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Investigating Reference Frame Utilization and Spatial Navigation Ability in the Context of Human Aging

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

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Aging-related cognitive decline, accompanied by an increasing older adult population, has spurred more recent research on the mechanisms of aging-related cognitive changes. Out of the various aging-affected cognitive processes, spatial navigation has been of increasing interest in studies, as impaired spatial navigation ability is shown to be one of the first indicators of aging-related cognitive decline and neurodegeneration. Although a general aging-related difference in spatial navigation ability has been observed, there remains a need to further understand the specific subprocesses during naturalistic spatial navigation that are most affected with advancing age. One potential contributor to differences in spatial navigation ability between younger and older adults is different *reference frame utilizations* (egocentric or allocentric). In this project, I pursued the following aims: (1) identify effects of *reference frame utilization* on naturalistic navigation ability (**Aim 1**), (2) identify effects of *aging* on naturalistic navigation ability (**Aim 2**), and (3) investigate *associations between reference frame utilization and aging* with naturalistic navigation ability (**Aim 3**). Overall, the results presented in this project encourages further investigation into the associations between reference frame utilization and aging with naturalistic spatial navigation ability, as considerable differences were found in navigation performance between individuals classified as egocentric and individuals classified as allocentric, older adults (OAs) had significantly decreased spatial navigation ability compared to younger adults (YAs), and finally, OAs classified as egocentric showed considerably decreased spatial navigation ability compared to OAs classified as allocentric, YAs classified as egocentric, and YAs classified as allocentric.

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Background

Human Aging

Aging in humans is a universal phenomenon that is consistently accompanied by significant decline in multiple cognitive processes. Within aging-related cognitive decline, aging impacts different cognitive abilities to various extents (Christensen, 2001; Antsey & Low, 2004; Buckner, 2004; Baghel et al., 2017). In fact, while crystallized cognitive abilities (i.e., retaining factual information, recalling how to perform an action) are not significantly affected with advancing age fluid cognitive abilities (i.e., problem solving, spatial manipulation, abstract thinking) are detrimentally impacted particularly after the age of 60 (Christensen, 2001; Baghel et al., 2017; Kievit et al., 2018). For example, aging leads to decline in executive function, such as the inability to effectively focus attention and juggle multiple tasks (Todorov et al., 2014; Baghel et al., 2017). Additionally, aging also impairs select memory systems, such as short-term declarative memory and episodic memory, while implicit or semantic memory systems are well-maintained (Christensen, 2001; Buckner, 2004; Mather, 2010). Furthermore, aging impairs cognitive speed, as perceptual processing and motor coordination slow down (Roberts & Allen, 2016; Monge & Madden, 2017). Aging impacts a wide variety of cognitive domains, and there is consistent cognitive decline among various cognitive processes that significantly affects quality of daily living and personal autonomy, making aging a worldwide human health concern.

Additionally, there is currently a worldwide increase in the aging population reflected in higher life expectancies (Ferrucci et al., 2008; Kanasi et al., 2016). In 1980, there was a global population of 382 million people over the age of 60; by 2006, there were almost 500 million; in 2030, there will be a projected 1 billion (Dobriansky et al., 2007; United Nations, 2017). Higher life expectancies are due to a general increase in population size, but also a decrease in mortality rates for the leading causes of mortality, including heart disease and cancer (Ferrucci et al., 2008). However, although average life span has been pushed to 85 years of age for all genders, with a current maximum lifespan of 122, average healthspan (the period where an individual is overall in good health) has not followed suit (Robine et al., 2019; Garmany et al., 2021; Olshansky et al., 2022). Although increasing life expectancy has been achieved, it is accompanied by an increased risk for a multitude of disabilities and chronic illnesses—often existing simultaneously. These comorbidities include, but are not limited to, dementia, osteoarthritis, and cardiovascular disease (Jaul & Barron, 2017; Barbé-Tuana et al., 2020). Traditionally, the focus in healthcare has previously been to increase the human lifespan; however, shifting the focus towards increasing the *healthspan* may prove to be more productive and beneficial, increasing independence, personal autonomy, and quality of life in the older population, in addition to increasing their age. Studies have suggested that increasing the human healthspan may result in an overall greater increase in the elderly population compared to efforts that target ameliorating individual diseases (Goldman et al., 2013; Seals et al., 2016; Olshansky et al., 2022). For example, in the Future Elderly Model by Goldman and colleagues, if individual diseases (i.e., cancer or heart disease) are delayed, there will be a projected 0.8-2.0% increase in elderly people by 2060. However, if the overall healthspan of individuals increased, there will be a projected 6.9% increase in the elderly population by 2060 (Goldman et al., 2013). Thus, with the goal of increasing the human healthspan in mind, along with the significantly increased presence of older adults around the world, there is an emerging emphasis to understand the mechanisms of the various aging-related cognitive changes, making aging an increasingly relevant global human health concern.

Aging & Spatial Navigation

Out of the various cognitive processes affected with advancing age, spatial navigation has been of increasing interest to aging-related studies. Spatial navigation refers to the ability to track changes in position and orientation in an environment and determine how to travel from one location to another (Gazova et al., 2012). It is a conserved behavior across species, reflecting its fundamental importance. Notably, based on qualitative, longitudinal, pathophysiological, and brain imaging studies, **impaired spatial navigation ability is shown to be one of the first indicators of aging-related cognitive decline and one of the earliest symptoms of neurodegenerative disorders**, such as Alzheimer's disease (AD). First, qualitative research has shown that subjective self-reports of memory impairment, especially memory complaints surrounding the inability to successfully navigate through unfamiliar environments, are more likely to be associated with objective decline in cognitive status (Amariglio et al., 2011). Additionally, on a Questionnaire of Cognitive Complaints, patients with higher self-reports of subjective cognitive decline and patients with mild cognitive impairment (MCI) reported higher spatial orientation difficulties compared to other subjective cognitive complaints (i.e., losing things, word finding difficulties, etc.) (Markova et al., 2019). Furthermore, in stark contrast with neurotypical control participants, patients with subjective cognitive decline, in addition to patients diagnosed with MCI and AD, were much more likely to express moderate to severe spatial navigation impairment (Cerman et al., 2018). These results indicate that spatial navigation complaints are one of the primary initial complaints in those who self-report cognitive decline.

Secondly, longitudinal studies have suggested that decreased spatial navigation ability, particularly decreased wayfinding ability, reliably predicts the progression of AD in preclinical patients (Allison, 2016; Levine et al., 2019). Spatial navigation deficits are also detected in patients with MCI before they are diagnosed with dementia (Gazova et al., 2012). Notably, in a prospective study, spatial navigation performance predicted predementia syndromes even in neurotypical individuals with no initial dementia-related symptoms (Verghese et al., 2017). These studies suggest that impaired spatial navigation performance is an effective predictor of later dementia symptoms and diagnoses in neurotypical individuals and patients with MCI.

Finally, aging-related pathological studies in humans have shown that deterioration in brain regions associated with navigation precede pathology in other brain regions (Lithfous et al., 2013; Vlček & Laczó, 2014). Particularly, a region that plays a crucial role in spatial navigation ability, showing preferential deterioration with aging, is the hippocampus within the medial temporal lobe (Gazova et al., 2012; Tondelli et al., 2012; Lovden et al., 2012; Coughlin et al., 2018). Hippocampal volume begins deteriorating even in early adulthood (30-40 years old), at annual decrements of around 0.37%-0.75% (DeLisi et al., 1997; Scahill et al., 2003; Lovden et al., 2012), and by age 70, the deterioration rate increases to annual decrements of 1.7% (Raz, 2004). Importantly, in a prospective study, neurotypical subjects who were later diagnosed with MCI or AD initially showed significantly reduced hippocampal volume compared to matched subjects who did not later develop cognitive impairment (Tondelli et al., 2012). Additionally, in AD, the hippocampus is identified as one of the brain regions that undergoes detrimental changes in the preclinical and prodromal stages of the disease (Coughlan et al., 2018). These studies highlight the fact that the hippocampus, a brain region involved in navigation and other forms of spatial cognition, is one of the first brain regions to deteriorate with aging, and its structural changes are involved in the initial cognitive decline of aging patients, who may later be diagnosed with dementias.

Therefore, a deeper understanding of aging-related deficits in spatial navigation ability and their neural correlates may be effective in pinpointing signs of cognitive decline at an early stage and predicting future neurodegeneration.

Measuring Spatial Navigation Ability

Since spatial navigation usually takes place in various external daily environments, it is difficult to measure in laboratory settings. Particularly, spatial navigation research lacks open-source tools that accurately measure spatial navigation performance in a lab setting that mimics real-world environments (i.e., **naturalistic spatial navigation**). Many navigational tasks that are utilized to measure navigation ability have traditionally been pen-and-paper cognitive spatial evaluations (e.g., the Porteus Maze test) or seated computer-based tasks that don't account for select visual, somatosensory, and motor cues usually associated with real-world navigation (Moffat, 2009; Laczó et al., 2014). Moreover, activities on a standard computer do not allow participants to achieve full engagement or presence during navigation tasks (Ijaz et al., 2019). These tasks do not adequately capture the way that humans navigate in the real-world.

