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MUDr. Martina Laczó

Early stages of neurodegenerative diseases and their diagnosis using experimental cognitive tests with a specific focus on spatial navigation

Časná stádia neurodegenerativních onemocnění a jejich diagnostika pomocí experimentálních kognitivních testů se specifickým zaměřením na prostorovou kognici

Dissertation Thesis

Supervisor: prof. MUDr. Jan Laczó, Ph.D.

Advisor: prof. MUDr. Jakub Hort, Ph.D.

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Abstract

This dissertation thesis is focused on early and differential diagnosis of Alzheimer's disease (AD) using experimental cognitive tests. AD starts as a preclinical stage, progresses to the mild cognitive impairment (MCI) and eventually to the dementia stage. It is crucial to diagnose AD very early to slow down its progression. However, the use of specific AD biomarkers, such as amyloid and tau positron emission tomography and cerebrospinal fluid (CSF) biomarkers, is very limited. Experimental spatial navigation and spatial pattern separation tests, unlike conventional cognitive tests, may have a strong diagnostic potential as they depend on brain regions affected early in AD. The first study in a virtual environment showed preference for word-centered navigation in cognitively normal older adults, while participants with early AD preferred body-centered strategy to compensate for neurodegeneration. Using a virtual navigation test, the second study showed different profiles of navigation impairment in MCI participants with AD and other (i.e., non-AD) etiologies and demonstrated that navigation assessment differentiated AD from non-AD participants. Various navigation strategies were associated with atrophy in different brain regions and CSF AD biomarkers. The third study showed that a spatial pattern separation test reliably detected early AD. The fourth study demonstrated that this assessment differentiates MCI participants with AD from those with non-AD etiology and showed that spatial pattern separation is supported by posterior medial temporal lobe regions and basal forebrain. In conclusion, spatial navigation and spatial pattern separation tests may be useful for early diagnosis of AD.

Key words

Alzheimer's disease, basal forebrain, body-centered spatial navigation, cerebrospinal fluid biomarkers, entorhinal cortex, hippocampus, mild cognitive impairment, parietal cortex, spatial pattern separation, world-centered spatial navigation

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Abstrakt

Tato disertační práce je zaměřena na časnou a diferenciální diagnostiku Alzheimerovy nemoci (AN) pomocí experimentálních kognitivních testů. AN začíná jako preklinické stadium, poté přechází do mírné kognitivní poruchy (MCI) a nakonec do stadia demence. Pro zpomalení progrese AN je zásadní časná diagnostika. Využití specifických biomarkerů AN, jako jsou amyloidová a tau pozitronová emisní tomografie a biomarkery AN v likvoru, je velmi limitované. Experimentální testy prostorové navigace a separace prostorových informací jsou závislé na oblastech mozku postižených v časných stadiích AN, a proto mají na rozdíl od tradičních kognitivních testů velký potenciál diagnostikovat AN. První studie ve virtuální realitě ukázala, že kognitivně zdraví starší senioři preferují navigaci závislou na okolním prostředí, zatímco účastníci s časnou AN preferují strategii závislou na poloze těla, čímž si kompenzují neurodegenerativní změny. Druhá studie používající navigační test ve virtuální realitě prokázala rozdílné profily poruch navigace u účastníků s MCI při AN a v důsledku jiné etiologie (tj. non-AN) a také prokázala, že vyšetření navigace odliší účastníky s AN od účastníků s non-AN. Různé navigační strategie byly spojeny s atrofií v odlišných oblastech mozku a likvorovými biomarkery AN. Třetí studie ukázala, že test separace prostorových informací spolehlivě odhalí časnou AN. Čtvrtá studie prokázala, že tento test odliší účastníky s MCI při AN od účastníků s non-AN a také, že separace prostorových informací závisí na oblastech zadního mediálního temporálního laloku a bazálního telencefala. Závěrem lze říci, že testy prostorové navigace a separace prostorových informací mohou být užitečné pro časnou diagnostiku AN.

Klíčová slova

Alzheimerova nemoc, bazální telencephalon, biomarkery v likvoru, entorhinální kůra, mírná kognitivní porucha, parietální kůra, navigace závislá na poloze těla, separace prostorových informací, navigace závislá na okolním prostředí

List of abbreviations

AD	Alzheimer´s disease
AD aMCI	amnestic mild cognitive impairment with Alzheimer´s disease
alEC	anterolateral entorhinal cortex
aMCI	amnestic mild cognitive impairment
amyloid-β	amyloid beta
ANOVA	analysis of variance
ANCOVA	analyses of covariance
APOE ε4	apolipoprotein E4
AUC	area under the ROC curve
BF	basal forebrain
CN	cognitively normal
CSF	cerebrospinal fluid
EC	entorhinal cortex
H-B	Holm-Bonferroni correction
MCI	mild cognitive impairment
MRI	magnetic resonance imaging
MTL	medial temporal lobe
NIA-AA	National Institute of Aging and the Alzheimer's Association
non-AD aMCI	amnestic mild cognitive impairment with non-Alzheimer's disease etiology
OR	odds ratio
p-tau	phosphorylated tau
PET	positron emission tomography
pmEC	posteromedial entorhinal cortex
RAVLT	Rey Auditory Verbal Learning Test
ROC	receiver operating characteristic
ROCFT	Rey-Osterrieth Complex Figure Test
RSC	rertrosplenial cortex
χ^2 test	chi-squared test

Content

1. INTRODUCTION

1.1. Neuropathology and stages of Alzheimer´s disease

In total, 7.45 million people in Europe suffer from dementia and this number is expected to increase to 9.9 million in 2030, thus dementia becomes an increasing socio economic problem (Hampel et al., 2021). Dementia is the latest stage of the so-called "continuum" of cognitive deficit in neurodegenerative diseases. The most common neurodegenerative disease is Alzheimer's disease (AD) (Grand et al., 2011). AD is in fact nearly life-long disease, because typical pathological processes start many decades before the first symptoms develop. Decades of ongoing undetected pathological processes are presumably the reason for a limited effect of medical treatment in a stage of fully developed AD.

The earliest stage is called "preclinical" and it is the longest stage of AD characterized by the presence of pathological processes while the cognitive functions are intact. This stage is followed by the mild cognitive impairment (MCI) stage where cognitive impairment is present but the patients are independent in activities of daily living. Dementia is the latest stage where cognitive impairment is so severe that it interferes with activities of daily living and patients are dependent on their caregivers. Although AD is the most common neurodegenerative disease, there is a variety of other neurodegenerative causes (e.g., frontotemporal lobar degeneration, dementia with Lewy bodies) and non-neurodegenerative diseases (e.g., depression, small vessel disease, intracranial hemorrhage or other brain lesions) that can lead to dementia. Recent postmortem studies reported that 12% and 23% patients diagnosed with AD based only on clinical and imaging assessment did not have evidence of AD pathology (Gaugler et al., 2013). Therefore, the diagnosis of AD should be done with caution to avoid misdiagnosis and subsequently also inappropriate treatment. Early and accurate diagnosis of AD is crucial in order to slow down the disease progression and to preserve functional abilities for a longer time and thus also to reduce the socio-economic impact. Although only symptomatic medication is currently available, the development of new disease modifying drugs in recent years creates a greater demand for accurate and early diagnosis. Emerging therapeutic interventions are likely to be effective only when performed in the earliest stages of the disease, before severe neuronal loss and irreversible cognitive impairment occur (Sperling et al., 2011b).

The pathological processes typical of AD occurring decades before the development of clinical symptoms include extracellular aggregation of amyloid-β (amyloid beta) plaques (Thal et al., 2002) and intracellular formation of neurofibrillary tangles (i.e., accumulation of abnormally phosphorylated tau protein) (Braak and Braak, 1995). Pathological accumulation of these proteins leads to progressive neuronal loss which is also referred to as neurodegeneration (Miller et al., 2013) and consequently leads to cognitive impairment (Green et al., 2000). Amyloid-β accumulation is initiated in the neocortical regions, and early spreads the posterior cortical regions, especially to the precuneus and posterior cingulate cortex, including the retrosplenial cortex (RSC) (Sojkova et al., 2011; Palmqvist et al., 2017) (Thal stage I). Amyloid-β pathology further spreads to the limbic regions including the hippocampus (Thal stage II), subcortical regions including the thalamus and basal ganglia (Thal stage III), specific brainstem regions (Thal stage IV), and finally to the cerebellum and remaining brainstem regions (Thal stage V) (Thal et al., 2002). Spread of tau pathology can be described by the consecutive Braak stages I-VI (Braak and Braak, 1995). In Braak stage I, tau pathology is initially present in the transentorhinal cortex (the region between the anterolateral entorhinal cortex [alEC] and the perirhinal cortex) and in Braak stage II, tau spreads to the posteromedial entorhinal cortex (pmEC) (Braak and Braak, 1995). Braak stages I-II clinically correspond to the preclinical stage of AD. In Braak stage III, tau pathology spreads to the hippocampus and in Braak stage IV tau spreads to the posterior cortical regions. Braak stages III-IV correspond to the early clinical stages (i.e., MCI). Eventually in Braak stages V and VI, tau pathology spreads to the entire neocortex (Braak and Braak, 1991), which is clinically the dementia stage. Brain atrophy progresses in parallel with the distribution of tau pathology (Whitwell et al., 2007). This is in contrast to amyloid-β accumulation, which is not directly linked to the progression of atrophy (Josephs et al., 2008).

1.2. Diagnosis of AD

The National Institute of Aging and the Alzheimer's Association (NIA-AA) created separate diagnostic recommendations for the preclinical stage (Sperling et al., 2011a), MCI stage (Albert et al., 2011) and the dementia stage of AD (McKhann et al., 2011) in 2011. Further, to increase the diagnostic certainty of AD etiology a new “research framework” for diagnosis of AD in a research setting based on evidence of AD biomarkers was established in 2018 (Jack et al., 2018).

1.2.1. Diagnosis of preclinical AD

Individuals in the preclinical stage of AD have biomarker evidence of AD pathology that can be detected as low CSF levels of amyloid-β or evidence of amyloid-β on amyloid positron emission tomography (PET) scan, while there is no detectable evidence of cognitive deficit. Amyloid-β accumulation initiates a cascade of other changes which include synaptic dysfunction and neuronal injury, which can be detected as elevated elevated tau or phosphorylated tau (p-tau), cortical hypometabolism and/or atrophy in temporoparietal regions on Fluorodeoxyglucose-PET and brain magnetic resonance imaging (MRI). In the very latest stages of preclinical AD, there may be evidence of subtle cognitive decline in comparison to individual´s previous cognitive levels or subtle cognitive impairment detected by challenging cognitive tests (Sperling et al., 2011a).

1.2.2. Diagnosis of MCI due to AD

Individuals with MCI are in spite of their cognitive deficit still independent in everyday functions, although they might have mild problems with performing complex functional tasks such as filling out forms, or using less familiar electronic devices. There should be evidence of change in cognition in comparison to the individual´s previous level in order to diagnose MCI. Information about the decline from the previous level can be provided by the patient, relatives or by a clinician who knows the patient well. Further, individuals with MCI have evidence of lower performance in one or more cognitive domains that is typically 1 to 1.5 standard deviations below the mean for their age and education matched peers. The lower performance can be in any of the cognitive domains, including memory, executive function, attention, language, and visuospatial skills. If the cognitive assessment is performed repeatedly, the decline in performance should be evident over time to increase the probability that cognitive deficit is due to AD. The MCI group can be subclassified into (amnestic MCI [aMCI]) when memory deficit is present and (non-amnestic MCI) when only non-memory cognitive functions are impaired (Albert et al., 2011).

1.2.3. Diagnosis of AD dementia

The diagnosis of dementia encompasses a spectrum of different stages ranging from mild dementia to the most severe stages. A typical feature of all dementia stages is impairment in activities of daily living leading to loss of independence in daily life. Evidence of loss of independence is a characteristic feature that differentiates dementia from MCI. In order to

diagnose dementia, it is important to have evidence of decline from the previous cognitive level. Information about cognitive decline can be obtained based on history-taking from a patient, relatives or a health professional, or from an objective cognitive assessment. Cognitive impairment in the dementia stage involves at least two cognitive domains. The diagnosis of dementia should not be done unless delirium or major psychiatric disorders are excluded (McKhann et al., 2011).

According to the NIA-AA criteria, when MCI or dementia syndromes are diagnosed, it is important to determine precisely the etiology of the cognitive deficit, which may be neurodegenerative, vascular, infectious, traumatic or combined. The use of specific biomarkers increases certainty of the diagnostic process and may reveal the underlying etiology. The recommended AD biomarkers include analysis of amyloid-β (i.e., levels of CSF amyloid-β or amyloid PET imaging) and biomarkers of neuronal injury (i.e., CSF total or p-tau, hippocampal or medial temporal lobe [MTL] atrophy on MRI, and temporoparietal hypometabolism or hypoperfusion on PET or single-photon emission computed tomography) (Albert et al., 2011; McKhann et al., 2011).

1.2.4. Biomarker evidence of AD

For the research purposes, the diagnosis of AD should be supported by evidence of AD-specific biomarkers according to the most recent diagnostic criteria (Jack et al., 2018). These criteria are also referred to as the AT(N) framework recognizing three groups of biomarkers which can be detected in vivo. The biomarkers denoted as "A" refer to aggregation of amyloid-β, which can be detected as a low level of amyloid-β_{42} or low amyloid-β_{42}/β_{40} ratio in CSF (Nutu et al., 2013) or visualization of amyloid-β by positron emission tomography (PET) imaging. "T" denotes aggregation of tau (neurofibrillary tangles) which can be detected as a high level of p-tau in CSF or visualized using tau PET imaging (Jack et al., 2018). And the last group of biomarkers "(N)" denotes neurodegeneration or neuronal injury, which can be detected as atrophy on MRI, high levels of total tau in CSF or hypometabolism on FDG-PET. In vivo detection of AD biomarkers has high sensitivity and specificity for the diagnosis of AD in early stages (Leuzy et al., 2018), however, these methods are available only at expert memory clinics and are limited for research purposes due to their invasiveness and high cost.

1.2.5. Conventional cognitive assessments of AD

The diagnosis of AD is commonly based on the evidence of deficit in conventional cognitive tests, especially episodic memory tests. Episodic memory deficits are associated with MTL atrophy, especially atrophy of the hippocampus. However, relying on episodic memory tests in the diagnosis of early stages of AD might be misleading because episodic memory deficits are not specific for AD as memory declines also in other neurodegenerative diseases (e.g., frontotemporal lobar degeneration and primary age-related tauopathy (Flanagan et al., 2016; Kim et al., 2019)). Also, episodic memory relies mainly on the MTL structures that undergo physiological age-related changes without an ongoing AD pathology (Park et al., 2003). Additionally, the hippocampus, the key region for episodic memory, is affected in the Braak III and IV stages of AD, which correspond to the fully developed MCI stage. Thus, episodic memory tests may not reliably detect the earliest stages of AD. A great limitation of conventional cognitive tests is that they are not able to reflect cognitive changes associated with the entorhinal cortex (EC), basal forebrain (BF) and RSC which are typically affected by AD pathology in the early stages. These tests may also be limited by ceiling effects. Therefore, lower performance in these tests can be compensated by an individual´s high occupational or educational attainment. The weakest point of conventional cognitive tests is their non-specificity for AD pathology and lack of specificity in distinguishing early AD stages from normal aging. Recently, two cognitive processes have been identified to decline very early in AD - pattern separation and spatial navigation. Evaluation of these cognitive processes thus has a potential to become useful in early diagnosis of AD. Our research is specifically focused on spatial navigation and spatial pattern separation and their potential to be early cognitive markers of AD.

1.3. Spatial navigation

1.3.1. Principles of spatial navigation

Spatial navigation is a cognitive process which allows us to move meaningfully in our environment and to find our way from one place to another (Coughlan et al., 2018). Spatial navigation is therefore very important for everyday life functioning. When navigating the environment, we combine visual, vestibular, proprioceptive, somatosensory and auditory information, therefore, spatial navigation is a multisensory integration process which combines multiple sources of information (Bates and Wolbers, 2014). Successful

navigation requires flexible use and switching between various navigation strategies. This cognitive process is supported by large brain networks which include the MTL structures (i.e., hippocampus, EC and parahippocampal cortex) (Ekstrom et al., 2003), posterior parietal cortex, precuneus (Maguire et al., 1998), posterior cingulate, RSC (Auger et al., 2012), frontal lobe regions (Moffat et al., 2007) and the subcortical structures (i.e., BF, caudate nucleus and thalamus (Hartley et al., 2003; Aggleton et al., 2012; Kerbler et al., 2015).

When navigating the environment, navigators can remember the traveled route in relation to their own bodies. This navigation strategy is referred to as body-centered (i.e., route learning, egocentric navigation). When learning routes, navigators can encode the sequence of body movements at decision points (e.g., right, left, straight) or form associations between direction changes and specific proximal landmarks (e.g., "Turn right at the shop") (Waller and Lippa, 2007). Using body-centered navigation also involves processing of visual information, and perception of navigator´s bodily distances and directions from the landmarks (Coughlan et al., 2018). This strategy can be used when walking or traveling the same route repetitively (Wolbers et al., 2004). One disadvantage of the body-centered strategy is its dependence on the navigator´s current position and therefore this type of navigation is less flexible, and cannot be used in novel environments or to create shortcuts along a known route. Body-centered navigation predominantly depends on the posterior parietal cortex (deIpolyi et al., 2007; Ruotolo et al., 2019), precuneus (Weniger et al., 2011; Saj et al., 2014) and the caudate nucleus (Iglói et al., 2010).

When navigating the environment, navigators can also remember the positions of places and objects in relation to other visible objects and features of the environment (i.e., environmental landmarks). This type of navigation is referred to as world-centered navigation (i.e., wayfinding, allocentric navigation). When using world-centered navigation, the navigator creates a cognitive map, which is an internal image of the environment carrying information about various places, landmarks and directions and distances between features of the environment. Unlike body-centered navigation, formation of the cognitive map is not dependent on the navigator´s position in the environment. A major advantage of cognitive maps is their flexibility, as the navigator can travel along the routes that have never been traversed before or create novel shortcuts

within the environment (Maguire et al., 1998). World-centered navigation depends on intact functions of the MTL structures, especially the hippocampus and the interconnected EC (Maguire et al., 1998; Nedelska et al., 2012; Laczó et al., 2017; Cholvin et al., 2021).

The MTL structures, in particular the hippocampus and EC have functional differences along the anterior-posterior axis in relation to spatial navigation. Hippocampus can be differentiated into the posterior hippocampus (i.e., the body and tail) and anterior hippocampus (i.e., the head). All hippocampal subregions have a specific role in spatial navigation, as the hippocampal body and tail are mainly involved in creating and using cognitive maps (Schinazi et al., 2013) while the hippocampal head is involved in navigation planning (Xu et al., 2010) and responding to novelty (Doeller et al., 2008). Further, the hippocampal subregions process spatial information at different levels of detail. The posterior hippocampus supports processing of details, while the anterior part processes coarse spatial information (Brunec et al., 2018). The EC also shows subregional differences in spatial navigation involvement. Spatial information processing is mainly supported by the pmEC (Reagh and Yassa, 2014), which is also involved in the formation of fine-grained spatial representations (Evensmoen et al., 2015). On the other hand, the alEC primarily processes object information (Reagh et al., 2018), however, it also supports spatial navigation by encoding distances between objects and locations (Chen et al., 2019).

