitudinal follow-up, and by situating it within converging evidence from recent longitudinal and meta-analytic work, the present study helps bridge the gap between experimental models of anosognosia and the more diffuse, emerging unawareness observed in preclinical and early-stage Alzheimer’s disease.

At the methodological level, the WLS study illustrates the value of integrating multiple, complementary sources of information within a large, population-based cohort. Beyond standard neuropsychological scores, the combination of self-report, informant-report, genetic stratification, and longitudinal follow-up offers a powerful

framework to characterise early unawareness. In practical terms, the study shows that a single, global question posed to a spouse or close informant can yield prognostic information that is not captured by the participant's own complaint or by baseline performance alone. By modelling SMC and SAM jointly, and by examining their interaction with APOE $\epsilon 4$ status, this work moves beyond purely cross-sectional descriptions of anosognosia to a more dynamic, trajectory-based approach. Methodologically, it also bridges clinical and epidemiological traditions: measures originally developed in memory clinic settings are here applied at scale in a community sample, thereby enhancing the external validity and potential generalisability of awareness indices.

At the clinical and translational level, these findings support the development of multi-layered screening strategies for individuals at high risk of Alzheimer's disease. In settings where large-scale biomarker assessment is not feasible, simple combinations of subjective measures (SMC and SAM), basic cognitive testing, and genetic information such as APOE $\epsilon 4$ carrier status could be used to enrich samples for underlying Alzheimer pathology before moving to more expensive or invasive assessments. This is particularly relevant in the context of preclinical and secondary prevention trials, where recruitment increasingly relies on amyloid and tau imaging or fluid biomarkers to identify cognitively unimpaired individuals with elevated pathological burden, but at the cost of substantial financial and logistical constraints. In such a framework, informant-based appraisals like SAM could help to pre-select those older adults who are both genetically at risk and already exhibiting subtle,

externally visible changes in daily life, thereby increasing the yield of subsequent amyloid or tau PET imaging and reducing unnecessary procedures.

More broadly, the ability to isolate very-high-risk subgroups using low-cost, widely accessible measures opens perspectives for earlier and more targeted therapeutic intervention. Individuals flagged by converging indicators, genetic vulnerability, declining objective performance over time, and increasingly negative informant reports despite relatively preserved self-appraisal, represent prime candidates for inclusion in observational cohorts with biomarker work-up and in early-intervention trials testing disease-modifying agents. By enriching such studies with participants who are still clinically unimpaired but already on a trajectory of decline, this approach may ultimately contribute to delaying, or in the best-case scenario preventing, progression to mild cognitive impairment or overt dementia. In this sense, the WLS findings do not merely refine the concept of early anosognosia; they also point towards concrete ways of integrating awareness measures into future risk-stratification and trial-design strategies in Alzheimer's disease.

Taken together, the WLS findings show that early anosognosia can be captured at the population level through the emerging discrepancy between self- and informant-based evaluations in genetically at-risk individuals, well before standard neuropsychological tests reveal clear-cut group differences. This first strand of work therefore anchors anosognosia in a longitudinal dynamic of failing self-updating in everyday life, with clear implications for risk stratification and trial design. The

second strand of the thesis adopts a complementary perspective: rather than focusing on large-scale epidemiological data, it turns to an experimental neuromodulation approach in healthy adults to probe the neural mechanisms that support metacognition and self-awareness. By targeting a key hub of the default mode network with deep TMS and examining its impact on both resting-state connectivity and confidence judgements, this second study seeks to test, in a more causal manner, some of the network-based hypotheses that have emerged from the clinical and imaging literature on anosognosia.

3.3.2 Probing network mechanisms of metacognition with deep TMS over the posterior cingulate cortex

The experimental deep TMS study reported in this thesis provides a complementary contribution to the understanding of anosognosia and metacognition by directly probing the causal role of large-scale brain networks underlying self-awareness. Converging neuroimaging evidence in Alzheimer's disease and mild cognitive impairment indicates that unawareness of deficits is not tied to a single cortical region, but rather to distributed alterations in cortical midline and medial temporal structures, particularly within the default mode network (DMN) and its medial temporal subnetwork (152,198,199). Systematic reviews have highlighted, across heterogeneous methods and samples, the recurrent implication of the posterior cingulate cortex, precuneus, medial prefrontal cortex, and medial temporal lobe in

impaired awareness and metamemory, together with changes in their functional coupling with frontoparietal control regions (198,199). In this context, the present deep TMS study was designed to test whether transiently perturbing a key DMN hub, the right posterior cingulate cortex, would be sufficient to alter metacognitive performance in healthy adults.

More fine-grained analyses of DMN organisation provide additional support for this network-based approach and help interpret our findings. Antoine et al. (2019) showed that anosognosia in Alzheimer's disease is consistently related to disconnection within the medial temporal subnetwork of the DMN, and to its connectivity with both the "core" self-referential subsystem and a dorsomedial subsystem thought to support access to person-related semantic knowledge (142). Tondelli et al. (2024) further reported that reduced awareness along the Alzheimer continuum is associated not only with DMN dysfunction, but also with broader dysregulation of salience and frontoparietal control networks(204). These data suggest that anosognosia emerges when multiple subsystems, episodic memory, self-referential processing, salience attribution, and cognitive control, are jointly compromised. Against this background, targeting the PCC with deep TMS provides a principled way to interfere with a central relay of these networks, while keeping other components structurally intact, thereby offering a controlled test of how far PCC-centered perturbation alone can impact metacognitive functioning.

The results of the deep TMS experiment speak directly to this question. By combining right PCC stimulation with resting-state fMRI, we showed that our protocol reliably induced network-level changes in DMN connectivity, in line with the idea that the PCC exerts a coordinating influence over distributed self-referential systems. In addition, when stimulation was combined with a memory task including trial-by-trial confidence ratings, we observed that deep TMS subtly altered the temporal dynamics of confidence judgements as a function of stimulus quality, suggesting that certain aspects of how evidence is evaluated and transformed into subjective confidence are indeed sensitive to PCC-centered perturbation. However, global recognition accuracy and standard indices of metacognitive efficiency remained largely preserved. In other words, we replicated the centrality of the PCC and the DMN highlighted by prior correlational work at a physiological level, but we did not observe the degree of behavioural disruption that might have been expected if PCC dysfunction alone were sufficient to generate anosognosia-like phenomena.

This apparent discrepancy with the literature is informative rather than contradictory. If, as suggested by Antoine et al. 2019 (142) and Tondelli et al. 2024 (204), anosognosia reflects a breakdown of coordinated activity across medial temporal, DMN, salience, and frontoparietal control networks, then transiently perturbing a single hub in a young, otherwise intact brain may be insufficient to produce overt unawareness. Our findings are more compatible with a model in which self-evaluation relies on a distributed and relatively resilient set of processes, in which

PCC-centered DMN activity interacts with other networks that can, at least under some conditions, compensate for a focal disturbance. From this perspective, the failure to elicit a robust metacognitive deficit in our study can be seen as evidence that anosognosia is not the immediate, reversible consequence of isolated PCC dysfunction, but rather the endpoint of more extensive and chronic network-level alterations that accumulate over time.

From a methodological standpoint, the deep TMS experiment nevertheless establishes a proof of feasibility for a neuromodulation paradigm targeting self-related networks in humans and helps refine current models by specifying what PCC/DMN disruption does and does not do in a healthy brain. Deep TMS over the right PCC, combined with resting-state fMRI, proved sensitive to network-level changes within the DMN, thereby validating the use of this approach as a tool for probing causal contributions of DMN nodes to self-referential processing. The addition of confidence-based measures highlighted that some components of metacognition here, the timing and scaling of confidence judgements in relation to stimulus difficulty, may be more vulnerable to perturbation than global metacognitive accuracy as captured by conventional signal-detection metrics. This pattern is consistent with recent reviews of non-invasive brain stimulation showing that neuromodulatory effects on self-referential processing tend to be subtle, task-dependent, and highly sensitive to stimulation parameters and outcome measures Bao et al. 2021(205). Thus, our findings both confirm this general conclusion and specify that, under the present conditions, PCC-targeted deep TMS primarily affects

the microstructure of confidence formation rather than leading to gross metacognitive failure.

Finally, the present results resonate with emerging work using alternative neuromodulation techniques that also target the DMN and, more specifically, the PCC. Pilot studies applying transcranial focused ultrasound to the PCC in healthy volunteers have reported changes in DMN connectivity accompanied by transient alterations in aspects of subjective experience such as attentional focus, mind-wandering, or the sense of self, without necessarily producing large, coarse-grained deficits on standard cognitive tasks(206). Our findings are closely aligned with this pattern: like these ultrasound studies, we observe clear modulation of DMN connectivity and discrete changes in the temporal profile of self-related judgements, but no collapse of overall task performance. In this light, the absence of a robust decrement in metacognitive efficiency in our protocol should not be interpreted as evidence against a role for the PCC in self-awareness, but rather as an indication that its contribution may be expressed through more nuanced shifts in the dynamics and phenomenology of self-related processing than those captured by our primary outcome measures. Taken together, the deep TMS experiment thus supports a network-based, rather than region-centric, view of anosognosia and metacognition, and points to future studies that will need to combine refined neuromodulation protocols with sensitive behavioural assays and multimodal imaging, ideally in populations closer to the clinical continuum of Alzheimer's disease.

3.4 Limitations of the present work and methodological perspectives

As with any research project, this thesis has several limitations that must be considered when interpreting the results and envisaging future directions.

Regarding the WLS study, a first limitation lies in the absence of systematic clinical diagnoses and Alzheimer's disease biomarkers for the entire cohort. APOE $\epsilon 4$ carrier status was used as a proxy for vulnerability, which is consistent with many epidemiological studies, but does not allow us to identify with certainty those participants with underlying amyloid or tau pathology. In addition, the study population comes from a relatively homogeneous cohort in terms of socio-educational background, which may limit the generalisability of the findings to other cultural contexts or to less advantaged populations.

With respect to the measurement of awareness of deficits, the awareness index used in the WLS relies on the comparison between subjective complaint and spouse evaluation. This approach has the advantage of being simple and applicable on a large scale, but it does not allow a fine-grained distinction between the different dimensions of anosognosia (cognitive vs. functional), nor does it dissociate lack of awareness from mechanisms of denial or emotional minimisation (207). Moreover, the validity of spouse ratings may be influenced by factors such as psychological

distress, caregiver burden, or relationship quality, which were not systematically modelled (18).

The neuromodulation studies are mainly limited by their preliminary nature and the relatively small sample sizes. Although comparable to other pilot work in neuromodulation, the number of participants reduces statistical power and the ability to detect subtle effects on metacognitive performance. Furthermore, stimulation of the PCC using a DB80 coil is not perfectly focal, and it is likely that more superficial portions adjacent to the PCC, such as the precuneus, were co-stimulated. This technical limitation makes it more difficult to attribute the observed effects precisely to a single region.

Accordingly, any null behavioural findings should be interpreted cautiously and should not be taken as evidence of absence. Under the conditions tested, stimulation-related effects may have been too small to detect reliably with the available sample size and task sensitivity, and future work will require larger samples and optimised paradigms to capture subtle changes in metacognitive performance.

In terms of the tasks used, metacognition was assessed through a relatively artificial visual memory task, involving decontextualised stimuli and a discrete confidence scale. While such paradigms are well suited to tight experimental control, they only imperfectly capture the complexity of self-evaluation in everyday life, where judgments concern rich, emotionally salient, and socially embedded situations. In addition, the marked practice and fatigue effects observed over the course of the

experimental session complicate interpretation and suggest that shorter, or more adaptive, designs would be better suited to the study of stimulation effects.

Finally, more generally, it is important to acknowledge the gap between experimental models developed in healthy participants and the clinical reality of patients with Alzheimer's disease. Anosognosia as observed in clinical practice occurs in a context of diffuse cognitive decline, possible psychiatric comorbidities, identity changes, and complex family dynamics. The experimental paradigms presented here do not claim to reproduce this complexity, but rather to isolate some basic mechanisms that will need to be re-examined in longitudinal studies combining biomarkers, metacognitive assessments, and ecological measures of awareness of deficits.

3.5 Clinical implications

3.5.1 Informant report as core diagnostic information

The findings of this thesis have several clinical implications. As detailed in section 3.3.1, the WLS study shows that spouse-rated memory abilities are not merely a subjective complement to the patient's self-report, but a particularly informative indicator of the person's cognitive status. In this community cohort, spouse appraisal is more closely aligned with objective performance than the subject's own complaint and better captures those individuals who have already entered a trajectory of

decline. Clinically, this means that the perspective of close informants should be treated as a core component of the diagnostic process rather than as ancillary information.

3.5.2 Prognostic value and the clinical relevance of discordance

Beyond this “snapshot” value at a given time point, the WLS data also demonstrate that informant evaluations carry prognostic information about subsequent cognitive trajectories, particularly in individuals at increased biological or genetic risk. From this perspective, the absence of complaint from the patient cannot be interpreted as reassuring when it contrasts with concern expressed by relatives. Discrepancy-based approaches that compare patient and informant reports are therefore highly relevant in practice, yet they remain underused in routine settings. Integrating such tools more systematically into clinical reasoning could improve early detection and risk stratification, especially in prodromal stages where standard neuropsychological tests are still within the normal range.

3.5.3 From evidence to practice: multi-source triage in routine care

From a practical standpoint, these findings support a more operational, multi-source approach to case-finding and triage. Rather than relying on a “complaint/no complaint” logic, clinicians may benefit from systematically combining: self-report,

informant report, objective testing and from explicitly interpreting self-informant discordance as clinically meaningful information.

This approach is consistent with meta-analytic evidence showing that reduced awareness of cognitive decline discriminates amnesic MCI and mild dementia more robustly than subjective decline alone (25), with longitudinal data indicating that informant-reported concerns (and their increase over time) are more strongly associated with incident dementia than participants' own complaints (202). Recent work further suggests that quantitative discordance measures on structured instruments (e.g., Everyday Cognition) can distinguish cognitively unimpaired individuals from those with MCI with high diagnostic specificity (203). In routine practice, integrating these elements more explicitly can improve risk stratification, guide the indication for biomarker work-up, and inform the intensity of follow-up, especially at prodromal stages where standard neuropsychological scores may remain within normal limits.

3.5.4 Informant-based screening in general practice and neurology

Given converging longitudinal evidence, including our findings, that informant-reported cognitive complaints predict subsequent decline more robustly than self-reported complaints, incorporating a brief informant component into routine consultations may improve early detection and triage (156,202,208). In practice, this can be implemented with short, validated instruments that take 2–5 minutes,

completed in the waiting room, digitally prior to the visit, or by phone when the informant cannot attend.

In general practice, brief tools designed for primary care can be particularly useful. The General Practitioner Assessment of Cognition (GPCOG) was specifically developed for this setting and explicitly includes an informant interview when the patient score is borderline, thereby operationalizing a pragmatic decision pathway that does not rely on patient complaint alone (209). In specialist settings, rapid informant scales such as the AD8 provide structured evidence of functional-cognitive change and have been evaluated in diagnostic accuracy studies and meta-analyses (210,211). The Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE, short form) is another option with good screening properties and relative insensitivity to education/language, although it can be influenced by informant characteristics (e.g., anxiety/depression, relationship quality), which should be checked clinically (212).

A pragmatic workflow is therefore to:

- 1) Encourage patients to attend with a knowledgeable informant whenever possible.
- 2) Collect a brief informant questionnaire systematically (especially when patient self-report appears reassuring but concerns exist)

- 3) Treat marked self-informant discordance as a signal prompting closer follow-up and objective assessment rather than reassurance.

Adapted from the GPCOG informant interview (Brodaty et al., 2002).

Instruction to the informant: “Compared to a few years ago...”

1. “Does the patient have more trouble remembering things that have happened recently?”
2. “Does he or she have more trouble recalling conversations a few days later?”
3. “When speaking, does the patient have more difficulty finding the right word or tend to use the wrong words more often?”

Suggested interpretation : Any “yes” response should prompt a more structured informant assessment (e.g., full GPCOG informant interview, AD8, or short IQCODE) and objective evaluation, rather than reassurance based on patient self-report alone.

3.5.5 Implications for clinical management

With respect to clinical management, the early identification of patients who underestimate the severity of their difficulties has direct implications for adherence

to interventions, risk management, and caregiver support. The presence of anosognosia or of marked patient–caregiver discordance should prompt adaptation of how the diagnosis is communicated, reinforcement of the role of relatives in implementing compensatory strategies, and anticipation of potential danger situations (e.g. driving, financial management, medication adherence). A better understanding of the cognitive and neurobiological mechanisms of anosognosia could ultimately contribute to the development of targeted interventions aimed at supporting the updating of self-knowledge and the online monitoring of performance, drawing on theoretical frameworks such as the Cognitive Awareness Model and dynamic approaches to self-awareness.

3.6 Research perspectives

From a research standpoint, several avenues emerge. First, the WLS results argue for the systematic integration of informant-based evaluation (spouse or close caregiver) in longitudinal studies of ageing and Alzheimer’s disease, treating it not merely as contextual information but as a genuine behavioural biomarker of risk for decline. In large cohorts, simple questions asked to relatives could be used to enrich samples for individuals most likely to benefit from further biomarker work-up or inclusion in preventive trials, thereby improving the efficiency of recruitment for costly procedures such as amyloid or tau PET imaging.

Second, the link between neuromodulation and metacognition warrants further exploration. As discussed in section 3.3.2, the deep TMS data indicate that it is possible to modulate the functional integrity of the DMN in healthy participants without, under the conditions tested, eliciting overt anosognosia or a massive breakdown of metacognition. Future work could extend this approach by targeting different nodes of self-awareness networks (such as the posterior cingulate cortex, temporoparietal junctions, or medial frontal regions), by combining stimulation with more sensitive indices of metacognitive efficiency (for example, meta-d' or M-ratio measures), and by using more ecological assessments of awareness of deficits in everyday life. Applying similar paradigms in populations closer to the Alzheimer's disease continuum would also help clarify how network-level vulnerability modulates the behavioural impact of focal perturbations.

