CHARLES UNIVERSITY

Second Faculty of Medicine

Summary of the Dissertation

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

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Content

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

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

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

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

1.   Background

Alzheimer's disease (AD) is the most common neurodegenerative disease with an increasing prevalence. AD forms a "continuum", where the earliest stage is called preclinical, followed by mild cognitive impairment (MCI) and final dementia stage. Early and accurate diagnosis of AD is crucial to maximize the effect of treatment and to slow down the disease progression. The pathological changes typical of AD include extracellular aggregation of amyloid-β 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 and consequently leads to cognitive impairment. Amyloid-β accumulation is initiated in neocortical regions and spreads to subcortical regions according to the Thal stages (Thal et al., 2002). Spread of tau pathology is defined by the Braak stages and initiates in the transentorhinal cortex, spreads to the entorhinal cortex (EC) and hippocampus and in later stages affects also the neocortical regions (Braak and Braak, 1995). Amyloid-β accumulation initiates a cascade of other pathologic changes including 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. Brain atrophy parallels the distribution of tau pathology (Whitwell et al., 2007), while there is no direct association between amyloid-β accumulation and the progression of atrophy.

1.1.   Diagnosis of AD

The National Institute of Aging and the Alzheimer's Association created separate diagnostic recommendations for the preclinical stage (Sperling et al., 2011), MCI (Albert et al., 2011) and dementia stage of AD (McKhann et al., 2011) in 2011. Further, a new "research framework" for diagnosis of AD in a research setting based on evidence of AD biomarkers (amyloid-β and p-tau) was created in 2018

(Jack et al., 2018). The preclinical stage is characterized by intact cognitive functions and evidence of AD pathology based on the assessment of specific biomarkers (Sperling et al., 2011). Individuals with MCI have evidence of cognitive decline from previous levels and they perform 1 to 1.5 standard deviation below the mean for their age and education matched peers in one or more cognitive domains. However, they are independent in everyday functions, although they might have mild problems with performing complex functional tasks. 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). In the dementia stage, the individuals are no longer independent in daily life and there is evidence of cognitive decline in at least two cognitive domains compared to their previous level (McKhann et al., 2011). The possible underlying etiologies of this cognitive deficit in any stage include neurodegeneration, vascular, infections, traumatic or combined. For research purposes, the diagnosis of AD should be supported by evidence of AD-specific biomarkers. The newest research diagnostic criteria are referred to as AT(N) framework recognizing three groups of biomarkers. The "A" refers to aggregation of amyloid-β which can be detected as low amyloid-β in CSF or visualized by amyloid-β positron emission tomography (PET) imaging. The "T" denotes aggregation of tau (neurofibrillary tangles) which can be detected as high concentration of phosphorylated tau (p-tau) in CSF or visualized using tau PET imaging (Jack et al., 2018). "(N)" indicates evidence of neurodegeneration or neuronal injury (i.e., atrophy on magnetic resonance imaging [MRI], elevation of total tau in CSF or cortical hypometabolism). The biomarkers can be detected in vivo and are highly specific and sensitive for the diagnosis of AD, however, their use is limited for expert memory clinics due to their high cost and invasiveness.

Cognitive assessment using conventional cognitive tests with a special focus on episodic memory assessment is a key part of the diagnostic process. However, these tests have a limited potential to detect early stages of AD, because episodic

memory declines in normal aging and also in other neurodegenerative diseases. Therefore, deficits in conventional cognitive tests may not be specific to AD. Recently, two cognitive processes have been identified to decline very early in AD - pattern separation and spatial navigation. Our research is specifically focused on spatial navigation and spatial pattern separation and their potential to be early cognitive markers of AD.

1.2. Spatial navigation

Spatial navigation is a cognitive process allowing us to move meaningfully in our environment and to find our way. Successful navigation requires a combination of various navigation strategies which are supported by different regions in the brain. The body-centered navigation involves encoding of body movements (e.g., turn right, left) or associations between direction changes and proximal landmarks (e.g., turn right at the shop). This strategy is useful when traveling along the same known route repetitively, but lacks the flexibility to navigate in novel environments or to create shortcuts. Body-centered navigation depends on the posterior parietal cortex, precuneus and the caudate nucleus (Weniger et al., 2011). The world-centered navigation involves encoding positions of places and landmarks and creating a cognitive map (i.e., internal image of the environment), which enables flexible creation of novel routes (Maguire et al., 1998). World-centered navigation depends on the medial temporal lobe (MTL), especially the hippocampus and the interconnected EC (Cholvin et al., 2021). The MTL structures have a functional differentiation along the anterior-posterior axis. The posterior hippocampus (i.e., the body and tail) is involved in creating and using cognitive maps and the anterior hippocampus (i.e., the head) is involved in navigation planning and responding to novelty. Further, the anterior hippocampus processes coarse spatial information, while the posterior regions process fine details (Brunec et al., 2018). The EC subregions include the posteromedial EC (pmEC) which supports spatial information processing and the anterolateral EC

(alEC), which supports object information processing. Further, world-centered navigation is supported by the basal forebrain (BF) consisting of multiple nuclei referred to as Ch1-4. The medial septal nucleus (Ch1) interconnected with the nucleus of the vertical limb of the diagonal band of Broca (Ch2) together with the posterior part of the nucleus basalis of Meynert (Ch4p), represent the major source of acetylcholine for the hippocampus and the EC (Mesulam et al., 1983). Also, the BF itself was found to support world-centered navigation (Kerbler et al., 2015). Another important aspect of spatial navigation is perspective taking, which allows the navigator to imagine spatial scenes from different perspectives (Marková et al., 2015) and this process is supported by the parietal cortex and MTL structures. Integration of different navigation strategies is necessary for successful real-life navigation, and this integration is supported by the retrosplenial cortex (RSC) which receives inputs from MTL and from the parietal regions (Auger et al., 2012).

1.3.   Spatial navigation in normal and pathological aging and in AD

Aging is associated with spatial navigation decline affecting specifically world-centered navigation as a consequence of age-related changes in the MTL (Moffat et al., 2006). Body-centered navigation remains intact because the posterior parietal regions remain preserved in aging (Maguire et al., 1998). Older adults preferentially use well known routes avoiding novel or less familiar places indicating preference of body-centered over world-centered strategy to compensate for world-centered navigation deficits (Rodgers et al., 2012). World-centered navigation deteriorates more severely with the progression of AD because the MTL regions are affected by AD pathology (Allison et al., 2016a). First signs of spatial navigation deficit occur already in the preclinical stage affecting world-centered navigation abilities while body-centered navigation remains intact (Allison et al., 2016b). In aMCI individuals, the studies showed world-centered and body-centered navigation deficits in virtual environments and real space

(Weniger et al., 2011; Laczó et al., 2012), and also deficit in perspective taking (Marková et al., 2015). Only a few studies used the AD-specific biomarkers to define the etiology of aMCI and compared spatial navigation performance in aMCI individuals with AD (i.e., AD aMCI) to the cognitively normal (CN) older adults and aMCI individuals without AD (i.e., non-AD aMCI). A study in real space showed that AD aMCI individuals had worse performance in body-centered and world-centered navigation tasks compared to cognitively normal (CN) older adults and non-AD aMCI individuals, who were similar to CN older adults in body-centered navigation (Schöberl et al., 2020). A study in a virtual environment showed that body-centered navigation performance differentiated patients with AD from those with other neurodegenerative diseases, while there were no differences in world-centered navigation between the groups (Tu et al., 2017). In another study, the path integration task in virtual reality differentiated the AD aMCI and non-AD aMCI individuals (Howett et al., 2019). This indicates that spatial navigation tests may be a promising diagnostic tool for AD, however, previous studies were not suitable for routine clinical settings as they required larger space to be performed.

1.4. Pattern separation

Pattern separation is a neural process of encoding similar inputs as non-overlapping representations (i.e., memories) so that they can be recalled separately, therefore, this process is important for accurate encoding of information which share similar features (Holden and Gilbert, 2012). This process is referred to as "object" pattern separation when discriminating similar objects or "spatial" pattern separation when discriminating spatial locations. Object information processing is supported by the hippocampal head and the alEC, while spatial processing is supported by the hippocampal body and tail and by the pmEC (Pihlajamäki et al., 2004; Lee et al., 2008). Additionally, higher levels of acetylcholine from the BF Ch1-2 support the pattern separation process in the hippocampus.

1.5. Pattern separation in normal and pathological aging and in AD

Aging is associated with worse object and spatial pattern separation as a consequence of age-related hippocampal changes (Holden and Gilbert, 2012). However, the recent studies indicated that spatial pattern separation decline is less pronounced than object pattern separation decline in normal aging (Reagh et al., 2016). Both object and spatial pattern separation impairment was reported in older adults with cognitive deficits including MCI individuals, where the etiology of the deficit was not determined (Holden and Gilbert, 2012). Only a few studies assessed pattern separation in individuals with biomarker evidence of amyloid-β and tau pathologies. These studies indicated that 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, which is found in different neurodegenerative diseases but may not be specific to AD. On the other hand, spatial pattern separation was not associated with tau pathology (Berron et al., 2019; Maass et al., 2019) but with amyloid-β in cortical regions (Maass et al., 2019), indicating that spatial pattern separation could be a reliable marker of early AD.