Further, world-centered navigation is supported by the BF. The BF is a heterogeneous structure consisting of separate nuclei which provide cholinergic input into many cortical and subcortical structures. The BF nuclei are referred to as Ch1-Ch4, the Ch1 is the medial septal nucleus which is interconnected with the nucleus of the vertical limb of the diagonal band of Broca (Ch2), Ch4p is the posterior part of the nucleus basalis of Meynert, and Ch4ai is the anterior-intermediate part of the nucleus basalis of Meynert (Mesulam et al., 1983a). These nuclei are the major sources of acetylcholine for brain regions supporting spatial navigation, the Ch1 and Ch2 nuclei project via fornix to the hippocampus, Ch4p projects to temporal cortical regions including the EC, and Ch4ai projects to medial regions of the hemispheres, amygdala and insular, parietal and prefrontal cortices (Teipel et al., 2005). The role of the BF in spatial navigation is supported by rodent studies showing that lesions of BF septo-hippocampal cholinergic projections disrupt processing of spatial information in the hippocampus (Ikonen et al., 2002). Further, previous studies

have shown association of BF and hippocampal atrophy with world-centered navigation impairment in individuals with AD (Kerbler et al., 2015).

For the research purposes, spatial navigation strategies are commonly assessed separately because they are supported by different brain regions. However, when navigating in real-life (i.e., traveling to work or going shopping) it is nearly impossible to rely only on one of the navigation strategies. Instead, integration of body-centered and world-centered spatial navigation is required. Integration of body-centered and world-centered navigation relies on the RSC, which is an integral part of the posterior cingulate region. The RSC receives inputs from the hippocampus and EC as well as from the parietal regions and thus integrates different navigation strategies (Auger et al., 2012; Clark et al., 2018).

Another spatial ability important for real-life navigation is perspective taking. When navigating along the well known routes, for example, when walking to work every day, we perceive scenes and landmarks (e.g., buildings along the way) always from the same viewpoint (i.e., perspective). However, in situations when we would not walk exactly the same route, the same scenes and landmarks would be seen from different perspectives. Therefore, we would need to imagine what the scenes would look like from different perspectives in order to find our way (Marková et al., 2015). This is referred to as perspective taking that is supported by the parietal cortex, temporal cortex and MTL structures (Zacks and Michelon, 2005; Lambrey et al., 2008).

1.3.2. Spatial navigation in normal aging

Aging is accompanied by discrete spatial navigation decline that specifically affects world-centered navigation (Moffat and Resnick, 2002; Iaria et al., 2009). In older adults, world-centered navigation impairment was found in real space (Gazova et al., 2013) as well as in virtual reality environments (Moffat and Resnick, 2002; Iaria et al., 2009). Functional neuroimaging studies showed association between reduced hippocampal activation and less accurate world-centered navigation task performance in older adults (Moffat et al., 2006; Antonova et al., 2009) supporting the notion that world-centered navigation decline in aging reflects age-related changes in the MTL structures. On the other hand, cognitively normal (CN) older adults had similar body-centered navigation performance as young participants in virtual reality environment and real space indicating that body-centered

spatial navigation does not decline with aging (Gazova et al., 2013). Body-centered navigation depends on posterior parietal lobe structures, which remain intact during aging (Maguire et al., 1998), thus this could explain why body-centered navigation remains unaffected in CN older adults.

When traveling or walking, one can use different strategies or different combinations of strategies that depend on the features of the environment and also on the navigator´s capabilities. Elderly individuals often avoid unfamiliar or less familiar ways and locations (Burns, 1999) and preferentially use well known routes and avoid formation of novel shortcuts to compensate for their spatial navigation decline. This change in navigational habits indicates preference of body-centered over world-centered strategy. Selective world-centered navigation decline in aging might be a reason why elderly individuals prefer body-centered navigation strategy to compensate for their deficit (Rodgers et al., 2012; Wiener et al., 2013). World-centered navigation depends on the hippocampus which undergoes structural changes in aging, therefore, choosing body-centered strategy indicates that older adults prefer extra-hippocampal strategy to avoid using dysfunctional brain regions (Moffat et al., 2006). When one strategy is chosen over the other (the more useful one) we call it a "compensatory strategy". Body-centered strategy is useful when the same route is repeated over and over again, but lacks the flexibility to navigate successfully in novel complex environments (Wolbers et al., 2004). Characterization of spatial navigation in healthy aging is an important first step in order to recognize spatial navigation changes that might occur during pathological aging.

1.3.3. Spatial navigation in pathological aging and AD

Spatial navigation deteriorates with the progression of AD (Hort et al., 2007; Allison et al., 2016; Levine et al., 2020). Spatial navigation deficits in AD are severe because AD pathology affects predominantly brain regions supporting spatial navigation (Auld et al., 2002). It is important to differentiate spatial navigation decline in normal aging from spatial navigation impairment characteristic of pathological aging as it might be a first sign of AD. Patients in the early stages of AD frequently report spatial navigation deficits, especially in less familiar places. In the later stages, patients with AD dementia become frequently disoriented even in well known environments, and may even get lost in less familiar places (Pai and Jacobs, 2004).

First signs of spatial navigation deficits are present already in preclinical stages of AD (Allison et al., 2016). CN older adults were classified based on the amyloid-β_{42} levels in CSF as preclinical AD (i.e., those who had low CSF amyloid-β levels) and CN older adults (i.e., those who had normal CSF amyloid-β levels). The preclinical AD group had less accurate world-centered navigation than CN older adults, while these two groups had similar performance in a body-centered navigation task (Allison et al., 2016). Spatial navigation has been also assessed in individuals with a genetic risk of developing AD. The APOE ε4 allele, which represents the strongest genetic risk of sporadic AD, was associated with worse spatial navigation performance even in CN young individuals (Kunz et al., 2015). Functional MRI analysis showed that worse performance in this group was associated with dysfunction of the EC (Kunz et al., 2015), which is one of the earliest regions affected by tau pathology in AD.

Individuals with aMCI frequently experience deficits in body-centered and world-centered navigation. These deficits have been documented both in virtual environments (Weniger et al., 2011; Laczó et al., 2012) and real space (DeIpolyi et al., 2007; Laczó et al., 2009), and also in perspective taking (Marková et al., 2015). Spatial navigation deficits in MCI cohorts were typically associated with lower volumes of the specific brain regions that are affected by AD pathology in the early stages. A study in a virtual environment showed spatial navigation deficits in both world-centered and body-centered navigation, where body-centered navigation impairment was associated with atrophy of the right-sided precuneus (Weniger et al., 2011). On the other hand, world-centered navigation impairment in aMCI was associated with atrophy of the hippocampus and BF, which are affected in early stages of AD (Nedelska et al., 2012; Kerbler et al., 2015). Another study using computerized and real life versions of the Morris water maze task found deficits in both world-centered and body-centered navigation in aMCI (Laczó et al., 2009). Spatial navigation deficits observed in individuals with aMCI are typically less pronounced than in patients with AD dementia (Laczó et al., 2009).

Most studies reporting spatial navigation deficits in MCI cohorts did not use the biomarkers to confirm that AD was the cause of MCI. There have been up to now only two studies with biomarker defined cohorts which compared aMCI individuals with AD (AD aMCI) and CN older adults. AD aMCI participants were impaired in world-centered and body-centered navigation in a large-scale real space environment consisting of extended

series of corridors (Schöberl et al., 2020). A computerized study reported deficits in scene recognition from a different perspective in AD aMCI individuals when the environment containing mountains was presented from a shifted viewpoint (Chan et al., 2016).

In addition to the early diagnosis of AD, it is also important to differentiate AD aMCI individuals from those with aMCI of other etiologies (i.e, non-AD aMCI). The potential of spatial navigation assessment in differentiating AD aMCI from non-AD aMCI individuals has been addressed only by a few studies. AD aMCI participants had worse body-centered and world-centered navigation performance in a complex real space environment compared to non-AD aMCI. While the non-AD aMCI showed impairment only in world-centered navigation and had comparable performance to the CN older adults in body-centered navigation (Schöberl et al., 2020). Similarly, body-centered navigation performance differentiated patients with AD from patients with other neurodegenerative diseases, while there were no differences in world-centered navigation between the groups (Tu et al., 2017). These results indicate that body-centered navigation tasks might detect AD-specific navigation deficits, whereas world-centered navigation tasks lack the specificity to differentiate AD from other diseases. Additionally, it is important to note that also path integration paradigm (i.e., task evaluating the ability to use self-motion to navigate) in immersive virtual reality differentiated older adults with MCI and positive AD biomarkers in CSF from those with AD negative biomarkers, as the AD group had less accurate performance (Howett et al., 2019). The previous studies indicated that spatial navigation may be a promising diagnostic tool to differentiate AD aMCI from non-AD aMCI (Howett et al., 2019; Schöberl et al., 2020), although, the limitation of these studies is that they require large scale space or movement in real space which is not optimal for clinical settings given common space constraints.

1.4. Pattern Separation

1.4.1. Principles of pattern separation

Pattern separation is an important process for accurate memory encoding allowing for subsequent recall of verbal and non-verbal information. It is a neural process of encoding similar inputs as non-overlapping representations (i.e., memories) so that they can be recalled separately from each other. Pattern separation process is needed when encoded information shares similar contextual features. Therefore, pattern separation is an

important mechanism for reducing potential interference among similar memory representations which enhances accuracy of memory (Holden and Gilbert, 2012). This process can be classified as "object pattern separation" when discriminating between visually similar objects and "spatial pattern separation" when discriminating between similar spatial information. A typical example of spatial pattern separation can be when we recall where we parked today versus where we parked yesterday in the same parking lot. However, spatial pattern separation process is not needed, when encoding very distinct spatial information, for example, when recalling where parking in front of your own house versus where parking in front of a shopping center.

The hippocampus, in particular the dentate gyrus, plays an important role in pattern separation processes. The hippocampus is functionally differentiated along its dorsoventral axis, such that spatial information is primarily processed in the posterior hippocampal subregions (i.e., hippocampal body and tail) and object information processing is primarily processed in the anterior hippocampus (i.e., hippocampal head) (Pihlajamäki et al., 2004; Lee et al., 2008). The hippocampus receives a strong input from the EC (Yassa et al., 2011b; Hunsaker and Kesner, 2013), which is functionally divided into the alEC and pmEC subregions. Spatial information is primarily conveyed from the pmEC, while object information is primarily conveyed from the alEC (Navarro Schröder et al., 2015). Hippocampal function is influenced by acetylcholine levels. Higher levels of acetylcholine increase the activity of the dentate gyrus and increase its responsiveness to stimulation from the EC (Giocomo and Hasselmo, 2007). Therefore, higher acetylcholine levels support the pattern separation processes. The major source of cerebral acetylcholine is the BF and especially its medial septal nuclei and vertical limb of the diagonal band of Broca (Ch 1-2 nuclei), which have direct cholinergic projections to the hippocampus (Mesulam et al., 1983a). Lesions of the cholinergic projections from the BF to the hippocampus lead to less accurate spatial pattern separation, highlighting the important modulatory role of the BF in this process (Ikonen et al., 2002).

1.4.2. Pattern separation in normal aging

Aging is associated with object and spatial pattern separation decline as a consequence of age-related hippocampal changes (Yassa et al., 2011a; Holden and Gilbert, 2012). Less efficient pattern separation may contribute to spatial and episodic memory deficits in aging

(Holden et al., 2012). Studies comparing object pattern separation performance in young and CN older adults mostly used the tasks consisting of a series of images featuring various objects. Participants were asked to classify these objects as "old" (i.e., same as previously seen), "similar" (i.e., similar to previously seen objects), or "unique" (i.e., objects not seen before). These studies consistently showed that the older participants incorrectly identified "similar" objects as "old" objects more frequently than the young participants, indicating object pattern separation deficits in the CN older participants (Toner et al., 2009; Yassa et al., 2011a; Stark et al., 2013). Studies comparing spatial pattern separation performance mostly used the tasks with pairs of objects located at different distances from each other (i.e., varying degrees of spatial interference). Participants were instructed to remember their locations and after a delay, they were presented again with the same objects and were asked whether the objects were in the same or different locations as seen initially. In these studies, the CN older adults had consistently less accurate performance compared to the young participants, indicating spatial pattern separation deficits (Holden et al., 2012; Reagh et al., 2014). However, the recent studies indicated that spatial pattern separation deficits are less pronounced than object pattern separation deficits in normal aging (Reagh et al., 2016; Güsten et al., 2021). Age-related pattern separation deficits are associated with functional changes in the hippocampus (Yassa et al., 2011a), disruption of functional connectivity between the EC and the hippocampus (Yassa et al., 2011b), and hippocampal atrophy (Shing et al., 2011). Additionally, studies indicated that age-related degeneration of cholinergic projections from the BF to the hippocampus leading to cholinergic deficit in the hippocampus could contribute to pattern separation deficits (Schliebs and Arendt, 2011).

1.4.3. Pattern separation in pathological aging and AD

Pathological aging is associated with pattern separation deficits that are more pronounced than those in normal aging. Specifically, object pattern separation is more disrupted in cognitively impaired older adults and aMCI individuals than in CN older adults (Yassa et al., 2010; Stark et al., 2013). In aMCI individuals, object pattern separation deficits were associated with functional changes in the hippocampus and EC (Yassa et al., 2010). Cognitively impaired older adults also had worse performance than CN older adults in spatial pattern separation tasks when classifying the objects as being at the same or different location (Stark et al., 2010; Holden et al., 2012). Worse spatial pattern separation

performance was further reported in older adults who were carriers of the APOE (Apolipoprotein) ε4 allele, which is the strongest genetic risk factor for sporadic AD, compared to the non-carriers (Sheppard et al., 2016). These studies clearly demonstrated object and spatial pattern separation deficits in cognitively impaired older adults and aMCI individuals compared to CN older adults. However, these studies did not use biomarkers to determine the etiology of cognitive impairment in the participants.

There are only a few studies that assessed pattern separation in individuals with biomarker evidence of amyloid-β and tau pathologies. In the recent studies, object pattern separation deficits were associated with tau accumulation in the anterior temporal regions on PET (Maass et al., 2019) and higher p-tau levels in CSF (Berron et al., 2019) in older adults suggesting that object pattern separation deficits may be a marker of tau pathology. The key regions for object pattern separation are the alEC and the anterior hippocampus (Pihlajamäki et al., 2004; Lee et al., 2008), the primary regions where accumulation of hyperphosphorylated tau commonly occurs in normal aging (Braak and Braak, 1997), primary age-related tauopathy (Crary et al., 2014) and also in AD (Braak and Braak, 1997). Together, these findings indicated that object pattern separation deficits could be a marker of tau pathology but may not be specific to AD.

Spatial pattern separation deficits do not seem to be associated with tau pathology as measured by p-tau levels in CSF (Berron et al., 2019) and tau accumulation on PET (Maass et al., 2019). On the other hand, spatial pattern separation deficits were associated with higher amyloid-β accumulation on PET in older adults (Webb et al., 2020). Specifically, amyloid-β depositions in the posterior-medial cortical regions were associated with more pronounced deficits (Maass et al., 2019). In AD, amyloid-β accumulates early in the precuneus, posterior cingulate and retrosplenial cortex (Pengas et al., 2010; Palmqvist et al., 2017), which are strongly interconnected with the posterior MTL regions. These regions include the posterior hippocampus (Aggleton et al., 2012) and the pmEC (Navarro Schröder et al., 2015), which are the key regions for spatial pattern separation. Together, these findings indicate that spatial pattern separation deficits may specifically reflect amyloid-β pathology and thus could be a reliable cognitive marker of early AD.

1.5. Experimental paradigms in spatial navigation and pattern separation assessments

Everyday life navigation takes place in real space in environments of various spatial scales. Environmental spatial scales can vary from vista-space (e.g., navigation within a room) to large-scale environments (e.g., navigation in a city). Navigation in real space involves processing of multiple sensory modalities, which include vestibular, somatosensory and visual input. Although there are spatial navigation paradigms in real space, their disadvantages include difficult standardization, limited options in experimental manipulation and space constraints of clinical settings. Therefore, various computerized tasks have been developed to assess spatial navigation in research settings. The disadvantage of these tasks is that real life multisensory stimulation during navigation is reduced and the sensory input is often provided only via visual system (Diersch and Wolbers, 2019), although there is positive correlation between navigating in virtual reality (VR) and real world navigation (Coutrot et al., 2019). Current computerized tasks are commonly developed in VR giving the researchers a tight control over the task features and allow detailed analysis of behavioral responses (Diersch and Wolbers, 2019). VR tasks are frequently based on behavioral paradigms previously used to study rodent behavior (e.g., human analogue of Morris Water Maze, radial maze or Y-maze) (Iaria et al., 2003; Hort et al., 2007; Rodgers et al., 2012) or in environments which resemble the real world (i.e., virtual cities, parks or landscapes) (Weniger et al., 2011; Chan et al., 2016). Navigation testing in a clinical or research setting needs to investigate specific aspects of navigation using paradigms which are timewise feasible, do not require extensive training and are ecologically valid (i.e., reflecting real world behavior).

2. AIMS AND HYPOTHESES

2.1. Study 1: The Effect of Alzheimer's Disease on Spatial Navigation Strategies

The early stages of AD are associated with spatial navigation deficits with the world-centered navigation impairment being the most prominent (Hort et al., 2007; Allison et al., 2016). This impairment is associated with neurodegeneration in the hippocampus and BF (Nedelska et al., 2012; Kerbler et al., 2015), the earliest structures affected in AD. Also aging is associated with less effective world-centered navigation (Moffat and Resnick, 2002) as a result of structural and functional changes in the MTL, especially the hippocampus (Nedelska et al., 2012). Body centered-navigation is not affected by aging, which may explain increasing preference for body-centered over world-centered navigation with aging (Rodgers et al., 2012; Wiener et al., 2013). This preference reflects the adoption of extra-hippocampal strategies to minimize involvement of the affected MTL regions (Moffat et al., 2006). The disruption of the hippocampus and its projections from the BF are greater in AD than in normal aging (Auld et al., 2002). However, it has not been investigated whether neurodegeneration in the hippocampus and BF is associated with adoption of the compensatory extra-hippocampal navigation strategies (i.e., increased preference for body-centered navigation) in the early clinical stages of AD (i.e., AD aMCI and mild AD dementia). For this purpose we used a virtual navigation task to assess navigation strategies preference and compare this preference to world-centered navigation performance in real space.

The aims of the study were to assess:

(1) the differences in spatial navigation strategy preference (world-centered vs. body-centered navigation) in the early clinical stages of AD (AD aMCI and mild AD dementia) compared to CN older adults;

(2) the association of strategy preference with world-centered navigation performance in real space; and

(3) the role of hippocampal and BF nuclei volumes in this association.

We hypothesized that:

(1) participants with mild AD dementia and AD aMCI would have a stronger preference for the body-centered navigation strategy compared to the CN participants;

(2) participants in the early clinical stages of AD with the body-centered strategy preference would have less accurate world-centered navigation performance; and
(3) hippocampal volume and volumes of BF nuclei would play important roles in the association between the body-centered strategy preference and less accurate world-centered navigation performance.

2.2. Study 2: Different Profiles of Spatial Navigation Deficits in Alzheimer's Disease Biomarker-positive versus Biomarker-Negative Older Adults with Amnestic Mild Cognitive Impairment

A vast majority of studies used spatial navigation paradigms to differentiate the CN older adults from individuals with MCI, however, the AD etiology of cognitive impairment in these MCI cohorts has not been supported by specific AD biomarkers (i.e., amyloid PET imaging or CSF analysis). Only a few studies indicated that spatial navigation in biomarker-defined aMCI individuals with AD may be worse than in those with non-AD etiology. However, none of the tests used in these studies was ideal for routine clinical assessment as they require movement in large space (Howett et al., 2019; Schöberl et al., 2020). In this study we used a virtual Navigation Test Suite to assess whether this test could differentiate individuals with AD aMCI from those with non-AD etiology. The Navigation Test Suite is a virtual realistic looking test which can be easily performed in clinical settings and evaluates different aspects of spatial navigation (i.e., body-centered, world-centered and world-centered navigation/perspective taking). In order to analyze the potential of this test to reflect the AD pathology, we explored the association of task performance with levels of CSF biomarkers (i.e., amyloid-$\beta_{1\text{-}42}$ and phosphorylated tau_{181} [p-tau_{181}]) and atrophy of specific brain regions that support spatial navigation.