Finally, it appears necessary to broaden the study of anosognosia beyond the strictly mnemonic domain, by examining other dimensions such as executive functioning, behaviour, and emotion, and by articulating more finely the neurological processes of unawareness with psychological mechanisms such as denial or minimisation. Integrating these different levels of analysis, neurobiological, cognitive, psychological, and social, represents a major challenge for the coming years. In this regard, the contributions of this thesis suggest that combining informant-based indicators, experimental metacognition paradigms, and targeted neuromodulation techniques may offer a particularly fruitful framework for better understanding and, ultimately, better managing anosognosia.

3.7 Ethical implication

A recurrent ethical concern in the literature, is whether it is appropriate to reduce anosognosia (i.e., enhance awareness) when Alzheimer's disease cannot be "cured." This question reflects a genuine tension between potential psychological costs and concrete benefits. On the one hand, impaired awareness has repeatedly been associated with lower depressive and anxiety symptoms in early Alzheimer's disease, suggesting that unawareness may sometimes be subjectively "protective" (213,214). On the other hand, low awareness can delay help-seeking, compromise safety, hinder adherence to interventions, and increase caregiver burden, outcomes that directly engage the ethical principles of beneficence and non-maleficence (215).

Importantly, enhancing awareness should not be framed as "confronting" patients with a deficit label, but as supporting functional insight, that is, the minimum level of shared understanding needed to enable safer decisions, acceptance of compensatory strategies, and collaborative care planning. This approach is consistent with relational and supported models of autonomy, which view autonomy as something that can be preserved through appropriate scaffolding and involvement of trusted care partners rather than as an all-or-nothing capacity (216).

Nevertheless, the potential for iatrogenic distress must be taken seriously. The period following diagnostic disclosure can be associated with heightened psychological vulnerability, including increased suicidal ideation in some subgroups, which

supports careful screening for depression/anxiety and structured post-disclosure support (217). Accordingly, ethically acceptable attempts to support awareness should be gradual, person-centered, and accompanied by emotional supports and follow-up, in line with compassionate disclosure guidance (218).

In practice, any attempt to support awareness should be implemented under appropriate conditions. This includes a person-centred and gradual approach, explicit screening for depressive and anxiety symptoms, and close follow-up after disclosure or any intervention likely to increase insight, with particular vigilance regarding suicidal ideation in psychologically vulnerable individuals (219). When clinically significant depressive symptoms emerge, they should be actively treated and monitored, as mood disturbances may both colour self-appraisal and amplify distress associated with increased awareness (213).

Finally, it should be acknowledged that the evidence base for interventions specifically aimed at increasing awareness in dementia remains limited and heterogeneous, which argues for prudence, individual tailoring, and outcome monitoring (220).

3.8 General conclusion

This work set out to shed light on the mechanisms underlying anosognosia in the context of brain ageing, by combining two complementary approaches: a large-scale observational study in the general population, and an experimental neuromodulation

model targeting a key node of self-awareness networks. Across chapters, it has become clear that impaired awareness of deficits cannot be treated as a mere epiphenomenon of cognitive decline; rather, it is a dynamic phenomenon situated at the interface between cognition, brain, and social environment.

The first major message emerging from this work concerns the central role of informant evaluation, particularly that provided by the spouse. Data from the Wisconsin Longitudinal Study show that spouse-rated memory abilities are not just an anecdotal counterpoint to the patient's own complaint: they provide a more faithful reflection of actual cognitive performance at the time of assessment. Crucially, this evaluation also has significant predictive value for subsequent cognitive decline several years later, especially in individuals at increased genetic risk. In other words, the way a spouse perceives the patient's memory is not only an indicator of the current situation, but also an early warning signal regarding the future trajectory. This finding invites a reconsideration of the place given to relatives in clinical practice: the absence of complaint from the patient, when it contrasts with concerns expressed by the spouse, should not be interpreted as a sign of preserved cognitive health, but rather as a possible marker of emerging anosognosia and increased vulnerability.

The second key message relates to the role of brain networks supporting self-awareness, and in particular the posterior cingulate cortex and the default mode

network. Neuromodulation studies in healthy participants show that inhibitory stimulation of the PCC transiently alters the functional organisation of the DMN, thereby confirming the strategic role of this region in the architecture of self-referential networks. However, under the experimental conditions tested, this acute perturbation is not sufficient to induce overt anosognosia or a massive breakdown of metacognition. This result suggests that, although the PCC and DMN represent privileged targets for understanding self-awareness, anosognosia in Alzheimer's disease most likely arises from progressive and combined disturbances across several networks, self-referential, mnemonic, and control, rather than from the isolated failure of a single node.

By bringing these two strands together, the thesis proposes an integrated view of anosognosia as a network phenomenon in a double sense: at the level of brain networks, involving complex interactions between medial temporal, posterior parietal, and frontal regions; and at the level of social networks, in which the patient's self-perception is continuously confronted with the views of relatives. The results underscore the importance, in both research and clinical settings, of articulating indicators derived from these two levels. On the one hand, spouse evaluation appears as a simple, low-cost, and robust behavioural biomarker for identifying at-risk profiles. On the other hand, targeted neuromodulation paradigms

provide a tool to experimentally probe the causal contribution of self-awareness networks and to open the way to future interventional strategies.

This thesis does not, of course, exhaust the complexity of anosognosia, but it highlights two structuring axes for future research: fully recognising the role of relatives as both actors and witnesses of awareness of deficits, and continuing to explore the brain networks of self-awareness through experimental paradigms combining neuromodulation, imaging, and fine-grained measures of metacognition.

Ultimately, the question of anosognosia leads back to a more fundamental issue: how a person can continue to experience themselves as the “same” individual while their brain, abilities, and relationships are being progressively reshaped. This question echoes the philosophical Ship of Theseus paradox, in which all the planks of a boat are gradually replaced while we continue to refer to it as the same vessel. The data presented in this thesis suggest that this subjective continuity of the self does not depend solely on the integrity of a particular region such as the posterior cingulate cortex, nor can it be reduced to an illusion detached from biological change. Rather, it emerges from the ongoing interplay between neurobiological processes, cognitive mechanisms of memory and metacognition, and the corrective or confirming feedback provided by others. In Alzheimer’s disease, anosognosia can thus be seen as the point where this balance fails: internal models that usually allow the individual to adjust their self-image to their actual abilities are no longer adequately updated by

experience or by the social environment. The person continues to carry forward an earlier version of themselves, while their brain and daily functioning have already changed. Understanding how these different levels, brain networks, cognitive monitoring, and interpersonal perspectives, jointly sustain or distort the feeling of “being oneself” will be essential, in future work, for grasping what is at stake when self-awareness breaks down in the course of brain ageing.

4 References

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5 Appendix

5.1 Spatial orientation as a behavioural marker of early entorhinal–hippocampal dysfunction

Beyond memory impairment and altered awareness of deficits, Alzheimer’s disease is also characterised, at an early stage, by difficulties in spatial orientation and navigation. It is from this line of questioning that the present doctoral work originally emerged, through an initial project centred on a virtual navigation task designed to probe different components of spatial orientation in young and middle-aged adults. The initial objective—beyond what is presented in this appendix—was to combine these behavioural data with molecular imaging (tau-PET), in order to examine the relationship between tau burden and navigation performance in clinically healthy individuals at increased risk of developing Alzheimer’s disease (in particular APOE ϵ 4 carriers). The aim was less to identify biomarkers in the strict sense than to contribute to a finer stratification of risk within populations that appear clinically intact.

A subsequent pilot study conducted in older patients, potentially at a prodromal stage, nevertheless revealed a marked floor effect: the navigation task proved too complex for this population, yielding performances that were difficult to interpret and compromising any meaningful analysis of relationships with biological markers.

Continuing the project would have required a substantial redesign of the paradigm, which was difficult to reconcile with the timeline and multicentre structure of the study. The work was not abandoned, however, but taken up and adapted by Lise Colmant, who made it the core of her own thesis by adjusting the task design to the specific constraints of older at-risk participants.

From a cognitive standpoint, spatial orientation relies on several systems: *path integration*, which allows one to update their position in the absence of external cues; *landmark-based navigation*, which exploits salient features of the environment; and *boundary-based navigation*, which relies on the overall configuration of the space. These systems are supported by partly distinct neural networks (entorhinal cortex and grid cells, hippocampus and place cells, parietal–retrosplenic regions), which are particularly vulnerable in the early phases of Alzheimer’s disease. Recent work has shown that subtle alterations in navigation can be detected at preclinical stages, especially in individuals at genetic or biological risk.

This line of enquiry complements the main thread of the thesis, focused on anosognosia and metacognition. Whereas self- and informant-based evaluation paradigms probe how individuals *represent* their difficulties, navigation tasks directly target the functioning of the entorhinal–hippocampal loop. The article reproduced in this appendix, “*Unmasking selective path integration deficits in Alzheimer’s disease risk carriers*”, illustrates this point: it demonstrates a selective deficit in path integration in APOE $\epsilon 4$ carriers in the absence of external cues, with

preserved performance when landmarks are available, suggesting compensatory recruitment of other regions. The aim of this appendix is therefore to place this initial project within its theoretical context and to show how spatial orientation, much like anosognosia, can be considered a potential tool for risk stratification in populations who are still functionally independent.

5.2 Unmasking selective path integration deficits in Alzheimer's disease risk carriers

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Abstract

Alzheimer's disease (AD) manifests with progressive memory loss and spatial disorientation. Neuropathological studies suggest early AD pathology in the entorhinal cortex (EC) of young adults at genetic risk for AD (APOE ϵ 4-carriers). Because the EC harbors grid cells, a likely neural substrate of path integration (PI), we examined PI performance in APOE ϵ 4-carriers during a virtual navigation task. We report a selective impairment in APOE ϵ 4-carriers specifically when recruitment of compensatory navigational strategies via supportive spatial cues was disabled. A separate fMRI study revealed that PI performance was associated with the strength of entorhinal grid-like representations when no compensatory strategies were available, suggesting grid cell dysfunction as a mechanistic explanation for PI deficits in APOE ϵ 4-carriers. Furthermore, posterior cingulate/retrosplenial cortex was involved in the recruitment of compensatory navigational strategies via supportive spatial cues. Our results provide evidence for selective PI deficits in AD risk carriers, decades before potential disease onset.

INTRODUCTION

Alzheimer's disease (AD), by far the most common form of dementia, is characterized by a progressive deterioration of cognitive functions, starting with episodic memory loss and spatial disorientation (1). No causal therapies for AD are currently available, possibly because drugs that would otherwise be effective are

applied too late (2). Hence, developing biomarkers and behavioral tests for identifying

subjects at risk for developing AD is a crucial goal of current AD research. The E4 allele of the apolipoprotein E (APOE) gene is the most important genetic risk factor for late-onset AD (3). Thus, it may provide an opportunity for assessing subclinical alterations of behavior, brain structure, and brain function at very early disease stages (1, 4). However, previous studies and meta-analyses on *APOE*-behavior relationships in young and middle-aged healthy participants showed divergent results: While some described cognitive impairments [e.g., (5)], others reported improved functioning [e.g., (6)]—suggesting antagonistic pleiotropy—or no effects [e.g., (4, 5, 7)]. Here, we hypothesized that these divergent findings occur because *APOE* ϵ 4 effects on behavior are mediated by subtle, preclinical AD pathology in confined brain regions, which may be compensated by increased recruitment of unaffected areas—possibly resulting in altered cognitive strategies. Postmortem brain studies revealed first signs of neurodegeneration in the form of neurofibrillary tangles in *APOE* ϵ 4-carriers already in early adulthood (8). Because tau-related neurodegeneration correlates closely with cognitive dysfunction (9), this very early tauopathy may manifest in subtle behavioral alterations. Among the first regions to be affected by neurofibrillary tangles is the entorhinal cortex (EC) (10), a hub for spatial navigation and memory. The EC contains spatially modulated cell types, including grid cells that fire at the vertices of equilateral triangles tiling the environment (11). In AD mouse models, early tauopathy in EC impairs grid cell

functioning and spatial memory performance (12). In humans, grid cell activity can be indirectly measured as “grid-like representations” (GLRs) via functional magnetic resonance imaging (fMRI) (13–15). These GLRs were described to be functionally relevant for memory and spatial navigation (4, 13, 16). We previously showed evidence for impaired GLRs in young *APOE* ϵ 4-carriers during virtual navigation in an arena (4). The spatial navigation performance of risk carriers was preserved, suggesting the use of compensatory strategies. Reduced GLRs were accompanied by relatively increased blood oxygenation level-dependent (BOLD) signals in the hippocampus (HC). Moreover, *APOE* ϵ 4-carriers navigated more often at the border of the arena, possibly in an attempt to stabilize their GLRs (4, 5, 17). Together, these findings suggest that subtle alterations in the neural and behavioral signatures of spatial navigation may occur in *APOE* ϵ 4-carriers already at an early age, and these changes may constitute prime candidates for neurocognitive markers of AD (1). However, to dissect navigational strategies, potential compensatory mechanisms, and their underlying neural computations, it is crucial to probe the navigational function of grid cells more directly. Theoretical considerations (11), computational models (18), and empirical studies in rodents (19) and humans (16) suggest that grid cells particularly support path integration (PI) processes. PI is the process of estimating one’s current position based on information about previous positions, heading direction, speed, and time elapsed. Specifically, grid cells may provide the computational basis for representing the integrated path (18) and for computing direct vectors to spatial goals (20, 21). Accurate goal vectors

are essential for the “incoming phase” of PI tasks in which subjects have to return to their home location. When only information from visual flow is available, PI inevitably leads to error accumulation (17, 18). This suggests that sparse environments and longer navigational routes unmask subtle PI deficits in *APOE* ϵ 4-carriers. By contrast, when supportive spatial cues such as boundaries or landmarks are available [as in most previous studies, see (4, 5)], additional brain regions could be recruited, in particular HC (4) and posterior cingulate/retrosplenial cortex (PC/RSC) (21, 22). This may enable *APOE* ϵ 4-carriers to perform on a par with control participants. In line with these predictions, our study unmasks a specific deficit of *APOE* ϵ 4-carriers in PI when no supportive spatial cues are available and identifies the neural mechanisms underlying this deficit.

RESULTS

Experimental task and study sample

We assessed PI performance with a novel task, the “Apple Game” (Fig. 1 and fig. S1; Methods; see also movie S1). In each trial, participants first navigated to a basket (“start phase”) whose location they were instructed to remember (“goal location”). Next, participants navigated toward a variable number of trees (“outgoing phase”), systematically varying outgoing distance (and thereby PI difficulty), until they found a tree with an apple (“retrieval location”). Basket and trees appeared consecutively

and disappeared as soon as the participant reached their locations. From the retrieval location, participants had to take the shortest route back to the basket location (incoming phase). In different subtasks, participants either had to rely purely on visual flow [“pure PI” (PPI)] or were provided with supportive spatial cues in the form of a boundary [“boundary-supported PI” (BPI)] or an intramaze landmark [“landmark-supported PI” (LPI)]. PI performance was quantified via the distance between response location and correct location of the basket (“drop error”; for alternative metrics, see Fig. 1D). We conducted the paradigm in $N = 267$ healthy participants genotyped for *APOE* across four different European sites (“*APOE* sample”; $n = 65$ *APOE* $\epsilon 3/\epsilon 4$ -carriers, risk group; $n = 202$ *APOE* $\epsilon 3/\epsilon 3$ -carriers, control group; age range, 18 to 75 years; mean age, 37.7 years; 38.6% male; table S1). Control and risk group did not differ regarding demographic characteristics or general cognitive status as indicated by mini-mental state examination [MMSE (23); table S2, fig. S1, and Supplementary Text]. All older participants (≥ 42 years, threshold established by means of kmeans clustering; see Methods) showed normal cognitive abilities (MMSE scores of ≥ 25). Additional analyses excluded an effect of *APOE* on time-counting strategies during the task, subjective navigation performance in everyday life, and pre-experimental navigation types (table S2 and Supplementary Text). For a subgroup of this sample, structural MRI (sMRI) data were available, allowing us to establish relationships between behavioral performance and brain structure (“sMRI sample”; $n = 99$ participants; n

= 23 risk carriers, $n = 76$ controls; table S3 and fig. S1). Furthermore, we recruited a separate group of $n = 35$ participants who completed a variant of the task inside the MR scanner (“fMRI sample”; table S1).

Determinants of PI performance

We analyzed PI performance as a function of *APOE* genotype, subtask, and path distance in the *APOE* sample. We used a series of linear mixed models with “subtask” (PPI, BPI, or LPI) and “path distance” as within-subject variables and “*APOE*” as between-subject variable. Path distance refers to either “outgoing distance” (i.e., the accumulated path distance during the outgoing phase; Fig. 1C, model 1a, and Table 1; Methods) or “incoming distance” (i.e., the Euclidean distance between retrieval location and goal location; Fig. 1C, model 1b). When reporting inference statistics, “all” or “both” always refer to the two models with either one of the two path distances. “Subject” and “site” were added as random factors, and “sex” and “age” as covariates (for main effects and for interactions with subtask and genotype). Post hoc comparisons were Tukey-corrected for multiple comparisons (number of subtasks). We found main effects of subtask and both path distance measures on PI performance (all $F \geq 466.42$, all $P < 0.001$ for the two models with incoming and outgoing distance; fig. S2). Pairwise comparisons showed that performance was worse in the PPI as compared to the BPI and LPI subtasks (all $z \geq 22.25$, all $P_{\text{Tukey}} < 0.001$). Performance in the BPI subtask was also worse than in the LPI subtask (both $z \geq 6.44$, both $P_{\text{Tukey}} < 0.001$). Notably, incoming distance

had a more pronounced effect in the PPI subtask than in the two other subtasks, as shown by a significant subtask by incoming distance interaction ($F = 99.17$, $P < 0.001$; fig. S2). This was not the case for outgoing distance ($F = 1.02$, $P = 0.361$). Post hoc analyses confirmed that incoming distance had a stronger effect on performance in the PPI subtask than in the two other subtasks (both $z \geq 9.80$, both $PTukey < 0.001$), with no difference between BPI and LPI ($z = 1.11$, $PTukey = 0.511$). Furthermore, we encountered significant main effects of sex and age (all $F \geq 63.49$, all $P < 0.001$): Younger age and male sex predicted better performance [see fig. S2 (also for interactions involving covariates)]. These results show that the absence of supportive spatial cues not only reduces PI performance but also increases the impact of longer incoming distances, which is presumably related to larger error accumulation (17, 18).