1. Objectives

Our overarching goal was to explore the potential of spatial assessment to differentiate individuals with early AD from CN older adults and individuals with cognitive deficits of non-AD etiologies.

1.1. Study 1: The Effect of Alzheimer´s Disease on Spatial Navigation Strategies

The aims of the study were to assess:

(1) spatial navigation strategy preferences (world-centered vs. body-centered) 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 spatial navigation performance in real space; and (3) the role of hippocampal and BF nuclei volumes in this association.

We hypothesized that:

(1) participants with AD aMCI and mild AD dementia would have a stronger preference for the body-centered strategy compared with the CN participants; (2) participants in the early stages of AD with the body-centered strategy preference would have less accurate world-centered navigation performance; and (3) lower hippocampal and BF nuclei volumes would be associated with higher body-centered preference and worse world-centered navigation performance.

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

The aims of the study were to assess:

(1) the differences in world-centered navigation, body-centered navigation and world-centered navigation/perspective taking performance 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, especially in the body-centered navigation task, (2) atrophy of the parietal regions would be associated with worse body-centered navigation; atrophy of the MTL regions (i.e., especially the posterior hippocampus and pmEC) would be associated with worse world-centered navigation; and worse wold-centered navigation/perspective taking would be associated with atrophy of both, the MTL and parietal regions and additionally with atrophy of the isthmus cingulate/RSC, and (3) lower levels of amyloid-$\beta_{1\text{-}42}$ in CSF would be associated with worse body-centered navigation, higher levels of CSF phosphorylated tau$_{181}$ (p-tau$_{181}$) would be associated with worse world-centered navigation, and both the low amyloid-$\beta_{1\text{-}42}$ and high p-tau$_{181}$ CSF levels would be associated with worse world-centered navigation/perspective taking.

1.3. Study 3: Spatial Pattern Separation in Early Alzheimer´s Disease

The aims of the study were to assess:

1) the differences in spatial pattern separation in the early clinical stages of AD (AD aMCI and mild AD dementia) compared to CN older adults, and 2) the association of spatial pattern separation performance with hippocampal and EC volumes, and volume of the BF Ch1-2 nuclei.

We hypothesized that:

(1) the participants in the early clinical stages of AD would have less accurate spatial pattern separation performance compared to the CN older adults, and (2) smaller volumes of the hippocampus, EC, and BF Ch1-2 nuclei would be associated with worse spatial pattern separation performance.

1.4. Study 4: Spatial Pattern Separation Testing Differentiates Alzheimer´s Disease Biomarker-Positive and Biomarker-Negative Older Adults with Amnestic Mild Cognitive Impairment

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 atrophy of specific hippocampal and EC subregions, specifically with the posterior hippocampus (i.e., tail and body) and the pmEC, and atrophy of the BF Ch1-2 nuclei.

2. Methods

3.1. Participants

Participants were recruited from the Czech Brain Aging Study (CBAS) cohort. All participants underwent clinical and laboratory evaluations, comprehensive cognitive assessment, brain MRI and spatial navigation and spatial pattern separation assessments.

(i) **CN participants** had cognitive performance within the normal range and did not have evidence of hippocampal atrophy on MRI.

(ii) **AD aMCI participants** met the clinical diagnostic criteria (Albert 2011). In Study 1 and 3, the participants had hippocampal atrophy and the subset had reduced amyloid-β_{1-42} in CSF. In Study 2 and 4, the

participants had positive CSF AD biomarkers (reduced amyloid-β_{1-42} and elevated p-tau$_{181}$) and/or positive amyloid PET imaging (positive visual read of 18F-flutemetamol PET scan).

(iii) **Non-AD aMCI participants** met the clinical diagnostic criteria (Albert et al., 2011) and had negative amyloid-β biomarkers defined as normal CSF amyloid-β_{1-42} and/or negative amyloid PET imaging.

(iv) **Mild AD dementia** participants met the clinical criteria for dementia (McKhann et al., 2011). In Study 1 and 3, the participants had hippocampal atrophy and the subset had reduced amyloid-β_{1-42} in CSF. In Study 2 and 4, the participants had positive CSF AD biomarkers (reduced amyloid-β_{1-42} and elevated p-tau$_{181}$) and/or positive amyloid PET imaging (positive visual read of 18F-flutemetamol PET scan).

Cognitive assessment included assessment of verbal and non-verbal memory, visuospatial functions, executive functions, attention and working memory, language functions and evaluation of global cognitive function, depressive symptoms and anxiety. AD biomarkers (i.e., amyloid-β_{1-42} , total tau and p-tau$_{181}$) were analyzed in CSF. Abnormal levels of CSF biomarkers were established according to the cut-off values: amyloid-β_{1-42} less than 665 pg/ml, p-tau$_{181}$ above 48 pg/ml and total tau above 358 pg/ml. Amyloid PET images were classified as positive or negative depending on whether accumulation in specific regions was present or absent. We used the established MRI protocol and depending on the study, hippocampal, EC and cortical and subcortical volumes and thicknesses were measured using volBrain volumetry system and FreeSurfer image analysis suite. Hippocampal and EC subregions were measured with the Advanced Normalization Tools package using the masks based on manually created templates from the CBAS study. The BF subregions were measured with SPM8 and VBM8-toolbox implemented in MatLab using the mask based on a cytoarchitectonic map of BF.

2.1. Study 1

2.1.1. Virtual Y-maze

The Y-maze consisted of 3 arms where participants navigated using a joystick. The task was used to identify preferred navigation strategy (body-centered versus world-centered). The Y-maze task consisted of 5 blocks where each block had 2 parts each: 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 landmarks). A circular area was at the end of each arm. In training trials, participants always started from the same circular area and had to find a goal (a pleasant sound in one of the remaining circular areas), when entering an incorrect circular area, they heard an unpleasant noxious buzzer. After reaching 5 times correctly to the goal, the final probe trial started. In the probe trial, they were placed in the area where the buzzer sound was previously located. The probe trial was designed to determine world-centered or body-centered strategy preference. Participants who, during the probe trial, followed the same route as they learned in training, regardless of absolute location (e.g., turned right), were classified for that block as using a body-centered strategy. Participants who moved to the same absolute location as trained in the training trials, even though it required taking a different route were classified as using a world-centered strategy for that trial. In order to classify preference of one strategy over another, the same strategy had to be chosen at least in 4 of the 5 blocks, otherwise their strategy preference was unspecific.
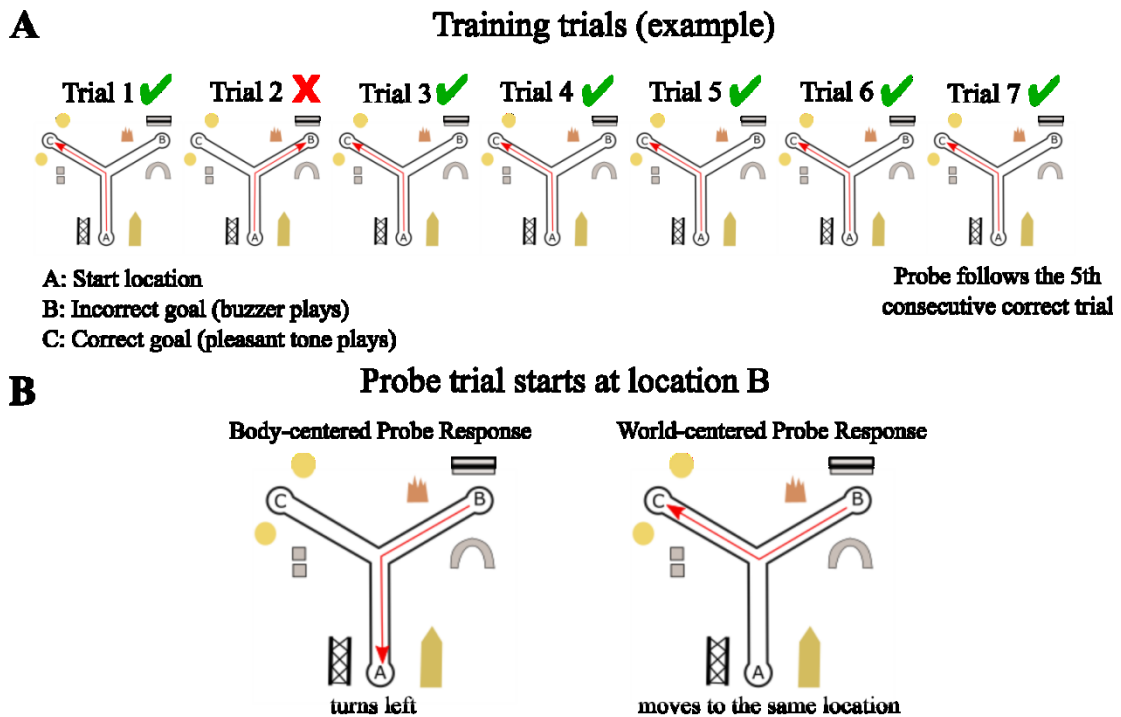
A Training trials (example)

Trial 1 ✔ Trial 2 ✘ Trial 3 ✔ Trial 4 ✔ Trial 5 ✔ Trial 6 ✔ Trial 7 ✔

A: Start location
B: Incorrect goal (buzzer plays)
C: Correct goal (pleasant tone plays)

Probe follows the 5th consecutive correct trial

B Probe trial starts at location B

Body-centered Probe Response

World-centered Probe Response

turns left

moves to the same location

Fig. 1 Virtual Y-maze strategy assessment. (A) Training trials: 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).