The aims of the study were to assess:
(1) the differences in body-centered navigation, world-centered navigation and world-centered navigation/perspective taking between the participants with AD aMCI and non-AD aMCI;
(2) the associations of spatial navigation performance with MRI measures of atrophy in the specific MTL, cortical and subcortical regions; and
(3) the associations of spatial navigation performance with CSF levels of AD biomarkers.

We hypothesized that:

(1) the participants with AD aMCI would perform worse in all three navigation tasks compared to the non-AD aMCI participants and the greatest differences would be observed in body-centered navigation performance;

(2) atrophy of the parietal regions including the precuneus and the posterior parietal cortex would be associated with worse body-centered navigation; atrophy of the MTL regions (i.e., the hippocampus and EC) and in particular their posterior parts would be associated with worse world-centered navigation; and worse performance in the world-centered navigation/perspective taking task would be associated with atrophy of both, the MTL and parietal regions and additionally with atrophy of the isthmus cingulate/RSC which supports integration of body-centered and world-centered navigation; and

(3) lower CSF levels of amyloid-$\beta_{1\text{-}42}$ would be associated with worse body-centered navigation given that amyloid-β accumulates predominantly in the parietal cortex; higher CSF levels of p-tau_{181} would be associated with worse world-centered navigation as tau protein accumulates in the MTL regions; and finally both, low amyloid-$\beta_{1\text{-}42}$ and high p-tau_{181}, CSF levels would be associated with the world-centered/perspective taking performance.

2.3. Study 3: Spatial Pattern Separation in Early Alzheimer's Disease

Spatial pattern separation deficits have been demonstrated in older adults with cognitive impairment including those with aMCI (Holden et al., 2012; Reagh et al., 2014). However, the underlying etiology of cognitive impairment has not been established as the participants did not undergo biomarker assessment. Spatial pattern separation strongly depends on the hippocampus and EC, which projects to the hippocampus (Marr, 1971; Hunsaker and Kesner, 2008, 2013). The spatial pattern separation processes in the hippocampus are supported by acetylcholine. The major source of acetylcholine are the BF Ch1-2 nuclei that provide cholinergic projections to the hippocampus (Mesulam et al., 1983a). This study examined the potential of spatial pattern separation assessment to diagnose AD aMCI and mild AD dementia, and to explore the association of spatial pattern separation performance with hippocampal and EC atrophy, and atrophy of BF Ch 1-2 nuclei.

The aims of the study were to assess:

1) the differences in spatial pattern separation in the biomarker-defined early clinical stages of AD (AD aMCI and mild AD dementia) compared to CN older adults; and

2) the associations of spatial pattern separation performance with hippocampal and EC volumes, and volumes of BF Ch1-2 nuclei.

We hypothesized that:

(1) the participants in the biomarker-defined early clinical stages of AD would have less accurate spatial pattern separation performance compared to the CN older adults, accuracy of the performance would be related to the disease severity, and differences in spatial pattern separation would not be explained by general memory or other cognitive deficits; and

(2) smaller volumes of the hippocampus, EC, and BF Ch1-2 nuclei would be associated with lower accuracy of spatial pattern separation above and beyond global brain atrophy and demographic characteristics.

2.4. Study 4: Spatial Pattern Separation Testing Differentiates Alzheimer's Disease Biomarker-positive and Biomaker-negative Older Adults with Amnestic Mild Cognitive Impairment

This study directly followed up Study 3 showing spatial pattern separation deficits in AD aMCI participants that were associated with hippocampal and EC atrophy (Parizkova et al., 2020). In the Study 4, we addressed this finding in more detail and analyzed the potential of the spatial pattern separation assessment to differentiate aMCI participants with AD from those with non-AD etiology. Further, we analyzed in more detail the associations of performance with volumes of the MTL, focusing on different hippocampal (i.e., head, body and tail) and EC (i.e., alEC and pmEC) subregions.

The aims of the study were to assess:

(1) the differences in spatial pattern separation performance between participants with AD aMCI and non-AD aMCI; and

(2) the associations of spatial pattern separation performance with volumes of specific hippocampal and EC subregions and BF Ch1- 2 nuclei.

We hypothesized that:

(1) the participants with AD aMCI would have less accurate spatial pattern separation performance than the participants with non-AD aMCI; and

(2) worse spatial pattern separation performance would be associated with smaller volumes of specific hippocampal and EC subregions, specifically with the posterior hippocampus (i.e., tail and body) and the pmEC, and smaller volumes of the BF Ch1-2 nuclei.

3. METHODS

3.1. Common methods for all studies (1-4)

3.1.1. Participant recruitment

Participants for all four studies were recruited from the Czech Brain Aging Study cohort (Sheardova et al., 2019) at the Memory Clinic of the Charles University, Second Faculty of Medicine and Motol University Hospital in Prague, Czechia and signed an informed consent approved by the local ethics committee, no. EK – 701/16 25.5.2016). The participants with cognitive deficits were referred to the Memory Clinic by general practitioners and neurologists for memory complaints reported by themselves and their informants. CN older adults were recruited from the University of the Third Age, senior centers (e.g., the Elpida center) and relatives of the participants and hospital staff. All participants underwent clinical and laboratory evaluations, comprehensive cognitive assessment, brain MRI and spatial navigation and spatial pattern separation assessments. The diagnostic criteria of the participant groups are outlined below.

3.1.1.1. All studies (1-4)

i) CN participants did not report any cognitive complaints, had cognitive performance within the normal range (i.e., score higher than 1.5 standard deviations below the age-and education-adjusted norms in all cognitive tests). In addition, they had no evidence of MTL atrophy on MRI and did not have family history of AD or other type of dementia in the first-degree relatives. These stringent criteria were applied to minimize the possibility of including participants with increased risk of AD and early clinical AD.

3.1.1.2. Studies 1 and 3

ii) AD aMCI participants met the clinical criteria for MCI due to AD according to the recommendations of the National Institute on Aging and Alzheimer's Association workgroups on diagnostic guidelines for AD (Albert et al., 2011). All participants had evidence of hippocampal atrophy on MRI. Hippocampal atrophy was assessed using the MTL atrophy visual scale (Scheltens et al., 1992) and served as an evidence of neuronal injury. The atrophy was defined using the age-specific cut-off scores (i.e., score > 2 in participants < 75 years and score >3 in participants ≥ 75 years) (Scheltens et al., 1992). These participants had an intermediate probability of AD. A subset of the participants also

underwent spinal tap with CSF analysis. Only participants with low levels of amyloid-$\beta_{1\text{-}42}$ were included and they were classified as having high probability of AD etiology (Parizkova et al., 2018, 2020).

iii) mild AD dementia participants met the clinical criteria for probable dementia due to AD based on recommendations of the National Institute on Aging and the Alzheimer's Association workgroups (McKhann et al., 2011). The AD etiology of dementia was supported by evidence of neuronal injury on MRI (pathological MTL atrophy score), indicating intermediate biomarker probability of AD etiology. In addition to neuronal injury on MRI, a subset of participants had low levels of beta amyloid in CSF, indicating high biomarker probability of AD etiology (Parizkova et al., 2018, 2020).

3.1.1.3. Studies 2 and 4

These studies included participant groups with aMCI and mild dementia who met the same clinical criteria as in Studies 1 and 3. The difference was that all participants with cognitive deficit underwent CSF or amyloid PET imaging and a subset of participants underwent both of these assessments. The CSF assessment included analysis of amyloid-$\beta_{1\text{-}42}$, total tau and p-tau$_{181}$. The mild AD dementia group consisted only of participants with AD confirmed by the biomarker assessment. Participants with aMCI were classified into two groups AD aMCI and non-AD aMCI depending on their biomarker profile.

ii) AD aMCI participants met the clinical criteria for aMCI (Albert et al., 2011). The participants had positive CSF AD biomarkers (reduced amyloid-$\beta_{1\text{-}42}$ and elevated p-tau$_{181}$) and/or positive amyloid PET imaging (positive visual read of 18F-flutemetamol PET scan).

(iii) Participants with non-AD aMCI met the clinical criteria for aMCI (Albert et al., 2011) and had negative amyloid-β biomarkers defined as normal CSF amyloid-$\beta_{1\text{-}42}$ and/or negative amyloid PET imaging.

(iv) Participants with mild AD dementia met the clinical criteria for dementia (McKhann et al., 2011). The participants had positive CSF AD biomarkers (reduced amyloid-$\beta_{1\text{-}42}$ and elevated p-tau$_{181}$) and/or positive amyloid PET imaging.

3.1.2. Exclusion criteria

Participants who met the exclusion criteria were not included in any of the studies. The following criteria applied across all groups included:

(i) depressive symptoms (≥ 6 points on the 15-item Geriatric Depression Scale [GDS-15])
(ii) anxiety (≥ 10 points on the Beck Anxiety Inventory [BAI])
(iii) low visual acuity < 20/40 [corrected] assessed using Snellen chart
(iv) vascular lesions on MRI (Fazekas score > 2 points, corresponding to moderate or severe changes)
(v) neurological or psychiatric disorders
(vi) inability to complete the spatial tasks
(vii) severe nausea caused by movement in virtual environment (in Study 1 and 2)

3.1.3. Cognitive assessment

The baseline cognitive assessment used in all studies included the following tests: (1) verbal memory measured with the Rey Auditory Verbal Learning Test (RAVLT) – trials 1–5 and 30-min Delayed Recall trial (Bezdicek et al., 2014); (2) non-verbal memory measured with the Rey-Osterrieth Complex Figure Test (ROCFT) – the Recall condition after 3 min (Drozdova et al., 2015); (3) visuospatial function measured with the ROCFT – the Copy condition (Drozdova et al., 2015); (4) executive function measured with the Trail Making Test B and Controlled Oral Word Association Test (Czech version with letters N, K, and P); (5) attention and working memory measured with the Forward and Backward Digit Spans and (Nikolai et al., 2018); and (6) language measured with the Boston Naming Test (30-item version) and Semantic Verbal Fluency test (Animals) (Nikolai et al., 2018). The Mini-Mental State Examination (Štěpánková et al., 2015) was administered to measure global cognitive function. The GDS-15 (Yesavage and Sheikh, 1986) and BAI (Beck et al., 1988) were used to assess depressive symptoms and anxiety among participants.

In addition, Study 1 used a 16-item picture version of the Enhanced Cued Recall Test (free and total recall scores) (Topinková et al., 2002) as a verbal memory test, Study 3 used the Logical Memory subtest of the Wechsler Memory Scale-Revised (Story I) – Immediate and Delayed Recall conditions (Nikolai et al., 2018) as verbal memory tests, and Studies 2,

3 and 4 used the Clock Drawing Test as a test of visuospatial functions (Mazancova et al., 2017).

3.1.4. CSF analysis of AD biomarkers

The 15 ml samples of CSF were obtained from the spinal tap. Initially taken 3ml of CSF were used for a routine biochemical and cytological analysis, and 10ml of CSF were centrifuged and stored at -80°C within 30 minutes after the sample was obtained from spinal tap. Processing and archiving of CSF was performed in accordance with European recommendations (Vanderstichele et al., 2012). CSF analysis was performed in the Cerebrospinal Fluid Laboratory, Institute of Immunology and Department of Neurology, Second Faculty of Medicine, Charles University and Motol University Hospital. CSF amyloid-$\beta_{1\text{-}42}$, total tau and p-tau_{181} were analyzed using ELISA (Innogenetics,Ghent, Belgium). Abnormal levels of CSF biomarkers were established according to the cut-off values: amyloid-$\beta_{1\text{-}42}$, less than 665 pg/ml, p-tau_{181} above 48 pg/ml and total tau above 358 pg/ml. The cutoff values were determined based on internal receiver operating characteristic (ROC) analyzes with validation against amyloid PET imaging results (Cerman et al., 2020).

3.1.5. Amyloid PET imaging (Study 2 and 4)

Amyloid PET examinations were performed on a PET/CT scanner (Biograph 40 TrueV HD from Siemens [Munich, Germany]) using a radiopharmaceutical flutemetamol (Vizamyl from GE Healthcare [Chicago, IL, USA]). Low-dose non-contrast CT scan was performed initially to correct for attenuation. Two phases of scanning were performed. The first phase of scanning (i.e., the perfusion phase) started immediately after administration of the radiopharmaceutical and represented perfusion of the gray matter. The second phase (i.e., the amyloid phase) was performed 90 minutes after radiopharmaceutical administration and visualized the white matter and beta-amyloid load in the gray matter. The images were visually reviewed by two experienced radiologists to assess the presence or absence of pathological radiopharmaceutical uptake in the gray matter in the amyloid phase (Cerman et al., 2020). Amyloid PET images were classified as positive or negative depending on whether accumulation in any of the eight specific brain regions was present or absent.

3.1.6. MRI acquisition and analysis

3.1.6.1. Image acquisition

We used the established MRI protocol (Parizkova et al., 2018) performed on a Siemens Avanto 1.5T scanner (Siemens AG, Erlangen, Germany). The T1-weighted 3-dimensional sequences were obtained for purposes of volumetric analysis. All scans underwent visual inspection and participants whose scans did not have sufficient quality were excluded from the study. Participants who had brain pathology, which could influence their cognitive functions (e.g., hydrocephalus, hematoma etc.) were also excluded from the studies.

3.1.6.2. Hippocampal volume analysis

Study 1: A fully automated volBrain volumetry system (Manjón and Coupé, 2016) was used to compute left and right hippocampal volumes. Volumes were adjusted for the total intracranial volume.

Study 2 and 4: First, manual segmentation of the hippocampus was performed for 26 CN older adults from the CBAS (Sheardova et al., 2019) to create a population-based template. The hippocampus was manually delineated and divided into three separate subregions (i.e., head, body and tail) according to the previously published segmentation protocol (Berron et al., 2017). Second, the population-based template was registered and diffeomorphically warped into participants' space using ANTs. The resulting warp field was used to transform ROI masks of the hippocampal subregions into the participants' space. The ROIs masks were subsequently masked with a gray matter ROI and their volumes were extracted.

Study 3: FreeSurfer image analysis suite (version 5.3; http://surfer.nmr.mgh.harvard.edu/) was used to compute left and right hippocampal volume. Volumes were summed into a single measure of total hippocampal volume and adjusted for total intracranial volume.

3.1.6.3. Entorhinal cortex measurements

Study 2 and 4: First, manual segmentation of the EC was performed for 26 CN older adults from the CBAS (Sheardova et al., 2019) to create a population-based template. The EC was manually delineated and divided into the alEC and pmEC subregions according to the previously published segmentation protocols (Berron et al., 2017; Olsen et al., 2017). Second, the template was registered and warped into participants' space using ANTs. The ROI masks of the EC subregions were transformed into the participant's space using the resulting warp field and subsequently masked with a gray matter ROI and their volumes were extracted.

Study 3: FreeSurfer image analysis suite (version 5.3; http://surfer.nmr.mgh.harvard.edu/) was used to compute left and right entorhinal cortical volume. Volumes were summed into a single measure of total entorhinal cortical volume and adjusted for total intracranial volume.

3.1.6.4. Basal forebrain measurements

Study 1, 3 and 4: Images were processed using statistical parametric mapping (SPM8) and the VBM8-toolbox implemented in MatLab R2015b. We used the BF mask based on a cytoarchitectonic map of BF cholinergic nuclei derived from combined histology and MRI of a postmortem brain (Teipel et al., 2005). The BF mask contains 6 regions (BF 1-6) which correspond to specific BF regions according to Mesulam´s nomenclature (Mesulam et al., 1983b). The BF1 region corresponds to the Ch4p sector (the posterior part of the nucleus basalis of Meynert), the BF2 corresponds to the Ch4a-i sectors (the anterior and intermediate parts of nucleus basalis of Meynert), the BF3 region corresponds to the Ch3 sector (horizontal limb of the diagonal band of Broca), the BF4 region corresponds to the nucleus subputaminalis (Šimić et al., 1999), the BF5 region corresponds to the interstitial nuclei, and the BF6 region corresponds to combined Ch1 and Ch2 sectors (the medial septal nuclei and vertical limb of the diagonal band of Broca). The images were nonlinearly registered into the MNI152 template, the resulting DARTEL parameters were used to warp the cytoarchitectonic map into individual brain images, and volumes of the BF nuclei were extracted. Left and right volumes were combined into a single measure of total volume for each BF nucleus and adjusted for total intracranial volume.

3.1.6.5. Measurements of other cortical and subcortical regions

Study 2: FreeSurfer image analysis suite (version 5.3; http://surfer.nmr.mgh.harvard.edu/) was used to calculate volumes of the right and left caudate nucleus and thickness of the right and left precuneus, isthmus cingulate and composite region of the posterior parietal cortex.

3.1.7. Statistical analysis

Analysis of variance (ANOVA) with post hoc Tukey's honestly significant difference test was performed to analyze differences in continuous variables including age, education, performance in cognitive tests and brain MRI measures between the diagnostic groups. For changes in proportion (gender), we used a χ^2 test. Other statistical analyses varied across the studies and are described specifically for each study within the Results section.

3.2. Specific methods for each study

3.2.1. Study 1 specifics

3.2.1.1. Study 1 participants

A total of 113 participants were recruited from the Czech Brain Aging Study cohort. After application of the exclusion criteria, the final sample included 69 participants who were classified into 3 groups: CN participants (n = 20), aMCI due to AD (n = 28) and mild AD dementia (n = 21).

3.2.1.2. Virtual Y-maze

The Y-maze is a virtual reality task with low immersion administered on a computer screen (Rodgers et al., 2012). Participants used a joystick to move through the environment. The task started with a familiarization training, in which participants traveled through a series of corridors to learn how to use the joystick. The Y-maze task was used to identify preferred navigation strategy (body-centered versus world-centered). The Y-maze consisted of 3 arms at 120° from each other connected in the center. Each arm had its floor slightly recessed below the surrounding surface and there was a small circular area at the end of each arm. The whole Y-maze was located in a large room containing various cues. Some of the cues were in a close proximity to the maze while the others were at a greater distance. All cues were clearly visible from inside of the maze, but participants could move

only within the maze (the software did not allow the participants to leave the Y-maze to move around the room). The Y-maze task consisted of 5 blocks where each block had 2 parts: 1) multiple training trials (Fig. 1A) and 2) a final probe trial (Fig. 1B). Each block had a different environment (i.e., different colors and different objects in the room) (Fig. 2).

In the training trials, participants always started from the same location in one of the circular areas at the end of one arm. The training consisted of multiple trials, in which the participants always started from the same location in the circular area at the end of one arm. Then the participants had to move to one of the two remaining circular areas at the end of the arms. A tone sounded after entering any of these areas. When participants entered the correct area (i.e., the goal area) they could hear a pleasant sound. If they entered an incorrect area they heard the sound of a noxious buzzer. It is important to note that in the first trial, the participants had to choose randomly which arm they entered. For the consecutive trials, they should know which arm was correct and which incorrect. The training trials continued repetitively until participants reached the correct goal area 5 times consecutively. Moving repetitively to the goal area ensured that the participants remembered this location.

The probe trial started immediately after completing the fifth correct training trial. However, participants were not placed into the same area as previously, and instead they were placed into an area where previously the noxious buzzer sounded. This time there was no buzzer at the entrance. Participants´ task was to find the goal again. The probe trail was designed to determine the world-centered or body-centered navigation strategy preference. Participants who followed the same route as they learned in the training regardless of orientation cues (e.g., turned right in the middle of the maze), were classified as using a body-centered navigation strategy. Whereas the participants who moved to the same absolute location based on the positions of the landmarks (despite moving in an opposite direction as during the training) were classified as using an world-centered navigation strategy.

The task consisted of five blocks to assess whether participants had consistent preference of one strategy over the other. Strategy preference was determined if the same strategy was chosen in at least 4 of the 5 blocks. Participants who chose one strategy in only 3 or 2 of

the 5 blocks were considered as having an inconsistent strategy preference and were excluded from the analysis. The instructions were modified for cognitively impaired participants and the paper diagram of an overhead view of a Y-maze was shown to the participants. Further, the participants were instructed to pay attention to the Y-maze and objects in its surroundings.