Therefore, in the Neural Plasticity Research Lab at the Emory Rehabilitation Hospital, we have aimed to address this inadequacy by creating an open-source virtual reality (VR) maze. Other studies have also approached navigation research through using VR to simulate a large-scale, navigational space (Cushman et al., 2008; Weniger, 2011; Ijaz et al., 2019). This VR maze is modeled off previously developed computer-based mazes (He et al., 2021) that allow participants to complete realistic navigation goals (i.e., finding buildings in a city) and has since been used to measure real-world-like naturalistic navigation deficits in human aging (**Figure 1A, 1B**). The city-like VR maze provides 360 degrees of visuospatial cues and allows participants to interact with their environment by using controllers to walk through the maze, providing participants with more visual, sensory, and motor cues than computer-based tasks. Our preliminary data suggest that older adults exhibit worsened spatial navigation performance than younger adults in the maze, with older adults exhibiting both increased mean completion times and distances traveled (**Figure 1C, 1D**). However, although this general aging-related difference in naturalistic navigation ability has been observed, there remains a need to further understand the specific subprocesses during active real-world spatial navigation that are most affected with advancing age, in order to most effectively target future cognitive neurorehabilitative treatments.

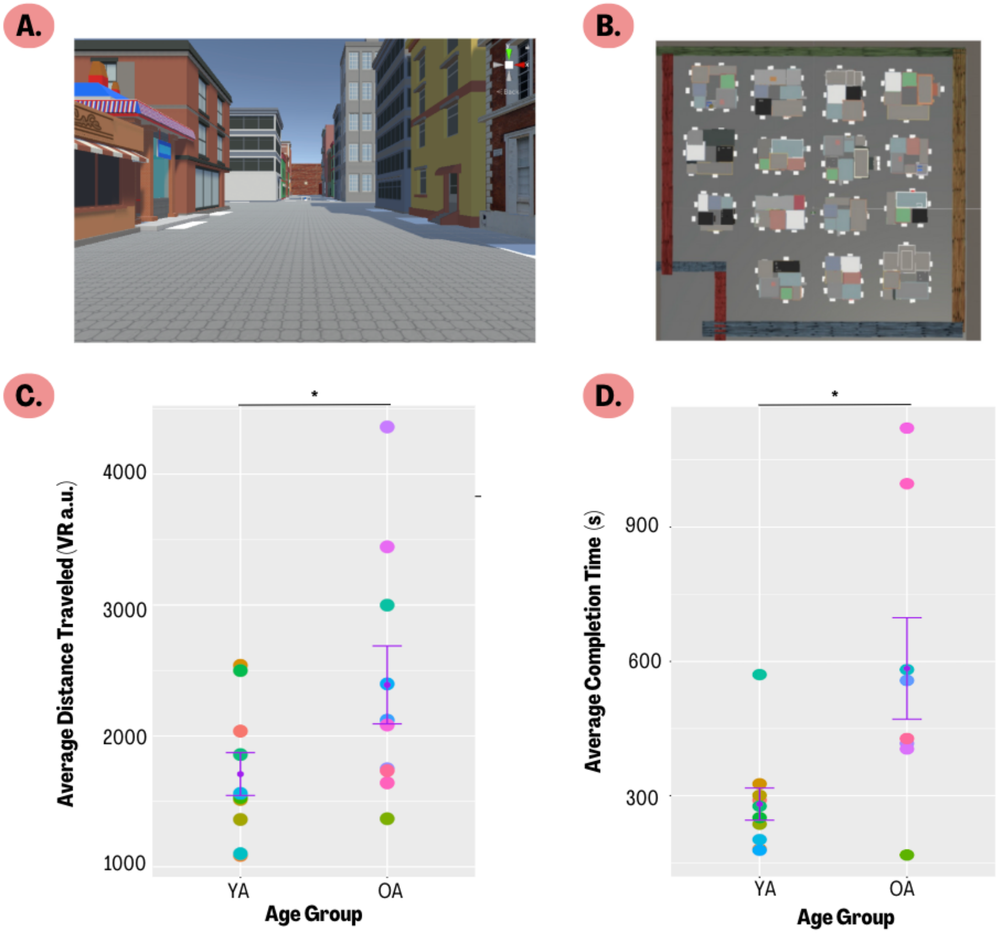


Figure 1. Preliminary data on aging effects on naturalistic navigation ability. (A) First person view and **(B)** Aerial view of city-like Virtual Reality (VR) maze. **(C)** One-way randomized ANOVA revealed significant group difference in mean distance traveled (p = 0.030) and **(D)** mean completion time in 3 maze repetitions (blocks) between younger (YAs) and older adults (OAs) (p = 0.013).

Aging & Reference Frame Utilization

One potential contributor to differences in spatial navigation ability between younger and older adults is different *reference frame utilization*. Past research has found that spatial information is encoded in the brain through either an egocentric reference frame or allocentric reference frame (Moffat, 2009; Rodgers et al., 2012; Lithfous et al., 2013; Vlček & Laczó, 2014; Coughlin et al., 2018). **Egocentric reference frames** refer to the use of first-person, route-centered information to navigate in unfamiliar places, where landmarks and external objects are related to the viewer (i.e., viewer-dependent). On the other hand, **allocentric reference frames** refer to the use of third-person, world-centered information to navigate, where landmarks are related to each other, independent of the viewer (i.e., viewer-independent) (Moffat, 2009; Coughlin et al., 2018). Studies have shown that the posterior parietal cortex is heavily involved in processing egocentric reference frame (Stein, 1992; Ciaramelli et al., 2010), while the hippocampus is crucial for processing allocentric reference frames (Astur et al., 2002, Iaria et al., 2007; Rodgers et al., 2012; Danjo, 2020). Additionally, a third brain region involved in reference frame spatial processing is the retrosplenial cortex, which mediates the two different types of representations and aids in the switching between egocentric and allocentric reference frame utilization (Stacho & Hanahan-Vaugh, 2022).

Past research has shown that, at the group level, younger adults can utilize and switch between reference frames to navigate. However, at the group level, older adults show *increased* utilization of egocentric reference frames and *decreased* utilization of allocentric reference frames, resulting in an egocentric **reference frame bias** (Iaria et al., 2009; Rodgers et al., 2012; Gazova, 2013). There are several neuroanatomical studies that suggest why this reference frame bias may emerge. First, functional neuroimaging studies have suggested that as people age, there is reduced hippocampal activation in older adults when performing navigational tasks compared to younger adults (Moffat, 2009; Konishi et al., 2013). Furthermore, it has also been shown that reduced right hippocampal volume is proportional to impaired spatial navigation ability (Nedelska et al., 2012).

In conclusion, it has been suggested that this reference frame bias may contribute to overall navigational impairment in older adults, such as the inability to effectively form a cognitive map while navigating (Iaria et al., 2009). **Therefore, this aging-related reference frame bias may offer a relevant navigation-based behavioral marker to identify potential aging-related cognitive decline** (Harris et al., 2012; Lithfous et al., 2014).

However, there is limited research exploring associations between naturalistic navigation performance and reference frame utilization. In past studies, the **y-maze task**, a robust behavioral assessment that has been widely utilized in both rodent and human models to assess spatial working memory and navigational preference, has been used to classify individuals' reference frame utilization (Ikonen et al., 1998; Rodgers et al., 2012; Kreuter et al., 2018; McHail et al., 2018; Parizkova et al., 2018). However, the implications of reference frame classification in the y-maze for real-world-like spatial navigation is still being explored. In human studies, there lacks substantial data on the potential differences in y-maze task performance for younger adults and older adults, and the sample size of older adults has been relatively small (He et al., 2021). Secondly, previous studies have largely only compared results from the y-maze to traditional navigational tasks (i.e., pen-and-paper and computerized tasks) or navigation tasks that did not capture real-world-like navigation ability (Rodgers et al., 2012; Parizkova et al., 2018).). **Therefore, I aimed to address these gaps by comparing both younger and older adults' reference frame classification, as measured by the y-maze task, to their naturalistic spatial navigation ability, as measured by performance on city like virtual reality maze.** This project provides the groundwork on how reference frame classification in the y-maze may be associated with human naturalistic spatial navigation ability.

Aims

In this project, I pursued the following aims: (1) identify effects of *reference frame utilization* with naturalistic navigation ability (**Aim 1**), (2) identify effects of *aging* on naturalistic navigation ability (**Aim 2**), and (3) investigate *associations between reference frame utilization and aging* with naturalistic navigation ability (**Aim 3**). I **hypothesized** that individuals classified as "egocentric," or those who have exhibited any egocentric reference frame utilization, will have *decreased* naturalistic navigation ability compared to individuals classified as "allocentric," or those who have exhibited exclusively allocentric reference frame utilization. (**Aim 1**). Following this first hypothesis, I predicted that participants classified as egocentric in the y-maze will have a higher completion time and distance traveled in the city-like maze compared to individuals classified as allocentric. Additionally, I **hypothesized** that older adults (OAs) will have *decreased* naturalistic navigation ability compared to younger adults (YAs) (**Aim 2**). Following this second hypothesis, I predicted that OAs will have a higher completion time and distance traveled in the city-like maze compared to YAs. Finally, I **hypothesized** that, as a group, a greater proportion of OAs will be classified as egocentric *and* have decreased naturalistic navigation ability compared to YAs (**Aim 3**). Following this third hypothesis, I predicted that, compared to YAs, a greater proportion of OAs will be classified as egocentric in the y-maze and will also have a higher completion time and distance traveled in the city-like maze.