Unmasking of *APOE* effects on PI in the absence of supportive spatial cues

We next analyzed effects of *APOE* on performance. No main (subtask-independent) effect of *APOE* was observed (both $F \leq 0.04$, both $P \geq 0.850$), in line with a previous study showing generally unimpaired PI performance in *APOE* $\epsilon 4$ -carriers (5). However, we observed a significant *APOE* by subtask interaction (both $F \geq 12.60$,

both $P < 0.001$; Fig. 2A), indicating different APOE effects on performance in the individual subtasks. The interaction was driven by worse performance of risk carriers as compared to controls specifically in the PPI subtask (both $z \geq 2.44$, both $PTukey \leq 0.039$), while there was no difference in the BPI (both $z \leq 0.05$, both $PTukey \geq 0.998$) or LPI subtasks (both $z \leq 0.98$, both $PTukey \geq 0.593$). To scrutinize this result, we compared performance as a function of APOE between the PPI subtask and either the BPI or LPI subtask (Fig. 2B). These contrasts measure a specific impairment of PI performance in the absence of supportive spatial cues. The performance of risk carriers declined more strongly than that of controls in the PPI subtask when compared to either the BPI subtask (both $z \geq 2.86$, both $PTukey \leq 0.012$) or the LPI subtask (both $z \geq 4.10$, both $PTukey < 0.001$). There was no APOE effect on the performance difference between the BPI and LPI subtask (both $z \leq 1.26$, both $PTukey \geq 0.410$). Post hoc analyses revealed that the effect of genotype on performance was driven by increased rotation errors (Fig. 1D, fig. S3, and Supplementary Text). Moreover, we observed an interaction between APOE and incoming distance ($F = 3.94$, $P = 0.047$; Fig. 2C), presumably reflecting stronger error accumulation (17, 18) in risk carriers than controls. This effect was not significant for outgoing distance ($F = 1.86$, $P = 0.173$). APOE effects were significantly stronger for incoming than outgoing distance (three-way interaction between outgoing distance, incoming distance, and APOE in a model including both distance types; $F = 4.68$, $P = 0.030$; model 1c). This indicates that performance in risk carriers is particularly affected by the incoming (i.e., Euclidean) distance

between retrieval and goal location. Because risk carriers' performance was on par with controls when supportive spatial cues were present, we hypothesized that the distance to these cues determines their performance more than in controls. We thus investigated PI performance as a function of "spatial cue distance" (i.e., the distance between the goal location and the boundary or the landmark; models 2a and 2b). Generally, higher distances from the boundary and lower distances from the landmark were associated with higher performance (main effects of goal-to-boundary distance and goal-to-landmark distance: both $F > 342.45$, both $P < 0.001$). Better PI performance in trials with higher distances between the goal location and the environmental boundary may be counterintuitive, because boundaries constitute an additional visual cue. However, goal locations in the center of the environment have a lower range of possible drop errors than goal locations in the periphery of the environment, leading to lower drop errors per se, as described previously (24). In addition, the performance of risk carriers declined more strongly with increasing goal-to-landmark distance than the performance of controls (interaction effect between goal-to-landmark distance and APOE: $F = 4.55$, $P = 0.033$; model 2b; Fig. 2D). By contrast, goal-to-boundary distance did not exhibit different effects in risk and control participants (interaction effect between goal-to-boundary distance and APOE: $F = 2.59$, $P = 0.107$; model 2a; Fig. 2D). Irrespective of PI performance, we investigated the mean distance of participants' navigation to the boundary or the landmark (only during the incoming phase, as movements during the outgoing phase were determined by the trees; models 2c and 2d). This revealed that risk carriers

showed a different navigational pattern: They navigated in closer proximity to the landmark ($F = 6.20$, $P = 0.013$; model 2d; Fig. 2E), suggesting that they used the landmark information to a higher degree to guide their behavior. By contrast, risk carriers navigated at similar distances to the boundary as controls ($F = 1.35$, $P = 0.245$; model 2c, Fig. 2E). In summary, a selective PI deficit in APOE $\epsilon 4$ -carriers was unmasked when environments lacked spatial cues and participants had to rely purely on PI. When environmental landmarks were available, risk carriers relied more strongly on their proximity.

Brain structural determinants of *APOE* effects on PI

. How is performance in the different subtasks related to brain volume in areas relevant for spatial navigation, and how are these relationships modulated by APOE? We analyzed PI performance as a function of relative gray matter volume in three regions of interest (ROIs): EC, HC, and PC/RSC. We used mixed models with subtask and path distance (outgoing distance, models 3a to 5a; or incoming distance, models 3b to 5b) as within-subject variables, APOE and “volume” of one of the three ROIs as between-subject variables, and subject as random factor (sMRI sample; Table 1 and Methods). Age and sex were added as covariates. Because these models contain two continuous predictors of interest (path distance and volume), post hoc comparisons were performed on quintiles of the path distance predictor (Tukey-corrected for number of compared quintiles). Qualitatively identical results were obtained when the path distance predictor was subdivided into quartiles or tertiles

(Supplementary Text). Risk carriers and controls did not differ regarding age, sex, whole-brain volume, and relative volume in any ROI (table S3 and fig. S1), in line with previous findings (25). Also, relative ROI volumes did not directly predict performance in any subtask, for either controls or risk carriers (all $z \leq 1.68$, all $P_{\text{Bonferroni}} \geq 0.279$). However, an effect of EC volume on performance in risk carriers was unmasked when analyzed in relationship to incoming distance: When we examined the effect of EC volume on performance (model 3b), we encountered a significant interaction with APOE and incoming distance ($F = 6.70$, $P_{\text{Bonferroni}} = 0.030$; Fig. 3A). While EC volumes did not predict performance for shorter incoming distances in either risk carriers or controls [800 to 2580 virtual meters (vm): all $z \leq 1.24$, all $P_{\text{TUkey}} \geq 0.214$], they exerted a significant effect for longer incoming distances specifically in risk carriers (4360 to 7920 vm: all $z \geq 2.17$, all $P_{\text{TUkey}} \leq 0.030$), but not in controls (all $z \leq 1.87$, all $P_{\text{TUkey}} \geq 0.061$). Effects in risk carriers were significantly larger than in controls at large incoming distances (7920 vm: $z = 2.56$, $P_{\text{TUkey}} = 0.044$), but not at lower incoming distances (800 to 6140 vm: all $z \leq 2.13$, all $P_{\text{TUkey}} \geq 0.126$). No comparable effects were found for outgoing distance (model 3a), in HC (model 4a/4b), or PC/RSC (model 5a/5b). These results show a selective influence of EC volume on performance in risk carriers for longer incoming distances, suggesting that effects of subtle structural degradation of the EC in risk carriers become visible in situations of large potential error accumulation.

Age-related modulations of *APOE* effects on PI performance

Age was included as a covariate in models 1a to 1c, and we described interactions with age in fig. S2. Notably, we found a significant subtask by *APOE* by age interaction ($F = 5.79$, $P = 0.003$; fig. S2F), suggesting that the difference between risk carriers and controls in the PPI condition might be modulated by age. However, risk carriers did not differ from controls with respect to age-related decline of performance in any of the subtasks (all $z < 1.79$, all $PTukey = 0.171$). We nevertheless aimed at a more detailed understanding of the age-related modulations of our *APOE* effects, which is crucial to evaluate the possible implications of our findings for preclinical dementia diagnosis. We therefore split the *APOE* sample into a “younger” subsample ($n = 163$; mean age $\pm$ SD, 24.32 ± 4.87) and an “older” subsample ($n = 104$; mean age $\pm$ SD, 58.71 ± 7.75) in a data-driven manner using MATLAB’s kmeans clustering algorithm. This method resulted in a cutoff age of 42 years. The proportion of risk carriers did not differ between the younger (risk group, $n = 35$; control group, $n = 128$) and older age group [$n = 30$; $n = 74$; $\chi^2(1) = 1.87$, $P = 0.190$]. The proportion of male and female participants also did not differ between the two age groups ($\chi^2 = 0.55$, $P = 0.269$). We then re-performed all analyses separately for the younger and older group. First, we examined the results of model 1 in detail. In both age groups, we found main effects of subtask and both path distance measures on PI performance (all $F \geq 77.76$, all $P < 0.001$). Pairwise

comparisons showed that performance in the PPI subtask was worse than in the BPI and LPI subtasks (all $z \geq 10.69$, all $PTukey < 0.001$). Performance was better in LPI than in BPI (both $z \geq 2.35$, both $PTukey \leq 0.049$). In both groups, we encountered a significant subtask by incoming distance interaction (both $F \geq 27.68$, both $P < 0.001$), but no subtask by outgoing distance interaction (both $F \leq 1.10$, both $P \geq 0.334$). Incoming distance had a stronger effect on performance in the PPI subtask than in the two other subtasks (all $z \geq 6.13$, all $PTukey < 0.001$), and there was no difference between BPI and LPI (both $z \leq 1.85$, both $PTukey \geq 0.154$). Moreover, we found a significant main effect of sex in both age groups (with females performing worse; all $F \geq 6.56$, all $P \leq 0.012$). Furthermore, we also found significant main effects of age in both subsamples (all $F \geq 4.99$, all $P \leq 0.028$). These effects replicate the findings in the entire sample and thus demonstrate that the age distribution of our sample did not significantly influence the main results of our study. In both age groups, we found no significant main effect of APOE (all $F \leq 0.10$, all $P \geq 0.756$) but observed significant APOE by subtask interactions on PI performance (all $F \geq 3.95$, all $P \leq 0.019$; Fig. 4A). These effects again replicate the findings in the entire sample. Pairwise comparisons revealed that the performance of risk carriers in the PPI subtask was significantly worse than the performance of controls only in the older age group (both $z \geq 2.72$, both $PTukey \leq 0.018$), but not in the younger age group (both $z \leq 1.70$, both $PTukey \geq 0.206$). This result suggests that the adverse effect of APOE $\epsilon 4$ on PI performance exacerbates with increasing age. In both age groups, we did not observe a difference between risk carriers and controls when

comparing PI performance in the BPI versus the LPI subtask (all $z \leq 1.86$, all PTukey ≥ 0.151). The younger age group showed no difference between risk carriers and controls when comparing PI performance in the PPI versus the BPI subtask (both $z \leq 1.72$, all PTukey ≥ 0.197 ; Fig. 4B). There was a trend for a difference between risk carriers and controls when comparing PI performance in the PPI versus the LPI subtask (both $z \geq 2.06$, both PTukey ≤ 0.099). In the older age group, risk carriers performed significantly worse in the PPI subtask as compared to both the BPI subtask and the LPI subtask (all $z \geq 2.96$, all PTukey ≤ 0.009 ; Fig. 4B). These results show again that the adverse effect of APOE $\epsilon 4$ on PI performance exacerbates with increasing age. We did not observe any APOE by incoming distance interactions in either age group (both $F \leq 0.61$, both $P \geq 0.436$; Fig. 4C), presumably due to reduced statistical power. Model 1c, in which we analyzed three-way interactions between outgoing distance, incoming distance, and APOE, was thus not applied. Next, we examined the results from model 2 in detail, which assessed the effects of boundary and landmark distance from the goal location on PI performance. Higher distances from the boundary and lower distances from the landmark were associated with better performance in both age groups (all $F \geq 198.03$, all $P < 0.001$), replicating our previous results. We did not observe the significant APOE by goal-to-landmark distance effect in either age group (both $F \leq 1.47$, both $P \geq 0.225$; Fig. 4D), presumably due to reduced statistical power. By contrast, we encountered a significant APOE by goal-to-boundary distance interaction in the young sample only ($F = 6.25$, $P = 0.012$; old sample: $F = 0.49$, $P = 0.485$; Fig. 4D), suggesting that young

risk carriers benefit more from central positions of goal locations. Last, young risk carriers navigated closer to the landmark ($F = 3.96$, $P = 0.047$) and older risk carriers showed a trend for this effect ($F = 3.02$, $P = 0.085$; Fig. 4E), largely replicating the result from the entire sample. We did not encounter differences in the movement-to-boundary distance between genotypes (both $F \leq 1.20$, both $P \geq 0.274$; Fig. 4E). We present a synopsis of all results in table S4. In addition, we tested whether there was an inverse U-shaped effect of APOE across the life span, by adding a quadratic age variable to models 1a and 1b (“age²”). Adding age² did not improve model fit [both $\chi^2(1) \leq 0.22$, both $P \geq 0.635$]. We found no main effect of age² (both $F \leq 0.81$, both $P \geq 0.370$) or interactions with subtask or APOE (all $F \leq 1.76$, all $P \geq 0.171$), suggesting that there were no inverse U-shaped effects of APOE across the life span. In summary, these analyses show that performance differences between subtasks, effects of incoming distance, and the interaction between subtask and incoming distance are present in both age groups. We found significant subtask by APOE interactions in both age groups, with a pattern of results replicating the findings in the entire cohort. We found that the adverse effect of APOE $\epsilon 4$ on PPI performance was present in the older subsample only, suggesting that this APOE effect may be mediated by age-dependent accumulation of preclinical AD pathology. This interpretation prompts future studies that assess whether PI deficits are related to AD biomarkers. Some effects did not replicate in the individual age groups: There was no interaction between APOE and incoming distance (i.e., a stronger reduction in performance with larger incoming distances for risk carriers as compared to

controls), and there was no interaction between APOE and goal-to-landmark distance (i.e., a stronger reduction in performance with larger distances between goal and landmark in risk carriers as compared to controls). This is probably due to lower statistical power in each subsample. The younger subsample showed a significant APOE by goal-to-boundary distance interaction that was not present in the entire cohort (i.e., risk carriers showed a more pronounced performance increase in trials with more central goal locations). Next, we re-performed the sMRI analysis after splitting the sMRI sample into two age groups. This was done according to the split that is apparent from the age histogram (young group: ≤ 28 years, $n = 48$; mean age $\pm$ SD, 22.42 ± 2.28 ; older group: ≥ 53 years, $n = 51$; mean age $\pm$ SD, 63.22 ± 5.42 ; fig. S1C). The proportion of risk carriers did not differ between the younger (risk group, $n = 9$; control group, $n = 39$) and older ($n = 14$; $n = 37$) age group [$\chi^2(1) = 1.05$, $P = 0.348$]. The proportion of male and female participants also did not differ between the two age groups [$\chi^2(1) = 0.01$, $P \approx 1.000$]. Similar to our main analysis, EC volume did not predict performance in either the older group or the younger group (all $F \leq 2.07$, all $P \geq 0.157$). In the older group, we observed a trend for a three-way interaction between incoming distance, APOE, and relative EC volume ($F = 3.60$, $P = 0.058$; Fig. 3B), similar to what we had found in the entire sample. This effect was not present in the younger group ($F = 0.29$, $P = 0.593$). To understand the data in detail, we conducted post hoc tests on quintiles of incoming distances. In control participants, PI performance did not depend on EC volume in either age group (all $t \leq 1.22$, all $P_{\text{Tukey}} \geq 0.221$). By contrast, and as in the entire sample, risk

carriers exhibited significant relationships between EC volume and performance at various incoming distances (see Fig. 3B). In younger risk carriers, EC volume was positively related to performance at high incoming distances (>4360 vm: both $t \geq 3.10$, both $PTukey \leq 0.002$). In older risk carriers, positive relationships between EC volume and performance occurred at almost all incoming distances (<7920 vm: all $t \geq 2.10$, all $PTukey \leq 0.036$). Because we did not observe a three-way interaction between EC volume, genotype, and incoming distance for either age group (see above), these differences should be considered cautiously, however. Thus, these analyses confirm that EC volume predicted PI performance specifically in risk carriers, underscoring that their EC integrity is of particular importance for PI performance. In conclusion, while most APOE effects occurred across the entire age range, the adverse effect of APOE $\epsilon 4$ on PPI seemed to be confined to older participants, underlining the relevance of our findings for detecting older persons at risk for cognitive vulnerability and AD.

fMRI correlates of integrated path and goal proximity during PI

. To understand the neural basis of PI and the impact of supportive spatial cues in our paradigm, we conducted an fMRI study with an adapted version of the task (fMRI sample; see Methods). Because we were interested in the physiological fMRI correlates of our PI task, the fMRI sample comprised a group of young and healthy participants, which were recruited irrespective of APOE genotype. First, we

analyzed neural representations of two fundamental metrics of PI: the instantaneous integrated path (i.e., the length of the travelled path during either the outgoing or incoming phase) and the instantaneous goal proximity (i.e., the distance between the subject's current location and the goal). We created a general linear model (GLM) with regressors for the entire time of movement during the outgoing and incoming phase, separately for each subtask (fig. S4). In two separate analyses, we included either integrated path or goal proximity as time-varying parametric modulators during movement (sampled at a temporal resolution of 5 Hz). We tested how these measures of PI were related to BOLD activity in the three ROIs (EC, HC, and PC/RSC). PC/RSC was defined as the posterior-ventral part of the cingulate gyrus [following (26, 27)], resulting in a mask slightly larger than RSC proper. P values were false discovery rate (FDR)–corrected for multiple comparisons (number of ROIs and number of contrasts). During the outgoing phase, EC and HC showed pronounced deactivation with increasing integrated path (both $t_{34} \geq 4.68$, both PFDR < 0.001 ; Fig. 5A). This was reversed during the incoming phase, where we observed increased activation in both areas with integrated path (both $t_{34} \geq 2.55$, both PFDR ≤ 0.046 ; Fig. 5A). When analyzed separately for the different subtasks, we found that EC and HC activity levels always differed significantly from zero in the PPI subtask (Fig. 5C and Supplementary Text). By contrast, integrated path did not predict activity in the LPI subtask, suggesting that this representation is less relevant in this subtask. Activity in PC/RSC did not show any relationship with integrated path. During both the outgoing and incoming phases, HC activity increased with goal

proximity (both $t_{34} \geq 2.96$, both $\text{PFDR} \leq 0.028$; Fig. 5B). In EC, we observed this effect only during the outgoing phase ($t_{34} = 2.86$, $\text{PFDR} = 0.029$), but not during the incoming phase ($t_{34} = 1.12$, $\text{PFDR} = 0.813$). No relationship between goal proximity and PC/RSC activity was observed. As integrated path and goal proximity are typically related in the real world and strongly correlated in our task as well ($\rho = -0.38$, $Z = 3.72$, $P < 0.001$), we included them in two separate GLMs. We conducted a control analysis to demonstrate that representations of integrated path were not simply side effects of goal proximity representations by including both parametric modulators into a single GLM (see Methods). Results were highly similar (Supplementary Text). Together, these results show that BOLD responses in EC and HC represent integrated path and goal proximity, two related variables that are crucial for solving PI tasks. This is in accordance with fMRI studies in humans [e.g., (20)], showing representations of goal direction and distance in EC and HC.

fMRI correlates of spatial information from boundaries and landmarks

Next, we analyzed which brain regions are recruited by supportive spatial cues. We used a GLM with “task phase” (start phase, outgoing phase, incoming phase, feedback) and subtask (PPI, BPI, and LPI) as regressors (fig. S5) and contrasted activity during the PPI subtask with activity during the BPI or LPI subtasks. In PC/RSC, activity increased during the LPI as compared to the PPI subtask ($t_{34} = 4.27$, $\text{PFDR} < 0.001$; Fig. 5D) and did not differ between the BPI versus the PPI

subtask ($t_{34} = 2.24$, PFDR = 0.095). Activity in EC and HC did not differ between the subtasks (all $t_{34} \leq 0.97$, all PFDR ≥ 0.385). An exploratory whole-brain analysis confirmed these results and additionally showed higher activity in V1 and adjacent visual areas during the BPI and LPI subtasks, probably due to the higher amount of visual information (Fig. 5E; for all clusters, see table S5). Hence, PC/RSC appears to be specifically involved in processing spatial information derived from environmental landmarks, in line with previous results (21, 22).