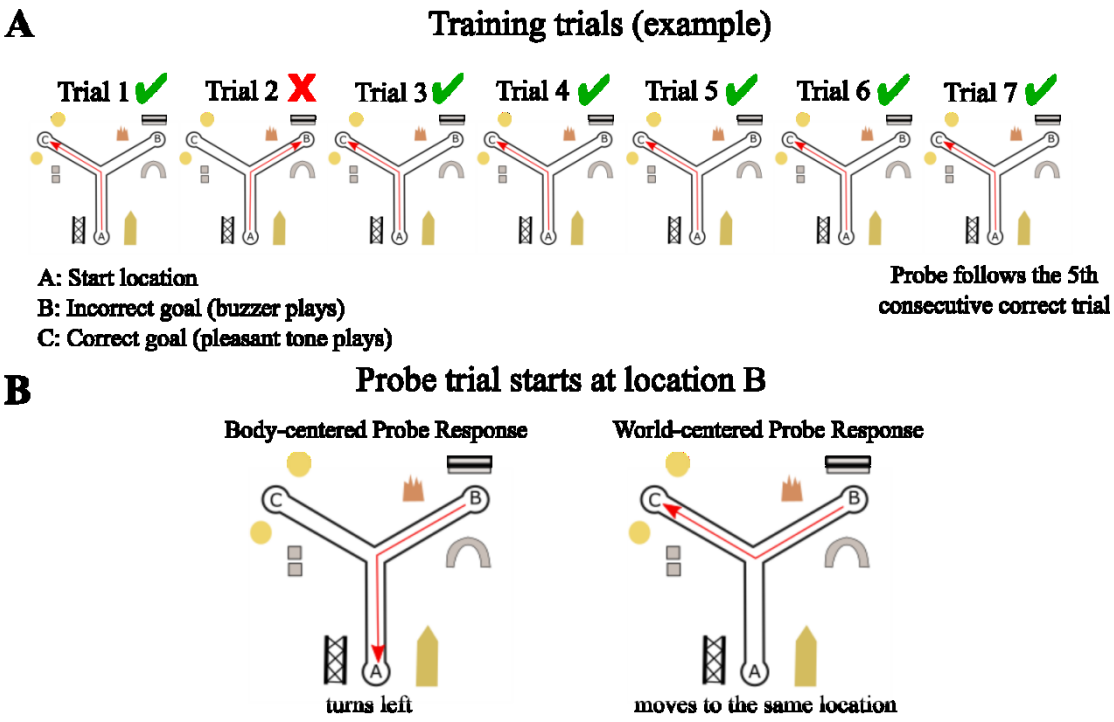


Fig. 1 Virtual Y-maze strategy assessment. (A) Training trials: participant starts at location A with location C being designated as the goal. In the first trial, the participant has a free choice and chooses correctly (as indicated by the "check mark"). In the second trial, the participant starts again in the same position and makes an incorrect choice (as indicated by "X"). The assessment continues until the participant travels to the correct goal location for 5 consecutive trials. Afterward, a probe trial starts. (B) Probe trial: the participant starts in the location that was neither the original starting location nor the designated goal location. The participant who uses the body-centered navigation strategy would turn left toward the location A, whereas the participant who prefers the world-centered strategy would move to the same absolute spatial location (location C).

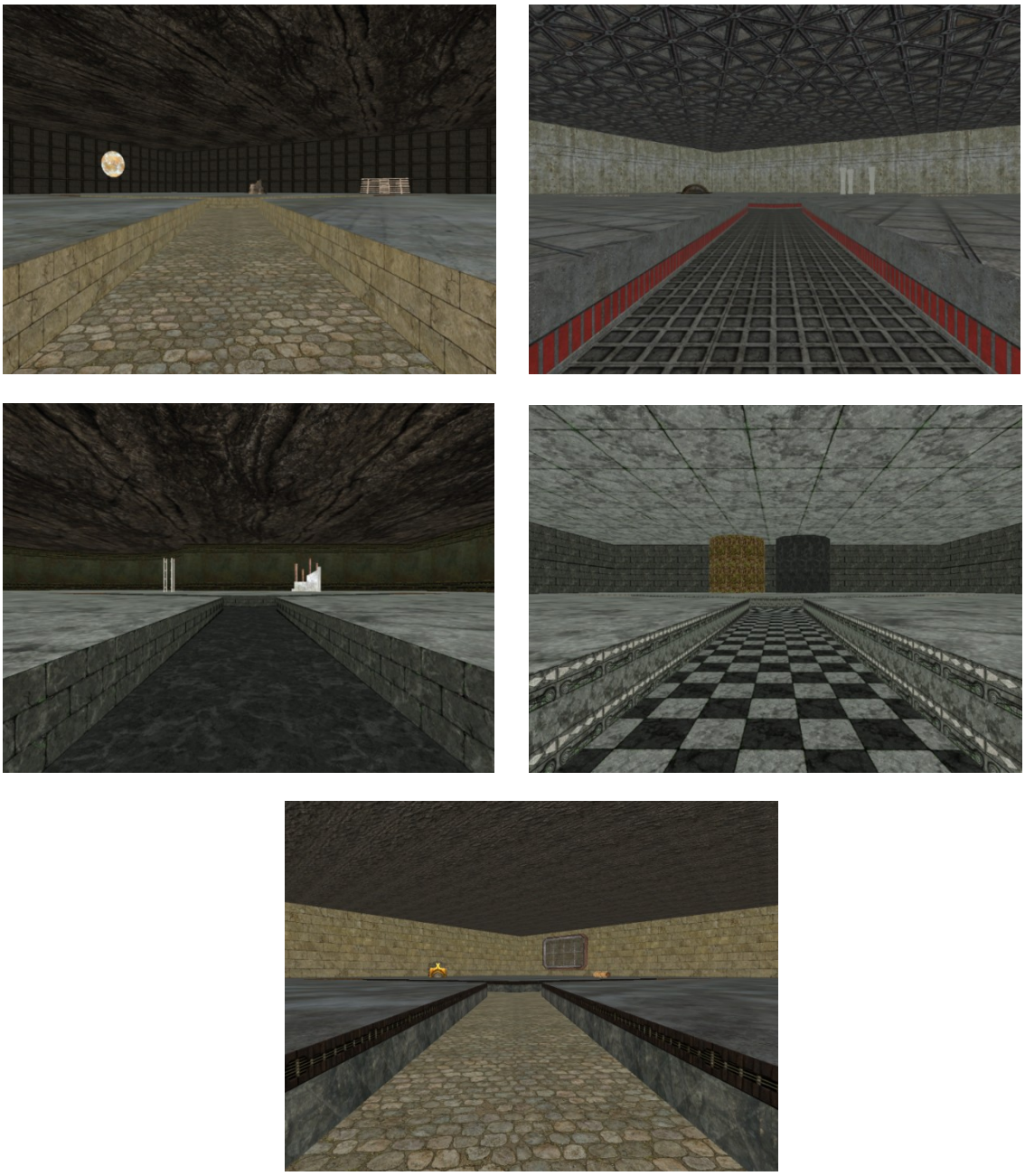


Fig. 2 Virtual Y-maze strategy assessment. Five different blocks of mazes with different designs.

3.2.1.3. Real space human analogue of the Morris Water Maze task

We used a human analogue of the Morris Water Maze (human MWM) task to assess world-centered spatial navigation. The human MWH was constructed on the same principle as the original version for animal models, where animals had to find a platform hidden under the water surface based on its spatial relation to orientation cues, when they were released from different positions in the pool (Vorhees and Williams, 2006). The

human MWM is a real space task taking place in a special enclosed circular arena measuring 2.8 meters in diameter and 2.9 meters high (Fig. 3A). This arena has a dark blue velvet curtain so that participants cannot see outside. There were two distinct visual cues on the wall of the arena and the participants had to find a hidden goal, which was in a constant distance and direction from each visual cue. Participants had eight trials, and in each trial a specific different starting position was assigned (Fig. 3B, 3C). From the starting position, they had to walk and place a special standing pole (a stick) on a place, where they thought the goal was located. Participants received feedback after each trial to facilitate learning. Performance was measured as distance error in centimeters (i.e., distance between the indicated position of the goal and the correct goal location) and was recorded by a computer. A total performance was calculated as a mean distance error across all eight trials.

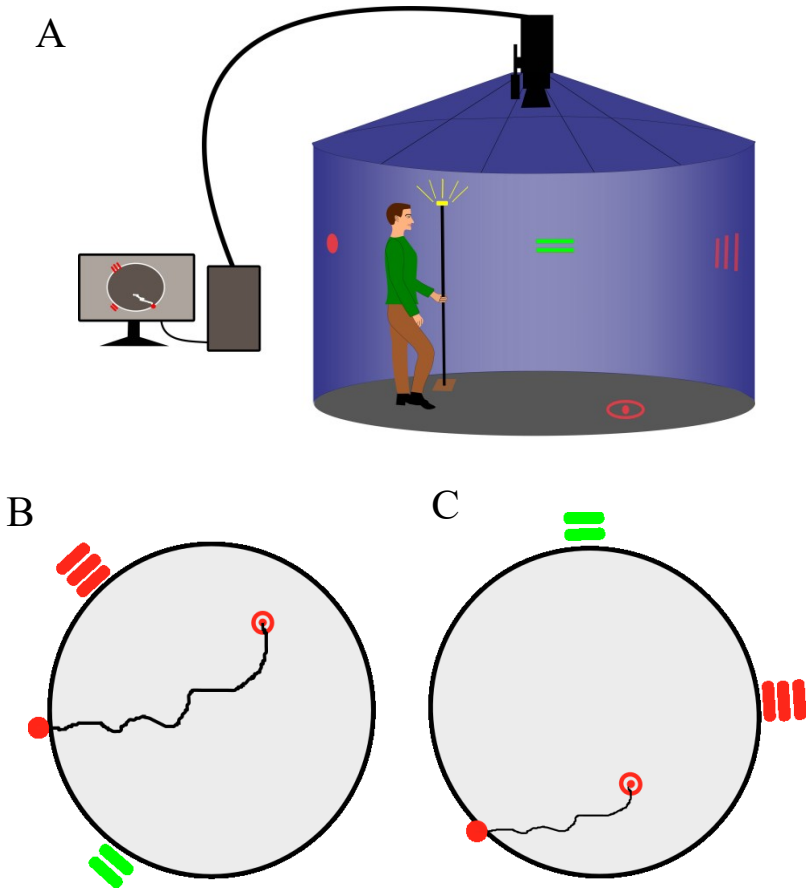


Fig. 3 Human analogue of the Morris water maze task. (A) The real space navigation setting. (B) The scheme of the task shows an aerial view of the arena (large circle) with starting point (red point), orientation cues (red and green lines), and goal (red circle). (C) An aerial view of the arena, where the orientation cues and the goal are rotated 90° from the previous trial shown in Fig. 3B.

3.2.2. Study 2 specifics

3.2.2.1. Study 2 participants

A total of 122 participants were recruited from the Czech Brain Aging Study cohort and they were classified into 4 groups: CN participants (n = 30), non-AD aMCI (n = 31) aMCI due to AD (n = 33), and mild AD dementia (n = 28).

3.2.2.2. The Navigation Test Suite

The Navigation Test Suite (Wiener et al., 2020) consists of three navigation tasks: the Route-repetition task (Fig. 4A), the Route-retracing task (Fig. 4A), and the Directional-approach task (Fig. 4B). The Navigation Test Suite is a VR task with low immersion administered on a computer screen which contains a realistic looking environment of the city suburb. All three tasks consist of streets with residential houses and four-way intersections. The streets are aligned by identical brick houses and intersections feature houses of different design and color. These distinct houses at the corners of intersections serve as navigation landmarks. Each task was preceded by its shorter version as a training to get familiar with the task principles (Wiener et al., 2020).

3.2.2.2.1. Route-repetition task

In the encoding phase, the participants were initially placed in the street next to the black car. Participants were instructed to remember the route, which they will travel. The computer transported them passively along the route with five intersections. While being transported along the route, participants turned right, left or continued straight at each intersection. There was a red telephone box at the end of this route. Identical brick houses were along the streets and each intersection featured four distinct houses at all four corners of each intersection. It is important to note that only one intersection was visible at one moment, as the ends of other streets were concealed in white fog. In the testing phase, the participants were again placed next to the black car and the computer passively transported them again along the same route as in the encoding phase. The movement stopped 20m before the center of each intersection and participants had to indicate in which direction the route continued. The options were right, left or straight. Participants did not get feedback during the task. Regardless of correct or incorrect answer their passive movement continued beyond the intersection correctly along the route giving them a chance to answer

correctly at the later intersections. Both encoding and testing phases were repeated three times (i.e., three sessions) in order to assess the effect of learning.

5.2.2.2.2. Route-retracing task

The encoding phase was similar to the Route-repetition task. The route featured different unique houses at the corners of intersections and the order of turns (right, left or straight) was different from that in the Route-repetition task. In the test phase, the participants had to navigate from the telephone box back to the start (i.e., to the black car). Therefore, they had to navigate along the route in the opposite direction as seen in the encoding phase. The Route-retracing task also consisted of three sessions which were performed immediately one after the other.

5.2.2.2.3. Directional-approach task

The Directional-approach task assessed participants´ ability to encode the configuration of houses (landmarks) at an intersection and assessed perspective taking and world-centered navigation (Wiener et al., 2013; de Condappa and Wiener, 2016). The task consisted of 15 separate intersections (i.e., trials) which did not have any relation one to the other. In the encoding phase of each trial, the participants were positioned in the street next to the black car. Participants were then passively transported to the intersection. The intersection featured two unique houses, which were diagonally in opposite corners of the street. All other houses at the intersection and along the streets were identical brick houses, which could not serve as reliable landmarks because of their uniform appearance. The movement stopped 20m before the center of the intersection and participants were asked to remember the street in which the car was located. In the testing phase, participants were transported towards the same intersection but from one of three other streets. Participants had to indicate from which direction they originally approached the intersections (i.e., in which street the car was located). The options were again right, left or straight. The car could not be seen from the intersection as it was hidden in the white fog.

The car was always parked in the south street. When approaching the intersection during the testing phase, the approach direction was from the east, north or west. Importantly, participants were not informed about cardinal directions in this task. When approaching the intersection in the encoding phase, participants always saw the intersection from a different

perspective and had to perform a perspective shift and imagine the intersection from a different direction to determine the position of the car. The perspective shift was the greatest when approaching the intersection from north (i.e., 180° shift). When approaching from east or west, the perspective shift was only 90°. The magnitude of perspective shift was important for task performance assessment.

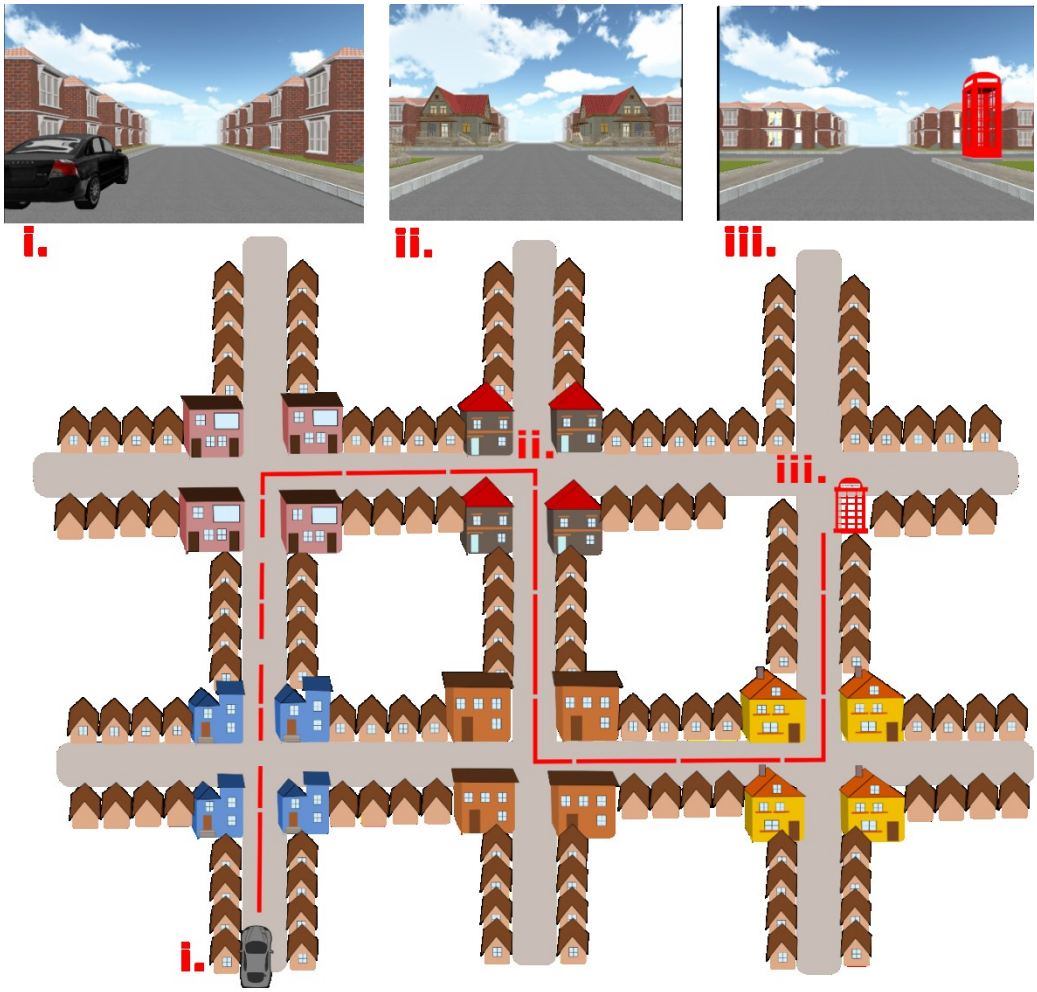


Fig. 4A The Navigation Test Suite with schematic aerial view and corresponding screenshots from the Route-repetition and the Route-retracing tasks. Three points on the map are labeled: (i.) The start location next to the car. (ii.) One of the intersections along the route with gray houses at the corners of the intersection. (iii.) The end of the route where the telephone box is present. In the Route-repetition task, the participants were passively transported through the city from the car to the telephone box during the encoding phase and in the test phase the participants had to reproduce the same route. The Route-retracing task was identical to the Route-repetition task with the exception that participants in the test phase had to find their way back from the telephone box to the car. The order of intersections and houses at each intersection had different design in each of these two tasks.