Materials and Methods

Methods Common to All Aims:

Participant Characteristics: Neurotypical YAs ($N = 15$; age: 18-35 years) and OAs ($N = 13$; age: ≥ 60 years) were recruited through lab participant databases and advertisements in the surrounding Atlanta community to recruit a diverse set of individuals from various backgrounds.

Eligibility Criteria: Participants were recruited for the study if they fulfilled the following criteria: (1) no history of musculoskeletal impairment, neurologic disease, major head trauma, epilepsy, or seizures; (2) no major psychiatric/sleep disorder or chronic fatigue; and (3) at least an 8th grade education; and (4) fluent English speaker to follow instructions. Once recruited, participants were excluded if they exhibited: (1) atypical cognitive impairment (score < 3 on the Mini-Cog Assessment); (2) recent history of substance abuse (<6 months); and (3) recent history of CNS-active drugs that may influence cortical excitability or learning.

Consideration of Relevant Biological & Sociological Variables: *(1) Sex:* Neurotypical adults of all sexes will be recruited, including male, female, and intersex. *(2) Gender*: Neurotypical adults of all gender identities will be recruited, including women, men, and non-binary/gender-fluid individuals. *(3) Racial & Cultural Identity:* Neurotypical adults of all racial identities, ethnic groups, and cultural backgrounds will be recruited. Notably, sex, gender, and racial identity were standardized self-reported variables.

Questionnaires: Once recruited, participants completed a set of questionnaires to identify potential factors that may affect cognitive performance and navigational ability, assessing daytime sleepiness, sleep quality, self-ratings of navigation ability, medical history, lifestyle, and gaming experience (**Table 1**).

Table 1. **Study-Specific Questionnaires**. A list of all questionaries and surveys that are completed by each participant to identify potential factors that may affect cognitive performance and navigational ability, including: sleepiness, memory, lifestyle, and gaming experience.

Questionnaire/Task Name	Questionnaire/Task Function
Study Questionnaire	Records medical history, mobility status, experience with virtual reality, and lifestyle components
Pittsburgh Sleep Quality Index	Records the sleeping habits and sleep quality of participants during the past month
Stanford Sleepiness Scale	7-point scale that records participants' level of sleepiness at the time of the study
Santa Barbara Sense of Direction Scale	7-level scale that records self-report of navigation ability and preferences
Mini COG	Cognition and memory test

Aim 1. Characterize effects of reference frame utilization with naturalistic navigation ability. To investigate effects of reference frame bias with naturalistic spatial navigation, participants completed the y-maze task and the VR city-like naturalistic maze.

Aim 1 Methods:

Y-Maze

The y-maze task is a well-established, robust navigational assessment that captures reference frame classification (Ikonen et al., 1998; Kreuter et al., 2018; McHail et al., 2018; Parizkova et al., 2018). To complete the y-maze for this study, participants were asked to take a seated position in a chair and utilize the arrow keys on a keyboard to complete the task on a desktop computer. At the beginning of the maze, the participants are placed in a virtual environment at the beginning of a corridor and are prompted to walk down the corridor and make navigational decisions at a fork, turning into either a left or right pathway. Both pathways had random, unique objects on either side of the path, serving as landmark indicators (**Figure 2A, 2B**). A positive auditory cue (i.e., a pleasant guitar chord) is associated with the end of one pathway, and a negative auditory cue is associated with the end of another (i.e., an unpleasant buzzer sound).

After reaching the end of an arm in the maze, participants are teleported back to the start location. Participants repeated the same task until they selected the pathway associated with the positive auditory cue 5 consecutive times *(completing 5 "**non-probe trials**").* Afterwards, participants were prompted to continue to the *"**probe trial,**"* where landmark indicators on each pathway arm rotated to the right by 120°, shifting one set of landmarks and revealing a new set. If the participant chose to navigate down the pathway with the shifted landmarks, it was referred to as a *"**landmark-based**" choice,* which implies allocentric reference frame utilization. However, if the participant chose to navigate down the same pathway associated with the positive auditory cue in the non-probe trial, it was referred to as a *"**route-based**" choice,* implying egocentric reference frame utilization (**Figure 2C)**. Crucially, neither pathway receives auditory feedback on the probe trial. It is also important to note that participants were not told that any of their decisions were "correct" or "incorrect;" they were simply asked to navigate in the maze in a way that they believe is most effective. After finishing the probe trial of the environment, participants are transported to a new environment. There are a total of 5 environments that participants are asked to complete for the complete y-maze task.

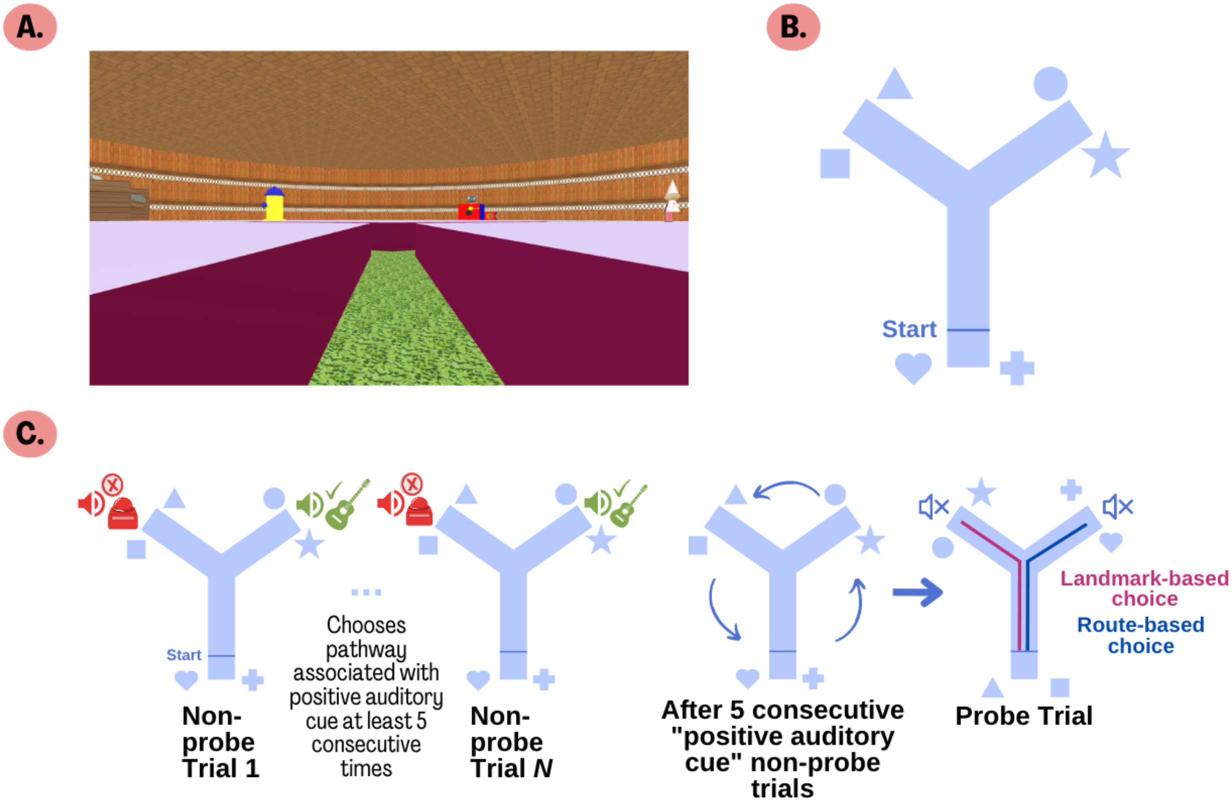


Figure 2. **Conceptual figures of the y-maze**. (**A**) First-person view and (**B**) Conceptual aerial view of the y-maze. **(C)** Visual explanation of the progression through non-probe and probe trials in the y-maze. Participants are prompted to navigate down a corridor and choose the left or right pathway. Both pathways had random, unique landmarks on either side of the path. A positive negative auditory cue is associated with the end of one pathway (i.e., a pleasant guitar chord), and a negative auditory cue is associated with the end of another (i.e., an unpleasant buzzer sound). Participants repeated the same y-maze until they selected the pathway associated with the positive auditory cue 5 consecutive *times (“**non-probe trials**”)*. Afterwards, participants were prompted to continue to the *“**probe trial,**”* where landmarks on each pathway arm rotated to the right by 120°, shifting one set of landmarks and revealing a new set. If the participant chose to navigate down the pathway with the shifted landmarks, it was referred to as a **“landmark-based” choice**, implying allocentric reference frame utilization. However, if the participant chose to navigate down the same pathway associated with the positive auditory cue in the non-probe trials, it was referred to as a **“route-based” choice**, implying egocentric reference frame utilization.