Support of PI computations by fMRI grid-like representations

Previous fMRI studies established a hexadirectional modulation of BOLD activity in the EC as a macroscopic signature of grid cell activity (4, 13, 14). We thus analyzed GLRs in our paradigm using a multi-voxel pattern approach (see Methods) (14). We focused on the posterior-medial EC (pmEC), the putative human homolog of the rodent medial EC (11, 14, 28). The analysis is based on the rationale that the hexadirectionally symmetric firing pattern of grid cells results in higher pattern similarity when movement directions are 60°, 120°, 180°, etc. apart from each other than when they have an offset of 30°, 90°, 150°, etc. (Fig. 6A). We observed a significant hexadirectional symmetry of pattern similarity in pmEC ($t_{34} = 2.56$, $P = 0.015$; Fig. 6B). A post hoc test revealed that the effect was confined to right pmEC ($t_{34} = 2.98$, $P = 0.005$), in accordance with previous studies on GLRs (4, 13). GLRs were not driven by a head direction signal: Eliminating movement directions with

angular differences of -15° to 15° still resulted in a hexadirectional signal (bilateral pmEC: $t_{34} = 2.90$, $P = 0.006$; right pmEC: $t_{34} = 3.36$, $P = 0.002$; Fig. 6B). We performed a series of control analyses to ensure validity and specificity of the GLRs (Fig. 6C and Supplementary Text).

Predictions of PI performance by neural representations of spatial features

Last, we aimed at establishing a mechanistic model that could explain PI performance (Fig. 7). We built an exploratory mixed linear model with subtask and incoming distance as within-subject predictors, subject as random factor, and the following fMRI-based between-subject predictors: (i) EC representations of integrated path during the incoming phase, (ii) HC representations of goal proximity during the incoming phase, (iii) GLRs in pmEC, and (iv) PC/RSC landmark representations (model 6, Table 1). The other potential fMRI-based predictors were not included into the model because they either caused multicollinearity or did not significantly improve model fit (for a detailed explanation of the model buildup, see Methods). We allowed interactions of the fMRI-based predictors with subtask or incoming distance, but not with the other fMRI-based predictors. Post hoc comparisons were Tukey-corrected for multiple comparisons (number of subtasks or number of compared quintiles). First, we found that EC representations of integrated path during the incoming phase predicted performance ($F = 16.19$, $P < 0.001$). This effect was more pronounced at high incoming distances, as shown by a significant

interaction with incoming distance ($F = 5.43$, $P = 0.020$): At higher incoming distances (quintiles 2 to 5), EC representations of integrated path showed a significant association with performance (all $z \geq 3.32$, all $PTukey < 0.005$). This was not the case at low incoming distances (quintile 1: $z = 1.97$, $PTukey = 0.221$). Second, while we did not encounter a significant main effect of HC representations of goal proximity during the incoming phase ($F = 1.39$, $P = 0.247$), we found a significant interaction with subtask ($F = 3.43$, $P = 0.032$). However, post hoc tests revealed no significant association between this fMRI predictor and performance in any subtask (all $z \leq 2.08$, all $PTukey \geq 0.109$). Third, the magnitude of GLRs had no main effect on performance ($F = 0.73$, $P = 0.398$) but showed a significant interaction with subtask ($F = 7.98$, $P < 0.001$). Post hoc comparisons revealed that GLRs predicted performance to a greater extent in PPI than in LPI ($z = 3.99$, $PTukey < 0.001$). No differences were encountered between the other subtask combinations (both $z \leq 2.14$, both $PTukey \geq 0.082$). When analyzed separately, we found that GLRs significantly predicted performance in the PPI subtask ($z = 2.52$, $PTukey = 0.035$), but not in the other subtasks (both $z \leq 0.96$, both $PTukey \geq 0.711$). These results indicate that the grid cell system in pmEC supports PI specifically in the absence of supportive spatial cues. Last, the magnitude of PC/RSC representations of landmarks showed no main effect on performance ($F = 0.07$, $P = 0.793$), but we encountered a significant two-way interaction with subtask ($F = 6.93$, $P = 0.001$) and a three-way interaction with subtask and incoming distance ($F = 4.36$, $P = 0.013$).

However, PC/RSC representations of landmarks predicted performance in none of the individual follow-up tests (all $z \leq 1.47$, all $PTukey \geq 0.543$).

DISCUSSION

Our behavioral results show that APOE $\epsilon 4$ -carriers perform as well as controls as long as they can use supportive spatial information from a boundary or a landmark. Their deficit in PI is unmasked, however, when potentials for error accumulation increase (i.e., with higher PI distance) and/or when no compensatory strategies can be used. In these conditions, PI performance relates to EC gray matter volume and to the strength of entorhinal GLRs. Supportive spatial cues improve performance, presumably via compensatory recruitment of other regions including the PC/RSC. We propose that changes in grid cell functioning underlie the impaired performance of risk carriers during PPI because various studies identified grid cells as the main neural substrate for PI (16, 19). In our fMRI study, we observed a positive relationship between the strength of GLRs and PI performance exclusively in the absence of supportive spatial cues that could reduce error accumulation during PI computations (17, 18). This indicates that the experimental condition in which risk carriers showed impaired PI performance relied most strongly on the grid cell system. Risk carriers may thus experience higher error accumulation during PPI due

to a compromised EC grid cell system. Furthermore, we found representations of integrated path and goal proximity in EC and HC during the outgoing as well as during the incoming phase. This result is in line with models suggesting that EC grid cells provide the computational basis for representing the integrated path (i.e., the distance and direction traveled) (18). A complementary model suggests that grid cells enable the computation of a direct vector to the goal (20, 21). Accordingly, we demonstrated that risk carriers' performance is particularly affected by high incoming distances and that this is associated with a higher dependency on EC volume in APOE ϵ 4-carriers. An environmental boundary or a landmark allowed risk carriers to perform as well as controls. This is consistent with a recent study showing similar PI performance in risk and control participants in rich virtual environments (5). This previous study examined the effect of APOE genotype on wayfinding distance and wayfinding duration during map-based navigation and on rotation errors during PI ("flare accuracy"). A particular strength of this study was the use of normative benchmark big data from the Sea Hero Quest (SHQ) game that was conducted by more than 27,000 participants worldwide: ϵ 4-carriers showed larger wayfinding distances than noncarriers, whereas wayfinding duration and flare accuracy were similar (5). Given that wayfinding distance may particularly rely on grid cell-based navigational processes, the study is in line with the notion that grid cell dysfunction may constitute a sensitive biomarker for AD risk, consistent with the current study. The new results presented here are complementary to this earlier study in several respects: First, effects of APOE ϵ 4 were observed during map-based

wayfinding in the study by Coughlan and colleagues, whereas the current study found a detrimental effect of APOE $\epsilon 4$ on PPI. Thus, different navigational processes were analyzed. Second, the current study applied an adapted version of the behavioral paradigm during fMRI scanning, which allowed us to measure the role of GLRs in our task and also to assess the recruitment of other navigational systems. In addition, structural neuroimaging allowed us to relate the behavioral results to EC volumes. We could thus identify a putative neural correlate for the PI deficit found in our study, i.e., GLRs in EC. In the future, it will be pivotal to directly compare the neurocognitive processes during the different experimental tasks and to further disentangle the effects of APOE genotype and AD pathology on different subcomponents of navigational behavior. Using fMRI, we demonstrated increased PC/RSC activity in response to an environmental landmark, consistent with previous studies (22). The PC/RSC provides a neural basis for viewpoint-dependent representations of local place and direction (21, 22), which may be at the core of compensatory strategies used by risk carriers. We propose that risk carriers recruited the PC/RSC in the landmark condition to a greater extent than controls to counteract error accumulation during PI and to compensate for a reduced reliability of GLRs, thus anchoring the cognitive map (21, 30). In support of this idea, risk carriers showed a stronger relationship between goal-to-landmark distance and performance, and navigated in closer proximity to the landmark during the incoming phase. However, future fMRI studies with genotyped participants are required to corroborate this idea. In the BPI subtask, the relationship between performance and

goal-to-boundary distance was not modulated by genotype, and risk carriers did not navigate closer to the boundary as compared to control participants. This null finding differs from previous findings showing preferred navigation of risk carriers along environmental boundaries (4, 5). This discrepancy may be explained by the specific layout of our BPI subtask: The boundary was at considerable distance to potential goal locations, and navigating toward the boundary led to a linear decrease in navigation speed. The exact effects of environmental boundaries on PI performance may be scrutinized in future studies by comparing different types of boundaries (squared and circular) and environmental layouts (distal and/or proximal landmarks).

AD is defined neuropathologically by two hallmarks: neurofibrillary tangles of hyperphosphorylated tau proteins and β -amyloid plaques (10, 31). While neurofibrillary tangles show a sequential spreading from EC to other limbic regions and eventually to multiple areas of the neocortex (32), amyloid deposition starts in neocortical areas, followed by a progression toward allocortical brain regions, subcortical structures, the brainstem, and the cerebellum (31). The accumulation of MTL tau seems to precede neocortical amyloid deposition in cognitively unimpaired older adults (33), although the exact sequence between pathologies and the interpretation to provide when only one pathology is observed is still highly debated. It is generally acknowledged that tau pathology is more closely related to cognitive deficits in preclinical and clinical stages of AD (33–35). Our and related previous findings in cognitively unimpaired samples at genetic risk for AD showed impaired performance (5, 36) and/or altered strategies (4) in navigational tasks that are

presumably MTL dependent, whereas performance in navigational tasks that depend primarily on regions outside of the MTL (such as landmark-oriented navigation) was not affected. This performance deficit may thus reflect initial occurrence of AD-related neurofibrillary tangles, rather than early amyloid deposition. However, positron emission tomography (PET) studies of amyloid and tau will be needed to determine the respective contributions of both AD pathologies, and their topographies, to the observed pattern of behavioral performance. Compensatory strategies of EC-dependent behavioral deficits in APOE ϵ 4-carriers may be relevant for the progression of AD pathology: It has been suggested that neural hyperactivity associated with these strategies leads to a progressive deterioration in structural integrity of relevant brain areas (37), perhaps by increasing β -amyloid (38) and/or tau deposition (34). Thus, potential compensatory strategies in risk carriers associated with higher activity in specific brain areas may contribute to AD progression (4). Consequently, at later stages of AD, patients should no longer be able to rely on compensatory mechanisms (39), leading to worse performance irrespective of whether supportive spatial cues are provided. In a recent study, mild cognitive impairment patients with positive AD biomarkers showed reduced PI performance irrespective of the availability of supportive spatial cues (36). At this stage, neural hyperactivity may even directly exert detrimental effects on behavior (40). Preventing hyperactivity might thus be a promising therapeutic strategy, possibly improving interneuron dysfunction and counteracting network abnormalities (41). Several limitations of the current study should be addressed.

First, our cohort may not be representative of the entire population as the participants were relatively highly educated (average years of education, 13.37 years). Previous studies showed that higher levels of education are associated with a reduced risk of dementia (42). Specifically, human PET data indicate a lower impact of tau pathology on neuronal function in AD patients with higher education, suggesting that the level of education might support resilience mechanisms (43) (“cognitive reserve hypothesis”; see also (42)). We therefore hypothesize that the APOE effects on PI performance we report might actually be more severe in the overall population—a question that should be investigated in future studies. Another limitation of the current study is that we conducted the fMRI study to reveal the neural mechanisms underlying our specific PI task in a group of participants without any particular impairments due to AD or aging—i.e., in young, healthy participants. Using this approach, we identified different neural processes related to our task. We hypothesize that these physiological neural processes are altered or impaired by age- and/or disease-related factors [e.g., (4, 16, 44)]. Future studies may thus examine the influence of APOE ϵ 4 and age on the physiological neural processes of our PI task. Last, based on the findings of the current study, we cannot conclude unambiguously that the detrimental effect of APOE ϵ 4 on PPI performance results from AD-related pathology. Instead, the selective impairment in PPI and the increased use of landmark-related strategies may reflect neurocognitive development evident even in the healthy state (45). Neuropathological studies showed increased AD-related neurofibrillary changes even in young APOE ϵ 4-carriers [mean age, 38 years; age

range, 22 to 46 years; (8)], which may speak in favor of the interpretation that impaired PI performance is a result of very early AD-related changes. Nevertheless, future studies are clearly needed to test for direct relationships between impaired PI performance and signs of AD neuropathology, e.g., using cerebrospinal fluid biomarkers and/or PET imaging.

CONCLUSION

EC-dependent functioning may prove particularly useful for predicting AD because of its early affection by AD-related neuropathological changes (10). Distinct navigational strategies may deteriorate at different preclinical and clinical stages of AD, and PPI may capture the behavioral manifestation of earliest neuropathological changes related to preclinical AD development.

METHODS

Participants

We recruited healthy participants at five European sites including Germany (two sites), Spain, Belgium, and Italy (table S1) to (i) examine the influence of APOE genotype on PI performance (APOE sample), (ii) elucidate the fMRI signatures of PI in our task (fMRI sample), and (iii) investigate associations between brain

structure and PI performance (sMRI sample). The study was performed in accordance with the Declaration of Helsinki and was approved by the respective institutional review boards at all sites. All participants gave their written informed consent

APOE sample

Four groups of participants (total, $N = 318$) were recruited at four different sites: Germany (“APOE sample 1,” $n = 112$; IfADo—Leibniz Research Centre for Working Environment and Human Factors at the Technical University Dortmund, Dortmund, Germany), Spain (“APOE sample 2,” $n = 114$; Pablo de Olavide University, Seville, Spain), Italy (“APOE sample 3,” $n = 68$; University of Parma, Parma, Italy), and Belgium (“APOE sample 4,” $n = 24$; Cliniques Universitaires Saint-Luc, Brussels, Belgium). Fifty-one participants were excluded because of genotypes other than $\epsilon 3/\epsilon 4$ or $\epsilon 3/\epsilon 3$ ($n = 46$), prior familiarity with the task ($n = 1$), or technical reasons ($n = 4$), resulting in a final sample of $n = 267$ participants (for demographics, see tables S1 and S2; fig. S1).

sMRI sample

The sMRI sample consisted of a subset of participants from APOE sample 2 for which sMRI scans were available ($n = 99$; for demographics, see tables S1 and S3; fig. S1).

fMRI sample

To elucidate the fMRI signatures of PI in our task, we recruited young healthy participants (n = 35; for demographics, see table S1) at Ruhr University Bochum, Bochum, Germany.

APOE genotyping

Four groups of participants (APOE samples 1 to 4) were analyzed for the APOE polymorphisms rs429358, a [C/T] substitution on chromosome 19q13.32 of the sequence

GCTGGGCGCGGACATGGAGGACGTG[C/T]GCGGCCGCCTGGTGCAGTAC
CGCGG, and rs7412, a [C/T] substitution of the sequence
CCGCGATGCCGATGACCTGCAGAAG[C/T]GCCTGGCAGTGTACCAGGCC
GGGGC.