2.1.2. Real space human analogue of the Morris Water Maze (hMWM) task

The hMWM tests world-centered spatial navigation in real space, specifically in an enclosed circular arena measuring 2.8 meters in diameter and 2.9 meters high (Fig. 2A). There were two distinct visual cues on the wall of the arena and the participants had to find a hidden goal, which was always in the same spatial relations to the visual cues on the walls (i.e., it was in a constant distance and direction from each of the visual cues). Participants always started from a specific starting location and had to indicate the goal location by placing a standing pole on the assumed position. The task had eight trials in which positions of cues changed while maintaining the same spatial relations among themselves (Fig. 2B and C). 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 recorded by a computer. A total performance was calculated as a mean distance error across all eight trials.
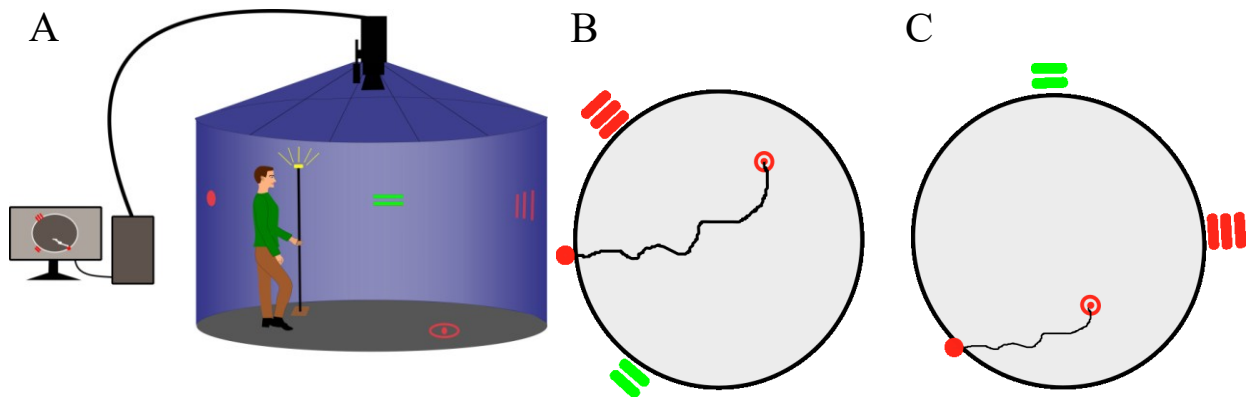

Fig. 2 Human analogue of the Morris water maze task. (A) The real space navigation setting. (B) The scheme of the task shows an aerial view of the arena (large circle) with starting point (red point), orientation cues (red and green lines), and goal (red circle). (C) An aerial view of the arena, where the orientation cues and the goal are rotated 90° from the previous trial shown in Fig. 2B.

2.2. Study 2

2.2.1. The Navigation Test Suite

The Navigation Test Suite (Wiener et al., 2020) in virtual reality is a realistic looking task with a series of streets with four-way intersections, which contains three navigation tasks: the route-repetition task, the route-retracing task, and the directional-approach task. The streets are aligned by identical brick houses and intersections feature distinct houses of different design and color (i.e., landmarks). Performance was measured as a percentage of correct responses in each of the tasks.

Route-repetition (body-centered navigation) task (Fig. 3A): participants were passively transported along a route with five intersections from the car to the

telephone box. In the test phase, the participants had to repeat the same route and were asked about the directions at each intersection. **Route-retracing (world-centered navigation) task (Fig. 3A):** Participants were passively transported along a route with five intersections from the car to the telephone box, the route was different than in the Route-repetition task. In the test phase, the participants had to navigate in the opposite direction (i.e., from the telephone box to the car). **Directional-approach (world-centered navigation/perspective taking) task (Fig. 3 B):** The task consisted of 15 separate intersections (i.e., trials) not related to each other. Participants were passively transported to an intersection where they encoded the configuration of houses (landmarks) at the corners. In the testing phase, they were transported to the same intersection from a different street (i.e., with 90° or 180° perspective shift) and the task was to indicate the original approach direction to the intersection.
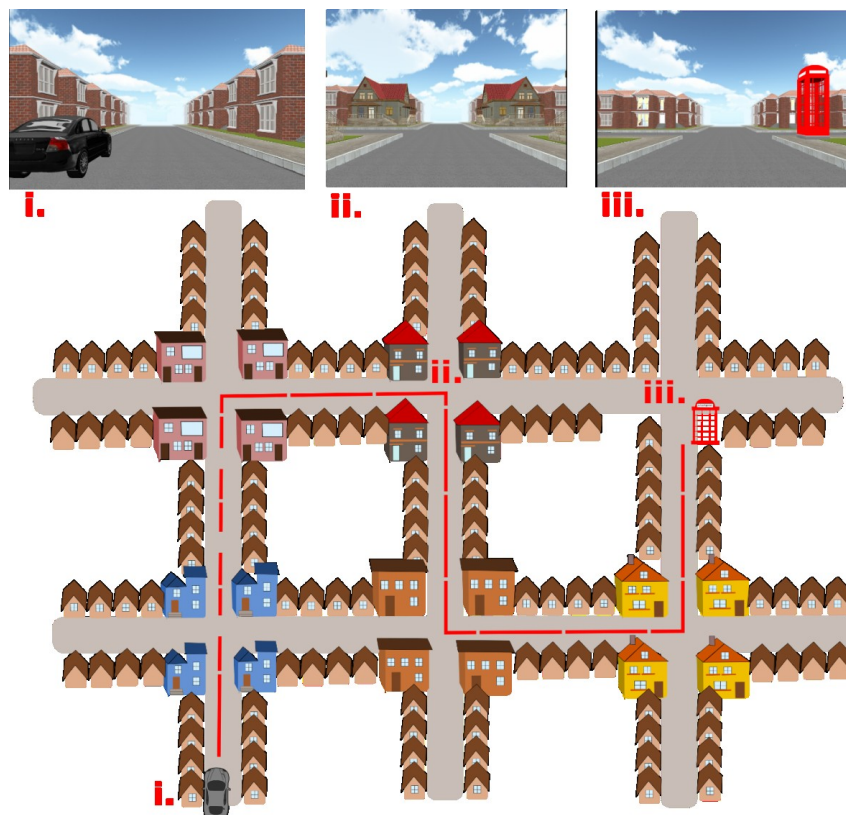

Fig. 3A The Navigation Test Suite with schematic aerial view of the Route-repetition and the Route-retracing tasks. In the Route-repetition task, the participants were passively transported through the city from the car to the telephone box during the encoding phase and in the test

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 had to find their way back from the telephone box to the car in the test phase.
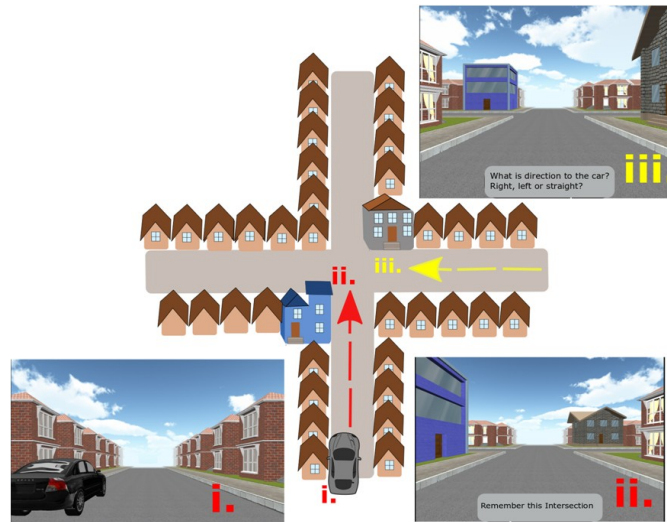

Fig. 3B The Navigation Test Suite with schematic view of the Directional-approach task: (i.) Participants started the task next to the car. (ii.) The encoding phase, where participants were passively transported towards the intersections featuring two unique houses. Participants had to remember where 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.