City-Like Virtual Reality (VR) Maze

Before and after participants enter the VR maze, they completed a *Simulator Sickness Questionnaire* that assessed 16 symptoms relating to current sickness level, including fatigue, nausea, blurred vision, vertigo, and difficulty focusing. Their sickness level (none, slight, moderate, severe) was recorded and scored on a scale of 0-60 (Kennedy et al., 1993). If participants scored above a 10 on the questionnaire, they were asked to provide additional consent before participating in the VR maze. This questionnaire indexes any VR-induced sickness that may have impacted navigational performance on the city-like VR maze.

The spatial navigation city-like VR maze task was modeled off previously developed computer-based mazes (He et al., 2021) and was designed and developed using Unity (V 2020.2.f), a virtual reality game development platform. Once participants were immersed in the VR environment, they were first placed in a **Familiarization Trial** to ensure comfortability and familiarization with headset and controller usage. The Familiarization Trial consisted of navigating to 3 easily identified buildings for participants to practice traveling in the VR maze. Each time the participant read target building descriptions at the top of the VR screen. After they successfully navigated to a building, they were teleported back to a start box so that the participant started in the same place each time they navigated to a new building (**Figure 3A)**.

After the Familiarization Trial, participants were then placed in the city-like maze. The city-like maze required navigation to 8 target buildings in a singular block. Example target buildings include the police department, fire station, pizzeria, and high school (**Figure 3B**). In each block, participants were asked to navigate to the same target buildings in the same order. Building instructions and teleportation to the start box were the same as the Familiarization Trial. Participants completed a total of 3 maze-repetitions (blocks) and were instructed to navigate to the target buildings as efficiently as possible.

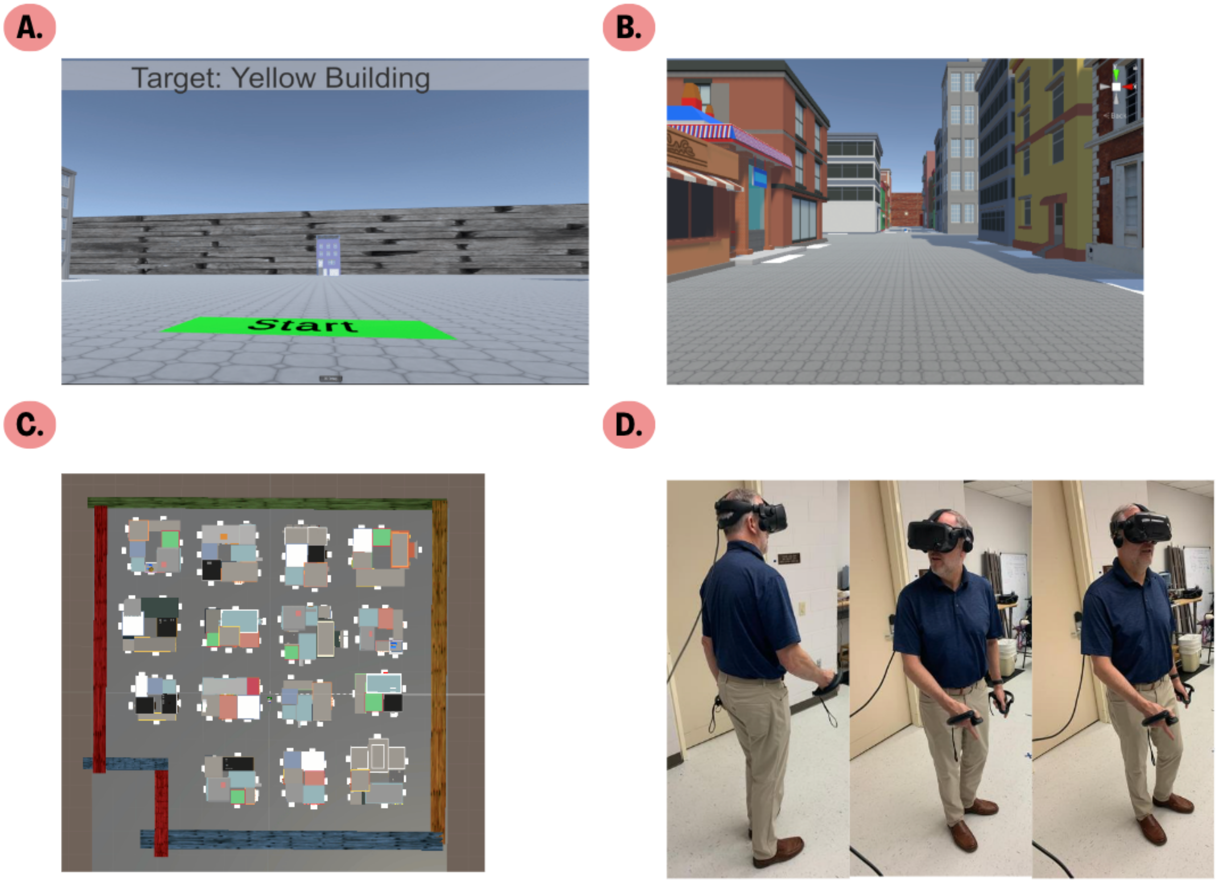


Figure 3. **Conceptual figures for the city-like VR maze.** (**A**) First-person view of the Familiarization Trial, (**B**) First-person view of the city-like VR maze, and (**C**) Aerial view of the city-like VR maze. (**D**) Representative participant using VR headset and controllers to navigate city-like VR maze.

Aim 2. Identify effects of aging on naturalistic spatial navigation.

To investigate aging effects on naturalistic spatial navigation, OAs and YAs completed the VR city-like naturalistic maze.

Aim 2 Methods:

Refer to City-Like VR Maze in Aim 1 Methods *(see above)*.

Aim 3. Investigate associations between reference frame utilization and aging with naturalistic spatial navigation.

To investigate associations between reference frame bias and aging with naturalistic spatial navigation, YA and OA performance on the VR city-like maze and y-maze were compared.

Aim 3 Methods:

Refer to Y-Maze and City-Like VR Maze in Aim 1 Methods *(see above).*

Y-Maze Performance Analysis

The primary outcome measure for the y-maze was the egocentric or allocentric reference frame classification for each individual. In each environment's probe trial, if the participant followed the shifted landmarks associated with the positive auditory cue, it was referred to as a "landmark-based choice," implying *allocentric* reference frame classification. On the other hand, if the participant chose the same path as previous trials even though landmarks have changed, it was referred to as a "route-based choice," implying egocentric reference frame utilization. Individuals who exhibited *any* route-based choice in the five y-maze environments were classified as egocentric. In contrast, individuals who exhibited landmark-based choices in all five y-maze environments were classified as allocentric. This classification was chosen because I was most interested in whether the appearance of any egocentric reference frame utilization had implications for decreased naturalistic spatial navigation ability, and even one instance of a route-based choice suggests that the individual made an egocentric reference frame-based choice to navigate at least some of the time.

Additional secondary outcome measures of the y-maze included average completion time, distance traveled, and speed, compared between non-probe trials and probe trials for each individual. Y-maze data analysis was performed using RStudio Cloud software (RStudio: Integrated Development for R. RStudio, PBC; Boston, MA). First, each matrix was down sampled so the sampling rate was between 0.2 to 0.3 seconds. Second, the total trial time and distance and average speed for all non-probe trials across all environments were calculated and stored. The same process was repeated for all probe trials across all environments. Then, an average non-probe trial time, distance, and speed was calculated by taking the average of all non-probe trials across all five environments. The same process was repeated to obtain average probe trial values across all five environments. Finally, a probe vs. non-probe ratio for trial time, distance, and speed was calculated to quantitatively compare probe and non-probe trials. It is important to note that for each participant, the probe vs. non-probe ratios are standardized to each participant. In other words, the ratio is calculated *independent* of how long, far, or fast a participant's performance in the y-maze is *compared to* other individuals—it is only based on how a participant performs in relation to themselves.

City-Like VR Maze Performance Analysis

The primary outcome measure for the VR city-like maze task was the average distance traveled, averaged over each block of the city-like maze. This value was quantified by taking the total distance traveled across all 3 blocks of the maze and dividing by the number of blocks (3).

Additionally, the secondary outcome measure of the city-like maze was average completion time per block for each individual. This value was quantified by taking the total completion time all 3 blocks of the maze and dividing by the number of blocks (3).

A higher average distance traveled and higher completion time implied poorer spatial navigation performance in the VR city-like maze.

Statistical Analysis

T-tests were performed for all statical analyses. Statistical analysis was performed using RStudio Cloud software (RStudio: Integrated Development for R. RStudio, PBC; Boston, MA). Outliers were evaluated using the following bounds: lower bound: *Q1 – 1.5IQR; upper bound: *Q3 + 1.5IQR. Outliers were excluded from subsequent analyses. Critical alpha level was set to $p < 0.05$ (uncorrected).