On the basis of the two single-nucleotide polymorphisms (SNPs), participants were assigned to one of the three alleles ϵ 2, ϵ 3, or ϵ 4. For APOE sample 1, venous blood was taken and DNA was isolated using the QIAamp DNA Blood Maxi Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA concentrations were determined using a NanoDrop ND-1000 UV/Vis spectrophotometer (PEQLAB Biotechnologie GMBH, Erlangen, Germany). Genotyping was performed on the ABI7500 Sequence Detection System with the use of TaqMan assays (assay ID: C_3084793_20 for rs429358 and assay ID: C_904973_10 for rs7412; Applied

Biosystems, Darmstadt, Germany). Analysis of data was performed according to the manufacturer's instructions. For APOE sample 2, genomic DNA was isolated from blood using a standard salting-out protocol. DNA concentration and purity were determined by UV spectrophotometric measurements. Genotyping was performed by real-time PCR using predesigned TaqMan SNP genotyping assays (rs429358 and rs7412). For APOE sample 3, genomic DNA was extracted from buccal brushes using the Gentra Puregene Buccal Cell Kit. SNPs were genotyped using an ABI PRISM 7700 Sequence Detector with TaqMan allele discrimination. For APOE sample 4, DNA was extracted from blood and analyzed for APOE polymorphisms using restriction enzyme isoform genotyping and TaqMan assays. Participants were not genetically preselected. Analyses focused on APOE ϵ_3/ϵ_3 -carriers ("control group," $n = 202$) and APOE ϵ_3/ϵ_4 -carriers ("risk group," $n = 65$). The prevalence of risk carriers (ϵ_3/ϵ_4) was 20.83% (ϵ_3/ϵ_3 , 64.74%; ϵ_3/ϵ_4 , 20.83%; ϵ_2/ϵ_3 , 10.58%; ϵ_2/ϵ_4 , 1.60%; ϵ_4/ϵ_4 , 1.60%; ϵ_2/ϵ_2 , 0.64%). The allele frequency of ϵ_4 was 12.82% (ϵ_3 allele, 80.45%; ϵ_4 allele, 12.82%; ϵ_2 allele, 6.73%), consistent with existing estimates (~14%). Risk and control groups did not differ in demographic characteristics. Participants from other genotypic subgroups were excluded (ϵ_2/ϵ_2 , $n = 2$; ϵ_2/ϵ_3 , $n = 33$; ϵ_2/ϵ_4 , $n = 5$; ϵ_4/ϵ_4 , $n = 5$). Participants and experimenters were blinded to genotype. Sample size was based on previous studies requiring >50 participants in the smaller subgroup. All participants reported normal or corrected vision and no neurological or psychiatric history.

Experimental task

Participants performed a PI task (the Apple Game) in a virtual environment implemented via Unreal Engine (Epic Games, version 4.11). The environment consisted of an endless grassy plane with a blue sky rendered at infinity. Trials were divided into three phases. During the start phase, participants navigated to a goal location, which was marked by a basket (Fig. 1, B and C). Participants were instructed to remember this location. When they had reached the goal location, the basket disappeared and a tree appeared in a different location (outgoing phase). Participants walked toward the tree, checked if it had an apple, and walked to the tree to make it disappear. If the tree did not have an apple, another tree appeared. Trials contained between one and five trees to systematically vary PI difficulty, corresponding to a variation of outgoing distance [Fig. 1C; see also the “Behavioral analyses” section; for similar tasks, see (16, 36)]. The final tree was marked by a red apple (retrieval location), indicating that the participants had to navigate back to the goal location (to place the apple into the basket; incoming phase). After pressing a button to indicate that this was the presumed location of the basket (“response location”), participants received feedback via zero to three stars, depending on the Euclidean distance between the response location and the correct goal location (drop error; Fig. 1D; <1600 vm for three stars, <3200 vm for two stars, and <6400 vm for one star; fig. S1). All phases were self-paced, and only the incoming phase had a time limit of 60 s before the next trial started (this time limit was never reached by any participant). Locations of baskets and trees were equally distributed across an

(invisible) grid of 8×8 squares (bin edge length, 800 vm) such that each participant visited all squares once in each environmental condition (fig. S1). Virtual meters are the metric to quantify distances in the virtual environment as given by Unreal Engine. The diameter of the arena was $\sim 13,576$ vm (fig. S1A). Therefore, the highest incoming distance of ~ 7920 vm corresponded to $\sim 58\%$ of the arena diameter. Virtual meters cannot be directly converted into real-world meters, but—assuming that the virtual character's eye height of 310 vm corresponds to ~ 1.65 real-world meters—it can be inferred that the virtual arena had a size of ~ 72 real-world meters. PI performance was tested in three environmental conditions that differed with regard to the presence or absence of supportive spatial cues. In the PPI condition, the virtual environment did not contain any landmarks or boundaries, and participants purely relied on visual flow to perform PI (Fig. 1A). In the BPI condition, a circular stonewall with a height of 2050 vm surrounded the environment at a radius of 6788 vm (Fig. 1A, middle; for location and radius, see also fig. S1). In the LPI condition, a lighthouse with a height of 1300 vm was present at $x = 1600$ vm and $y = 800$ vm, serving as an intramaze landmark (Fig. 1A; for the location, see also fig. S1). No distal cues outside the boundary were present, which is different from previous studies examining the influence of APOE on navigational behavior (4, 5). We chose this design to specifically assess the effects of the boundary and the landmark. Participants navigated the virtual environments using a joystick (behavioral experiment, Trust GXT 555 Predator; fMRI experiment, MR-compatible joystick from Nata Technologies, Coquitlam, Canada), allowing them to move forward, turn

left, or turn right. Moving backward was not possible so that movement direction was equivalent with heading direction. In each subtask, participants' speed was attenuated when their distance from the center of the arena was larger than 5657 vm and linearly decreased to zero at 6788 vm, ensuring a constant movement radius in subtasks with and without a visible boundary (fig. S1). In this "speed reduction zone," participants could navigate at full speed when heading toward the center of the arena. The position of the participant was logged every 200 ms, which allowed us to extract movement periods, movement speed, and movement direction.

Behavioral experiment

The paradigm was subdivided into subtasks of 16 trials each. Subtasks varied with respect to the layout of the virtual environment. Participants started the first trial of each subtask in the center of the virtual environment ($x = 0$ vm, $y = 0$ vm). The outgoing phase of each subtask contained either one tree (three trials), two trees (three trials), three trees (four trials), four trees (three trials), or five trees (three trials) in randomized order (including the tree marked by an apple). This trial procedure allowed a perfect balancing of locations during the experiment so that each participant visited all 64 squares once in each subtask (fig. S1). Before the beginning of the task, all participants completed eight trials in the PPI condition to practice the paradigm. As we encountered systematic within-subject effects of fixed trial and location sequences in a subgroup of participants (for the exact number of participants, refer to table S2), we fully randomized trials and locations for later

participants. The randomization neither showed a main effect on performance ($F = 0.74$, $P = 0.477$) nor did any of the results change when we added randomization version as a covariate to the model (subtask: $F = 799.09$, $P < 0.001$; incoming distance: $F = 4139.47$, $P < 0.001$; “APOE $\times$ subtask”: $F = 10.89$, $P < 0.001$; performance difference in PPI between risk carriers and controls: $z = 2.08$, $P_{\text{Tukey}} = 0.037$). Participants in Parma, Seville, and 20 participants in Brussels completed a long version of the paradigm with six subtasks ($2 \times \text{PPI}$, $2 \times \text{BPI}$, and $2 \times \text{LPI}$; Fig. 1E), with the order of the subtasks being pseudorandomized such that the same subtask would not follow each other and the three different subtasks would be equally distributed across the experimental halves. This resulted in a total of 96 experimental trials. Participants could take breaks between the subtasks. The experiment lasted 107.44 ± 33.15 min (mean $\pm$ SD) in total. Participants in Dortmund and four participants in Brussels completed a shorter version of the paradigm with three subtasks ($1 \times \text{PPI}$, $1 \times \text{BPI}$, and $1 \times \text{LPI}$; Fig. 1E) with the order of the subtasks being randomized, resulting in 48 experimental trials. Including breaks between the subtasks and practice trials, the short version of the experiment lasted 52.83 ± 12.08 min (mean $\pm$ SD) in total.

fMRI experiment

The fMRI experiment consisted of a practice run, which was conducted during the structural scan (6 min), and two functional runs. The practice run comprised a maximum of nine trials (three trials in each condition) and ended when the structural

scan was over. Each functional run had a duration of 22.32 ± 3.18 min (mean $\pm$ SD), which corresponds to 536 ± 76 (mean $\pm$ SD) fMRI volumes. The duration of the functional runs varied between participants, because the entire task was self-paced. In between runs, participants could take short breaks. Each run consisted of six blocks containing four trials of each of the three environmental conditions (Fig. 1F), resulting in 16 trials per condition and thus 48 trials in total across the experiment. The order of the blocks was pseudorandomized such that the same subtask would not follow each other and that the three different subtasks would be equally distributed across the two experimental runs. Before every new trial, participants viewed a fixation crosshair with a variable duration of 5 to 7.5 s (randomly distributed).

Data acquisition

Data were acquired according to a standardized protocol (with regard to experimental setup, instructions, behavior of the investigator, and breaks), ensuring comparability across the five recording sites.

Behavioral data acquisition (APOE sample)

The paradigm was presented on laptops with a screen diagonal of 45 cm, a resolution of 1920×1080 pixels, and a frame rate of 60 frames/s. We attached the joystick to the table by means of a custom-made frame (identical for all sites), which also served as an armrest, and placed the laptop at a distance of 50 cm in front of the participant.

MRI data acquisition (sMRI sample)

Structural brain images were acquired at the Neuroimaging Service of the Pablo de Olavide University (Seville, Spain) using a 3T Philips Ingenia CX MRI scanner equipped with a 32-channel receiver head coil (Philips, Best, The Netherlands). Head motion was minimized by placing foam padding around the subject's head. One high-resolution three-dimensional (3D) T1-weighted magnetization-prepared rapid gradient echo (MP-RAGE) sequence was acquired in the sagittal plane. Acquisition parameters were empirically optimized to enhance the gray/white matter (WM) contrast with repetition time (TR)/echo time (TE) = 2600/4.7 ms, flip angle (FA) = 9°, voxel resolution = 0.65 mm isotropic, acquisition matrix = 384 mm × 384 mm, resulting in 282 contiguous slices without gap between adjacent slices, acceleration factor (SENSE) = 1.7, and field of view (FOV) = 250 mm × 250 mm × 183 mm.

fMRI data acquisition (fMRI sample)

The fMRI recordings were conducted at the Bergmannsheil hospital in Bochum using a 3 T Philips Achieva scanner (Best, The Netherlands) with a 32-channel head coil. High-resolution whole-brain structural brain scans of participants were acquired using a T1-weighted sequence at 1-mm isotropic resolution, an FOV of 240 mm × 240 mm, and 220 transversally oriented slices during a total acquisition time (TA) of 6 min 2 s. Blood oxygenation level-dependent (BOLD) contrast images were

measured with a T2*-weighted gradient echo EPI sequence with 2.5-mm isotropic resolution, TR = 2500 ms, TE = 30 ms, FA = 90°, FOV = 96 mm × 96 mm, 46 transversal slices in interleaved order without slice gap, and TA = 22.32 ± 3.18 min (mean ± SD), corresponding to 536 ± 76 volumes (mean ± SD). We discarded the first five images of each session to allow signal steady-state transition. Participants viewed the virtual environment via MR-compatible liquid crystal display (LCD) goggles (VisuaStim Digital, Resonance Technology Inc., Northridge, CA, USA) with a resolution of 800 × 600 pixels, and they navigated the virtual environment by means of an MR-compatible joystick.

Data analysis

We extracted behavioral data from logfiles using MATLAB (2018a, The MathWorks Inc., Massachusetts) including the Parallel Computing Toolbox (v6.12) and the CircStat Toolbox (47). ROIs were created using FreeSurfer (v6.0.0). We used SPM12 (www.fil.ion.ucl.ac.uk/spm) for all fMRI analyses. Statistics were done in R (48) (3.5.0) using the lme4 (49) (v1.1-17) and emmeans (40) (v1.2.2) packages.

Behavioral analyses

We determined three different performance measures from the behavioral logfiles (Fig. 1D): drop error, “distance error”, and “rotation error.” The drop error was defined as the Euclidean distance between the goal location and the response

location. We computed the distance error as the absolute difference between two distance measures: distance error = $D_{\text{correct}} - D_{\text{response}}$, where D_{correct} is the distance between the retrieval and the goal location (i.e., the distance of the correct incoming path) and D_{response} is the distance between the retrieval and the response location (i.e., the distance of the incoming path chosen by the participant). Rotation error was computed correspondingly as the absolute difference between two angular measures: rotation error = $R_{\text{correct}} - R_{\text{response}}$, where R_{correct} is the heading direction of the correct incoming path and R_{response} is the heading direction of the response path. Only for visualization and enhanced readability, we converted the error measures to performance measures of participant i (performance $_i$) using a linear transformation: performance $_i = [\max(\text{error}) - \text{error}_i + \min(\text{error})] / \max(\text{error}) \times 1000$, where $\max(\text{error})$ and $\min(\text{error})$ correspond to the maximum and minimum error across all participants, respectively, and error_i corresponds to the error in participant i . The formula simply reverses the errors and maps them into the range between 0 and 1000. Note that the linear transformation does not affect the statistical results. Thus, “performance” refers to the drop error, “performance based on distance error” refers to the distance error, and “performance based on rotation error” refers to the rotation error. To analyze performance as a function of path distance, we calculated incoming and outgoing distance (Fig. 1C): outgoing distance was determined as the cumulated path distance during the outgoing phase, i.e., we summed the Euclidean distances between “goal,” “tree without apple,” and “retrieval” locations. Incoming distance was defined as the Euclidean distance

between the retrieval location and the goal location. Outgoing and incoming distances reflect different subcomponents of PI (keeping track of the traveled path with regard to the goal location and computing a straight line in relation to the goal location, respectively) but are putatively both subject to cumulative error accumulation (18). The precision of PI processes can be improved if sensory information is available to recalibrate the ideothetic coding process (17). We thus investigated the influence of spatial cue distance, i.e., the distance between the goal location and the boundary (“goal-to-boundary” distance) or the landmark (“goal-to-landmark” distance) on performance. Last, we examined navigational strategy, irrespective of performance. To this end, we computed the mean Euclidean distance from the boundary or from the landmark across all time points of the incoming phase (this was not computed during the outgoing phase, because navigation during this period was determined by the consecutive trees).

Statistical analysis

To assess potential demographic differences between risk carriers and controls, we performed two-tailed t tests, Wilcoxon tests, and chi-square tests. To test for significant relationships between fMRI representations, we performed bivariate Pearson or Spearman correlations. To disentangle the various factors driving PI performance in the different subtasks of our paradigm, we implemented a mixed linear model using the lme4 package in R (48, 49). We were both interested in fixed effects of predictors varying within subjects and in effects of predictors varying

between subjects. As within-subject predictors, we included subtask (i.e., PPI, LPI, and BPI) and two different types of distance predictors: a path distance predictor that could be quantified in all three subtasks (i.e., outgoing distance or incoming distance), and a spatial cue distance predictor that was only defined in the corresponding subtask (goal-to-boundary distance in the BPI subtask and goal-to-landmark distance in the LPI subtask). In addition, we considered between-subject effects: genotype (i.e., APOE ϵ 4-carriers and APOE ϵ 4-noncarriers), which was defined for all subjects in the APOE sample; volume in EC, HC, or PC/RSC, which was only defined for subjects with available sMRI data (sMRI sample); and representations of integrated path, goal proximity, boundary, landmark, and GLRs, which were only defined for subjects with available fMRI data (fMRI sample). Continuous variables were centered on the grand mean (between-subject predictors) or on the subject mean (within-subject predictors). We included age and sex as covariates to control for main effects and interactions on subtask, genotype, and gray matter volume. Site and subject were included as random factors. We tested separate models for each outcome variable (performance, performance based on distance error, and performance based on rotation error). Because it is most appropriate for model building and preferable in terms of power (52), we always used type II sum of squares (SS) to test fixed effects. For post hoc pairwise comparisons, we used Tukey-adjusted Fisher's tests as implemented in the emmeans package (50). All statistical tests were two-tailed at an alpha level of $\alpha < 0.05$. We did not report regression coefficients and degrees of freedom, as both are not intuitively

interpretable in mixed linear models of high complexity. We reported all significant main effects and interactions and nonsignificant effects, which were of interest for our hypotheses. Thus, effects that are not reported in the results section were not significant. We did not report significant interactions with covariates (but see fig. S2). All mixed linear models, except model 6, were built in a hypothesis-driven way (see Table 1 for an overview of all models): Models 1a and 1b were built to test for effects of subtask, genotype, and the two path distance regressors on performance. We used separate models for the two path distance predictors (“a,” outgoing distance; “b,” incoming distance), because they were positively correlated and would have caused multicollinearity. However, to scrutinize whether the two predictors showed significantly different interactions with genotype, we built model 1c. This model contained one regressor that accounted for the effects of the path distance predictors irrespective of the type of path distance and a binary regressor that accounted for the type of path distance (0, outgoing distance; 1, incoming distance). Models 2a and 2b served to analyze the effects of spatial cue distance, i.e., the subtask-specific measures of the distance between the goal and either the landmark (LPI subtask) or the boundary (BPI subtask). In models 2a and 2b, we analyzed the effects of genotype and goal-to-boundary or goal-to-landmark distance on performance in the respective subtasks. Models 2c and 2d were the only models in which we did not test for factors influencing PI performance but tested whether APOE genotype predicted whether subjects navigated closer to the boundary (BPI subtask) or the landmark (LPI subtask), similar to previous studies (4, 5). Thus, we

did not use performance as criterion (i.e., dependent variable) but distance-to-boundary (BPI subtask) or distance-to-landmark (LPI subtask), respectively. These distance measures were obtained by averaging the Euclidean distances across all time points of the incoming phase of each trial of the respective subtasks. Models 3 to 5 tested for effects of EC, HC, and PC/RSC volumes on PI performance, respectively. These between-subject variables were included in addition to genotype, subtask, and one of the path distance measures. We corrected for multiple comparisons (i.e., the number of ROIs tested) using Bonferroni correction. We conducted additional analyses to gain a more in-depth and comprehensive understanding of how our genotype effects might vary between different age groups. In detail, we split the sample into two age groups in a data-driven way using MATLAB's kmeans clustering algorithm. The resulting cutoff was at 42 years, which divided the overall sample into a younger group ($n = 163$; mean age $\pm$ SD, 24.32 ± 4.87) and an older group ($n = 104$; mean age $\pm$ SD, 58.71 ± 7.75). The sMRI sample was split up in accordance with the gap in the age histogram (young group: ≤ 28 years, $n = 48$; mean age $\pm$ SD, 22.42 ± 2.28 ; older group: ≥ 53 years, $n = 51$; mean age $\pm$ SD, 3.22 ± 5.42 ; fig. S1C). We performed the analyses of models 1 to 3 in the two age groups. Last, model 6 served to predict performance by means of incoming distance, subtask, and different fMRI representations. As we did not have specific hypotheses about the predictors, this model was built in an exploratory way with the restrictions that overfitting and multicollinearity had to be avoided. The following fMRI representations were considered as model predictors because of

significant effects in previous analyses (Figs. 5 and 6): (1) GLRs in bilateral pmEC; (2) representations of integrated path during outgoing phase in EC; (3) representations of integrated path during incoming phase in EC; (4) representations of integrated path during outgoing phase in HC; (5) representations of integrated path during incoming phase in HC; (6) representations of goal proximity during outgoing phase in EC; (7) representations of goal proximity during outgoing phase in HC; (8) representations of goal proximity during incoming phase in HC; (9) landmark representations in PC/RSC. We included (1) in the model as it has been suggested that the grid cell system is particularly involved in PI (11). We included (3) in the model because it was significantly correlated with mean drop error across participants ($r_{34} = -0.541$, $P = 0.001$). Because (2), (5), and (7) were correlated with (1) ($r_{34} = 0.50$, $P = 0.002$), (1) ($p_{34} = -0.38$, $P = 0.024$), and (3) ($r_{34} = -0.341$, $P = 0.045$), respectively, they were excluded to avoid multicollinearity. Consequently, we started the model buildup using predictors (1), (3), (4), (6), (8), and (9). These predictors were not correlated (all r_{34} or $p_{34} \leq 0.32$, all $P \geq 0.061$). We allowed interactions between the fMRI representations and incoming distance and subtask, but not interactions between the fMRI representations themselves. In the next step, we aimed at excluding predictors from the model that were not relevant for predicting PI performance to build a parsimonious model (49): We started selecting predictors by applying an arbitrary significance threshold of $\alpha = 0.1$, which resulted in removing (4) and (6). We proceeded with the following procedure: We started with the highest-order interaction term and removed it if it was not significant at $\alpha =$

0.05. We calculated a new model and successively continued removing lower order terms (as long as they were not part of a higher-order interaction) until there was no nonsignificant term that could be removed. This resulted in a final model including subtask, incoming distance, and (1), (3), (8), (9), as well as their interactions with subtask and incoming distance as predictors (model 6).