2.3. Study 3 and 4

2.3.1. Spatial pattern separation task

The Spatial pattern separation task is a computerized task (Fig. 4). First, the participants had 5 seconds to remember the position of a small blue circle on the screen with white background. Afterwards, the circle disappeared and participants were instructed to read aloud random numbers appearing in the middle of the screen to prevent them from fixating vision on the location where the circle was seen. The numbers were appearing for 10 or 20 seconds (in Study 3) and for 20 seconds only in Study 4. After the delay of 10 or 20 s, 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 indicated using the buttons which circle was in the original position. The task had 64 trials in the Study 3 (32 with a 10 s delay and 32 with a 20 s delay), the Study 4, included only 32 trials all with a 20 s delay. 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.
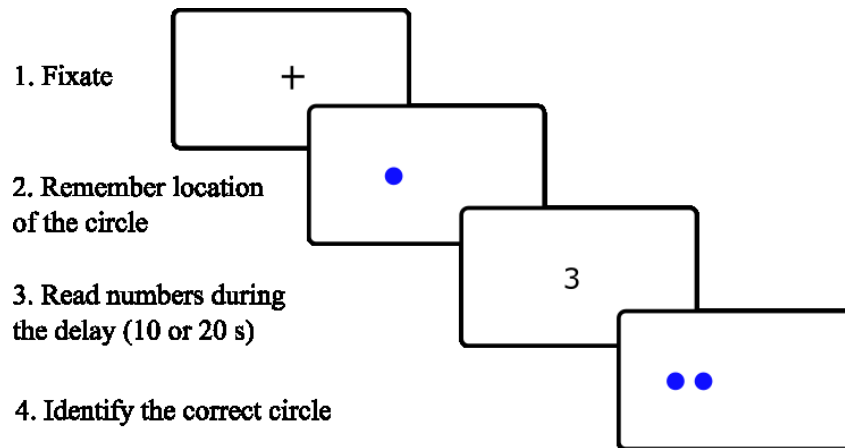

Fig. 4 Example of a spatial pattern separation task trial as seen by participants on the computer screen.

3. Results

3.1. Study 1

The χ^2 test showed that navigation strategy preference varied between the groups ($\chi^2 = 11.9$, p = 0.003). Participants in the CN group preferred the world-centered strategy (39% body-centered, 61% world-centered), while participants with early AD including the AD aMCI (67% body-centered, 33% world-centered) and mild AD dementia (94% body-centered, 6% world-centered) groups preferred body-centered strategy. The 3 (CN vs. aMCI vs. dementia) x 2 (male vs. female) x 2 (body-centered vs. world-centered Y-maze strategy preference) analysis of variance (ANOVA) with world-centered navigation distance error as a dependent measure showed a main effect of the group on world-centered navigation performance [F(2) = 21.35, p < 0.001]. The AD aMCI and mild AD dementia groups had less accurate world-centered navigation performance than the CN

group (p < 0.001). Participants in the AD aMCI group who preferred the body-centered strategy had less accurate performance in the world-centered navigation task than those who preferred the world-centered strategy (p = 0.003). Lower total, right and left hippocampal and BF Ch1-2 nuclei volumes correlated with less accurate world-centered navigation performance (r ≥ 0.366, p ≤ 0.004). In the AD aMCI group, total hippocampal volume accounted for 14%, left hippocampus for 9% and the right hippocampus for 20% of the association between strategy preference and world-centered navigation performance. The Ch4p (posterior part of the nucleus basalis of Meynert) and Ch1-2 (the medial septal nuclei and vertical limb of the diagonal band of Broca) accounted for 24% and 25% of this association, respectively.
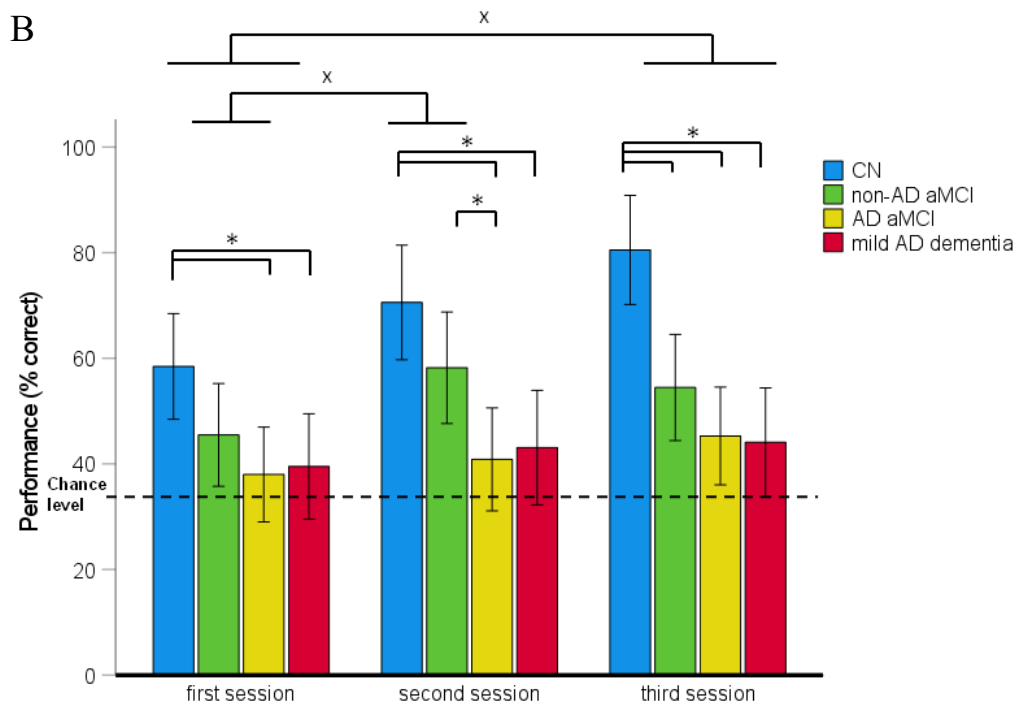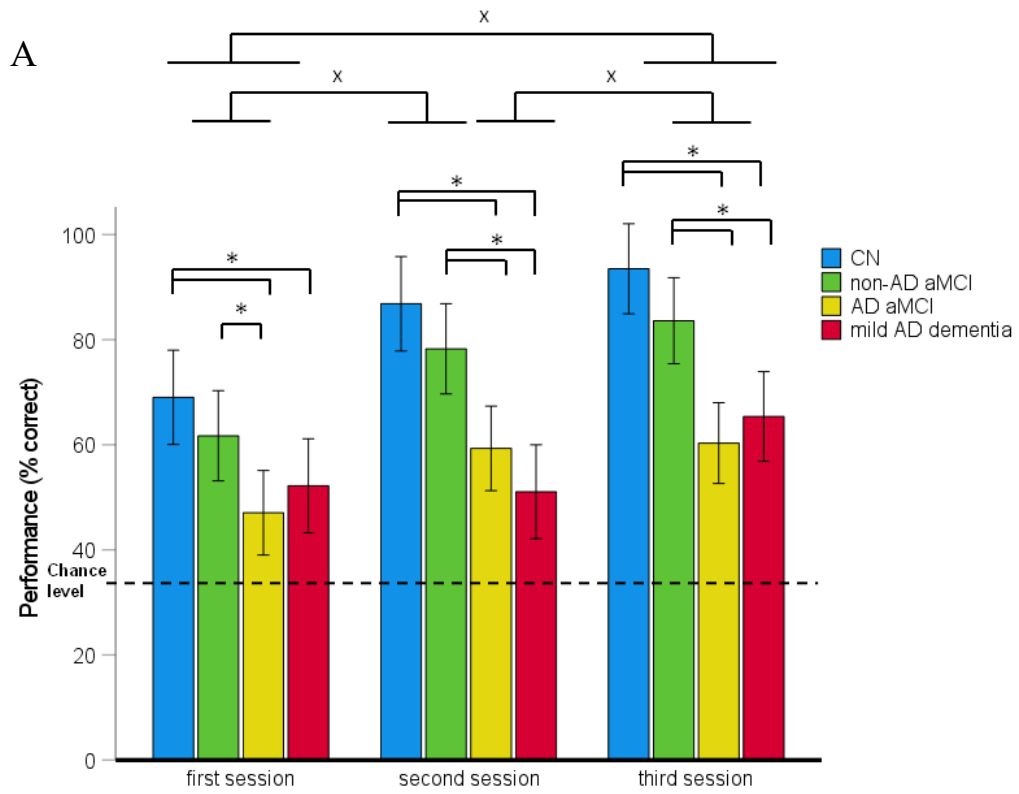
3.2. Study 2

Spatial navigation performance measured as the mean percentage of correct responses in Navigation tests Suite is presented in Fig. 5.

Route-repetition task performance: The AD aMCI group performed worse than the non-AD aMCI group (p < .001, 95% CI [-29.76, -8.17]) and the CN group (p < .001, 95% CI [-38.69, -16.44]). The non-AD aMCI group had comparable performance to the CN group (p = 0.272, 95% CI [-20.29, 3.09]). The groups improved across the sessions (i.e., second v.s first (p < 0.001) and third vs. second (p = .020)). Performance of the groups in all sessions was above the chance level (p ≤ .005). According to the ROC analysis, the Route-repetition task differentiated the non-AD aMCI from the AD aMCI group with AUC values of 0.78 (p < 0.001).