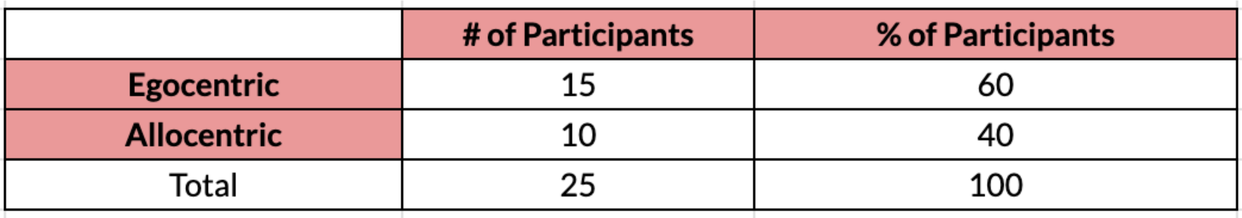
* Q1 = 25th percentile; Q3 = 75th percentile; IQR (interquartile range) = Q3 – Q1

Results

Aim 1: Individuals classified as egocentric in the y-maze had significantly increased completion time but not distance traveled in the city-like VR maze compared to individuals classified as allocentric.

To characterize effects between reference frame classification and naturalistic spatial navigation ability, participants completed the y-maze task and three blocks of the city-like VR maze task. Participants were classified as egocentric if they made a route-based choice on *any* of the probe trials. On the other hand, participants were classified as allocentric *only if* they made a landmark-based choice on *all* probe trials. The number and percentage of individuals classified as egocentric and allocentric is shown in **Table 2**.

Table 2. **Proportion of participants with egocentric or allocentric reference frame classification.**



	# of Participants	**% of Participants**
Egocentric	15	60
Allocentric	10	40
Total	25	100

No significant difference was found for the average distance traveled in the city-like maze between egocentric and allocentric individuals (t_{21}: 1.829; p=0.084) (**Figure 4A**). However, a significant difference was found in the average completion time between the two reference frame classification groups (t_{24}: 2.699; p=0.014) (**Figure 4B**).

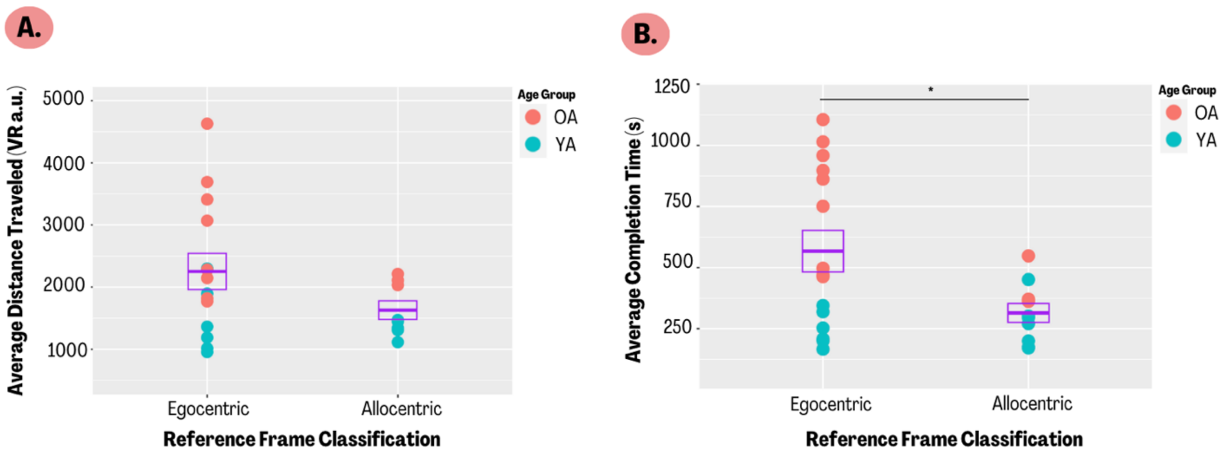


Figure 4. Effects of reference frame classification and naturalistic spatial navigation ability. (A) Average distance traveled was measured in arbitrary VR units (VR a.u.). No significant difference was found for the average distance traveled in the city-like maze between individuals classified as egocentric and those classified as allocentric (t_{21}: 1.829; p = 0.084). Two individuals classified as allocentric and one individual classified as egocentric were removed due to being outliers (see Materials and Methods: Statistical Analyses for how outliers were determined). Egocentric: N = 14; Allocentric: N = 8. (**B**) A significant difference was found for the average completion time between individuals classified as egocentric and allocentric (t_{24}: 2.699; p = 0.0142). No outliers were determined in average completion time data. Egocentric: N = 15; Allocentric: N = 10. Red dots indicate older adult participants (**OAs**); turquoise dots indicate younger adult participants (**YAs**).

Next, to further analyze y-maze performance of individuals classified as egocentric and allocentric by comparing probe and non-probe trial performance, a ratio was calculated for average completion time, distance traveled, and speed in probe vs. non-probe trials (**Figure 5A, 5B, 5C**). Significant differences were not found for the average completion time ratios (t_{24}: -0.673; p = 0.509), average distance traveled ratios (t_{25}: 0.610; p = 0.554), nor average speed ratios (t_{25}: 0.734; p = 0.472).

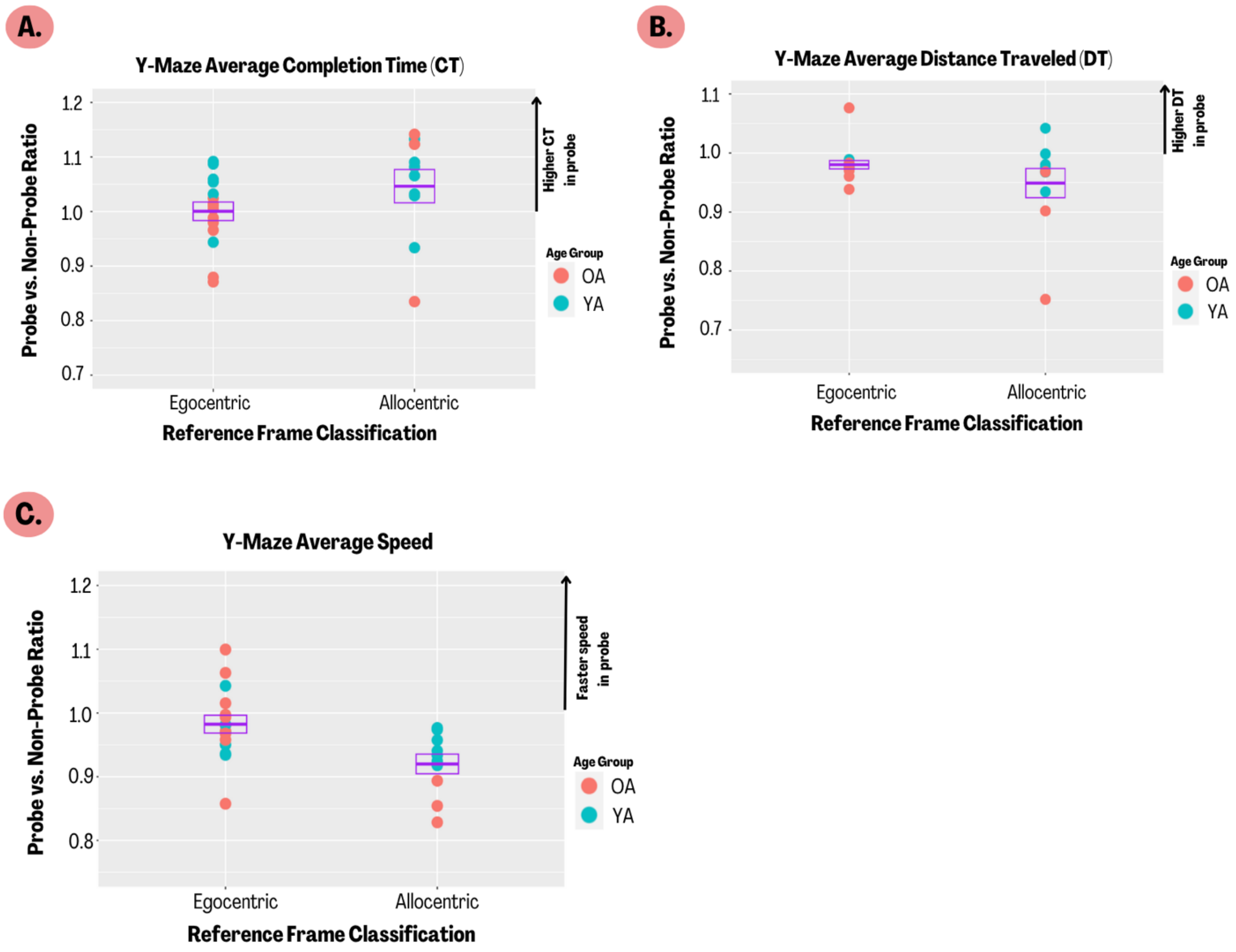


Figure 5. Y-maze probe vs. non-probe trial ratios. (A) Probe vs. non-probe ratio for average completion time in the y-maze across all five environments (t_{24}: -0.673; p = 0.509). One individual classified as allocentric and two individuals classified as egocentric were removed due to being outliers (see Materials and Methods: Statistical Analyses for how outliers were determined). Egocentric: N = 15; Allocentric: N = 10. (**B**) Probe vs. non-probe ratio for average distance traveled in the y-maze across all five environments (t_{25}: 0.610; p = 0.554). One individual classified as allocentric (same outlier as in A.) and one individual classified as egocentric were removed due to being outliers. Egocentric: N = 16; Allocentric: N = 10. (**C**) Probe vs. non-probe ratio for average speed in the y-maze across all five environments. None of the probe vs. non-probe ratios were significantly different between individuals classified as egocentric and allocentric (t_{25}: 0.734; p = 0.472). One individual classified as allocentric (same outlier as in A. and B.) and one individual classified as egocentric (same outlier as one of the outliers in A.) were removed due to being outliers. Egocentric: N = 16; Allocentric: N = 10. Red dots indicate older adult participants (**OAs**); turquoise dots indicate younger adult participants (**YAs**).