Creation of ROI masks and sMRI analysis

Anatomical ROIs were created using a semiautomatic approach as implemented in FreeSurfer (<https://surfer.nmr.mgh.harvard.edu/>). Briefly, sMRIs were processed using the analysis pipeline of FreeSurfer v6.0 that involves intensity normalization, registration to Talairach space, skull stripping, WM segmentation, tessellation of the WM boundary, and automatic correction of topological defects (53). Pial surface misplacements and erroneous WM segmentation were manually corrected on a slice-by-slice basis to enhance the reliability of cortical thickness and hippocampal volume measurements. Segmented brain images were parcellated into cortical and subcortical regions (27, 54). This allowed us to obtain volume measurements of HC and EC. For the PC/RSC ROI, we used the posterior-ventral part of the cingulate gyrus derived from the Destrieux atlas [following (26), which used this ROI as an approximation for RSC]. Human EC contains structurally and functionally distinct subparts (28). Therefore, we subdivided the EC using a template of anterior-lateral EC (alEC) and pmEC based on functional connectivity with these subregions, which represent the human homolog of rodent lateral and medial EC (28), respectively. To

obtain subject-specific masks, the template was mapped from Montreal Neurological Institute (MNI) space into individual subject's native space. To ensure that only voxels located in gray matter were analyzed, the ROI masks were thresholded and intersected with a gray matter mask based on FreeSurfer's cortical parcellation. Each mask was manually inspected to ensure anatomical correctness. To perform fMRI ROI analyses, ROIs were coregistered to the mean functional image of the respective participant so that all ROI analyses were performed in native space. For the sMRI analysis, we calculated relative gray matter volume by dividing the volume of the ROI by the whole-brain volume of the respective participant (FreeSurfer variable: "BrainSegVolNotVent").

fMRI analysis

Preprocessing. Preprocessing of fMRI data was performed using SPM12 and included slice time correction and spatial realignment. For the whole-brain analysis, we normalized fMRI scans to MNI space using parameters from the normalization procedure of the segmented structural T1 image. Spatial smoothing with a 5-mm isotropic Gaussian kernel was applied to the normalized fMRI data and the fMRI data in native space. Normalized functional images were used to perform whole-brain analyses, while functional images remained in native space for the ROI analyses. **General linear models.** Functional images were analyzed via two separate GLMs. In the PI model (fig. S4), we used four different regressors to model the start phase, the outgoing phase, the incoming phase, and time periods of no movement,

separately for each of the three subtasks and each of the two runs (24 regressors). Time periods of no movement were defined as those time periods when movement speed was at zero or minimally above zero (<1 percentile of the subject-specific movement speeds). To include parametric regressors for the moment-to-moment changes in integrated path and goal distance (see below), the regressors for outgoing phase and incoming phase had separate onsets at each movement time point (every 200 ms) and a duration of zero. We hypothesized that regions involved in PI would represent the integrated path and/or the goal proximity of the current location. We further hypothesized that this representation should be more relevant, and thus more pronounced, when no supportive spatial cues were available (i.e., in the PPI subtask). To test this, we included one of two parametric modulators during the movement periods of the outgoing and incoming phases: integrated path (i.e., the cumulative distance that has been traveled at each time point during the outgoing or incoming phase) or goal distance (i.e., the instantaneous Euclidean distance to the goal during the outgoing or incoming phase). We assumed that goal proximity and integrated path were correlated, because this is often the case in the real world. To test this assumption empirically, we correlated goal proximity and integrated path across the entire time of the experiment within each participant. We tested each participant's empirical Spearman ρ value against a surrogate distribution of ρ values established by shuffling the data time series for 10,000 times. We transformed the resulting P value into a z value. The mean empirical z value across participants was tested against a surrogate distribution of mean z values established by random sign flips for

10,000 times. As expected, we found that goal proximity and integrated path were related in our task ($\rho = 0.38$, $Z = 3.72$, $P < 0.001$). Therefore, we built a third GLM that included both parametric modulators at the same time. Goal proximity was entered first, and integrated path was entered as a second orthogonalized parametric modulator. We then correlated the β values for the integrated path regressor from this GLM with the β values from the GLM that only included the integrated path as parametric modulator to show that representations of integrated path were not a side effect of goal proximity representations. In the subtask model (fig. S5), we modeled start phase, outgoing phase, incoming phase, and “feedback” separately for each of the three subtasks and each of the two runs (24 regressors). The duration of these regressors corresponded to the duration of the respective phases in each trial. In addition, in the outgoing phase, we included “PI difficulty” as a parametric modulator, reflecting the number of distractor trees in each trial (with values of 1 to 5). This parametric modulator resembles the outgoing distance in the behavioral analyses and was included to reduce error variance in the GLM. We tested two contrasts: “BPI > PPI” and “LPI > PPI”. All parametric modulators were normalized to values between 0 and 1 and mean centered. All regressors were convolved with the hemodynamic response function before entering the GLM. As nuisance regressors, we included motion parameters as estimated in the realignment procedure and mean value of WM and corticospinal fluid. We did not model temporal derivatives. ROI analysis. For the ROI analysis, we extracted mean β values (SPM con-files) from our three ROIs (EC, HC, and PC/RSC) in native space and compared

them against zero across participants by means of t tests for normally distributed data or Wilcoxon's ranked sum tests otherwise. To compare mean β values across subtasks, we used repeated-measures analysis of variance (ANOVA) or Friedman ANOVA if data did not fulfill normality assumption. To test for differences between two conditions post hoc, we used t tests (or Wilcoxon ranked sum tests if normality assumption was violated). Whenever we used the same model for several ROIs or several contrasts, we corrected for multiple comparisons using FDR, corrected for the number of contrasts and the number of ROIs ($\alpha < 0.05$) as implemented in R, and adjusted the P values accordingly. For the PI model, we corrected for six multiple comparisons when assessing the relationship between mean β values and the parametric modulators (integrated path or goal proximity, respectively). These six comparisons correspond to three ROIs (EC, HC, and PC/RSC) and two contrasts against zero (one parametric modulator during the outgoing phase and one parametric modulator during the incoming phase). Only if a contrast tested against zero showed a significant effect for an ROI (corrected for six multiple comparisons), we tested for differences between subtasks (one-way repeated-measures ANOVA with subtask as within-subjects factor) and again corrected for the number of ROIs. We performed two types of post hoc comparisons: (i) we compared the subtasks against each other and corrected for three comparisons (PPI versus BPI, PPI versus LPI, and BPI versus LPI), and (ii) we tested every subtask against zero and likewise corrected for three comparisons (PPI versus 0, BPI versus 0, and LPI versus 0). For the subtask model, we assessed the influence of either a boundary or a landmark in

the environment on BOLD activity and thus corrected for six multiple comparisons (three ROIs: EC, HC, and PC/RSC; two contrasts: BPI > PPI and LPI > PPI). We did not directly compare the boundary and landmark condition. Whole-brain analysis. In the subtask model, we performed whole-brain analyses on the contrasts between subtasks. Contrast images from the first-level analysis of each participant were entered into a GLM, treating “subjects” as a random effect. Statistical parametric maps were initially thresholded at a family-wise error (FWE)–corrected α level of $P < 0.05$ across the whole brain. We considered clusters significant at $P < 0.05$, FWE-corrected (extent threshold of five voxels). For all significant clusters, we provide maximum probability tissue labels with MNI coordinates derived from the Neuromorphometrics atlas as implemented in SPM12. Given our hypothesis that EC, HC, and PC/RSC would be involved in our task, we used small volume correction with a mask of the EC, HC, and PC/RSC. We derived these masks from FreeSurfer by warping subject-specific ROIs to standard space, averaging the mask across subjects, and thresholding the composite mask such that a selected voxel was included in the masks of at least 50% of the participants. Thereby, we ensured that results of the ROI analysis and the whole-brain analysis would correspond as closely as possible. Grid-like representations. To detect GLRs in the fMRI data, we performed a representational similarity analysis following a previous study (14). The rationale underlying this analysis is that the activity of a grid cell population should be similar during movements that are $n60^\circ$ offset from each other (due to the sixfold rotational symmetry of the grid pattern), where $n = \{0, 1, \dots, 6\}$. In other words, the

activity of a grid cell population should be relatively similar at angular steps of 60° . By contrast, the activity of a grid cell population should be relatively dissimilar during movements that are $n60^\circ + 30^\circ$ offset from each other. In the following, the former condition is termed “ $\text{mod}(\alpha, 60^\circ) = 0^\circ$ ” and the latter condition is termed “ $\text{mod}(\alpha, 60^\circ) = 30^\circ$ ” (where α refers to the angular difference between movement directions and “mod” indicates the modulo operator; Fig. 6A). Analysis steps comprised (i) extracting the voxelwise signal within the ROIs for every fMRI volume; (ii) calculating the mean orientation, speed, and trial number associated with each fMRI volume; (iii) excluding volumes associated with slow movement ($<33\%$ average movement speed); (iv) creating means of volumes within bins of 5° orientation based on the information about mean orientation; (v) calculating the angular difference of movement directions between volume bins; (vi) calculating Fisher z-transformed Pearson correlations between the volume bins; and (vii) deleting correlations of the same trial to reduce effects of temporal autocorrelations between subsequent fMRI volumes. We compared pattern similarities of angular differences of $\pm 15^\circ$ from the $\text{mod}(\alpha, 60^\circ) = 0^\circ$ condition, for which we expected higher pattern similarity, against angular differences of $\pm 15^\circ$ from the $\text{mod}(\alpha, 60^\circ) = 30^\circ$ condition, for which we expected lower pattern similarity. As a control, we re-performed the analysis after excluding correlations based on angular differences of $\pm 15^\circ$ from 0° to exclude the possibility that GLRs were driven by similarities of activity during movements in the same direction (in 360° space), possibly reflecting a head direction signal. To examine the specificity of sixfold rotational symmetry,

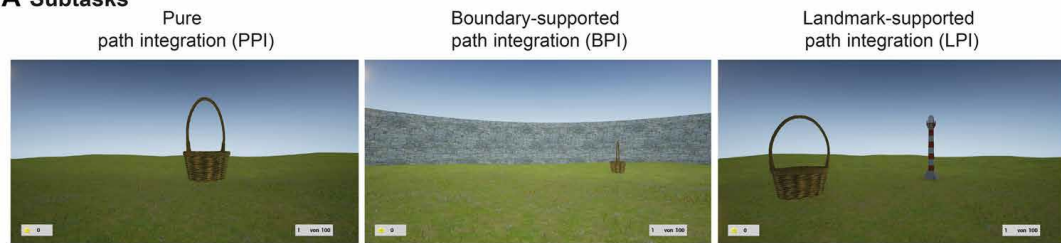
we performed further control analyses that tested for other types of rotational symmetry (fourfold, fivefold, sevenfold, and eightfold rotational symmetry). To exclude possible effects of autocorrelations as a confound, we tested whether the temporal proximity between fMRI volumes assigned to the two conditions was similar. We thus calculated the mean TR difference between every possible combination of angular differences and averaged within the $\text{mod}(\alpha, 60^\circ) = 0^\circ$ condition and the $\text{mod}(\alpha, 60^\circ) = 30^\circ$ condition for every participant. We calculated a paired t test across participants to ensure that the temporal proximity did not differ between the two conditions. Last, for each participant separately, we checked for uniform sampling of movement directions in 360° and 60° spaces. To this end, we transformed the movement directions (in 360° space or in 60° space) into bins of 5° and tested if the orientation bins deviated from uniformity by means of a Rayleigh test. This resulted in an empirical z value that we tested against a surrogate distribution established by shuffling the orientation bins 10,000 times. Comparing the empirical z value against the distribution of surrogate z values resulted in one P value for each participant reflecting how normal or extreme the empirical z value of each participant was. We calculated spatial and temporal signal-to-noise ratios (SNRs) [following (14)] by extracting the voxelwise signal of aIEC and pmEC for each hemisphere and either calculating the mean and the SD across time points (temporal SNR) or across voxels (spatial SNR). We compared SNRs between EC subregions (aIEC versus pmEC) and between hemispheres (left versus right) using a

two-way ANOVA. By means of a Pearson correlation, we tested whether the strength of GLRs was significantly related to the SNRs of pmEC.

Tables and Figures

Figure 1.

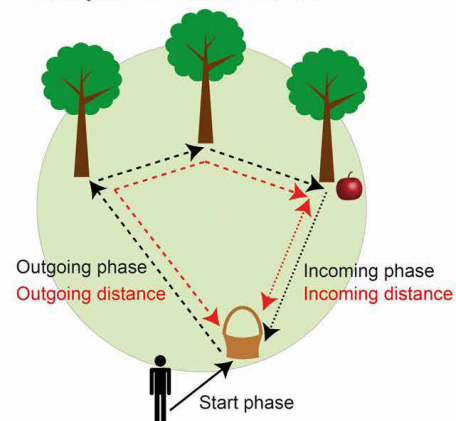
A Subtasks



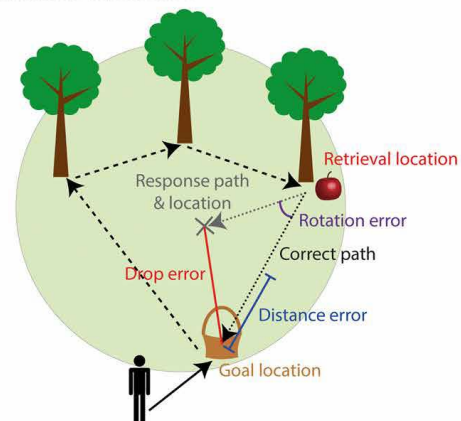
B Trial procedure



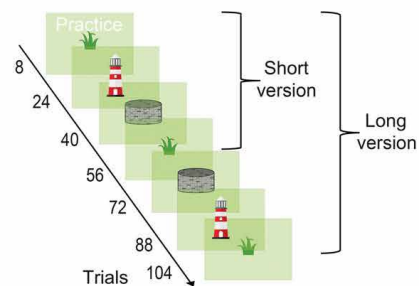
C Task phases and distances



D Performance measures



E Behavioral experiment



F fMRI experiment

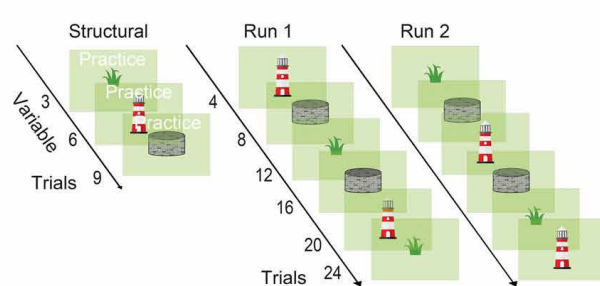


Fig. 1. Experimental paradigm. (A) Participants performed a novel PI task (the Apple Game) in a virtual environment. The task comprised three subtasks that differed with regard to the presence or absence of supportive spatial cues: the PPI subtask without any supportive cue, the BPI subtask with a circular boundary, and the LPI subtask with an intramaze landmark (lighthouse) close to the center of the environment. (B) In each trial, participants collected a basket (start phase) and tried to remember its location (goal location). After navigating toward a variable number of trees (1 to 5; outgoing phase), which disappeared after having been reached, participants had to find their way back to the goal location (incoming phase). Last, they received feedback via different numbers of stars, depending on response accuracy. (C) Outgoing distance refers to the cumulated distance during the outgoing phase, and incoming distance refers to the Euclidean distance between retrieval location (tree with apple) and goal location (basket). (D) PI performance was assessed as the distance between the correct goal location and the response location (drop error). The drop error can be separated into the distance error (i.e., the difference between the retrieval-to-goal distance and the retrieval-to-response distance) and the rotation error (i.e., the difference between the retrieval-to-goal rotation and the retrieval-to-response rotation). (E) The behavioral task comprised 8 practice trials followed by 16 trials in each subtask (short version; in the long version, all subtasks were performed twice, resulting in

32 trials in each subtask). (F) The fMRI task consisted of up to nine practice trials during the structural scan, followed by two functional runs with six blocks of four trials each. See also fig. S1 and movie S1.

Tables 1.