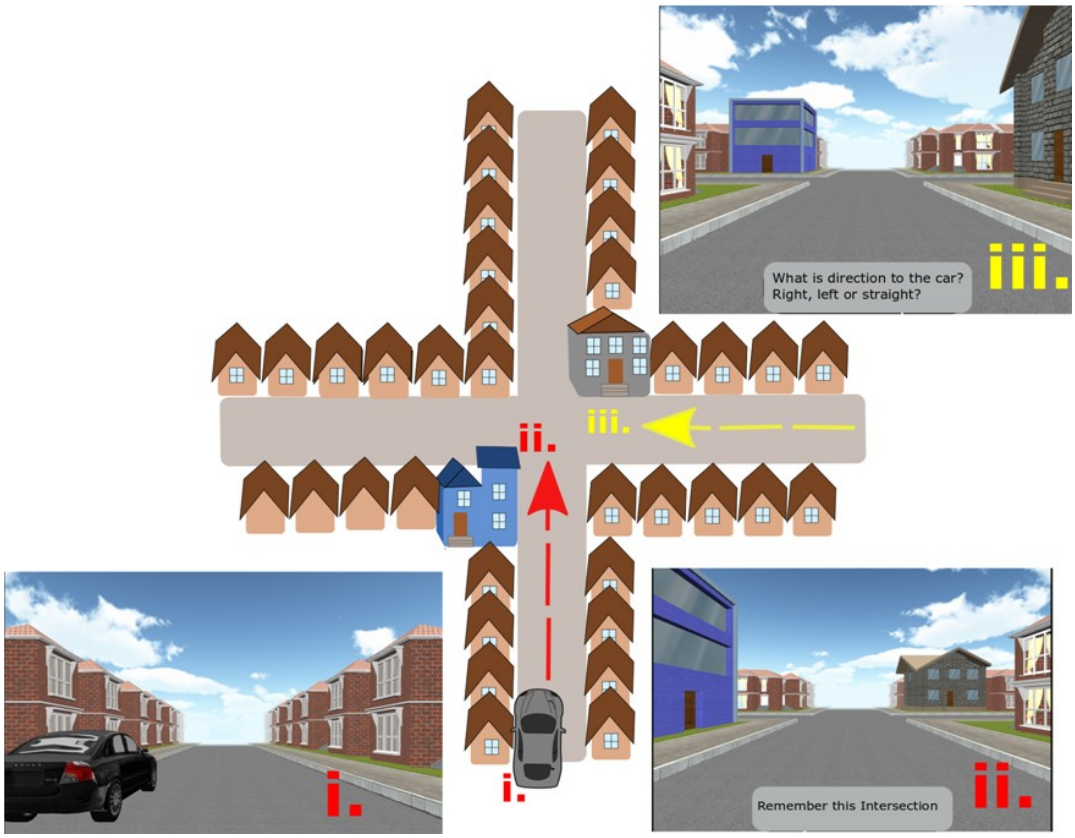


Fig. 4B The Navigation Test Suite with schematic aerial view and corresponding screenshots from the Directional-approach task: (i.) Participants started the task next to the car. (ii.) The encoding phase, where participants were passively transported towards one of the intersections featuring two unique houses. Participants had to remember where the car was parked. (iii.) The test phase, where participants approached the intersection from a different direction (here from east) and had to indicate direction to the car.

3.2.2.3. Nausea assessment questionnaire

Nausea assessment questionnaire was performed after the Y-maze and Navigation Test Suite tasks. This questionnaire used in previous studies (Moffat and Resnick, 2002) was administered after completing the tasks in virtual reality to identify participants with severe nausea.

3.2.3. Study 3 and 4 specifics

3.2.3.1. Study 3 participants

A total of 98 participants were recruited from the Czech Brain Aging Study cohort and they were classified into 3 groups: CN participants (n = 23), AD aMCI (n = 44) and mild AD dementia (n = 31).

3.2.3.2. Study 4 participants

A total of 122 participants were recruited from the Czech Brain Aging Study cohort and they were classified into 4 groups: CN participants (n = 30), non-AD aMCI (n = 31), AD aMCI (n = 33), and mild AD dementia (n = 28).

3.2.3.3. Spatial pattern separation task

The Spatial pattern separation task was performed on a wide screen monitor (Fig. 5). The screen background was white during the whole task. First, participants saw a blue circle (measuring 2 cm in diameter) and they had 5 seconds to remember its position on the screen. Afterwards, the circle disappeared and random numbers started appearing in the middle of the screen. Participants were instructed to read these numbers aloud. This was done to prevent them from fixating their vision on the original position of the circle. The numbers appeared for 10 or 20 seconds in Study 3 and for 20 seconds only in Study 4. The paradigm was modified in Study 4 based on the results from Study 3, because the results for 20s delay were superior to those for 10s delay. After the delay, two identical circles appeared on the screen and one of them (i.e., the correct one) was in the original position, while the other circle was 0 (edges of the circles were touching), 0.5, 1.0, and 1.5 cm away from the correct circle. Participants were holding two buttons, one in the right hand and one in the left hand. They indicated using these buttons which circle (i.e., the right or left) was the correct one. A small black cross appeared in the middle of the screen, after the decision was made to separate individual trials. There was no feedback provided. The task had 64 trials in the Study 3 and these trials included 32 trials with 10 s delay and 32 trials with 20s delay. In Study 4, the task included only 32 trials, all with 20 s delay. There was always a short break after 16 trials to reduce the fatigue in both studies. Four different separation distances (i.e., 0, 0.5, 1.0 and 1.5cm) were used to assess the effect of spatial separation distance on performance. Trials in both studies contained the same proportional number of individual separation distances. Participants completed a short training in the

beginning of the assessment. Training was identical to the testing part with the exception that participants got feedback after giving their responses.

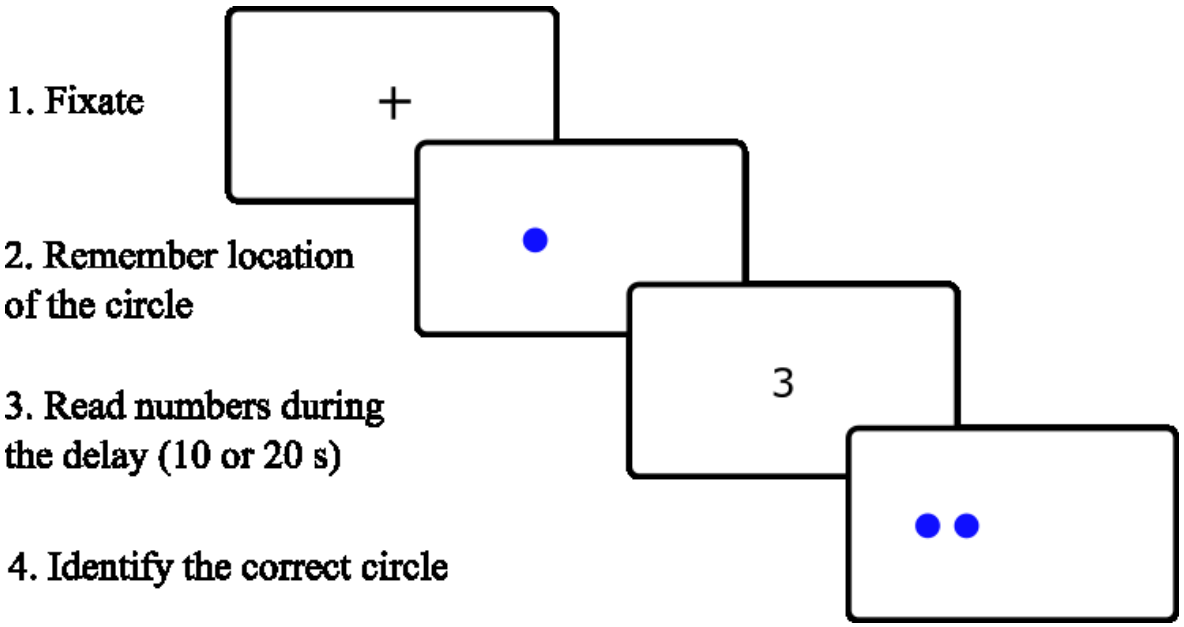


Fig. 5 Example of a spatial pattern separation task trial as seen by participants on the computer screen.

4. RESULTS

4.1. Study 1 results

4.1.1. Navigation strategy preference

From 69 participants who entered the study, 59 participants showed consistent strategy selection (i.e., choosing the same strategy, world-centered or body-centered, in at least 4 of the 5 blocks). Remaining 10 participants with inconsistent strategy were excluded from the analysis. According to the stepwise regression analysis, among demographic characteristics, only gender was associated with strategy selection (odds ratio = 5.99, $p = 0.032$), therefore was further included in the main analyses (i.e., analysis of variance [ANOVA]).

The χ^2 test was performed to investigate differences in strategy preference between the groups. Preference for navigation strategy in Y-maze differed across the groups ($\chi^2 = 11.9$, $p = 0.003$). Participants in the CN group preferred the world-centered navigation strategy (39% body-centered, 61% world-centered), while participants in the AD aMCI group preferred the body-centered navigation strategy (67% body-centered, 33% world-centered), and preference for body-centered navigation strategy was even stronger in participants with the mild AD dementia (94% body-centered, 6% world-centered). Strategy selection did not differ between males and females ($\chi^2 = 2.32$, $p = 0.128$).

4.1.2. The effect of strategy preference on allocentric navigation performance

To assess the effect of Y-maze strategy preference on world-centered navigation performance in real space, we performed a 3 (CN vs. aMCI vs. dementia) x 2 (male vs. female) x 2 (body-centered vs. world-centered Y-maze strategy preference) ANOVA with world-centered navigation distance error as a dependent measure. There was a main effect of the group on world-centered navigation performance [$F(2) = 21.35$, $p < 0.001$], where the AD aMCI and mild AD dementia groups had less accurate world-centered navigation performance than the CN group ($p < 0.001$). Further, the dementia group had worse world-centered navigation performance than the aMCI group ($p < 0.001$). There was no significant main effect of strategy selection [$F(1) = 0.01$, $p = 0.935$] or gender [$F(1) = 0.64$, $p = 0.428$] on world-centered navigation performance. However, there was a significant interaction between the group and strategy selection [$F(2) = 5.13$, $p = 0.010$]. Specifically,

participants in the AD aMCI group who preferred the body-centered navigation strategy had less accurate performance in the real space world-centered navigation task than those who preferred the world-centered navigation strategy ($p = 0.003$). Other interactions were not significant.

4.1.3. The role of hippocampal and BF volumes in the association between strategy selection and world-centered navigation performance

In the correlation analyses (with Holm-Bonferroni [H-B] correction for multiple comparisons), lower total, right, and left hippocampal volumes and lower BF Ch1-2 nuclei volumes correlated with less accurate world-centered navigation performance ($r \geq 0.366$, $p \leq 0.004$). To investigate the role of hippocampal and BF volumes in the association between strategy selection and world-centered navigation performance in real space, we used the 3 (group) x 2 (gender) x 2 (strategy preference) analyses of covariance (ANCOVAs) adjusted for hippocampal and BF volumes. Further, the proportion of the differences accounted for by volumes of the hippocampus and BF was calculated by the formula: % accounted for = (adjusted mean difference $_{\text{basic model}}$ - adjusted mean difference $_{\text{model with volumetric measurements}}$/adjusted mean difference basic model) x 100. In the AD aMCI group, total hippocampal volume accounted for 14%, left hippocampal volume for 9%, and right hippocampal volume for 20% of the association between strategy preference and world-centered navigation performance. Volumes of the Ch4p (posterior part of the nucleus basalis of Meynert) and Ch 1-2 (the medial septal nuclei and vertical limb of the diagonal band of Broca) nuclei accounted for 24% and 25% of this association, respectively.

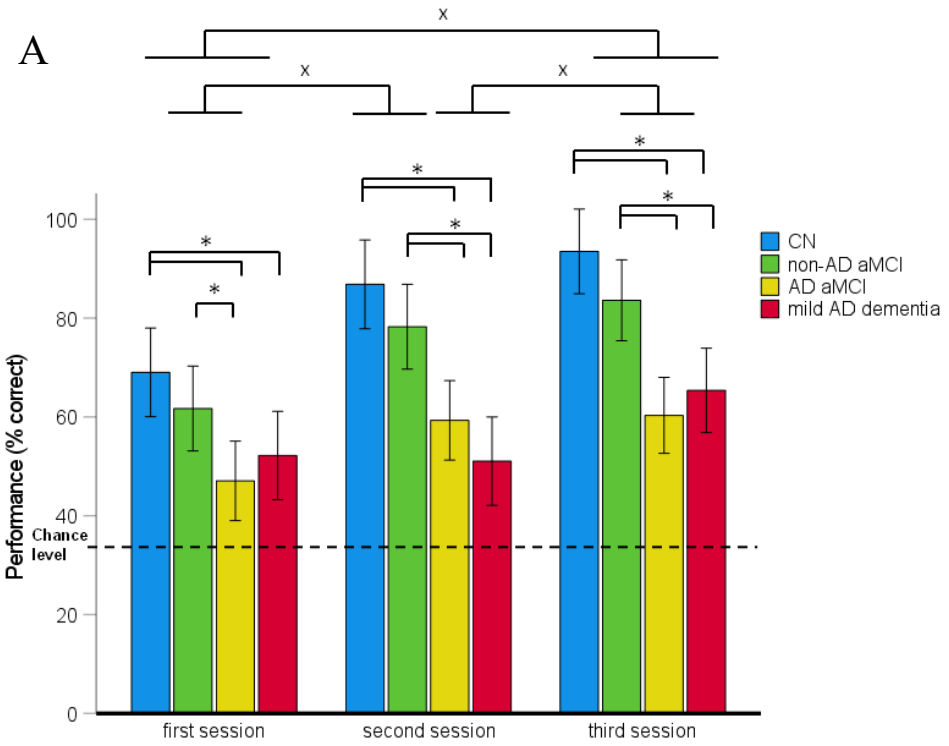
4.2. Study 2 results

4.2.1. Statistical methods and demographic characteristics

Spatial navigation performance was measured as the mean percentage of correct responses in each Navigation Test Suite task in the CN, non-AD aMCI, AD aMCI and mild AD dementia groups. The results are presented in Fig. 5. We analyzed performance in specific sessions in the Route-repetition (Fig. 6A) and Route retracing (Fig 6B) tasks and performance in specific approach directions in the Directional-approach task (Fig. 6C). The mixed model ANCOVA with the diagnostic group (CN vs. non-AD aMCI vs. AD aMCI vs. mild AD dementia) as between-subject factor and the session (1st vs. 2nd vs.

3rd) or the approach direction (east vs. north vs. west) as the within-subjects factor was performed to assess the effect of the diagnostic group and session on performance (i.e., the dependent variable). The analysis was controlled for age (mean-centered), years of education (mean-centered) and gender. The differences between the individual groups and sessions or approach directions were assessed using the post hoc Sidak's test. Further, the between-group differences in each session or approach direction were assessed using the post hoc pairwise comparisons with the H-B correction. A one-sample t-test was used to compare performance to the chance level performance (i.e., 33.33%) at each session and approach direction. The ROC analysis (including areas under the ROC curves [AUCs], sensitivity and specificity analyses at optimal cut-off values) was performed to evaluate the potential of each task to differentiate non-AD aMCI from AD aMCI. Pearson's correlation coefficients with the H-B correction were calculated to analyze the association between regional brain atrophy on MRI and each spatial navigation task. In the next step, separate linear regression models adjusted for age, years of education and gender were used to explore significance of associations controlling for demographic factors.

The CN group was younger than the mild AD dementia group ($p = 0.008$) and more educated than the non-AD aMCI group ($p = 0.003$). Otherwise, no differences in demographic characteristics were found between the groups. Importantly, the AD aMCI and non-AD aMCI groups did not differ in cognitive performance measured by conventional neuropsychological tests (all $p \geq 0.100$).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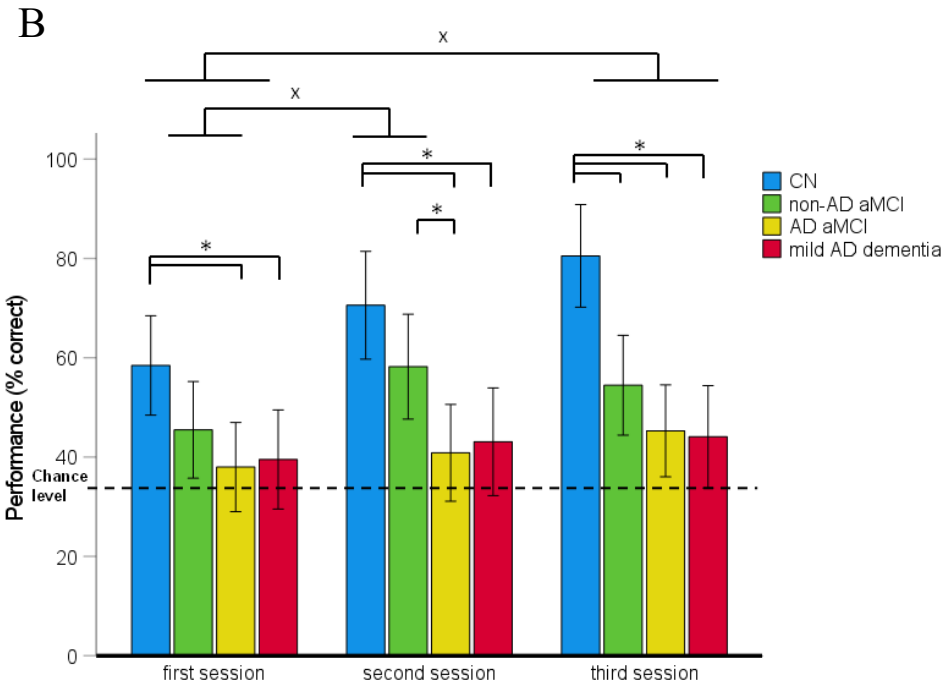
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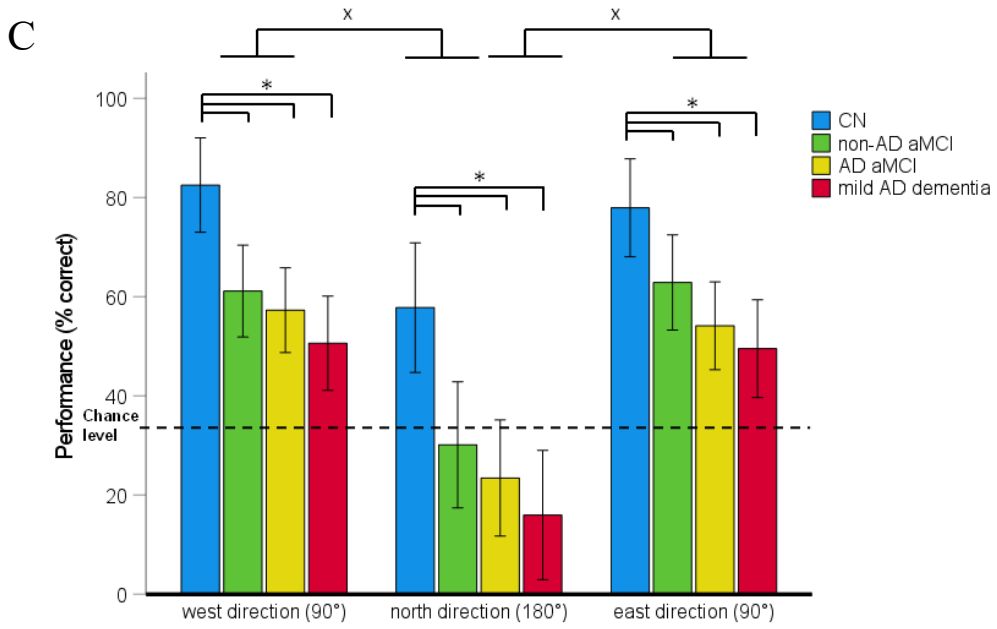


Fig. 6 Navigation Test Suite task performance: A) Route-repetition task, B) Route-retracing task, C) Directional-approach task —spatial navigation performance as mean percentage of correct responses in each session (95% CI). * $p < 0.05$ indicating the differences between the groups; $^{\chi}$ $p < 0.05$ indicating the differences between the sessions; CN, cognitively normal; non-AD aMCI, amnestic mild cognitive impairment with negative AD biomarkers; AD aMCI, amnestic mild cognitive impairment with Alzheimer's disease; mild AD dementia, mild dementia with Alzheimer's disease; CI, confidence interval.

4.2.2. Navigation Test Suite performance

4.2.2.1. Route-repetition task performance

There was a significant effect of the diagnostic group ($F[3, 122] = 20.67$, $p < 0.001$) and session ($F[2, 244] = 26.96$, $p < 0.001$), while the session-by-group interaction was not significant ($F[6, 244] = 1.98$, $p = 0.069$). In general, the AD aMCI group performed worse than the non-AD aMCI group ($p < 0.001$, 95% CI [-29.76, -8.17]) and the CN group ($p < 0.001$, 95% CI [-38.69, -16.44]), while having comparable performance to the mild AD dementia group ($p = 1.00$, 95% CI [-11.58, 10.27]). The non-AD aMCI group had similar performance to the CN group ($p = 0.272$, 95% CI [-20.29, 3.09]). The group performance improved across the sessions (i.e., second v.s first ($p < 0.001$) and third vs. second ($p = 0.020$)). The groups performed above the chance level in all sessions ($p \leq 0.005$). The AD aMCI group had worse performance than the non-AD aMCI (all $p_{H\text{-}Bcorrected} \leq 0.031$) and CN (all $p_{H\text{-}Bcorrected} \leq 0.002$) groups in all three sessions. According to the ROC analysis,

the Route-repetition task differentiated the non-AD aMCI from the AD aMCI group with an AUC value of 0.78 ($p < 0.001$).