Aim 2: Individuals in the OA age group (≥ 60 years of age) displayed both increased average distance traveled and average completion time in the city-like maze compared to individuals in the YA age group (18-35 years of age).

To explore aging effects on naturalistic spatial navigation ability, the city-like VR maze data was analyzed for YAs and OAs.

A significant difference in average distance traveled in the city-like maze was found (t_{23}: 3.436; $p = 0.004$). Additionally, there was a significant difference in average completion time in the city-like maze (t_{24}: 5.358; $p < 0.0005$) (**Figure 6A, 6B**). Furthermore, Additionally, the average distance traveled and completion time values of OAs have considerably higher variability than YAs.

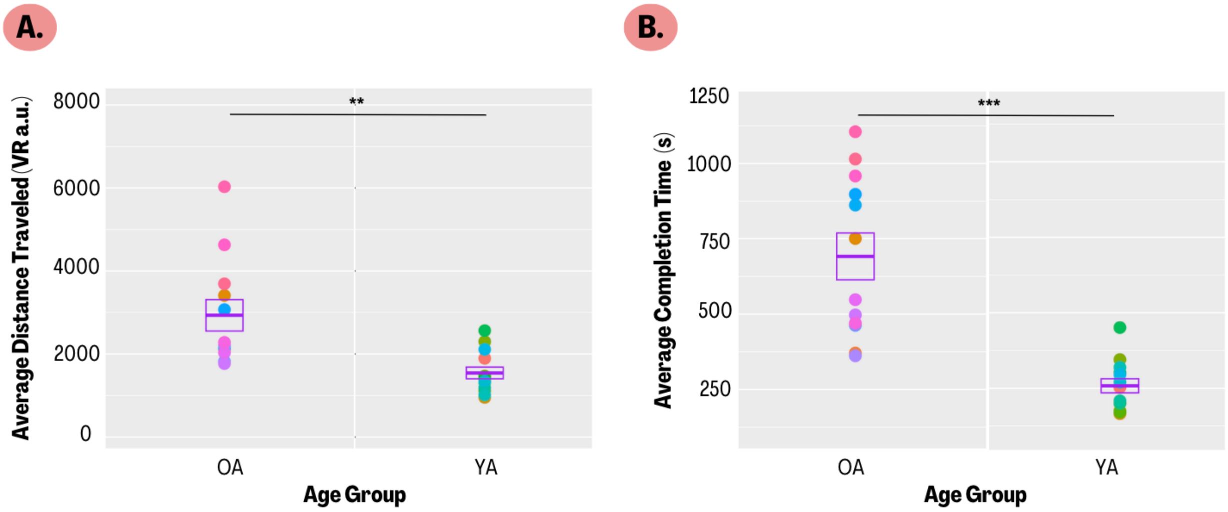
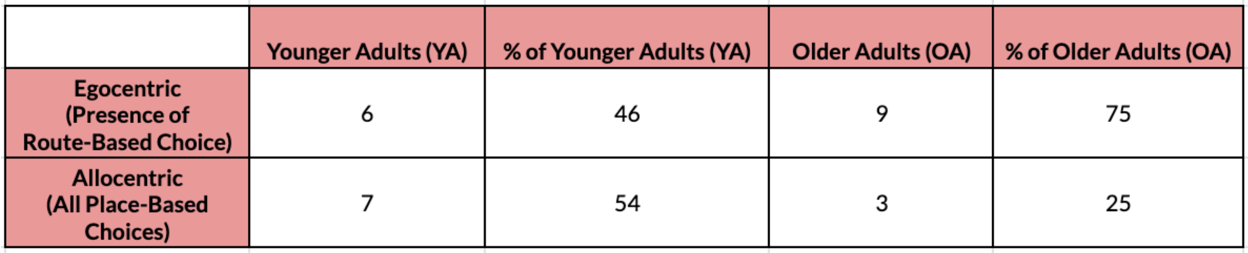


Figure 6. Aging effects on naturalistic spatial navigation ability. (A) Average distance traveled was measured in arbitrary virtual-reality units. Average distance traveled in the city-like maze was significantly higher for older adults (OAs) than younger adults (YAs) (t_{23}: 3.436; $p = 0.004$). One OA was removed due to being an outlier. OAs: N = 11; YAs: N = 13. (**B**) Average completion time in the city-like maze was also significantly higher for OAs than YAs (t_{24}: 5.358; $p < 0.0005$). No outliers were removed. OAs N = 12; YAs: N = 13. Each colored dot represents a participant.

Aim 3: A greater proportion of OAs were classified as egocentric compared to YAs and exhibited the highest average distance traveled and completion time in the city-like maze compared to OAs classified as allocentric and both YA reference frame groups.

To explore associations between reference frame classification and aging with naturalistic spatial navigation ability, participants were classified into four groups based on their age group and reference frame classification: OA-Egocentric, OA-Allocentric, YA-Egocentric, YA-Allocentric. All individuals who exhibited *at least one instance* of a route-based choice in the y-maze was classified as egocentric, while individuals who exhibited *all* landmark-based choices was classified as allocentric. The number and proportion of individuals classified as egocentric and allocentric in both age groups are displayed in **Table 3**. Out of the OAs, there was a greater proportion of individuals classified as egocentric (75%) compared to individuals classified as allocentric (25%). In contrast, out of the YAs, there was around an equal proportion of individuals classified as egocentric and allocentric (46% and 54%, respectively) (**Table 3**).

Table 3. **Number and proportion of OA and YA individuals classified as egocentric and allocentric**.



	Younger Adults (YA)	% of Younger Adults (YA)	Older Adults (OA)	% of Older Adults (OA)
Egocentric (Presence of Route-Based Choice)	6	46	9	75
Allocentric (All Place-Based Choices)	7	54	3	25

No statistical analyses between the four groups were appropriate due to insufficient sample size of OAs classified as allocentric and the unequal group distributions. However, preliminary data shows that although YAs classified as allocentric and egocentric had similar average distance traveled and completion time in the city-like maze, OAs classified as egocentric had a considerably higher average distance traveled and completion time than OAs classified as allocentric. Additionally, the average distance traveled and completion time values of OAs classified as egocentric have considerably higher variability than OAs classified as allocentric (**Figure 7A, 7B**).

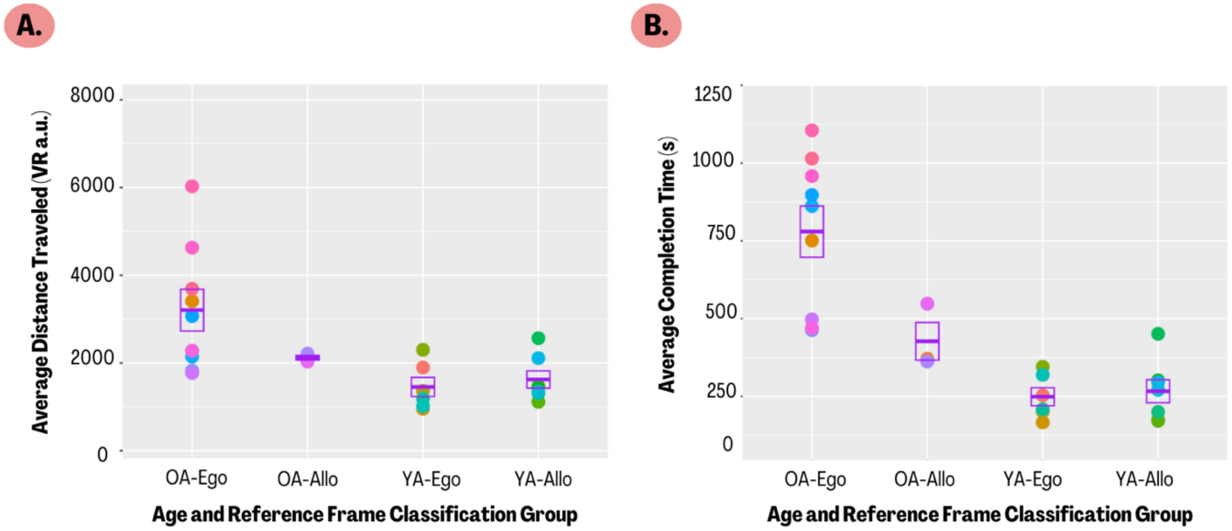


Figure 7. Preliminary data on associations between reference frame classification and aging with naturalistic spatial navigation ability. Although no statistical analyses performed due to insufficient sample size of older adults (OAs) classified as allocentric, preliminary data suggests there is no difference in city-like VR maze performance between younger adult (YA) individuals classified as egocentric or allocentric. However, there is considerable difference in maze performance between OAs classified as egocentric or allocentric; OAs classified as egocentric display (**A**) higher average distance traveled and (**B**) higher average completion time. Average distance traveled was measured in arbitrary VR units (VR a.u. OAs classified as egocentric are abbreviated "**OA-Ego**," OAs classified as allocentric are abbreviated "**OA-Allo**," YAs classified as egocentric are abbreviated "**YA-Ego**," and YAs classified as allocentric are abbreviated "**YA-Allo**." OA-Ego: N = 9; OA-Allo: N = 3; YA-Ego: N = 6; YA-Allo: N = 7. Each colored dot represents a participant.