Route-retracing task performance: In general, the AD aMCI group had worse performance than the CN group (p < .001, 95% CI [-41.88, -15.07]) and did not differ from the non-AD aMCI (p = .128, 95% CI [-24.48, 1.80]) and mild AD dementia (p = 1.00, 95% CI [-14.02, 12.31]) groups. The AD aMCI group had worse performance than non-AD aMCI in the second session ($p_{H-Bcorrected}$ = .032)

and worse performance than the CN group in all three sessions (all $p_{\text{H-Bcorrected}} \leq$ .016). The non-AD aMCI group had similar performance as the CN group with the exception of the third session where their performance was worse ($p_{\text{H-Bcorrected}} =$ .003). The CN and non-AD aMCI groups performed above the chance level in all sessions ($p \leq .009$). In contrast, performance of the AD aMCI group at the first and second session did not differ from the chance level ($p \geq .223$) and exceeded chance level performance only at the third session ($p = .026$). According to the ROC analysis, the task differentiated non-AD aMCI from the AD aMCI with AUC values of 0.64 ($p = 0.041$). The groups improved across the sessions (i.e., second vs. first ($p = .021$) and third vs. first ($p < .001$)).

Directional-approach task performance: In general, the cognitively impaired groups (i.e., non-AD aMCI, AD aMCI and mild AD dementia) had worse performance than the CN group ($p \leq .001$) and did not differ between each other. All groups had worse performance when the approach direction was from north (i.e., 180° perspective shift) compared to the conditions when approach direction was from the west and east (i.e., 90° perspective shift) ($p < .001$). The CN group outperformed all remaining groups at all approach directions (all $p_{\text{H-Bcorrected}} \leq$ .044), while there was no difference between the non-AD aMCI and AD aMCI groups in any approach direction. The CN group performed above the chance level in all approach directions (all $p \leq .011$). All three other groups performed above the chance level only when approaching from the west and east ($p \leq .003$). According to the ROC analysis, the task differentiated the CN group from the cognitively impaired groups with AUC values of ≥ 0.717 ($p \leq 0.001$) but did not differentiate the non-AD aMCI and the AD aMCI groups.
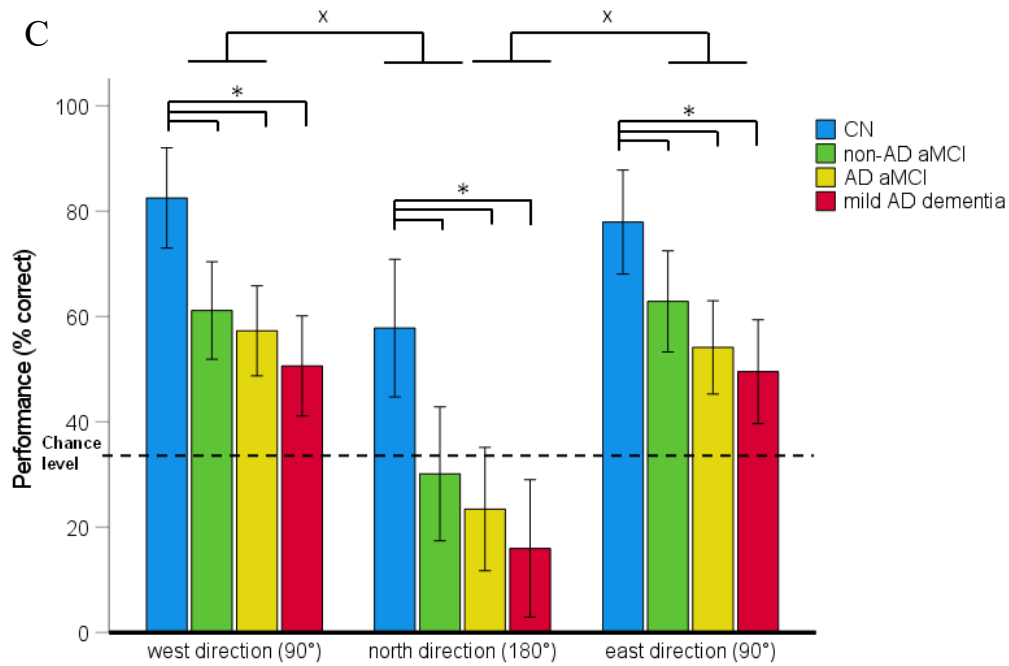
Fig. 5 Navigation Test Suite task performance: A) Route-repetition task, B) Route-retracing task, C) Directional-approach task —spatial navigation performance as mean percentage of correct responses in each session (95% Cl). *p < 0.05 indicating the differences between the groups; *x* p < 0.05 indicating the differences between the sessions; CI, confidence interval.

Worse Route-repetition task performance was associated with lower thickness of the right and left precuneus and posterior parietal cortex and lower volume of the right alEC. Worse Route-retracing task performance was associated with lower volumes of the right hippocampal body and the right and left pmEC. Worse Directional-approach task performance was associated with lower volumes of the left hippocampal body, the right hippocampal tail, the right and left pmEC, and thickness of the right isthmus cingulate/RSC, the right and left precuneus and posterior parietal cortex (all ß ≥ 0.24, p ≤ .030). Lower CSF levels of amyloid-β_{1-42} correlated with worse performance in the Route-repetition and Directional-approach tasks (both r ≥ 0.31, p ≤ 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 ≥ -0.30, p ≤ 0.043).

3.3. Study 3

The mean percentage of correct performance for each diagnostic group for time delay of 10 s and 20 s are presented in Fig. 6. The $3 \times 2 \times 4$ mixed factorial ANOVA with diagnostic group (CN vs. aMCI vs. mild 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 with the post hoc Sidak´s test found effect of diagnostic group ($F[2, 95] = 75.65$, $p < 0.001$). 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. There was no effect of time delay (10 s vs. 20 s) on spatial pattern separation performance ($F[1, 95] = 0.09$, $p = 0.761$). Spatial separation distance had a significant effect on performance ($F[3, 285] = 20.12$, $p < 0.001$). Performance linearly increased with increasing spatial separation (i.e., with increasing distance between the circles) ($F[1,95] = 52.46$, $p < 0.001$). According to the ROC analysis, the task differentiated the CN group from the AD aMCI group with sensitivity of 77% and specificity of 82% for 10 s time delay (AUC = 0.84, p < 0.001) and with sensitivity of 82% and specificity of 82% for 20 s time delay (AUC = 0.92, p < 0.001). According to the multivariate linear regression analysis controlled for total brain volume and demographic factors, lower total hippocampal, EC, and Ch 1-2 nuclei volumes were associated with less accurate spatial pattern separation performance after 10 s and 20 s ($\beta \geq 0.25$, $p \leq 0.018$).
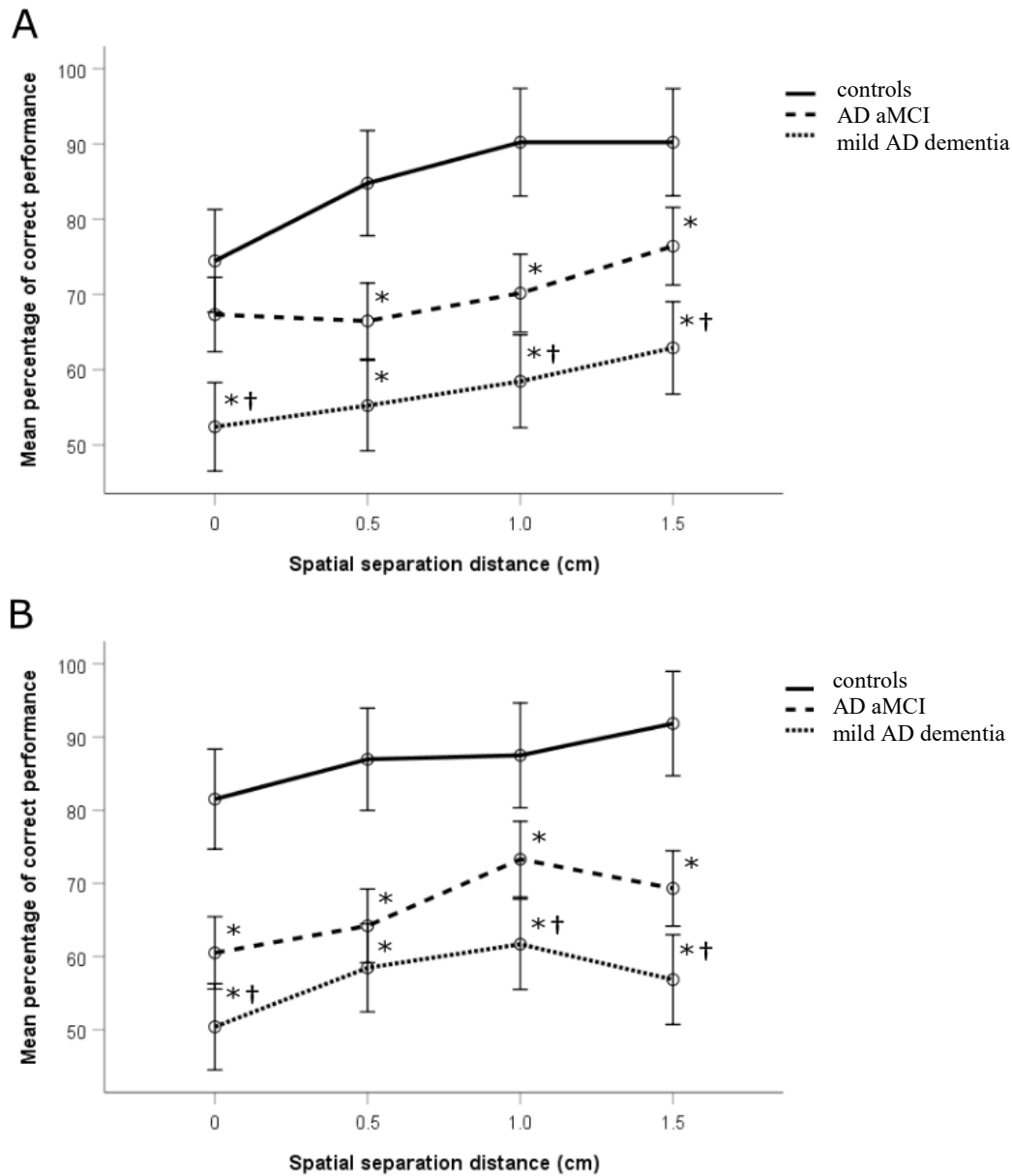
Fig. 6 Spatial pattern separation performance A) Mean percentage of correct performance for each spatial separation for time delay of 10 s (±1 SE). B) Mean percentage of correct performance for each spatial separation for time delay of 20 s (±1 SE). *p < 0.05 compared to the CN group; †p < 0.05 compared to the AD aMCI group.