4.2.2.2. Route-retracing task performance

There was a significant effect of the diagnostic group ($F[3, 121] = 12.83$, $p < 0.001$) and session ($F[2, 242] = 7.47$, $p < 0.001$), while the session-by-group interaction was not significant ($F[6, 242] = 1.16$, $p = 0.331$). In general, the AD aMCI group had worse performance than the CN group ($p < 0.001$, 95% CI [-41.88, -15.07]) and did not differ from the non-AD aMCI ($p = 0.128$, 95% CI [-24.48, 1.80]) and mild AD dementia ($p = 1.00$, 95% CI [-14.02, 12.31]) groups. The non-AD aMCI group performed worse than the CN group ($p = 0.009$, 95% CI [-31.30, -2.96]). In the analysis of individual sessions, the AD aMCI group had worse performance than the non-AD aMCI group in the second session ($p_{H\text{-}Bcorrected} = 0.032$) and worse performance than the CN group in all three sessions (all $p_{H\text{-}Bcorrected} \leq 0.016$), while the non-AD aMCI group was similar to the CN group with $\leq$ 0.016), while the non-AD aMCI group was similar to the CN group with the exception of worse performance in the third session ($p_{H\text{-}Bcorrected} = 0.003$). The CN and non-AD aMCI groups performed above the chance level in all sessions ($p \leq 0.009$). In contrast, performance of the AD aMCI group did not differ from the chance level in the first and second session ($p \geq 0.223$) and exceeded the chance level only in the third session ($p = 0.026$). According to the ROC analysis, the Route-retracing task differentiated the non-AD aMCI from the AD aMCI group with a AUC value of 0.64 ($p = 0.041$). The group performance improved across the sessions (i.e., second v.s first ($p = 0.021$) and third vs. first ($p < 0.001$)).

4.2.2.3. Directional-approach task performance

There was a significant effect of the diagnostic group ($F[3, 121] = 14.16$, $p < 0.001$) and approach direction ($F[2, 242] = 64.19$, $p < 0.001$), while the approach direction-by-diagnostic group interaction was not significant ($F[6, 242] = 0.26$, $p = 0.955$). In general, the groups with cognitive impairment (i.e., non-AD aMCI, AD aMCI and mild AD dementia) had worse performance than the CN group ($p \leq 0.001$) and did not differ between each other. The groups had worse performance when the approach direction was from north (i.e., 180° perspective shift) compared to the conditions when the approach direction was from the west and east (i.e., 90° perspective shift) ($p < 0.001$). The CN group

outperformed all remaining groups at each approach direction (all $p_{H\text{-}Bcorrected} \leq 0.044$), while there was no difference between the non-AD aMCI and AD aMCI groups in any approach direction. Further, the CN group performed above the chance level in all approach directions (all $p \leq 0.011$). All three other groups performed above the chance level only when approaching from the west and the east ($p \leq 0.003$) and at or below the chance level when approaching from the north. According to the ROC analysis, the Directional-approach task did not differentiate the non-AD aMCI from the AD aMCI group (AUC value of 0.62, $p = 0.109$). However, the task differentiated the CN group from the cognitively impaired groups with AUC values of ≥ 0.717 ($p \leq 0.001$).

4.2.2.4. Association between regional brain atrophy and spatial navigation performance

The correlation analysis with H-B correction showed that worse performance in the Route-repetition task was associated with reduced thickness of the right and left precuneus and posterior parietal cortex and smaller volume of the right alEC (all $r \geq 0.38$, $p \leq 0.001$). Worse performance in the Route-retracing task was associated with the smaller volumes of the right hippocampal body and the right and left pmEC (all $r \geq 0.34$, $p \leq 0.001$). Finally, worse performance in the Directional-approach task was associated with smaller volumes of the left hippocampal body, the right hippocampal tail and alEC, the right and left pmEC, and reduced thickness of the right isthmus cingulate/RSC, the right and left precuneus and posterior parietal cortex (all $r \geq 0.32$, $p \leq 0.001$). The associations (except the one of right alEC volume and Directional-approach task performance) remained significant in the regression analyses adjusted for age, education and gender (all $ß \geq 0.24$, $p \leq 0.030$).

4.2.2.5. Association between CSF biomarkers and spatial navigation performance

According to the correlation analysis, lower CSF levels of amyloid-$\beta_{1\text{-}42}$ correlated with worse performance in the Route-repetition and Directional-approach tasks (both $r \geq 0.31$, $p \leq 0.032$), higher CSF levels of total tau correlated with worse performance in the Directional-approach task ($r = -0.31$, $p = 0.041$), and higher CSF levels of p-tau_{181} correlated with worse performance in the Route-retracing and Directional-approach tasks (both $r \geq -0.30$, $p \leq 0.043$). The subsequent regression analyses controlled for demographic factors (i.e., age, education and gender) showed that lower amyloid-$\beta_{1\text{-}42}$ CSF levels were associated with less accurate performance in the Route-repetition task ($ß = 0.39$, $p = 0.005$)

and higher p-tau$_{181}$ CSF levels were associated with less accurate performance in the Route-retracing (ß = -0.28, p = 0.041) and Directional-approach (ß = -0.29, p = 0.037) tasks. Other associations in the regression analyses were not significant.

4.3. Study 3 results

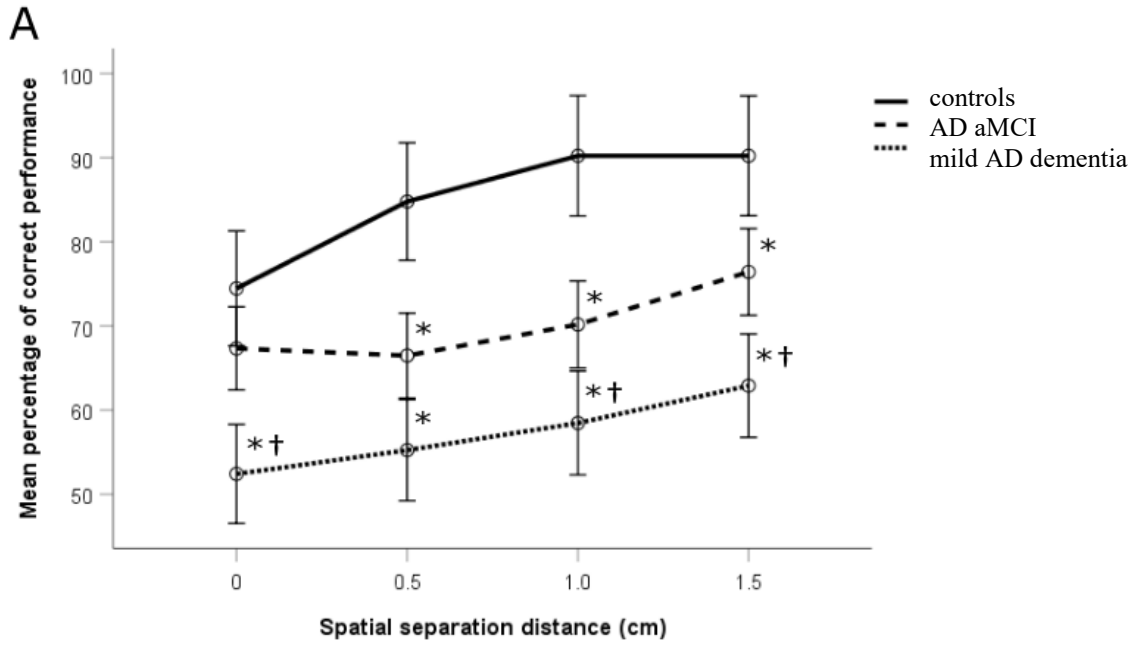
4.3.1. Demographic characteristics

The CN group was younger than the AD aMCI and mild AD dementia groups ($p < 0.001$). The mild AD dementia group had less years of education compared to the CN group and there were no differences in gender proportion between the groups. The AD aMCI had worse performance in most of the cognitive tests compared to the CN group ($p < 0.001$).

4.3.2. Spatial pattern separation performance

Spatial pattern separation performance was measured as the mean percentage of correct responses in the CN, AD aMCI and mild AD dementia groups for time delay of 10 s and 20 s. The results are presented in Fig. 7. The 3 × 2 × 4 mixed factorial ANOVA with diagnostic group (CN vs. AD aMCI vs. mild AD dementia) as the between-subjects factor and time delay (10 s vs. 20 s) and spatial separation (0 vs. 0.5 vs. 1.0 vs. 1.5 cm) as the within-subjects factors was used to analyze accuracy of spatial pattern separation performance measured as percentage of correct responses, which was a dependent variable. The post hoc Sidak´s test was used to assess the between-group differences. We observed a significant main effect of the diagnostic group ($F[2, 95] = 75.65$, $p < 0.001$), where on average, the AD aMCI ($p < 0.001$, 95% CI [12.18, 22.76]) and mild AD dementia ($p < 0.001$, 95% CI [23.22, 34.54]) groups had worse spatial pattern separation performance compared to the CN group. Specifically, the CN group outperformed the AD aMCI and mild AD dementia groups in all spatial separations ($p \leq 0.002$) and the differences remained significant after the H-B correction. The overall performance of the mild AD dementia group was worse compared to the AD aMCI group ($p < 0.001$, 95% CI [6.56, 16.23]). Specifically, the AD aMCI group had better performance than the mild AD dementia group at the 0, 1.0, and 1.5 cm spatial separations ($p \leq 0.010$) and these findings remained significant after the H-B correction.

There was no effect of time delay (10 s vs. 20 s) on spatial pattern separation performance ($F[1, 95] = 0.09$, $p = 0.761$), and the interaction between the diagnostic group and time delay was also not significant ($F[2, 95] = 0.77$, $p = 0.465$). However, spatial separation distance had a significant effect on performance ($F[3, 285] = 20.12$, $p < 0.001$). Specifically, spatial separation distance had a significant linear effect on spatial navigation ($F[1,95] = 52.46$, $p < 0.001$), where performance was linearly increasing with increasing spatial separation (i.e., with increasing distance between the circles). Other interactions were not significant.



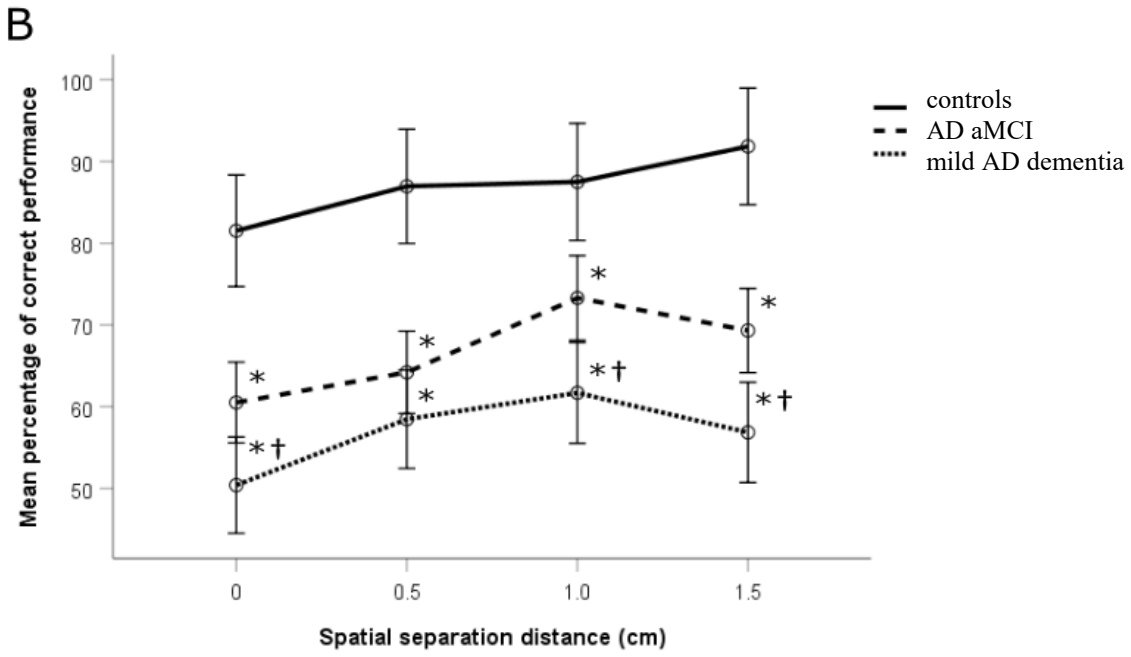


Fig. 7 Spatial pattern separation performance A) Mean percentage of correct performance for each spatial separation for time delay of 10 s (±1 SE). B) Mean percentage of correct performance for each spatial separation for time delay of 20 s (±1 SE). *$p < 0.05$ compared to the CN group; †$p < 0.05$ compared to the AD aMCI group. AD aMCI, amnestic mild cognitive impairment with Alzheimer's disease; mild AD dementia, mild Alzheimer's disease dementia.

The 3×2×4 mixed model ANCOVA controlling for neuropsychological tests scores, which correlated with spatial pattern separation performance (i.e., ROCFT Recall, logical memory, and RAVLT Delayed Recall), was performed to analyze whether the differences in spatial pattern separation between the groups could be explained by general cognitive decline. Next, the ANCOVA analysis controlling for demographic characteristics (i.e., age, education and gender) was performed to assess the potential influence of these factors on performance. After controlling for performance in neuropsychological tests, the effect of the diagnostic group ($F[2, 94] \geq 15.42$, $p < 0.001$) and spatial separation ($F[3, 282] \geq 4.96$, $p \leq 0.002$) remained significant. After controlling for demographic factors, the main effect of the diagnostic group remained significant as well ($F[2, 90] = 47.27$, $p < 0.001$).

The ROC analysis was performed to determine the potential of the spatial pattern separation task to discriminate the CN group (reference) from the AD aMCI group, separately for 10 s and 20 s time delay. The AUC value for 10 s time delay was 0.84 (95% CI [0.75, 0.94], $p < 0.001$) and for 20 s time delay was 0.92 (95% CI [0.85, 0.99], $p < 0.001$). According to the Youden's index, for the 10 s delay, the optimal cut-off value was 26 (out of 32) correct responses with sensitivity of 77% and specificity of 82%. For the 20 s delay, the optimal cut-off value was 25 (out of 32) correct responses with specificity of 82% and sensitivity of 82%. Pattern separation performance after 10 s and 20 s delay had comparable AUCs (AUC difference = 0.08, $p = 0.173$).

4.3.3. Association between regional brain atrophy and spatial pattern separation performance

Pearson's correlation with the H-B correction was used to assess the associations between hippocampal, EC and Ch1-2 nuclei volumes and spatial pattern separation performance after 10 s and 20 s delay. Lower hippocampal, EC, and Ch 1-2 nuclei volumes correlated with less accurate spatial pattern separation performance after 10 s and 20 s delay ($r \geq 0.25$, $p \leq 0.020$). Next, we performed a multivariate linear regression analysis controlled for total brain volume and demographic characteristics (i.e., age, education and gender). All these

associations between lower hippocampal, EC, and Ch 1-2 nuclei volumes and less accurate spatial pattern separation performance after 10 s and 20 s remained significant in the analysis ($\beta \geq 0.25$, $p \leq 0.018$).

4.4. Study 4 results

4.4.1. Demographic characteristics

The CN group was more educated than the mild AD dementia group ($p = 0.007$) and there were more women in the CN and mild AD dementia groups than in the non-AD aMCI and AD aMCI groups (79 and 73% vs. 46% and 51%). The non-AD and AD aMCI groups did not differ in cognitive performance in conventional neuropsychological tests.

4.4.2. Spatial pattern separation performance

The results of the spatial pattern separation performance are presented in Fig. 8. The 4 × 4 mixed factorial ANOVA with diagnostic group (CN vs. non-AD aMCI vs. AD aMCI vs. mild AD dementia) as the between-subjects factor and spatial separation (0 vs. 0.5 vs. 1.0 vs. 1.5 cm) as the within-subjects factor was used to analyze spatial pattern separation performance measured as the percentage of correct responses (i.e., the dependent variable). The post hoc Sidak´s test was used to assess the between-group differences. The main effect of the diagnostic group was significant ($F[3,114] = 22.29$, $p < 0.001$). On average, the AD aMCI group had worse performance in spatial pattern separation compared to the non-AD aMCI ($p = 0.039$, 95% CI [–18.80, –0.31]) and CN ($p < 0.001$, 95% CI [–29.21, –11.29]) groups, while having similar performance to the mild AD dementia group ($p = 0.190$, 95% CI [–1.86, 16.64]). The non-AD aMCI group had less accurate performance than the CN group ($p = 0.024$, 95% CI [–20.45, –0.94]) and more accurate performance than the mild AD dementia group ($p < 0.001$, 95% CI [6.93, 26.97]). Further, there was a significant main effect of spatial separation ($F[1.91,218.01] = 6.05$, $p = 0.003$). Specifically, there was a significant linear effect of spatial separation ($F[1,114] = 11.88$, $p = 0.001$) where, on average, as the distance in spatial separation increased, the performance improved. The spatial separation-by-diagnostic group interaction was not significant ($F[5.74,218.01] = 0.79$, $p = 0.571$).

Next, we assessed the differences in spatial pattern separation performance between

individual groups for each spatial separation using post hoc pairwise comparisons with the H-B correction. The AD aMCI group had less accurate performance than the non-AD aMCI group at the 1.5 cm spatial separation ($p = 0.008$) and the CN group at each spatial separation ($p \leq 0.007$). The AD aMCI group did not differ from the mild AD dementia group at any spatial separation ($p \geq 0.101$). The non-AD aMCI group had less accurate performance than the mild AD dementia group at the 0.5, 1.0, and 1.5 cm spatial separations ($p \leq 0.028$) and did not differ from the CN group at any spatial separation ($p \geq 0.070$). A one-sample t-test was used to assess differences from the chance level performance (i.e., 50%). The CN, non-AD aMCI, and AD aMCI groups performed above the chance level in the task overall and at each spatial separation ($p \leq 0.005$), while the mild AD dementia group performed at the chance level at 0.0 and 1.0 cm spatial separations ($p \geq 0.098$).