Discussion

Overall, there were three aims that were addressed in this study: (**Aim 1**) characterize effects of reference frame utilization on naturalistic spatial navigation ability; (**Aim 2**) characterize effects of aging on naturalistic spatial navigation ability; (**Aim 3**) characterize associations between reference frame utilization with naturalistic spatial navigation ability. There was no significant difference in naturalistic spatial navigation ability between individuals classified as egocentric or allocentric (**Aim 1**). However, OAs displayed significantly decreased naturalistic spatial navigation ability compared to YAs (**Aim 2**). Additionally, a greater proportion of OAs were classified as egocentric and displayed considerably decreased naturalistic spatial navigation ability compared to both OAs classified as allocentric and YAs of both reference frame classifications (**Aim 3**).

Aim 1: Individuals classified as egocentric showed trends of decreased naturalistic spatial navigation ability compared to those classified as allocentric.

To characterize effects of reference frame classification on naturalistic spatial navigation ability, participants completed the y-maze task and three blocks of the city-like VR maze task, respectively. Past studies suggested that loss of the utilization of an allocentric reference frame in OAs has led to decreasing spatial navigation ability (Iaria et al., 2009; Moffat, 2009), so I hypothesized that individuals classified as egocentric would have decreased naturalistic navigation ability compared to individuals classified as allocentric. Participants were classified as egocentric if they made a route-based choice on *any* of the probe trials and allocentric *only if* they made a landmark-based choice on *all* probe trials. Again, this classification was chosen because I was most interested in whether the appearance of any egocentric reference frame utilization had implications for decreased naturalistic spatial navigation ability, and even one instance of a route-based choice suggests that the individual made an egocentric reference frame-based choice to navigate at least some of the time.

Although a significant difference was found in average completion time with individuals classified as egocentric exhibiting higher average completion time, no significant difference was found in average distance traveled (**Figure 4A, 4B**). These data suggest that egocentric individuals may have taken longer to decide where to navigate in the city-like maze than allocentric individuals, which suggests some uncertainty. Furthermore, though no significant difference was found in average distance traveled (p = 0.084), there was a trend for a difference in average distance traveled that showed the same relationship as average completion time. Based on this data, my hypothesis that individuals classified as egocentric would exhibit decreased naturalistic spatial navigation ability is tentatively supported. These data suggest that egocentric reference frame utilization may be an indicator of decreased naturalistic spatial navigation ability. Other studies have also shown that impaired allocentric spatial processing (and therefore an increased utilization of egocentric reference frames) has been associated with decreased spatial navigation ability (Moffat & Resnick, 2002; Iaira et al., 2009).

Next, the data analysis on the secondary outcome measures of the y-maze aimed to explore whether there was a difference in y-maze performance between individuals classified as egocentric and individuals classified as allocentric individuals. Specifically, I aimed to explore whether there was a difference in average completion time, distance traveled, and average difference in speed between y-maze probe trials compared to non-probe trials across all environments. In the *non-probe trials*, due to the positive and negative auditory cues, participants are primed to choose the pathway that is associated with the positive cue after the first non-probe trial in each y-maze environment. However, since there is *only one probe trial* per environment, where the landmarks shift 120 ° and a new set of landmarks is revealed, the participant does not have positive or negative auditory cues to rely on and must choose a pathway by either following the shifted landmarks (*landmark-based choice*) or staying with the same pathway that was associated with the positive auditory cue in the non-probe trials (*route-based choice*). It can be reasonably inferred that if an individual exhibited longer completion time, greater distance traveled, or slower speed in their probe trials compared to their non-probe trials, then there was active processing of the shifted landmarks during the probe trial. Therefore, I hypothesized that individuals classified as allocentric would have a higher average completion time and distance traveled but slower speed in the probe trials compared to the non-probe trials, as higher values suggest a higher degree of exploration and lower navigation certainty during the probe trial. Following my hypothesis, I predicted that the probe vs. non-probe ratios for average completion time and distance traveled would be greater than 1 for individuals classified as allocentric. I also predicted that the probe vs. non-probe ratios per individual for average speed would be less than 1 for individuals classified as allocentric. In contrast, I predicted that the probe vs. non-probe ratios for average time, distance, and speed of individuals classified as egocentric would be near 1, indicating no difference in performance between the probe and non-probe trials. Again, each probe vs. non-probe ratio was standardized to each participant (see Methods: Y-Maze Performance Analysis).

No significant difference in average completion time, distance traveled, nor speed were found between individuals classified as allocentric or egocentric (**Figure 5A, 5B, 5C)**. However, intriguingly, for the average speed ratios, all allocentric individuals exhibited a ratio of less than 1, indicating that they were all, on average, slower in their probe trials compared to their non-probe trials, implying active processing of the shifted landmarks in the y-maze probe trials (**Figure 5C**). In contrast, for individuals classified as egocentric, around half exhibited a ratio of a slightly higher than 1, while the other half exhibited a ratio of slightly less than 1, indicating that, on a group level, the probe vs. non-probe ratio is around 1 (**Figure 5C**). These data suggest that, at the group level, individuals classified as egocentric had no considerable difference in probe and non-probe trial average speed, implying that there was no evidence to suggest active processing of the shifted landmarks in the probe trials.

These data are one of the first to explore differences between y-maze non-probe and probe trial performance for individuals classified as egocentric and individuals classified as allocentric. Further analysis of y-maze performance can allow us to further examine behavioral differences and interpret individuals' degree of exploration and navigation certainty in the y-maze (see Limitations).

Aim 2: Individuals in the OA age group (≥ 60 years of age) displayed significantly decreased naturalistic spatial navigation ability compared to individuals in the YA age group (18-35 years of age).

To investigate aging effects on naturalistic spatial navigation ability, the city-like VR maze data for both YAs and OAs was analyzed. An emphasis was placed on studying *naturalistic* spatial navigation ability through VR technology because traditional seated pen-and-paper and computerized tasks do not sufficiently capture how humans navigate in the real world. For example, visual, somatosensory, and motor cues usually associated with real-world navigation are not present in seated tasks (Moffat, 2009; Laczó et al., 2014). I hypothesized that OAs would exhibit decreased naturalistic spatial navigation ability compared to YAs. The results support my hypothesis, as both average distance traveled and completion time were significantly higher in OAs than in YAs (**Figure 6A, 6B**). Furthermore, these data support previous preliminary data done in the lab (**Figure 1C**, **1D**). These data validate the aging effects that have been observed in traditional navigational tasks (Barrash, 1994; Moffat & Resnick, 2002; Iaria et al., 2009) in a real-world-like environment, even though more visuospatial, somatosensory, and motor cues were provided. Therefore, these data suggest that the observed aging effects related to spatial navigation ability may not be due to the amount of sensory information provided; a more relevant contributor is the difference in active processing of spatial information between OAs and YAs during the task.

Aim 3: A greater proportion of OAs were classified as egocentric compared to YAs and displayed considerably decreased naturalistic spatial navigation ability compared to OAs classified as allocentric and YAs classified as either egocentric or allocentric.

To investigate associations between reference frame classification and aging with naturalistic navigation ability, participants were assigned into one of four groups based on their age and reference frame classification: OA-Egocentric, OA-Allocentric, YA-Egocentric, and OA-Allocentric. Based on previous studies that have suggested that older adults lose the ability to utilize an allocentric reference frame to navigate (Iaria et al., 2009; Rodgers et al., 2012; Gazova, 2013), I hypothesized that, at the group level, a greater proportion of OAs would be egocentric, and that these individuals would exhibit the worst spatial navigation ability compared to individuals classified into the other three groups. In line with the first hypothesis, I also hypothesized that, at the group level, there would be an equal proportion of YA individuals classified as egocentric or allocentric, and that YAs classified as allocentric would exhibit the best spatial navigation ability out of the four groups.

Although it was not appropriate to perform statistical analyses given the small sample size of OAs classified as allocentric and the unequal group distributions, the preliminary data showed intriguing trends. First, out of the OAs, the proportion of individuals classified as egocentric (75%) was considerably greater than the proportion of individuals classified as allocentric (25%). On the other hand, the proportion of YAs classified as egocentric and those classified as allocentric was around the same (46% and 54%, respectively) (**Table 3).** These data are in line with my hypothesis that a greater proportion of OAs would be classified as egocentric, and that there would be a roughly equal proportion of YAs classified as either reference frame group. There appears to be an aging-related difference in the proportion of reference frame utilization across individuals, which may reflect a shift in reference frame utilization with aging. Previous studies have also provided results that suggest this egocentric reference frame bias emergence in OAs (Iaria et al., 2009; Rodgers et al., 2012; Gazova, 2013). Furthermore, inability to perform statistical analyses due to insufficient number of OAs classified as allocentric further suggests that as individuals age, there is a shift towards egocentric reference frame utilization.