3.4. Study 4

The mean percentage of correct performance for each spatial separation in each of the diagnostic groups is presented in Fig. 7. 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 as the percentage of correct responses (i.e., the dependent variable). The post hoc Sidak′s test was to analyze the effect of the diagnostic group on performance. The AD aMCI group had worse performance in spatial pattern separation than the non-AD aMCI group (p = 0.039, 95% CI [–18.80, –0.31]) and the CN group (p < 0.001, 95% CI [–29.21, –11.29]), while having similar performance as 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]). According to the ROC analysis, the task differentiated the non-AD aMCI and the AD aMCI group with an AUC value of 0.67 (p = 0.024). The regression analysis controlled for demographic factors showed that lower volumes of the hippocampal tail and body, pmEC and BF Ch1-2 nuclei were associated with worse spatial pattern separation performance (β ≥ 0.26, p ≤ 0.017).
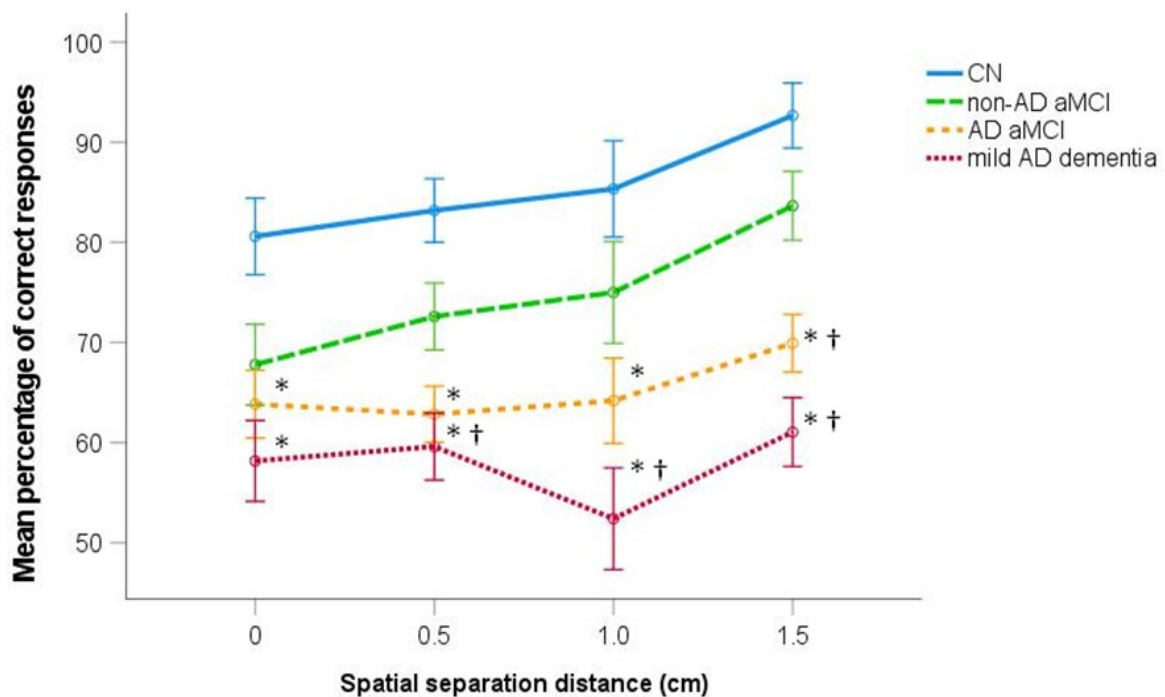
Fig. 7 Spatial pattern separation performance. Mean percentage of correct performance for each spatial separation (±1 SE). ∗p < 0.05 compared to the CN group; †p < 0.05 compared to the non-AD aMCI group. CN, cognitively normal; non-AD aMCI, amnestic mild cognitive impairment with non-Alzheimer's pathologic change; AD aMCI, amnestic mild cognitive impairment with Alzheimer's disease; mild AD dementia, mild dementia with Alzheimer's disease.

4. Discussion

5.1. Study 1

In the virtual Y-maze task, CN older adults preferred world-centered navigation strategy and individuals with early AD preferred body-centered navigation strategy, the preference of which increased with the disease severity. Cognitively impaired participants also had less accurate world-centered navigation performance in real space and this deficit was more pronounced in the participants with mild AD dementia than those with AD aMCI, which is consistent with previous findings (Weniger et al., 2011; Allison et al., 2016b). 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 result indicated that world-centered navigation deficit in AD aMCI led to the change in strategy preference, specifically to the recruitment of compensatory extra-hippocampal strategy (i.e. body-centered), which corresponds to the previous findings in older adults (Colombo et al., 2017). The association between strategy preference and world-centered navigation performance in AD aMCI was explained by the right and left hippocampal atrophy from 22% and 9%, respectively. The hippocampus is impaired in early AD and is a key region for world-centered navigation (Nedelska et al., 2012). These results indicate that AD-related neurodegenerative changes in the hippocampus lead to increased tendency to use extra-hippocampal navigation strategies as a compensation for neurodegenerative changes. Further, atrophy of the BF Ch4p and Ch1-2 nuclei 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 inclination towards extra-hippocampal strategies may also be a consequence of AD-related changes in the BF.

5.2. Study 2

A virtual realistic-looking Navigation Test Suite was used to characterize different profiles of spatial navigation impairment in AD aMCI and non-AD aMCI participants, who had similar performance in conventional cognitive tests. The participants with AD aMCI had worse body-centered navigation than those with non-AD aMCI, who had similar performance as the CN older adults. These findings are in agreement with previous studies indicating that body-centered navigation can distinguish CN older adults from individuals with AD (Tu et al., 2015). Body-centered navigation was associated with atrophy of the precuneus and posterior parietal cortex, which was consistent with previous studies (Wolbers and Wiener, 2014). Lower CSF levels of amyloid-β (i.e., greater burden of AD pathology) were associated with worse body-centered navigation performance, consistent with previous findings (Maass et al., 2019). Next, this study showed world-centered navigation deficits in AD aMCI and non-AD aMCI participants and differences between these two groups, where the AD aMCI participants had a tendency to perform worse in some aspects of world-centered navigation. Previous research provided inconsistent results about the potential of world-centered tasks to differentiate AD aMCI and non-AD aMCI individuals. One study found that AD aMCI individuals were worse than those with non-AD aMCI in finding novel shortcuts (Schöberl et al., 2020). Another study reported that a world-centered task, which involved indicating locations on the map, failed to distinguish individuals with AD from those with other neurodegenerative diseases (Tu et al., 2017). These inconsistent findings indicate that the potential of world-centered tasks to detect AD depends on the specific features of the tasks. This study also showed that world-centered navigation deficits are associated with atrophy in the posterior

hippocampal regions and pmEC. Further, worse performance was associated with higher CSF levels of p-tau$_{181}$, which is in line with a previous study (Allison et al., 2019).