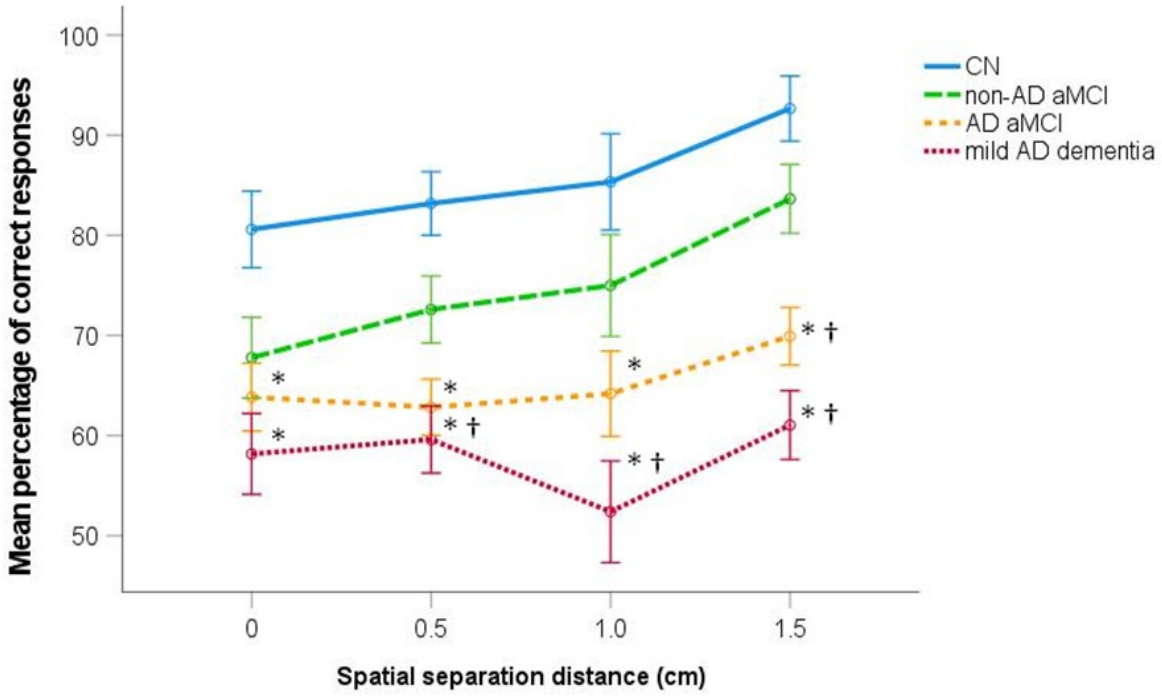


Fig. 8 Spatial pattern separation performance. Mean percentage of correct performance for each spatial separation (±1 SE). ∗p < 0.05 compared to the CN group; †p < 0.05 compared to the non-AD aMCI group. CN, cognitively normal; non-AD aMCI, amnestic mild cognitive impairment with non-Alzheimer's pathologic change; AD aMCI, amnestic mild cognitive impairment with Alzheimer's disease; mild AD dementia, mild dementia with Alzheimer's disease.

The 4 x 4 mixed factorial ANCOVA controlling for demographic characteristics (age, education and gender) was performed to assess a potential influence of demographic factors on performance. After controlling for demographic factors, the main effect of the

diagnostic group remained significant ($F[3,113] = 21.57$, $p < 0.001$. Specifically, the AD aMCI group had worse performance than the non-AD aMCI ($p < 0.050$, 95% CI [–18.48,–0.01]) and CN ($p < 0.001$, 95% CI [–28.38, –10.30]) groups, while having similar performance to the mild AD dementia group ($p = 0.183$, 95% CI [– 1.79, 16.64]). The non-AD aMCI group had better performance compared to the mild AD dementia group ($p < 0.001$, 95% CI [5.25, 25.23]). However, the differences between the non-AD aMCI and CN groups were no longer significant ($p = 0.080$, 95% CI [–19.29, 0.66]).

The ROC analysis was performed to determine the potential of the spatial pattern separation task to discriminate the CN group (reference) from the groups with cognitive impairment and to differentiate the non-AD aMCI group (reference) from the AD aMCI group. Spatial pattern separation task differentiated the CN group from all cognitively impaired groups including the non-AD aMCI, AD aMCI and mild AD dementia groups with AUC values of 0.76 (95% CI [0.63, 0.89], $p = 0.001$), 0.88 (95% CI [0.81, 0.96], $p < 0.001$), and 0.94 (95% CI [0.87, 1.00], $p < 0.001$), respectively. Further, the task was able to differentiate the non-AD aMCI from the AD aMCI group with a AUC value of 0.67 (95% CI [0.53, 0.80], $p = 0.024$).

4.4.3. Association between regional brain atrophy and spatial pattern separation performance

The associations between spatial pattern separation performance and volumes of the hippocampal and EC subregions and the BF Ch1-2 nuclei were assessed using Pearson's correlation with H-B correction. Lower volumes of the hippocampal tail and body, pmEC and BF Ch1-2 nuclei correlated with less accurate spatial pattern separation performance ($r \geq 0.28$, $p \leq 0.006$). Next, the linear regression models controlled for age, education and gender were performed to assess a potential contribution of demographic factors on these associations. All these associations between lower volumes of the hippocampal tail and body, pmEC and BFCh1-2 nuclei and less accurate spatial pattern separation performance remained significant in the analyses ($\beta \geq 0.26$, $p \leq 0.017$).

5. DISCUSSION

5.1. Study 1

5.1.1. Spatial navigation strategy preference and world-centered navigation performance

This study assessed spatial navigation strategy preferences and their relation to spatial navigation performance in the early clinical stages of AD. Strategy preferences were tested using a virtual Y-maze task and spatial navigation performance was assessed in real space. Consistent with our hypothesis, participants with AD preferred body-centered navigation more than the CN older adults and this preference for body-centered strategy increased with the severity of the disease. Specifically, the participants with mild AD dementia had even stronger preference for body-centered strategy than the participants with AD aMCI. Cognitively impaired participants also had less accurate world-centered navigation performance consistently with the previous findings (Laczó et al., 2011; Weniger et al., 2011; Allison et al., 2016) and this deficit was more pronounced in the participants with mild AD dementia than those with AD aMCI. In contrast to the previous studies (Rodgers et al., 2012) the CN participants in the current study had a more pronounced preference world-centered navigation strategy, which could be caused by specific modifications of our task. The modifications included simplification of instructions for cognitively impaired participants, where a paper diagram showing an overhead view of the Y-maze was presented before the assessment and the participants were instructed to pay attention to the Y-maze and the objects in the surroundings. This modification may have increased preference of world-centered strategy in the CN participants. However, it did not increase the preference for world-centered strategy in cognitively impaired groups. World-centered navigation strategy relies predominantly on the MTL structures and their cholinergic input from the BF, which undergo neurodegenerative changes in AD (Maguire et al., 1998; Nedelska et al., 2012; Kerbler et al., 2015). On the other hand, body-centered navigation relies on extra-hippocampal brain regions, especially the posterior parietal cortex and the caudate nucleus, where neurodegeneration occurs in the later stages of AD (Maguire et al., 1998). Therefore, increasing preference for body-centered strategy in AD indicates involvement of extra-hippocampal brain regions and reliance on compensatory navigation strategies (Iaria et al., 2009). Next, the low preference for the world-centered navigation strategy was associated with worse world-centered navigation performance in real space in the participants with AD aMCI. This corresponds to previous research, which showed that

world-centered navigation deficits in older adults lead to the preferential use of extra-hippocampal strategies (Colombo et al., 2017). Our results suggest that world-centered navigation deficits in early AD (i.e., individuals with AD aMCI) lead to the change in strategy preference, especially the recruitment of compensatory extra-hippocampal strategies.

5.1.2. The associations of spatial navigation strategies with brain atrophy

Further, we explored the roles of specific brain regions affected early in AD in the association between strategy preference and world-centered navigation performance in real space. A typical feature of AD is degeneration of the hippocampus, which occurs in the early stages (Braak and Braak, 1995). This study supports previous findings of the association between hippocampal atrophy and world-centered navigation deficits in AD (Nedelska et al., 2012). Further, this study showed that right and left hippocampal atrophy explained 22% and 9%, respectively, of the association between strategy preference and world-centered navigation performance in AD aMCI participants. These results indicate that AD-related neurodegenerative changes in the hippocampus lead to decline in world-centered navigation and increased tendency towards the use of extra-hippocampal navigation strategies as a compensation for neurodegenerative changes.

Additionally, the role of the BF in the association between strategy preference and world-centered navigation was explored. The BF is among the first structures affected by AD (Mufson et al., 2003; Schliebs and Arendt, 2006) and provides acetylcholine for the hippocampus and EC which are strongly interconnected and essential for world-centered navigation (Hasselmo and McGaughy, 2004). The most important BF regions are the Ch1 (medial septum nucleus) together with Ch2 (vertical limb of the diagonal band) which represent the main cholinergic input to the hippocampus (Ikonen et al., 2002) and Ch4p (nucleus basalis of Meynert) which is the main input to the EC (Mesulam et al., 1983a). This study showed association of smaller Ch1-2 and Ch4p with worse world-centered navigation and atrophy of the Ch4p and Ch1-2 explained 24% and 25%, respectively, of the association between strategy preference and world-centered navigation in AD aMCI participants. This finding supports our hypothesis that worse world-centered navigation and inclination towards extra-hippocampal strategies may also be a consequence of AD-related changes in the BF.

5.2. Study 2

5.2.1. The main findings of the study

In this study we explored spatial navigation differences between AD positive and negative aMCI participants in various virtual realistic-looking spatial navigation tasks. The participants with AD aMCI and non-AD aMCI had similar performance in conventional cognitive tests, and we evaluated whether spatial navigation assessment could be used to specifically detect AD-related cognitive impairment and thus whether the test has a potential to be used in early and differential diagnosis of AD. We also explored the relationship between spatial navigation performance and MRI measures of atrophy of specific MTL, parietal and subcortical regions. Finally, we investigated the association of spatial navigation performance with CSF levels of AD biomarkers. We found that the AD aMCI participants had worse body-centered performance compared to the non-AD aMCI participants and that the AD aMCI participants also had a tendency to perform worse in some aspects of world-centered navigation. Worse body-centered navigation performance was associated with lower thickness of the parietal regions (i.e., the precuneus and posterior parietal cortex), while worse world-centered navigation was associated with lower volume of the MTL, especially the right posterior hippocampus and pmEC. Worse performance in the world-centered navigation/perspective taking task was associated with lower thickness of the parietal regions and right isthmus cingulate/RSC and lower volume of the MTL, especially the posterior hippocampus and the pmEC. Further, worse body-centered navigation performance was associated with lower levels of amyloid-$_{\beta 1\text{-}42}$, while worse world-centered navigation and performance in the world-centered navigation/perspective taking task were associated with higher levels of p-tau$_{181}$.

5.2.2. Route-repetition task (body-centered navigation)

5.2.2.1. Body-centered navigation performance in AD aMCI and non-AD aMCI participants

Consistent with our hypothesis, the AD aMCI participants had worse body-centered navigation compared to the participants with non-AD aMCI. The AD aMCI participants had similar performance as those with mild AD dementia and the participants with non-AD aMCI had similar performance to the CN older adults. These findings are consistent with previous research, which indicated that body-centered navigation assessment can

discriminate the CN older adults from participants with AD (Tu et al., 2015, 2017; Schöberl et al., 2020). On the other hand, body-centered navigation assessment did not differentiate preclinical AD (i.e., CN older participants with amyloid-β pathology), from CN older adults without amyloid-β pathology (Allison et al., 2016). These results indicate that body-centered navigation deficit might be specific for early clinical stages of AD (i.e., MCI), but not for the preclinical stages. Consistently with our recent findings (Laczó et al., 2021), all participants performed above the change level, thus no floor effect was observed in none of the groups, and all groups showed the effect of learning across all three experimental sessions.

5.2.2.2. The association of body-centered navigation with brain atrophy and CSF biomarkers

Worse body-centered navigation was associated with cortical thinning of the precuneus and posterior parietal cortex which was consistent with our hypothesis and with previous studies showing the association between body-centered navigation deficits and atrophy of the parietal regions (Weniger et al., 2011; Wolbers and Wiener, 2014). These findings indicate that body-centered spatial navigation impairment reflects neurodegeneration in the parietal cortex, which is typical for early AD (Landau et al., 2011). Worse body-centered navigation was also associated with lower volume of the right alEC. There is evidence that the alEC is involved in processing distance information from landmarks (Chen et al., 2019) and our study suggests that the alEC could be also involved in encoding directional information from proximal landmarks, however, more research is needed to investigate the role of the alEC. The current study also showed that greater burden of amyloid-β pathology, measured as low levels of amyloid-β in CSF, was associated with worse body-centered navigation. This result is consistent with previous findings of increased cortical amyloid-β pathology accumulation and worse scene recognition from a constant first person viewpoint in early AD (Maass et al., 2019).

5.2.3. Route-retracing task (world-centered navigation)

5.2.3.1. World-centered navigation performance in AD aMCI and non-AD aMCI participants

This study showed worse world-centered navigation in both AD aMCI and non-AD aMCI groups in comparison to the CN group. These findings are consistent with our hypothesis

and with the world-centered real space navigation deficits in amyloid-β positive and negative aMCI participants (Schöberl et al., 2020). Contrary to our hypothesis, there was no difference in overall performance between the participants with AD aMCI and non-AD aMCI. However, further analyses revealed the differences between the groups as the AD aMCI participants performed above the chance level only in the third session and had a chance level performance in first and second session, while the non-AD aMCI participants performed above the chance level in all three sessions. Performance at the chance level indicates a possible presence of the floor effect, which could lead to non-significant overall differences between the groups. Furthermore, the AD aMCI participants had worse performance than the non-AD aMCI participants in the second session of the task. In general, these results indicate that the participants with AD aMCI had a tendency to underperform in the world-centered task compared to the participants with non-AD aMCI. World-centered navigation testing has been previously shown as a reliable method for identification of preclinical AD (Allison et al., 2016, 2019), however, there were inconsistent results in a potential of world-centered navigation testing to differentiate cognitively impaired individuals with AD from those of other etiologies. On one hand, the large-scale real space paradigm which required creating novel routes, differentiated amyloid-β positive from amyloid-β negative aMCI participants (Schöberl et al., 2020). On the other hand, another task focused on world-centered navigation which required location identification on a map failed to discriminate participants with AD from those of other neurodegenerative diseases (Tu et al., 2017). In general, these studies indicate that world-centered navigation deficit is characteristic of preclinical AD (Allison et al., 2016, 2019) and further declines as the disease progresses to its clinical stages (i.e., aMCI and dementia) (Hort et al., 2007; Levine et al., 2020). However, world-centered navigation assessment has a limited potential to differentiate cognitively impaired participants with AD from non-AD etiologies. The potential for differential diagnosis of cognitively impaired individuals might depend on specific features of the navigation task and whether the floor effect is present or not.

5.2.3.2. The association of world-centered navigation with brain atrophy and CSF biomarkers

Consistently with the hypothesis, less accurate world-centered navigation was associated with lower volumes of the right posterior hippocampus (i.e., hippocampal body) and the pmEC. These results are consistent with a notion that posterior regions of the MTL, which

are early affected by AD pathology (Scahill et al., 2002; Du et al., 2004; Tapiola et al., 2008), support world-centered navigation. The previous studies showed that the right hippocampus (Maguire et al., 1998; Nedelska et al., 2012; Laczó et al., 2017) and especially its posterior region is involved in creation and use of cognitive maps (Doeller et al., 2008; Schinazi et al., 2013). Similarly, the pmEC was previously shown to be important for world-centered navigation (Chadwick et al., 2015) and processing of spatial information (Berron et al., 2018). However, neurodegeneration in the MTL region is observed also in neurodegenerative diseases other than AD (Jack Jr et al., 1992; Nelson et al., 2019), which may explain a limited potential of world-centered navigation assessment in differentiating individuals with AD and non-AD aMCI. Furthermore, this study showed the association of less accurate world-centered navigation with higher CSF levels of p-tau$_{181}$. This result complements a previous finding in CN older adults of the association between higher p-tau$_{181}$ levels in CSF and less accurate world-centered navigation performance (Allison et al., 2019). Tau pathology in the MTL (Braak and Braak, 1995) together with neocortical amyloid-β accumulation are the major pathological markers of AD. However, tau pathology in MTL (without amyloid-β accumulation) is present also in other neurodegenerative diseases including primary age-related tauopathy (Crary et al., 2014) and argyrophilic grain disease (Ferrer et al., 2008). Therefore, the association between tau pathology and world-centered navigation may not be that specific for AD as the association of amyloid-β and body-centered navigation.

5.2.4. Directional-approach task (world-centered navigation/perspective taking)

5.2.4.1. World-centered/perspective taking performance in AD aMCI and non-AD aMCI participants

Consistent with the hypothesis, all cognitively impaired participants (i.e., non-AD aMCI, AD aMCI and mild AD dementia) had worse performance than CN older adults. These findings complement previous results of perspective taking deficits that were observed in cognitively impaired participants, where the etiology of cognitive deficit was not determined (i.e., AD biomarkers were not used) (Marková et al., 2015; Laczó et al., 2021). Performance in the current task was worse when the perspective shift was greater (i.e., 180°) compared to the trials with smaller perspective shift (i.e., 90% shift). Contrary to the hypothesis, we did not find significant differences in the overall performance between the AD aMCI and non-AD aMCI participants. All groups performed above the chance level in

trials with 90° perspective shift. Whereas, all cognitively impairmed groups performed at the chance level in trials with 180° perspective shift, indicating that these trials put great demand on perspective taking and thus are prone to the floor effect. In contrast, one study found worse recognition of topographical layouts of mountains scenarios in aMCI individuals with positive AD biomarkers compared to those with negative biomarkers (Chan et al., 2016). However, the degree of perspective shift ranged from 15° to 90°, which is in contrast to our task, where the perspective shift was 90° and 180°. Our task thus puts greater demands on perspective taking and is therefore more difficult for cognitively impaired individuals, which could explain the discrepancy in the results.

5.2.4.2. The association of world-centered navigation/perspective taking with brain atrophy and CSF biomarkers

Consistently with the hypothesis, worse performance in the world-centered navigation/perspective taking task was associated with lower volumes of the posterior MTL regions (i.e., the right hippocampal tail and left hippocampal body and pmEC), thinning of the precuneus, the posterior parietal cortex and right isthmus cingulate/RSC. Therefore, these results indicate that world-centered navigation/perspective taking deficits are associated with neurodegeneration in multiple brain regions, which is in line with previous findings showing the association of perspective taking with the parietal regions (Zacks and Michelon, 2005) and the posterior hippocampus (Schinazi et al., 2013), as well as world-centered navigation with the pmEC (Chadwick et al., 2015). Further, the results are consistent with involvement of the RSC in processing of landmark information for navigation (Auger et al., 2012) and the role of the RSC in integration of body-centered and world-centered navigation (Clark et al., 2018), which are both needed for this task. Further, in agreement with the hypothesis, worse performance was associated with higher levels of p-tau$_{181}$ and total tau and lower levels of amyloid-β$_{1-42}$ in CSF. Previous research showed that lower amyloid-β$_{1-42}$ and higher total tau in CSF were associated with worse recognition of different topographical layouts of the same scenario (Wood et al., 2016) and that higher p-tau$_{181}$ and lower amyloid-β$_{1-42}$ in CSF were associated with less accurate world-centered navigation (Allison et al., 2019). Overall, our and the previous studies suggest that tau and amyloid-β pathologies contribute to deficits in world-centered navigation and perspective taking.

5.2.5. Study 3

5.2.6. Spatial pattern separation performance in early AD

In this study we used a computerized spatial pattern separation task to evaluate its potential to differentiate individuals in the early stages of AD from CN older participants, and to evaluate the association of spatial pattern separation performance with hippocampal, EC and BF Ch1-2 nuclei volumes in early AD. We found that AD aMCI participants had worse spatial pattern separation performance than CN older adults and that participants with mild AD dementia had even worse performance than the AD aMCI participants. These findings indicate that spatial pattern separation abilities deteriorate with the progression of the disease. The task consisted of trials with different spatial separations (0, 0.5, 1.0 and 1.5 cm) providing a varying degree of spatial interference. We found that performance of the AD aMCI and CN participants declined as the distance between the original and the second circle was getting smaller. This finding is in agreement with our hypothesis and with previous research, which showed that smaller distances between the circles represent greater spatial interference and create a greater demand for the spatial pattern separation processes to form and maintain non-overlapping representations of spatial locations (Yassa and Stark, 2011). Importantly, the differences between the participant groups remained significant after controlling for performance in conventional cognitive tests. Therefore, worse performance of cognitively impaired participants could not be explained by general cognitive deficit, but rather points to a specific disruption of the spatial pattern separation processes in early AD. The analysis was also controlled for demographic factors, including age, gender and education. Age-related decline was previously reported in various discrimination tasks (Yassa et al., 2011a; Stark et al., 2013), however, our analysis did not show an influence of age or other demographic factors on performance indicating that spatial pattern separation performance is the most strongly associated with the cognitive status (i.e., the diagnostic group). This result is concordant with previous spatial pattern separation studies that compared performance between young and older adults, where the difference between young and older adults became non-significant after excluding cognitively impaired participants (Stark et al., 2010; Holden et al., 2012; Reagh et al., 2014). Further, the results showed that the spatial pattern separation task discriminated the CN older adults from participants with AD aMCI due to AD with up to 82% sensitivity and 82% specificity. Previous studies reported spatial pattern separation deficits in older adults with worse memory (Holden and Gilbert, 2012; Reagh et al., 2014; Sheppard et al., 2016) that were even reinforced by the presence of APOE ε4 allele

(Sheppard et al., 2016). Our study extends the previous findings by showing that spatial pattern separation can be used to reliably distinguish individuals in the early stages of AD from CN older adults with high sensitivity and specificity.