Table 1. Statistical models.					
Model	Criterion	Predictors (within subject)	Predictors (between subject)	Covariates	Random effects
Effects of APOE, subtask, and path distance on performance					
1a	Performance	Subtask, outgoing distance	APOE	Age, sex	Subject, site
1b	Performance	Subtask, incoming distance	APOE	Age, sex	Subject, site
1c	Performance	Subtask, (outgoing and incoming) path distance, type of distance	APOE	Age, sex	Subject, site
Effects of landmark and boundary					
2a	Performance	Goal-to-boundary distance	APOE		Subject, site
2b	Performance	Goal-to-landmark distance	APOE		Subject, site
2c	Distance to boundary		APOE		Subject, site
2d	Distance to landmark		APOE		Subject, site
Effects of EC volume, APOE, subtask, and path distance on performance					
3a	Performance	Subtask, outgoing distance	APOE, relative EC volume	Age, sex	Subject
3b	Performance	Subtask, incoming distance	APOE, relative EC volume	Age, sex	Subject
Effects of HC volume, APOE, subtask, and path distance on performance					
4a	Performance	Subtask, outgoing distance	APOE, relative HC volume	Age, sex	Subject
4b	Performance	Subtask, incoming distance	APOE, relative HC volume	Age, sex	Subject
Effects of PC/RSC volume, APOE, subtask, and distance on performance					
5a	Performance	Subtask, outgoing distance	APOE, relative PC/RSC volume	Age, sex	Subject
5b	Performance	Subtask, incoming distance	APOE, relative PC/RSC volume	Age, sex	Subject
Mechanistic model to explain path integration performance					
6	Performance	Subtask, incoming distance	EC integrated path representations during incoming phase, HC goal proximity representations during incoming phase, pmEC GLRs, PC/RSC landmark representations		Subject

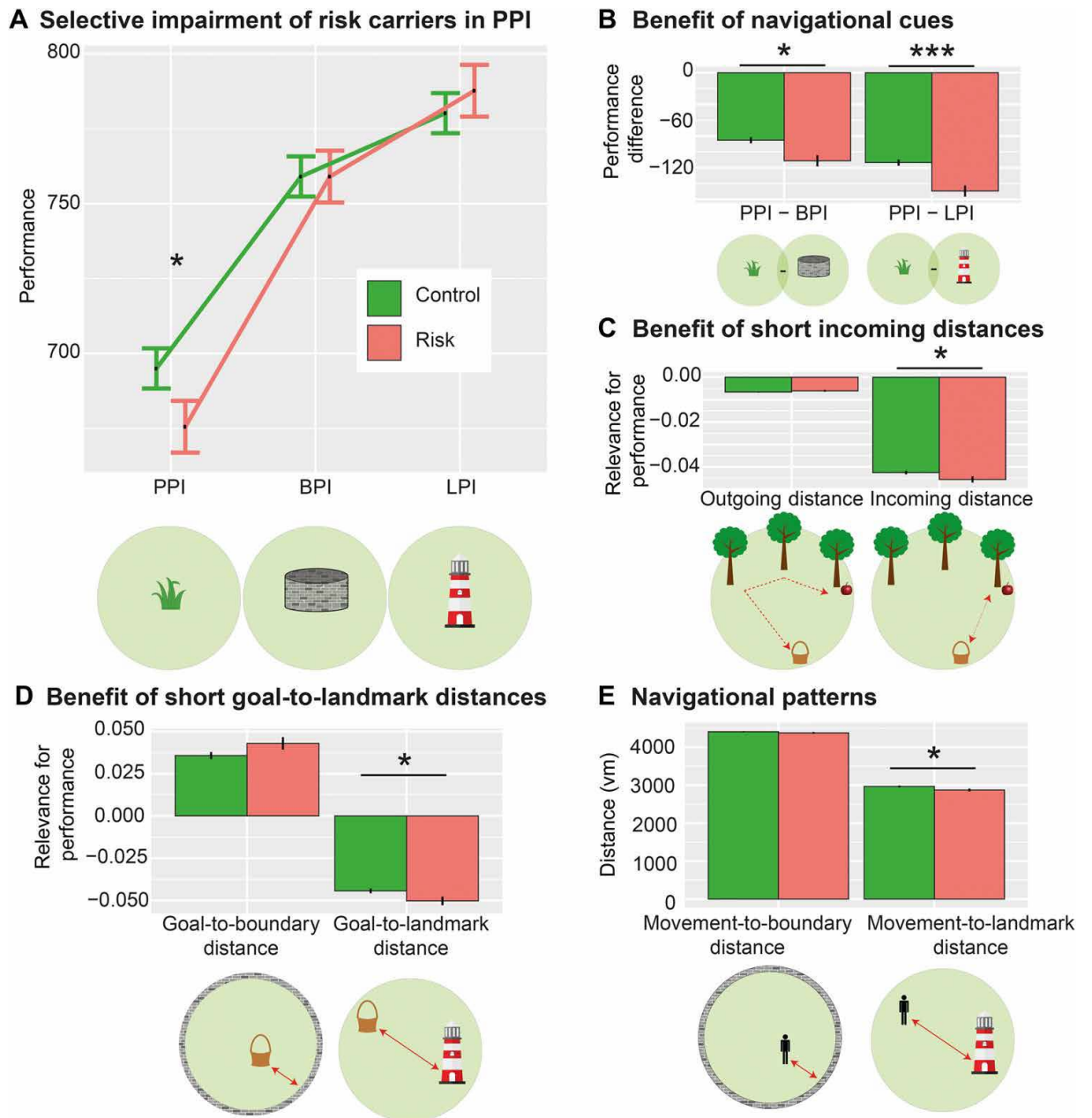


Figure 2. Performance as a function of genotype, distances, and subtask. (A)

Performance (which is inversely related to drop error) is specifically impaired in risk

carriers when no supportive spatial cues are available, i.e., in the PPI subtask. (B) Risk carriers benefit more from environmental landmarks and boundaries than controls. (A) and (B) depict results from model 1b; results from model 1a are statistically equivalent. (C) Incoming (but not outgoing) distance is more closely related to spatial memory performance in risk carriers than controls (model 1c). (D) Goal-to-landmark distance (but not goal-to-boundary distance) is more relevant in risk carriers than controls (models 2a and 2b). (E) Movement-to-landmark distance (but not movement-to-boundary distance) is significantly lower in risk carriers than controls (models 2c and 2d). *Y* axes show parameter estimates resulting from the different models; error bars, SEM; * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. Control, *APOE* $\epsilon 3/\epsilon 3$ -carriers; Risk, *APOE* $\epsilon 3/\epsilon 4$ -carriers; PI, path integration; BPI, boundary-supported PI; LPI, landmark-supported PI; vm, virtual meters. See also figs. S1 to S3 and tables S1 and S2.

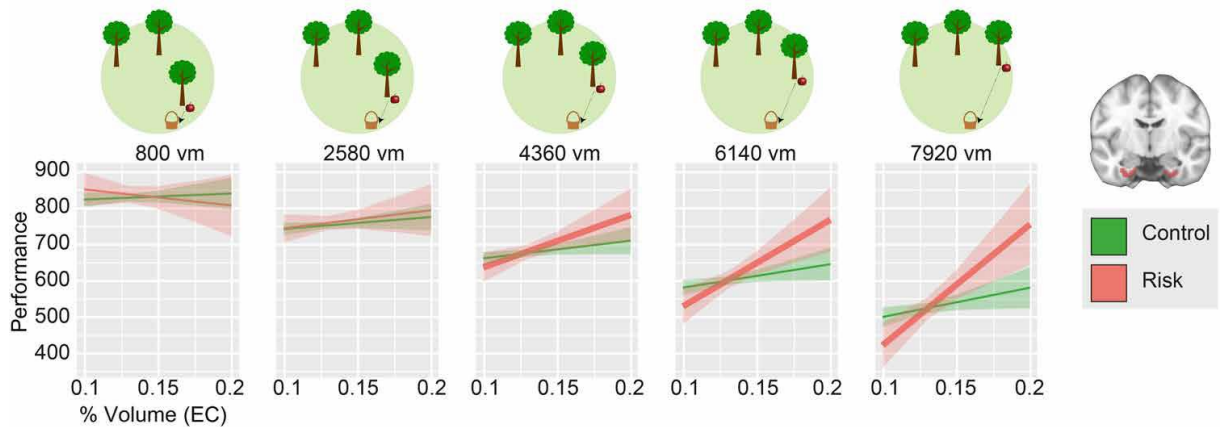
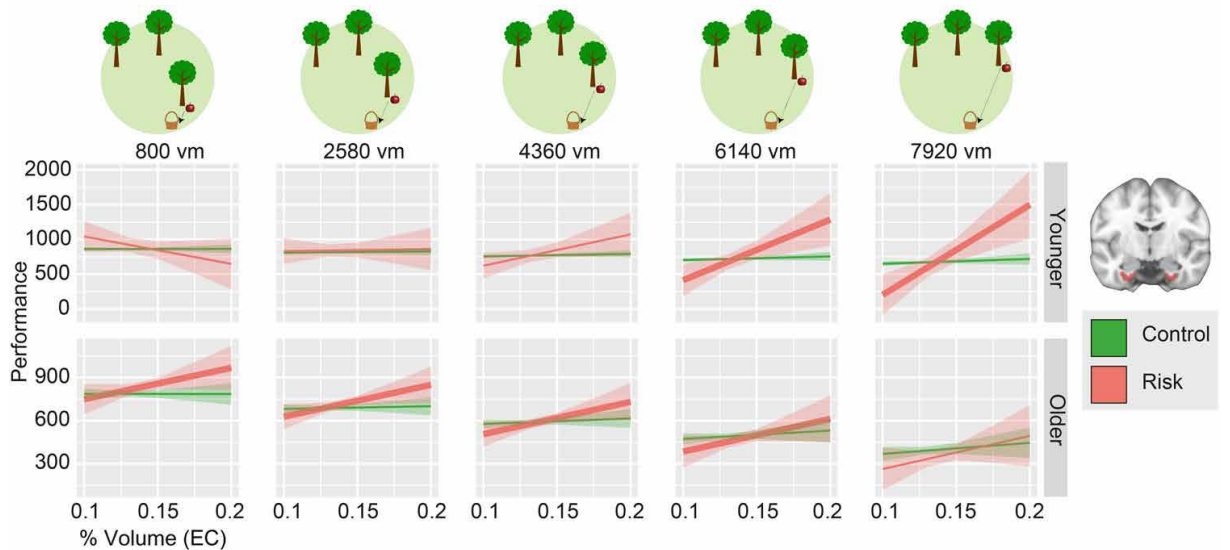
A Risk carriers' EC volume predicts performance at long incoming distances**B Risk carriers' EC volume predicts performance in both age groups**

Figure 3. Performance as a function of genotype, age, incoming distance, and EC gray matter volume. (A) EC gray matter volume predicts performance only during PI with long incoming distances (middle to right panels) and only in risk carriers (model 3b). **(B)** In younger risk carriers, EC gray matter volume predicted performance during PI trials with long incoming distances. In older risk carriers, EC

gray matter volume predicted performance during the majority of all trials (model 3b). The young group comprises subjects aged 18 to 28 years; the older group contains subjects aged 53 to 75 years (see age histogram in fig. S1C). As the models contained two continuous predictors, one of them (incoming distance) was discretized into quintiles for post hoc tests and graphical depiction. Thicker lines mark slopes that are significantly different from zero. *Y* axes show parameter estimates resulting from the different models; shaded areas, SEM. Control, *APOE* ϵ 3/ ϵ 3-carriers; Risk, *APOE* ϵ 3/ ϵ 4-carriers;

% volume, percent of whole-brain volume. See also fig. S1 and tables S1 and S3.

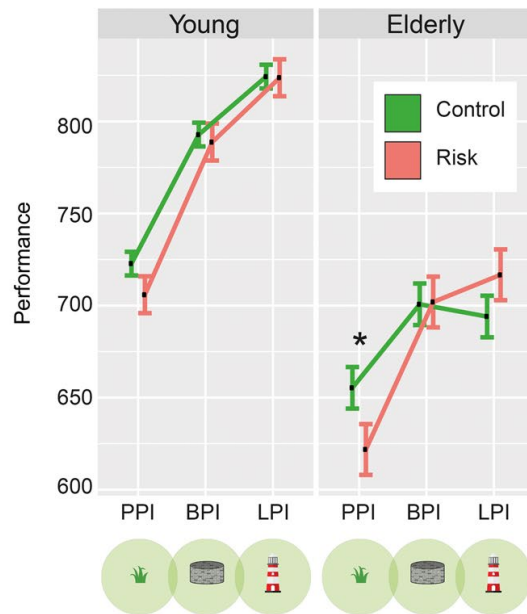
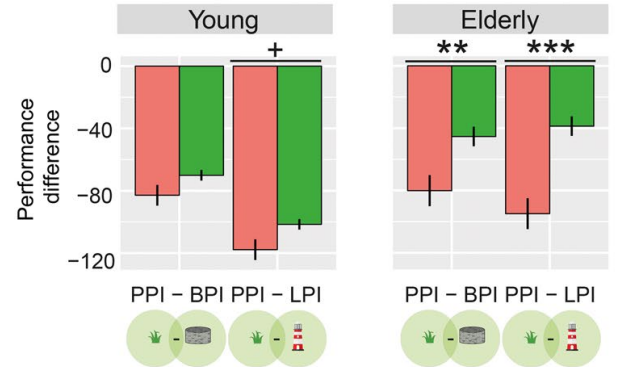
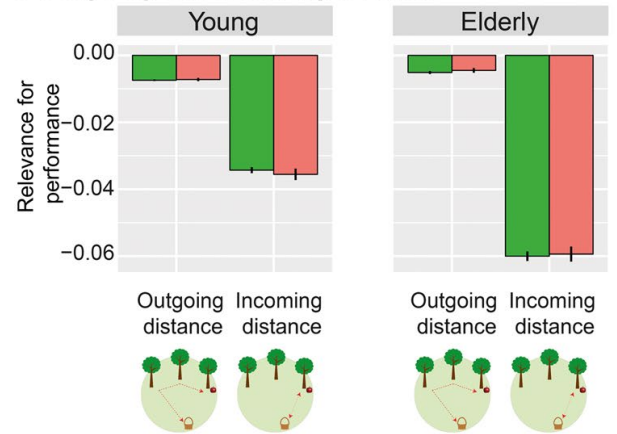
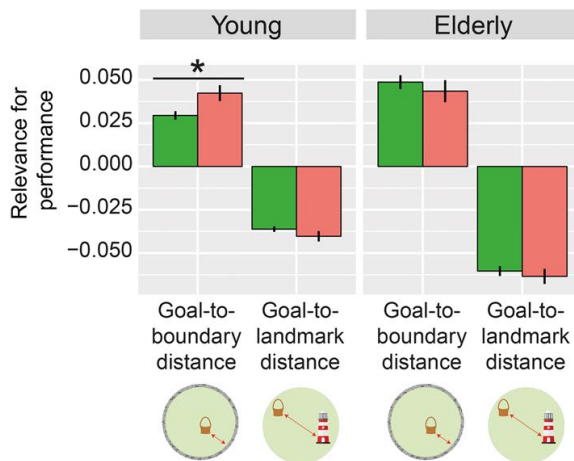
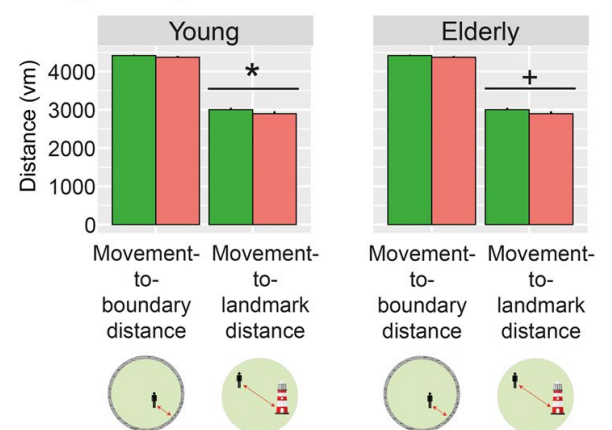
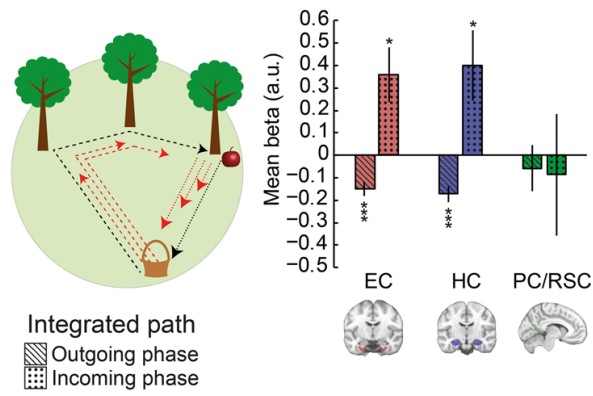
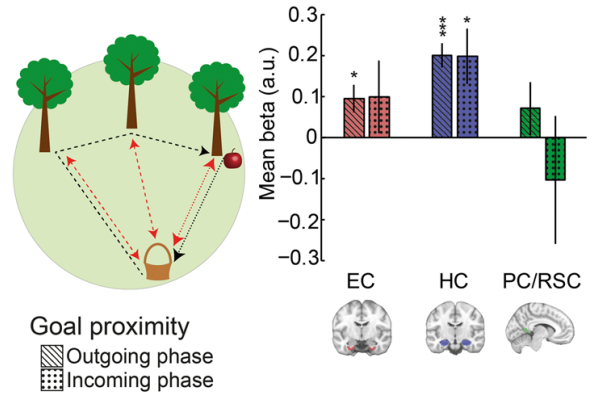
A Selective impairment of risk carriers in PPI**B Benefit of navigational cues****C Outgoing and incoming distance****D Goal-to-boundary and goal-to-landmark distance****E Navigational patterns**

Figure 4. Performance as a function of genotype, distances, and subtask split by age groups. The younger age group comprises subjects aged 18 to 41 ($n = 163$), and the older age group comprises subjects aged 42 to 75 ($n = 104$). (A) Performance (which is inversely related to drop error) is specifically impaired in older risk carriers when no supportive spatial cues are available, i.e., in the PPI subtask. (B) In older participants, risk carriers benefit more from environmental landmarks and boundaries than controls. (A) and (B) depict results from model 1b; results from model 1a are statistically equivalent. (C) In both age groups, neither incoming nor outgoing distance is more closely related to spatial memory performance in risk carriers than controls. (D) In the younger age group, goal-to-boundary distance is more relevant in risk carriers than in controls (models 2a and 2b). (E) In younger participants, movement-to-landmark distance (but not movement-to-boundary distance) is significantly lower in risk carriers than in controls (models 2c and 2d). Y axes show parameter estimates resulting from the different models; error bars, SEM. $+P < 0.10$, $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. Control, *APOE* $\epsilon 3/\epsilon 3$ -carriers; Risk, *APOE* $\epsilon 3/\epsilon 4$ -carriers. See also figs. S1 to S3 and tables S1, S2, and S4.