Finally, in the world-centered navigation/perspective taking task, all cognitively impaired participants (i.e., non-AD aMCI, AD aMCI and mild AD dementia) performed worse than the CN older adults. Performance was worse when the perspective shift was greater. No significant differences were found between the AD aMCI and non-AD aMCI participants. The previous study reported differences in perspective taking between aMCI individuals with positive and negative AD biomarkers (Chan et al., 2016), however, there was a considerably smaller perspective shift, which might explain the discrepancy with our results. Further, worse performance in this task was associated with atrophy of the posterior MTL regions (i.e., right hippocampal tail and left hippocampal body and pmEC), precuneus, posterior parietal cortex and right isthmus cingulate/RSC. Worse performance in the task was also associated with higher CSF levels of p-tau$_{181}$ and total tau and lower levels of amyloid-β_{1-42}, consistent with previous studies (Allison et al., 2019).

5.3. Study 3

This study showed that the spatial pattern separation test differentiates participants in the early stages of AD from CN older adults and that performance declines with the severity of the disease. These results support and further extend the findings of previous studies, which indicated that spatial pattern separation tests can differentiate CN and cognitively impaired older adults, although the etiology of cognitive impairment was not determined in these studies (Reagh et al., 2014). The results also showed that the spatial pattern separation task discriminates CN older adults from participants with AD aMCI due to AD with up to 82% sensitivity and 82% specificity. Next, we found that performance declined as the distance between

the original and the second circle was getting smaller indicating that this test actually assesses spatial pattern separation processes (Holden et al., 2012). 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. The reason may be that rapid forgetting occurs between 5 and 10 s and does not accelerate when the time delay increases from 10 s to 20 s (Kesner and Hopkins, 2006). Further, this study showed that the spatial pattern separation deficits are associated with atrophy in the hippocampus, EC and BF Ch1-2 nuclei, which are the regions affected early by AD pathology.

5.4. Study 4

This study was a direct follow-up to Study 3 and found that the AD aMCI participants had worse spatial pattern separation performance than those with non-AD aMCI. Specifically, the task differentiated aMCI participants with AD from those with aMCI of other etiology with high diagnostic sensitivity (>80%). It should be mentioned that the AD and non-AD aMCI participants had similar performance in conventional cognitive tests. These results complement and further extend previous findings indicating that worse performance in a scene discrimination task (Maass et al., 2019) and a task combining spatial and object discrimination (Webb et al., 2020) is associated with cortical amyloid-β accumulation, which is typical for early AD (Palmqvist et al., 2017). Together, these findings indicate that spatial pattern separation assessment may have a potential to detect the AD-related cognitive changes. Further, this study showed that spatial pattern separation deficits in older adults are associated with atrophy in the specific MTL regions including the posterior hippocampus (i.e., the body and tail) and the pmEC, which were previously shown to be involved in spatial information processing (Lee et al., 2008).

6. Conclusion

Our results indicated that spatial navigation and spatial pattern separation assessments can reliably detect AD-related cognitive deficits. Specifically, these assessments can differentiate individuals with AD aMCI from CN older adults and also individuals with non-AD aMCI. Further, spatial navigation and spatial pattern separation deficits are associated with atrophy in specific brain regions that are affected in early AD including the hippocampus (especially the posterior regions), EC (especially the pmEC), BF nuclei and the parietal regions. In addition, deficits in various aspects of spatial navigation reflect different AD pathologies (i.e., amyloid-β and tau). Spatial navigation and spatial pattern separation assessments thus could complement conventional cognitive tests, which lack the diagnostic sensitivity for differentiating AD from other neurodegenerative diseases and may not reliably reflect the underlying AD pathology. 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.

9. References

1. Albert, M. S., DeKosky, S. T., Dickson, D., Dubois, B., Feldman, H. H., Fox, N. C., et al. (2011). The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers. Dement.* 7, 270–9.

2. Allison, S. L., Fagan, A. M., Morris, J. C., and Head, D. (2016a). Spatial Navigation in Preclinical Alzheimer's Disease. *J. Alzheimers. Dis.* 52, 77–90.

3. Allison, S. L., Fagan, A. M., Morris, J. C., and Head, D. (2016b). Spatial Navigation in Preclinical Alzheimer's Disease. *J. Alzheimers. Dis.* 52, 77–90.

4. Allison, S. L., Rodebaugh, T. L., Johnston, C., Fagan, A. M., Morris, J. C., and Head, D. (2019). Developing a Spatial Navigation Screening Tool Sensitive to the Preclinical Alzheimer Disease Continuum. *Arch. Clin. Neuropsychol.* 34, 1138–1155.

5. Auger, S. D., Mullally, S. L., and Maguire, E. A. (2012). Retrosplenial cortex codes for permanent landmarks. *PLoS One* 7, e43620.

6. Berron, D., Cardenas-Blanco, A., Bittner, D., Metzger, C. D., Spottke, A., Heneka, M. T., et al. (2019). Higher CSF Tau Levels Are Related to Hippocampal Hyperactivity and Object Mnemonic Discrimination in Older Adults. *J. Neurosci.* 39, 8788–8798.

7. Braak, H., and Braak, E. (1995). Staging of Alzheimer's disease-related neurofibrillary changes. *Neurobiol. Aging* 16, 271–284.

8. Brunec, I. K., Bellana, B., Ozubko, J. D., Man, V., Robin, J., Liu, Z. X., et al. (2018). Multiple Scales of Representation along the Hippocampal Anteroposterior Axis in Humans. *Curr. Biol.* 28, 2129-2135.e6.

9. Chan, D., Gallaher, L. M., Moodley, K., Minati, L., Burgess, N., and Hartley, T. (2016). The 4 Mountains Test: A Short Test of Spatial Memory with High Sensitivity for the Diagnosis of Pre-dementia Alzheimer's Disease. *J. Vis. Exp.* 116, 54454.

10. Cholvin, T., Hainmueller, T., and Bartos, M. (2021). The hippocampus converts dynamic entorhinal inputs into stable spatial maps. *Neuron* 109, 3135–3148.e7.

11. Colombo, D., Serino, S., Tuena, C., Pedroli, E., Dakanalis, A., Cipresso, P., et al. (2017). Egocentric and allocentric spatial reference frames in aging: A systematic review. *Neurosci. Biobehav. Rev.* 80, 605–621.

12. Holden, H. M., and Gilbert, P. E. (2012). Less efficient pattern separation may contribute to age-related spatial memory deficits. *Front. Aging Neurosci.* 4, 9.

13. Holden, H. M., Hoebel, C., Loftis, K., and Gilbert, P. E. (2012). Spatial pattern separation in cognitively normal young and older adults. *Hippocampus* 22, 1826–1832.

14. Howett, D., Castegnaro, A., Krzywicka, K., Hagman, J., Marchment, D., Henson, R., et al. (2019). Differentiation of mild cognitive impairment using an entorhinal cortex-based test of virtual reality navigation. *Brain* 142, 1751–1766.

15. Jack, C. R., Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Haeberlein, S. B., et al. (2018). NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimer's Dement.* 14, 535–562.

16. Kerbler, G., Nedelska, Z., Fripp, J., Laczó, J., Vyhnalek, M., Lisý, J., et al. (2015). Basal Forebrain Atrophy Contributes to Allocentric Navigation Impairment in Alzheimer's Disease Patients. *Front. Aging Neurosci.* 7, 185.

17. Kesner, R. P., and Hopkins, R. O. (2006). Mnemonic functions of the hippocampus: A comparison between animals and humans. *Biol. Psychol.* 73, 3–18.

18. Laczó, J., Andel, R., Vyhnalek, M., Vlcek, K., Magerova, H., Varjassyova, A., et al. (2012). From Morris Water Maze to Computer Tests in the Prediction of Alzheimer's Disease. *Neurodegener. Dis* 10, 153–157.

19. Lee, A. C., Scahill, V. L., and Graham, K. S. (2008). Activating the medial

temporal lobe during oddity judgment for faces and scenes. *Cereb. Cortex* 18, 683–696.

20. Maass, A., Berron, D., Harrison, T. M., Adams, J. N., La Joie, R., Baker, S., et al. (2019). Alzheimer's pathology targets distinct memory networks in the ageing brain. *Brain* 142, 2492–2509.

21. Maguire, E., Burgess, N., Donnett, J., Frackowiak, R., Frith, C., and O'Keefe, J. (1998). Knowing where and getting there: a human navigation network. *Science* 280, 921–924.

22. Marková, H., Laczó, J., Andel, R., Hort, J., and Vlček, K. (2015). Perspective taking abilities in amnestic mild cognitive impairment and Alzheimer's disease. *Behav. Brain Res.* 281, 229–238.

23. McKhann, G. M., Knopman, D. S., Chertkow, H., Hyman, B. T., Jack Jr, C. R., Kawas, C. H., et al. (2011). The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers. Dement.* 7, 263–269.

24. Mesulam, M. M., Mufson, E. J., Levey, A. I., and Wainer, B. H. (1983). Cholinergic innervation of cortex by the basal forebrain: cytochemistry and cortical connections of the septal area, diagonal band nuclei, nucleus basalis (substantia innominata), and hypothalamus in the rhesus monkey. *J. Comp. Neurol.* 214, 170–197.