An unexpected finding was that spatial pattern separation performance did not depend on the time delay (i.e., 10 or 20 s) between the presentation and recall. This is in contrast to the previous studies, which reported the decline of performance with increasing delay between presentation and recall (Ally et al., 2013; Roberts et al., 2014). However, the difference between our and the previous studies might be a consequence of different experimental designs. Specifically, the influence of time delay was found in paradigms, which used different distractions between the presentation and recall. The distraction in our study included presentation of random numbers during the time delay with no memory or pattern separation requirements, whereas other experimental paradigms included an increasing amount of similar objects during the delay, which could disrupt the pattern separation processes (Kuhl et al., 2010). Another study showed similar spatial pattern separation deficits for time delays of 10, 20 and 30 s in amnestic participants with post-hypoxic hippocampal damage, who had performance comparable to CN participants after a time delay of 5 s, indicating that rapid forgetting occurs between 5 and 10 s. This finding is congruent with our results and indicates the process of forgetting in the spatial pattern separation task does not accelerate when time delay increases from 10 s to 20 s. However, it should be noted that according to the ROC analysis the 20 s time delay distinguished better the AD aMCI from CN participants compared to the 10 s delay (AD aMCI versus CN for 10 s delay [AUC = 0.84] and for 20 s [AUC = 0.92]), although, the difference between the AUC values was not statistically significant.

5.2.7. The associations of spatial pattern separation with brain atrophy

Furthermore, our aim was to evaluate structural brain changes underlying spatial pattern separation deficits. We analyzed hippocampal and EC volumes because these regions play a major role in the spatial pattern separation processes (Yassa and Stark, 2011) and are affected early by AD pathology (Braak and Braak, 1995). Additionally, the BF Ch1-2 nuclei were selected for the analysis because the BF is also among the structures affected early by AD pathology (Schmitz et al., 2016) and the BF Ch1-2 nuclei are the major source of acetylcholine in the hippocampus (Mesulam et al., 1983a) where acetylcholine

modulates the pattern separation processes (Giocomo and Hasselmo, 2007; Hunsaker and Kesner, 2013). This is the first study to investigate the association between structural BF changes and spatial pattern separation performance. Our results showed that lower hippocampal, EC and BF Ch1-2 nuclei volumes were associated with worse spatial pattern separation performance in early AD in both the AD aMCI and mild AD dementia participants. This association remained significant after controlling for demographic factors and total brain volume indicating that worse spatial pattern separation performance in early AD was linked to atrophy of specific brain regions. Our results complement previous findings, which showed the associations of hippocampal atrophy and functional changes in the EC and hippocampal subregions (dentate gyrus and CA3 region) with worse object pattern separation in aMCI participants (Yassa et al., 2010) and the associations of functional changes in the hippocampus and EC with object pattern separation in CN older adults (Marks et al., 2017). Our study further extended these findings showing the associations between spatial pattern separation performance and hippocampal, EC and BF Ch1-2 atrophy in the early clinical stages of AD.

5.3. Study 4

5.3.1. Spatial pattern separation performance in AD aMCI and non-AD aMCI participants

We examined spatial pattern separation performance in AD MCI and non-AD aMCI participants to assess whether spatial pattern separation assessment can be used for differential diagnosis of AD (i.e., to differentiate individuals with AD from those with non-AD etiology). Next, we analyzed in detail the associations of spatial pattern separation performance with specific structural brain changes in the hippocampal and EC subregions and the BF Ch1-2 nuclei. Based on the findings from the previous Study 3 (Parizkova et al., 2020), we modified the spatial pattern separation task and used only a 20 s time delay, as the results were superior to those for 10 s time delay.

Consistent with the hypothesis, the AD aMCI participants had worse spatial pattern separation performance than the non-AD aMCI participants and CN older adults. It should be mentioned that the AD and non-AD aMCI participants had similar performance in conventional cognitive tests. The difference in performance between the AD aMCI and non-AD aMCI participants remained significant after controlling for demographic factors.

On the other hand, the non-AD aMCI participants had similar performance to the CN older adults when controlled for demographic characteristics. Our results support the findings from our previous study (Study 3, (Parizkova et al., 2020)) where performance in the spatial pattern separation task differentiated participants with early AD from the CN participants. In addition, the current study showed that the task can also differentiate aMCI participants with AD from those with aMCI of other etiology with high diagnostic sensitivity (>80%). These findings are in accordance with other studies, which found differences in spatial navigation performance between amyloid-β positive and amyloid-β negative aMCI cohorts in real space (Schöberl et al., 2020) and virtual environments (Howett et al., 2019; Laczó et al., 2022). Our findings also complement previous work, which showed the association of cortical amyloid-β with lower performance in a scene discrimination task (Maass et al., 2019), as well as in a spatial and object discrimination task (Webb et al., 2020). However, these mnemonic discrimination tasks evaluated the ability to differentiate between similar objects or between similar locations of different objects on a computer screen, therefore, all of them involved object pattern separation to some extent. The paradigm used in our study was specifically designed to avoid any object separation processes (as only the same looking blue circles were presented) and thus the task assessed only the spatial pattern separation processes. This is an important feature of our paradigm, because object pattern separation decline occurs in normal aging and may not be specific for AD (Reagh et al., 2016). Therefore, a purely spatial pattern separation task could have a greater potential for the diagnosis of early AD. The current study also confirmed previous findings (Kesner and Hopkins, 2006) that spatial pattern separation performance declines when spatial interference is higher (i.e., the distance between the original and second circle is smaller).

5.3.2. The associations of spatial pattern separation with brain atrophy

Further, this study explored the associations of spatial pattern separation performance with structural brain changes. We previously showed that worse spatial pattern separation performance is associated with smaller hippocampal, EC and BF Ch1-2 nuclei volumes (Parizkova et al., 2020). This study extended these findings focusing on the analysis of hippocampal and EC subregions in relation to spatial pattern separation performance. Consistent with the hypothesis, worse spatial pattern separation performance was associated with smaller volume of the posterior hippocampus (i.e., body and tail) and

pmEC, while no association with anterior hippocampus (i.e., head) or alEC was observed. This is the first evidence that spatial pattern separation is associated with smaller posterior hippocampal and pmEC volumes. Current results complement previous findings of association between functional changes in the posterior hippocampus (Lee et al., 2008), the pmEC (Berron et al., 2018) and performance in spatial discrimination tasks. Our results are also in line with the concept of hippocampal functional differentiation along the anterior-posterior longitudinal axis where the posterior regions process fine-grained information (in this case location of the circles), while the anterior part processes of coarse spatial information (Pihlajamäki et al., 2004; Nadel et al., 2013). Similarly, our findings correspond to the functional differentiation of the EC, where the pmEC is involved in spatial information processing, while the alEC was reported to support object information processing (Maass et al., 2015; Navarro Schröder et al., 2015). Consistent with our previous research (Study 3, (Parizkova et al., 2020)), lower BF Ch1-2 nuclei volume was associated with worse spatial pattern separation performance.

6. CONCLUSION

Our studies explored the potential of spatial navigation and spatial pattern separation assessments to help in the early diagnosis of AD. We characterized deficits in spatial navigation and spatial pattern separation abilities in early AD and evaluated whether assessment of these abilities could differentiate participants with early AD from participants with other etiologies of cognitive impairment (i.e., non-AD). Next, we explored the associations of spatial navigation and spatial pattern separation performance with volumetric changes in selected brain regions. Finally, we evaluated associations of spatial navigation abilities with CSF levels of AD biomarkers. The author of the dissertation thesis was involved in the design of the experiments, preparation of experimental assessments, selection of participants for the assessment, and data collection. Further, the author was involved in the analysis and interpretation of behavioral data and their relation to MRI and CSF measures.

The first study analyzed spatial navigation strategy preferences in the early clinical stages of AD and the effect of strategy preference on world-centered navigation performance in real space. Additionally, we evaluated the impact of hippocampal and BF atrophy on the association of strategy preference with world-centered navigation performance. This study showed that the CN older adults prefer a world-centered navigation strategy to navigate the environment, whereas the participants with early AD prefer body-centered navigation strategy and their tendency to use this strategy increases with the severity of AD. The lower preference for world-centered navigation strategy was associated with worse world-centered navigation performance in AD aMCI participants and this association was explained by hippocampal and BF nuclei atrophy by up to 25%. These results indicate that neurodegenerative changes in the hippocampus and BF cause world-centered navigation deficits and lead to the compensatory recruitment of body-centered (i.e., extrahippocampal) strategy. This study showed that assessment of spatial navigation strategies preferences might be a valuable tool for identification of individuals in the early stages of AD.

The second study was the first study up to date to comprehensively examine spatial navigation abilities (i.e., body-centered, world-centered navigation and perspective taking) in individuals with AD aMCI and non-AD aMCI using an ecologically valid virtual realistic-looking test. Furthermore, the relation between spatial navigation profiles,

regional brain atrophy and CSF levels of AD biomarkers was investigated. Using the Navigation Test Suite we showed different profiles of spatial navigation impairment in the participants with AD aMCI and non-AD aMCI. Specifically, the AD aMCI participants had worse body-centered navigation compared to the non-AD aMCI group and they also had a tendency to perform worse in the world-centered navigation task. Deficit in the task combining world-centered navigation with perspective taking was present in both aMCI groups regardless of the etiology (i.e., biomarker status). Next, this study showed that different spatial navigation deficits were associated with neurodegeneration in specific brain regions. Specifically, worse body-centered navigation was associated with parietal atrophy, worse world-centered navigation with atrophy of the posterior MTL regions, and worse performance in the world-centered navigation/perspective taking task was associated with atrophy in multiple brain regions (i.e., the MTL, parietal cortex and isthmus cingulate/RSC). Finally, the analysis of the associations between spatial navigation performance and CSF levels of AD biomarkers demonstrated that spatial navigation deficits reflect different aspects of AD pathology. Specifically, worse body-centered navigation was associated with amyloid-β pathology, worse world-centered navigation with tau pathology, and worse performance in the world-centered navigation/perspective taking task with both amyloid-β and tau pathology measured in CSF. This study showed that a complex assessment of spatial navigation abilities using the Navigation Test Suite has a potential to improve early diagnosis of AD in clinical settings and may complement conventional cognitive tests.

Further, we evaluated the potential contribution of spatial pattern separation assessment to the early diagnosis of AD. The Study 3 was the first study to examine spatial pattern separation in biomarker-defined early stages of AD (i.e., AD aMCI and mild AD dementia). Our findings indicated that spatial pattern separation abilities were impaired in the early stages of AD and deteriorated with the severity of the disease. Spatial pattern separation performance declined with increasing spatial interference and performance was not affected by the length of time delay. This study also explored the associations between spatial pattern separation performance and volumetric changes in brain regions affected early in AD, which also support the spatial pattern separation processes. According to our results, the spatial pattern separation deficits were related to hippocampal, EC and BF Ch1-2 nuclei atrophy. Therefore, spatial pattern separation appears to be a useful cognitive test for diagnosis of AD-related cognitive decline, although, there has been no evidence of the

potential of the task to differentiate cognitively impaired participants with AD from those with cognitive impairment of other etiology.

Therefore, the Study 4 followed up to examine the potential of spatial pattern separation assessment to differentiate cognitively impaired participants with AD from cognitively impaired participants of other etiology. We found that AD aMCI had worse performance than the non-AD aMCI participants while having similar cognitive performance in conventional cognitive tests. Further, we analyzed in detail the associations of spatial pattern separation performance with specific structural brain changes in the hippocampal and EC subregions and the BF Ch1-2 nuclei. Our results showed that spatial pattern separation deficits were associated with atrophy of the posterior hippocampus, pmEC, and BF Ch1-2 nuclei, indicating that worse performance in the task reflected neurodegeneration in specific brain regions that are affected in the early stages of AD. Therefore, spatial pattern separation assessment may aid to distinguish individuals with early AD from those with cognitive deficits caused by other neurodegenerative diseases.

Together, our results indicated that spatial navigation and spatial pattern separation assessments could complement conventional cognitive tests, which lack the diagnostic sensitivity for differentiating AD from other neurodegenerative diseases (Flanagan et al., 2016; Coughlan et al., 2018). Spatial navigation and spatial pattern separation assessments could also help as screening tools to detect individuals at risk of AD. The advantage of spatial abilities assessments is that they can be easily performed in clinical settings and can be available for a large proportion of the population, unlike other diagnostic methods such as amyloid PET imaging or CSF biomarker analysis, which are expensive and invasive methods limited to research settings and expert clinics.

7. SUMMARY

With rapidly growing number of people with AD, the demands for early and accurate diagnosis and treatment increase. Our studies explored the utility of experimental spatial navigation and spatial pattern separation tests for the early and differential diagnosis of AD. An ideal cognitive test should be easy to administer and reliably detect AD-related cognitive deficits. Previous research showed that assessment of spatial navigation and spatial pattern separation can distinguish cognitively impaired and CN older adults and that these cognitive processes depend on the brain regions affected in early AD. However, the etiology of cognitive impairment was not determined by AD biomarkers. Our studies with AD biomarkers compared spatial navigation and spatial pattern separation performance in participants with AD aMCI versus CN older adults and those with non-AD aMCI. We aimed to determine whether these spatial tests could contribute to the early and differential diagnosis of AD. The first study in a virtual Y-maze showed preference for body-centered navigation strategy in participants with early AD that increased with disease severity and was associated with world-centered navigation deficits in real space. Preference for body-centered (i.e., extra-hippocampal) navigation strategy was a compensation for AD-related neurodegenerative changes in the MTL regions and BF, which support world-centered navigation. The second study used a virtual realistic-looking navigation test to characterize different profiles of navigation impairment in AD aMCI and non-AD aMCI participants. The greatest difference was observed in body-centered navigation, where the AD aMCI participants performed worse than those with non-AD aMCI, who were similar to CN participants. The differences between AD aMCI and non-AD aMCI participants in world-centered navigation were less pronounced. Body-centered navigation deficits were associated with atrophy in the precuneus and posterior parietal cortex and amyloid-β pathology, while world-centered navigation deficits were associated with atrophy in the posterior MTL regions and tau pathology. The third study showed that the spatial pattern separation test reliably detected individuals with early AD. The fourth study showed that spatial pattern separation assessment can differentiate AD aMCI from non-AD aMCI participants and that worse performance is associated with atrophy of the posterior hippocampus, pmEC and BF Ch1-2 nuclei. In conclusion, our studies showed that spatial navigation and spatial pattern separation tests may be useful for early and differential diagnosis of AD. These tests are convenient for clinical settings and could be used for a population-wide screening to detect individuals with early AD.

8. SOUHRN

S narůstajícím počtem lidí s Alzheimerovou nemocí (AN) se zvyšují nároky na její časnou a přesnou diagnostiku a léčbu. Naše studie zkoumaly přínos experimentálních testů prostorové navigace a separace prostorových informací pro časnou a diferenciální diagnostiku AN. Ideální kognitivní test by měl být snadno proveditelný a spolehlivě odhalit kognitivní postižení související s AN. Předchozí výzkum ukázal, že vyšetření prostorové navigace a separace prostorových informací odliší kognitivně postižené od kognitivně zdravých seniorů a také, že tyto kognitivní procesy závisí na oblastech mozku postižených v časných stadiích AN. Etiologie kognitivního deficitu však v těchto studiích nebyla určena pomocí specifických biomarkerů. Naše studie používající biomarkery AN porovnávaly výkon v testech prostorové navigace a separace prostorových informací mezi účastníky s AN aMCI, kognitivně zdravými seniory a účastníky s non-AN aMCI. Naším cílem bylo zjistit, zda tyto prostorové testy mohou přispět k časné a diferenciální diagnostice AN. První studie ve virtuálním Y-bludišti ukázala u účastníků s AN vyšší preferenci navigační strategie závislé na poloze těla, která se zvyšovala s tíží onemocnění a byla spojena s horším výkonem v navigaci závislé na okolním prostředí v reálném prostoru. Preference navigační strategie závislé na poloze těla (tj. nehipokampální) u AN kompenzovala neurodegenerativní změny v oblastech MTL a BF, které jsou důležité pro navigaci závislé na okolním prostředí. Druhá studie použila navigační test ve virtuální realitě k určení různých profilů narušení navigace u účastníků s AN aMCI a non-AN aMCI. Největší rozdíly byly nalezeny v navigaci závislé na poloze těla, kde účastníci s AN aMCI měli horší výkon než účastníci s non-AN aMCI, kteří měli podobný výkon jako kognitivně zdraví senioři. Méně významné rozdíly mezi účastníky s AN aMCI a non-AN aMCI byly v navigaci závislé na okolním prostředí. Postižení navigace závislé na poloze těla souviselo s atrofií precuneu a zadní parietální kůry a patologií amyloidu-β, zatímco postižení navigace závislé na okolním prostředí souviselo s atrofií zadních oblastí MTL a tau patologií. Třetí studie ukázala, že test separace prostorových informací spolehlivě odhalí účastníky s časnou AN. Čtvrtá studie ukázala, že hodnocení separace prostorových informací odliší účastníky s AN aMCI a non-AN aMCI a že horší výkon je spojen s atrofií zadního hipokampu, pmEC a jader BF Ch1-2. Závěrem lze říci, že naše studie prokázaly potenciál testů prostorové navigace a separace prostorových informací pro časnou a diferenciální diagnostiku AN. Tyto testy jsou vhodné pro klinická pracoviště a mohou být použity i pro celopopulační screening k odhalení jedinců s časnou AN.

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10. OVERVIEW OF AUTHOR´S PUBLICATIONS

10.1. Original scientific works, which are the basis of the dissertation

Parizkova, M., Lerch, O., Moffat, S. D., Andel, R., Mazancova, A. F., Nedelska, Z., et al. (2018). The effect of Alzheimer's disease on spatial navigation strategies. *Neurobiol. Aging* 64, 107–115. IF $_{2018}$ **4.398**

Laczó, M., Martinkovic, L., Lech, O., Wiener, J. M., Kalinova, J., Matuskova, V., et al. (2022). Different profiles of spatial navigation deficits in Alzheimer's disease biomarker-positive versus biomarker-negative older adults with amnestic mild cognitive impairment. *Front. Aging Neurosci.*, In press. IF$_{2020}$ **5.750**

Parizkova, M., Lerch, O., Andel, R., Kalinova, J., Markova, H., Vyhnalek, M., et al. (2020). Spatial Pattern Separation in Early Alzheimer's Disease. *J. Alzheimers. Dis.* 76, 121–138. IF$_{2020}$ **4.472**

Laczó, M., Lerch, O., Martinkovic, L., Kalinova, J., Markova, H., Vyhnalek, M., et al. (2021). Spatial Pattern Separation Testing Differentiates Alzheimer's Disease Biomarker-Positive and Biomarker-Negative Older Adults With Amnestic Mild Cognitive Impairment. *Front. Aging Neurosci.* 13, 774600. IF$_{2020}$ **5.750**

10.2. Original scientific works related to the topic of the dissertation

Pařízková, M., Andel, R., Lerch, O., Marková, H., Gažová, I., Vyhnálek, M., et al. (2017). Homocysteine and Real-Space Navigation Performance among Non-Demented Older Adults. *J. Alzheimers. Dis.* 55, 951–964. IF$_{2017}$ **3.476**

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10.3. Original scientific works without relation to the topic of the dissertation

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11. APPENDIX

Original scientific works, which are the basis of the dissertation