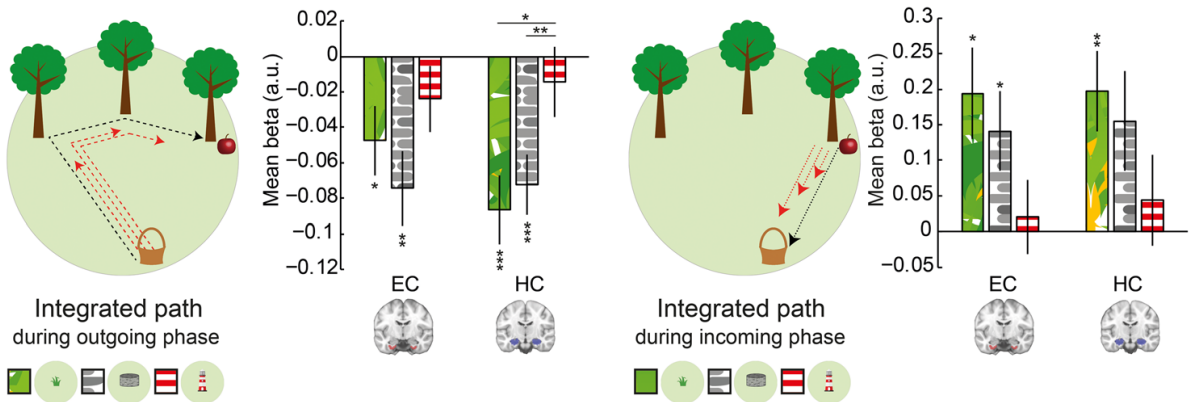
A Representation of integrated path



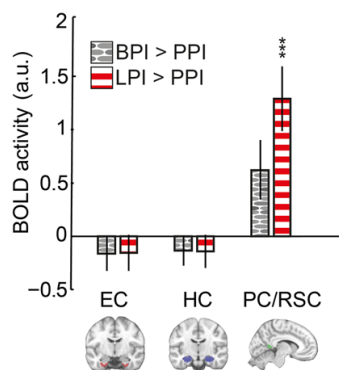
B Representation of goal proximity



C Representation of integrated path: Subtask dependence



D BOLD activity in response to navigational cues: ROI analysis



E BOLD activity in response to navigational cues: Whole brain results

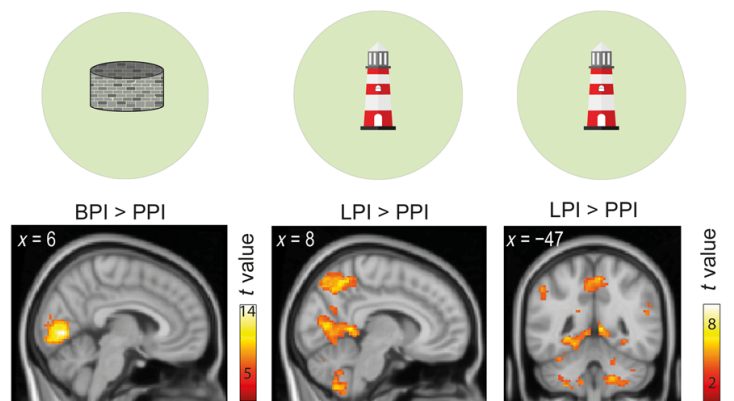


Figure 5. fMRI results: Neural determinants of integrated path, goal proximity, and subtask. (A) Representation of integrated path in EC and HC: Deactivation during navigation at longer integrated paths during outgoing phase and activation during incoming phase. (B) Representation of goal proximity: Activation for navigation closer to the goal in HC in both trial phases and in EC during the outgoing phase. (C) Subtask dependence of integrated path representations: HC deactivation during navigation at longer integrated paths during the outgoing phase was modulated by subtask. During the incoming phase, we observed a statistical trend for a similar result in EC and HC. (D) Significantly higher activity in PC/RSC during the LPI as compared to the PPI subtask. (E) Confirmatory whole-brain analyses: (left) activation of visual areas during the BPI as compared to the PPI subtask and (right) higher PC/RSC activity in the LPI than in the PPI subtask. *P* values are FDR-corrected for the number of contrasts and for the number of ROIs in all ROI analyses (A, B, and D). For post hoc comparisons between subtasks in (C), *P* values are FDR-corrected for number of subtasks. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. Error bars (A to D), SEM. Statistical parametric maps in (E) are thresholded at *P* < 0.05, family-wise error (FWE)-corrected for whole brain (left) and small volume-corrected for PC/RSC (middle and right), and clusters are considered significant at *P* < 0.05, FWE-corrected. FDR, false discovery rate; a.u., arbitrary units. See also figs. S4 and S5 and table S5.

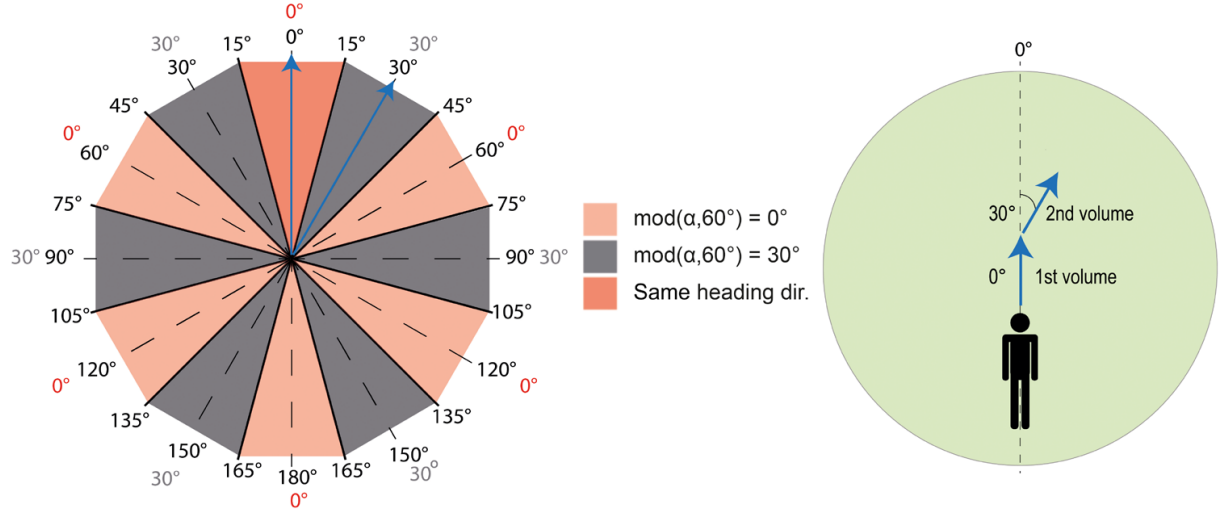
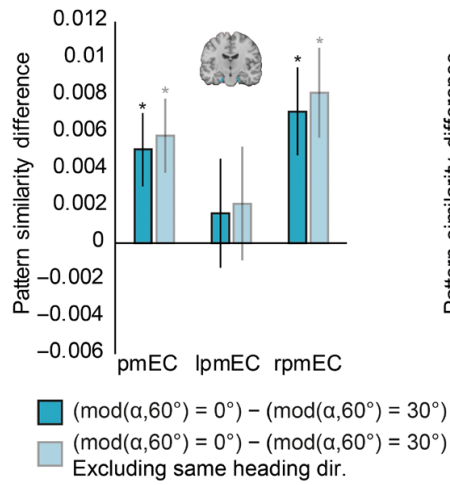
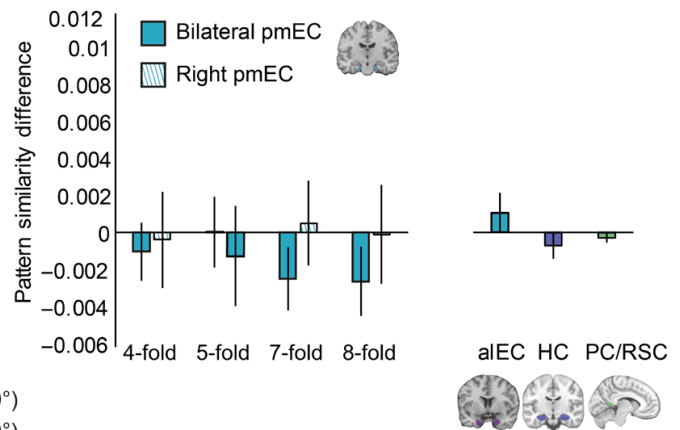
A Schematic depiction of analysis strategy**B GLRs in (right) pmEC****C Control analyses**

Figure 6. Grid-like representations in pmEC. (A) Left: Schematic depiction of angular differences in 360° space (inner numbers) and in 60° space (outer numbers). We expected higher pattern similarity for angular differences of $\text{mod}(\alpha, 60^\circ) = 0^\circ$ (rose, where α is the angular difference between two movement directions) as compared to angular differences of $\text{mod}(\alpha, 60^\circ) = 30^\circ$ (gray). We expected the same

result when excluding pattern similarities of the same heading direction (dark rose). Right: Movement directions of two exemplary fMRI volumes (blue arrows). In the first volume, the subject navigates at an angle of 0° with respect to the reference axis. In the second volume, the subject navigates at an angle of 30° . This results in an angular difference of 30° (in 360° and 60° spaces) and thus $\text{mod}(\square, 60^\circ) = 30^\circ$ (compare to blue arrows on the left). (B) In bilateral and in right pmEC, pattern similarity for angular differences of $\text{mod}(\square, 60^\circ) = 0^\circ$ was significantly higher than pattern similarity for angular differences of $\text{mod}(\square, 60^\circ) = 30^\circ$, suggesting a hexadirectional symmetry of pattern similarities (dark blue bars). Same result when removing movements with similar heading directions in 360° space (light blue bars). (C) Control analyses. Left: No evidence for fourfold, fivefold, sevenfold, or eightfold rotational symmetry of pattern similarity in bilateral or right pmEC. Right: No evidence for sixfold rotational symmetry of pattern similarity in aLEC, HC, and PC/RSC. Error bars (B and C), SEM. $*P < 0.05$. mod, modulus; dir., direction; pmEC, posterior-medial entorhinal cortex; aLEC, anterior-lateral entorhinal cortex; HC, hippocampus.

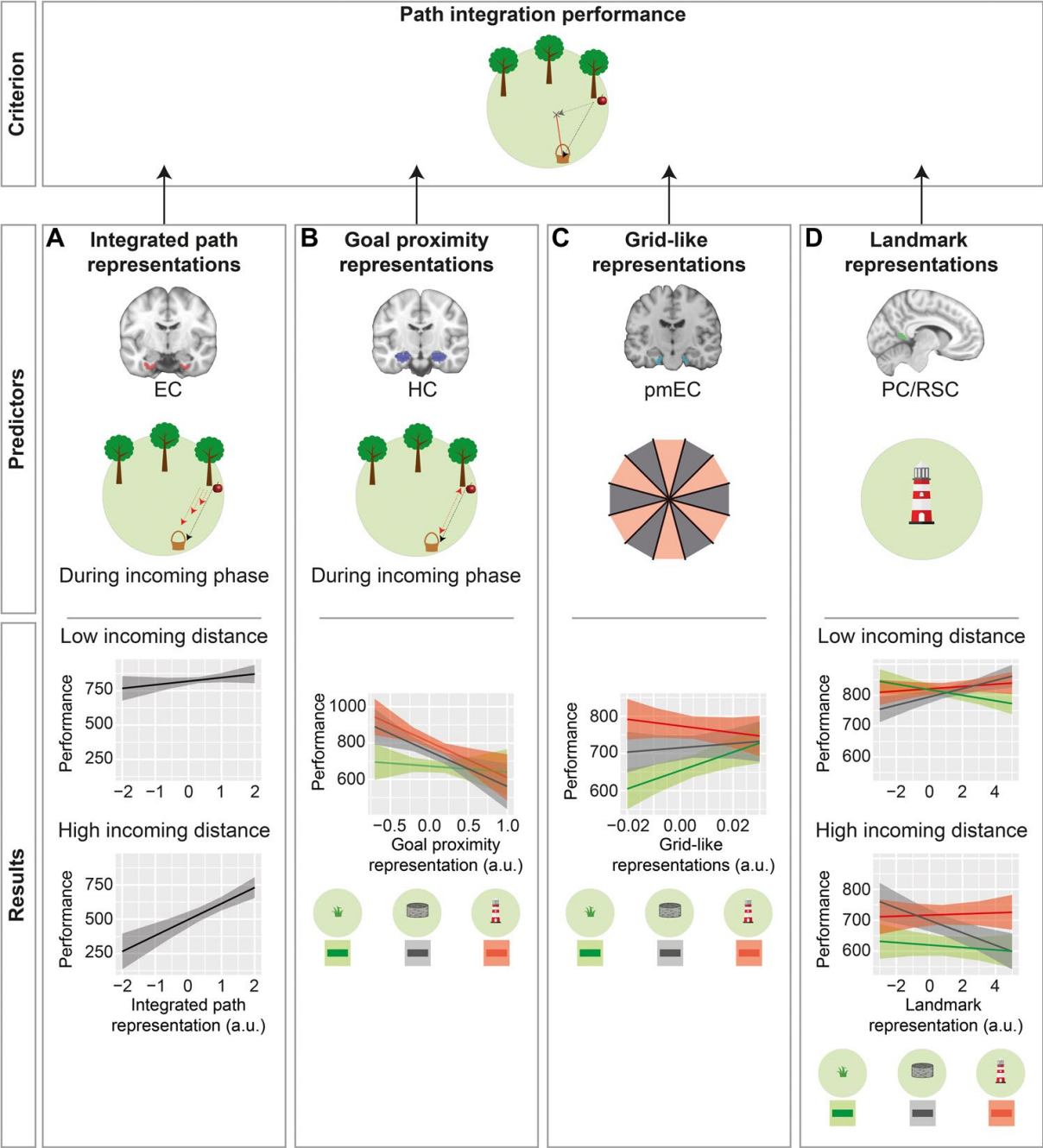


Figure 7. Mechanistic model to predict PI performance. We aimed at predicting PI performance as a function of fMRI-based representations of spatial features in combination with subtask and incoming distance (model 6). (A) Integrated path representations in EC interacted with incoming distance in predicting PI performance: At higher incoming distances, stronger integrated path representations in EC were associated with better performance. (B) Goal proximity representations in HC interacted with subtask in predicting PI performance. In none of the subtasks was the prediction significant by itself. (C) GLRs in pmEC interacted with subtask in predicting PI performance: Only in PPI, higher GLRs in pmEC were associated with better performance. (D) Landmark representations in PC/RSC interacted with subtask and incoming distance in predicting PI performance. In none of the individual subtask by incoming distance combinations was the prediction significant by itself. As the model contained two continuous predictors, one of them (incoming distance) was discretized into quintiles for post hoc tests and for graphical depiction; only quintiles 1 and 5 are depicted (A and D). Y axes show parameter estimates for performance; shaded areas, SEM.

5.3 Conclusion; Anosognosia and spatial orientation as tools for risk stratification

The data presented in this appendix suggest that spatial orientation, and path integration in particular, may constitute a sensitive indicator of early entorhinal–hippocampal dysfunction in individuals at risk for Alzheimer’s disease. The reproduced study shows that, in the absence of external cues, APOE ϵ 4 carriers exhibit a specific path integration deficit, whereas their performance remains preserved in navigation conditions rich in landmarks and their overall cognitive profile falls within the normal range. These findings argue for a targeted vulnerability affecting specific components of navigation, detectable before the emergence of clinically manifest symptoms.

This profile echoes the central results of the thesis regarding anosognosia. In the WLS cohort, the discrepancy between self-evaluation and informant evaluation proves more sensitive than the subject’s own complaint in predicting future cognitive decline, particularly in APOE ϵ 4 carriers. Just as landmark-free navigation brings to light otherwise hidden difficulties, the confrontation between the subject’s perspective and that of relatives reveals early alterations in awareness of deficits in a context where performance remains ostensibly normal.

These two approaches show clear parallels and strong complementarity. They rely on paradigms that limit opportunities for compensation (absence of external cues; tension between self-image and external feedback) and recruit brain networks that are particularly implicated in Alzheimer's disease (the entorhinal–hippocampal system on the one hand, the default mode network on the other). They thus open the way to a finer stratification of ageing trajectories: some individuals may show navigation deficits with preserved awareness, others signs of anosognosia with intact navigation, and others still a combination of these vulnerabilities.

In this context, it is important to recall that the initial project of coupling navigation performance with tau-PET in at-risk individuals could not be completed within the framework of this thesis, due to the floor effect observed in the pilot study among older patients in a possibly prodromal phase. The complexity of the task would have required a major redesign of the paradigm, incompatible with the timeline and multicentre structure of the study. This work nonetheless found a natural continuation in the thesis of Lise Colmant, who adapted the design to this population and further developed the investigation of links between navigation, cognitive vulnerability, and biological markers.

Rather than claiming to have identified neuropsychological biomarkers in the strict sense, this appendix highlights how anosognosia and spatial orientation can contribute to stratifying an otherwise autonomous population along gradients of

vulnerability. When combined with clinical, imaging, and biological data, these behavioural and metacognitive approaches represent important stepping stones towards a finer understanding of the heterogeneity of trajectories that do, or do not, lead to clinical Alzheimer's disease, and towards more targeted selection of participants for future early therapeutic trials.

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