25. Moffat, S. D., Elkins, W., and Resnick, S. M. (2006). Age differences in the neural systems supporting human allocentric spatial navigation. *Neurobiol. Aging* 27, 965–972.

26. Nedelska, Z., Andel, R., Laczó, J., Vlcek, K., Horinek, D., Lisy, J., et al. (2012). Spatial navigation impairment is proportional to right hippocampal volume. *Proc. Natl. Acad. Sci. U. S. A.* 109, 2590–2594.

27. Palmqvist, S., Schöll, M., Strandberg, O., Mattsson, N., Stomrud, E., Zetterberg, H., et al. (2017). Earliest accumulation of β-amyloid occurs within

the default-mode network and concurrently affects brain connectivity. *Nat. Commun.* 8, 1214.

28. Pihlajamäki, M., Tanila, H., Könönen, M., Hänninen, T., Hämäläinen, A., Soininen, H., et al. (2004). Visual presentation of novel objects and new spatial arrangements of objects differentially activates the medial temporal lobe subareas in humans. *Eur. J. Neurosci.* 19, 1939–1949.

29. Reagh, Z. M., Ho, H. D., Leal, S. L., Noche, J. A., Chun, A., Murray, E. A., et al. (2016). Greater loss of object than spatial mnemonic discrimination in aged adults. *Hippocampus* 26, 417–422.

30. Reagh, Z. M., Roberts, J. M., Ly, M., Diprospero, N., Murray, E., and Yassa, M. A. (2014). Spatial discrimination deficits as a function of mnemonic interference in aged adults with and without memory impairment. *Hippocampus* 24, 303–314.

31. Rodgers, M. K., Sindone, J. A., and Moffat, S. D. (2012). Effects of age on navigation strategy. *Neurobiol. Aging* 33, 202.e15-202.e22.

32. Schöberl, F., Pradhan, C., Irving, S., Buerger, K., Xiong, G., Kugler, G., et al. (2020). Real-space navigation testing differentiates between amyloid-positive and -negative aMCI. *Neurology* 94, e861–e873.

33. Sperling, R. A., Aisen, P. S., Beckett, L. A., Bennett, D. A., Craft, S., Fagan, A. M., et al. (2011). Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers. Dement.* 7, 280–292.

34. Thal, D. R., Rüb, U., Orantes, M., and Braak, H. (2002). Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology* 58, 1791–1800.

35. Tu, S., Spiers, H. J., Hodges, J. R., Piguet, O., and Hornberger, M. (2017). Egocentric versus Allocentric Spatial Memory in Behavioral Variant Frontotemporal Dementia and Alzheimer's Disease. *J. Alzheimer's Dis.* 59,

883–892.

36. Tu, S., Wong, S., Hodges, J. R., Irish, M., Piguet, O., and Hornberger, M. (2015). Lost in spatial translation - A novel tool to objectively assess spatial disorientation in Alzheimer's disease and frontotemporal dementia. *Cortex* 67, 83–94.

37. Webb, C. E., Foster, C. M., Horn, M. M., Kennedy, K. M., and Rodrigue, K. M. (2020). Beta-amyloid burden predicts poorer mnemonic discrimination in cognitively normal older adults. *Neuroimage* 221, 117199.

38. Weniger, G., Ruhleder, M., Lange, C., Wolf, S., and Irle, E. (2011). Egocentric and allocentric memory as assessed by virtual reality in individuals with amnestic mild cognitive impairment. *Neuropsychologia* 49, 518–527.

39. Whitwell, J. L., Przybelski, S. A., Weigand, S. D., Knopman, D. S., Boeve, B. F., Petersen, R. C., et al. (2007). 3D maps from multiple MRI illustrate changing atrophy patterns as subjects progress from mild cognitive impairment to Alzheimer's disease. *Brain* 130, 1777–1786.

40. Wiener, J. M., Caroll, D., Moeller, S., Bibi, I., Ivanova, D., Allen, P., et al. (2020). A novel virtual-reality-based route-learning test suite: Assessing the effects of cognitive aging on navigation. *Behav. Res. Methods* 52, 630–640.

41. Wolbers, T., and Wiener, J. M. (2014). Challenges for identifying the neural mechanisms that support spatial navigation: the impact of spatial scale. *Front. Hum. Neurosci.* 8.

10. Overview of author´s publications

Cumulative IF = 67.77, H-index 6, total number of citations (according to WOS) 96, without self-citations 85

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**

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**

Laczó, J., Markova, H., Lobellova, V., Gazova, I., **Parizkova, M.**, Cerman, J., et al. (2017). Scopolamine disrupts place navigation in rats and humans: a translational validation of the Hidden Goal Task in the Morris water maze and a real maze for humans. *Psychopharmacology (Berl).* 234, 535–547. IF$_{2017}$ **3.222**

Laczó, J., **Parizkova, M.**, and Moffat, S. D. (2018). Spatial navigation, aging and Alzheimer's disease. *Aging (Albany. NY).* 10, 3050–3051. IF$_{2017}$ **5.515**

Pappas, C., Small, B. J., Andel, R., Laczó, J., **Parizkova, M.**, Lerch, O., et al. (2019). Blood Glucose Levels May Exacerbate Executive Function Deficits in Older Adults with Cognitive Impairment. *J. Alzheimers. Dis.* 67, 81–89. IF$_{2020}$ **4.472**

Laczó, J., Cechova, K., **Parizkova, M.**, Lerch, O., Andel, R., Matoska, V., et al. (2020). The Combined Effect of APOE and BDNF Val66Met Polymorphisms on Spatial Navigation in Older Adults. *J. Alzheimers. Dis.* 78, 1473–1492. IF$_{2020}$ **4.472**

Laczó, M., Wiener, J. M., Kalinova, J., Matuskova, V., Vyhnalek, M., Hort, J., et al. (2021). Spatial Navigation and Visuospatial Strategies in Typical and Atypical Aging. Brain Sci. 11, 1421. IF$_{2020}$ **3.394**

Amlerova, J., Laczó, J., Nedelska, Z., **Laczó, M.**, Vyhnálek, M., Zhang, B., et al. (2022). Emotional prosody recognition is impaired in Alzheimer's disease. *Alzheimers. Res. Ther.* 14, 50. IF$_{2020}$ **6.982**

Lerch, O., **Laczó, M.**, Vyhnálek, M., Nedelská, Z., Hort, J., and Laczó, J. (2022). APOEε4 Allele Moderates the Association Between Basal Forebrain Nuclei Volumes and Allocentric Navigation in Older Adults Without Dementia. *J. Alzheimers. Dis.* 86, 155–171. IF$_{2020}$ **4.472**

Original scientific without relation to the topic of the dissertation
Kubová, H., Folbergrová, J., Rejchrtová, J., Tsenov, G., **Pařízková, M.**, Burchfiel, J., et al. (2018). The Free Radical Scavenger N-Tert-Butyl-α-Phenylnitrone (PBN) Administered to Immature Rats During Status Epilepticus Alters Neurogenesis and Has Variable Effects, Both Beneficial and Detrimental, on Long-Term Outcomes. *Front. Cell. Neurosci.* 12. IF$_{2018}$ **3.900**

Fabera, P., **Parizkova, M.**, Uttl, L., Vondrakova, K., Kubova, H., Tsenov, G., et al. (2019). Adenosine A1 Receptor Agonist 2-chloro-N6-cyclopentyladenosine and Hippocampal Excitability During Brain Development in Rats. *Front. Pharmacol.* 10. IF$_{2019}$ **4.225**

Lectures and poster presentations at professional meetings

7 lectures between 2017-2022, selected examples

Parizkova M. (2018). Virtual navigation assessment in early Alzheimer´s Disease. 2nd DZNE Interdisciplinary Symposium on Spatial Cognition in Aging & Neurodegeneration, Magdeburg, Germany

Pařízková M., Kalinová J., Laczó J. (2020). Early diagnosis of Alzheimer´s disease using spatial navigation assessment. 17th International Medical Postgraduate Conference, virtual.

35 first-author or coauthor posters, selected examples:

Parizkova M., Kalinova J., Vyhnalek M., Hort J., Laczó J. (2018). Right-left discrimination and mental rotation in patients with Alzheimer´s disease. 3rd Congress of the European Academy of Neurology, Vienna, Austria.

Parizkova M., Kalinova J., Vyhnalek M., Hort J., Wiener JM., Laczó J. (2019). Virtual and real-space navigation assessment in early Alzheimer´s disease. Alzheimer's and Parkinson's Diseases Conference, Lisbon, Portugal

Parizkova M., Kalinova J., Vyhnalek M., Hort J., Laczó J. (2020). Virtual navigation assessment and eye-tracking assessment in Alzheimer´s disease. Alzheimer's Association International Conference, virtual

Parizkova M., Kalinova J., Vyhnalek M., Hort J., Wiener JM., Laczó J. (2021). Spatial navigation and scene exploration in biomarker-defined early Alzheimer's disease. 6th Congress of the European Academy of Neurology, virtual