Importantly, OAs classified as egocentric demonstrated increased average distance traveled and completion time in the city-like VR maze compared to OAs classified as allocentric; yet YAs classified as egocentric and YAs classified as allocentric appear to have similar average distance traveled and completion time (**Figure 7A, 7B**). These preliminary data suggest, interestingly, that reference frame utilization in YAs may not be associated with spatial navigation ability; however, in contrast, reference frame utilization in OAs may be one contributing factor to aging-related declines in naturalistic spatial navigation ability. Therefore, it may be that aging, coupled with the increased utilization of egocentric reference frames, is associated with decreased spatial navigation ability. These preliminary data support other studies that have suggested older adults with a preference for egocentric reference frame utilization have decreased spatial navigation ability (Moffat & Resnick, 2002; Iaria et al., 2009; Rodgers et al., 2012).

Limitations

One limitation in this study is the unrepresentative method of calculating average distance traveled, completion time, and speed probe vs. non-probe ratios in the y-maze analysis of **Aim 1** (**Figure 5**). To calculate the ratio, the average of *all* non-probe trials across all five environments was used for each individual. Although this ratio is standardized to the individual (see Methods: Y-Maze Performance Analysis), this average may not accurately characterize the difference in probe and non-probe trial performance, as individuals may take longer, travel farther, and be slower during the first few non-probe trials compared to the last few non-probe trials per environment. This trend is present because individuals would still be familiarizing themselves on how to navigate down the corridor, choose a pathway, and how to interpret the positive or negative auditory cues the first time they enter a new environment. With the goal of capturing the non-probe trials that most accurately depict an individual's y-maze performance when they are at their highest certainty, the average of the last non-probe trials *before* the probe trial across all environments may be more informative. At the last non-probe trial before the probe trial, an individual is most certain in which path to choose, as it would be the fifth consecutive time that they will have chosen the same pathway associated with the positive auditory cue (**Figure 2C**). Furthermore, to provide even further accuracy, the average completion time, distance traveled, and speed ratio for each of the five y-maze environments could be calculated first, then the average of the five ratios could be calculated. This strategy would consider any inter-environmental differences in y-maze performance. Thus, the probe vs. non-probe ratios would more accurately characterize the difference in probe and non-probe trial performance if both data analysis revisions were implemented.

Additionally, a second limitation is that, although there is a Familiarization Trial for the city-like VR maze task that allows individuals to be comfortable with using the VR headset and controllers, there is not a Familiarization Trial in the y-maze. This limitation may have caused some participants to have a longer completion time, travel farther, or have a slower speed in the y-maze because they may not have been as familiar as others in using the arrow keys on the keyboard to navigate down the corridor and turn to choose a pathway. However, this is not a major limitation since all y-maze performance analyses were standardized to the participant, so even if y-maze performance differed between individuals because of motor coordination or familiarity in controlling keyboard arrow keys, this would not have skewed their non-probe vs. probe ratio.

Furthermore, the sample size of the OAs classified as allocentric is a limitation as it was not large enough to perform statistical analyses to investigate associations between reference frame classification and aging with naturalistic spatial navigation ability. Although the data shows interesting trends, the difference in navigation performance could not be objectively compared statistically. It is important to note that one OA was not able to complete the 3 blocks of the city-like VR maze and the y-maze due to mobility challenges, so their data was excluded altogether. Moreover, the city-like VR maze data of two YAs were not properly collected due to a data collection malfunction, so their city-like maze data was not used, but their y-maze data was used for the probe vs. non-probe ratios (**Figure 5**).

Future Directions

The data in this study support further exploration of reference frame classification and its potential associations with naturalistic spatial navigation ability. First, a larger sample size with at least N = 25 YAs and N = 25 OAs could allow for statistical analyses to be performed across the four age group-reference frame classification groups (OA-Egocentric, OA-Allocentric, YA-Egocentric, YA-Allocentric). At the Neural Plasticity Research Lab, we are presently continuing to recruit participants and collect data.

Additionally, instead of a discrete age group classification, the age of an individual could be used instead. This would provide more insight into how navigation performance differs within the YAs (i.e. between an 18 year-old and 35 year-old) and within the OAs (i.e. between a 60 year-old and 90 year-old), and could provide a more gradual display of the emergence of increased egocentric reference frame utilization and spatial navigation decline.

Thirdly, the demographics of the individuals could be incorporated into data analysis so that associations between sex and ethnicity differences and naturalistic spatial navigation ability could be explored in addition to age group and reference frame classification. Additionally, differences in VR experience among individuals could be explored to investigate whether there is a difference in spatial navigation performance in the city-like VR maze. Furthermore, the data from the Simulator Sickness Questionnaire pre-VR and post-VR could be analyzed to ensure there was no significant VR-related sickness involved in the city-like VR maze task.

Finally, a long-term goal relating to implications of reference frame classification by the y-maze is the utilization of the y-maze as an easily administered tool in clinical settings to identify early indicators of aging-related cognitive decline.

Conclusion

Aging effects seen in traditional navigational tasks were likewise observed in a real-world-like environment that measured naturalistic spatial navigation ability. Additionally, aging, coupled with the increased utilization of egocentric reference frames, was found to be associated with decreased spatial navigation ability. Overall, the data presented in this project encourages further investigation into reference frame utilization and its implications for naturalistic spatial navigation ability in the context of human aging.

Supplemental Materials

Reference Frame Classification: Addition of Mixed Reference Frame Group

While performing data analysis on reference frame classification of individuals, a considerable number of individuals exhibited both “landmark-based” and “route-based” choices in the y-maze. While they were all classified as egocentric in the study, supplementary data analysis was performed by alternatively classifying these individuals as “mixed,” (i.e., exhibiting both route-based and landmark-based choices in the y-maze). **Table S1** shows the number and proportion of individuals classified as egocentric, mixed, or allocentric. Additionally, **Figure S1** displays a gradient showing individuals classified by the proportion of route-based choices (i.e., egocentric reference frame utilization) to landmark-based choices (i.e., allocentric reference frame utilization) they exhibited in the y-maze.

Table S1. Number and proportion of OA and YA individuals classified as egocentric, mixed, or allocentric.

	Young Adults (YA)	% of Young Adults (YA)	Older Adults (OA)	% of Older Adults (YA)
Egocentric	3	23	5	42
Mixed	3	23	4	33
Allocentric	7	54	3	25

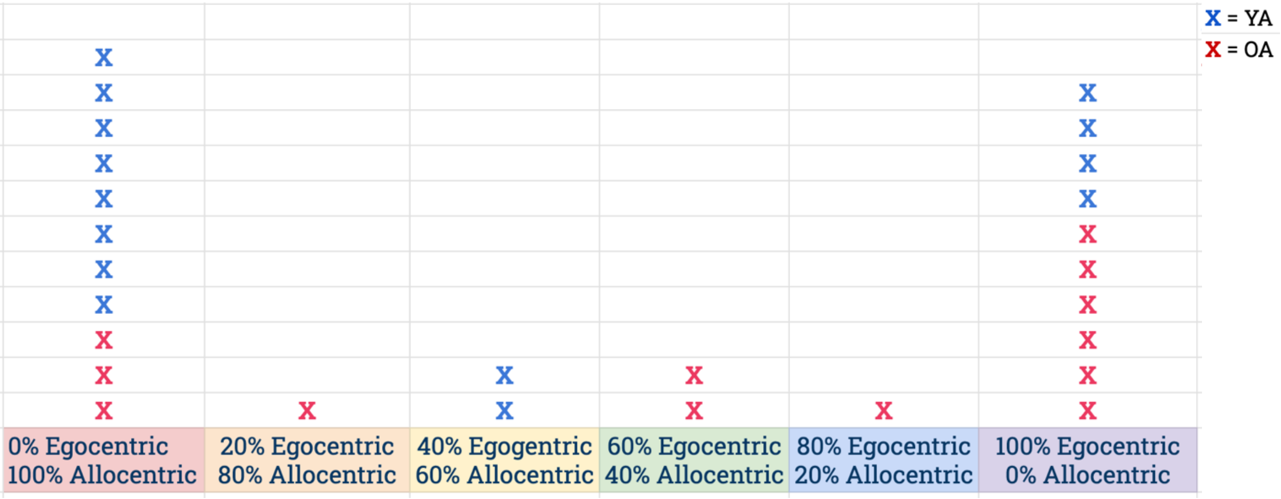


Figure S1. Individuals classified by the proportion of route-based choices (egocentric reference frame utilization) to landmark-based choices (allocentric reference frame utilization) exhibited in the y-maze.

First, effects of reference frame classification on spatial navigation ability were explored (**Figure S2**). There was no significant difference in average distance traveled or completion time between the three groups.

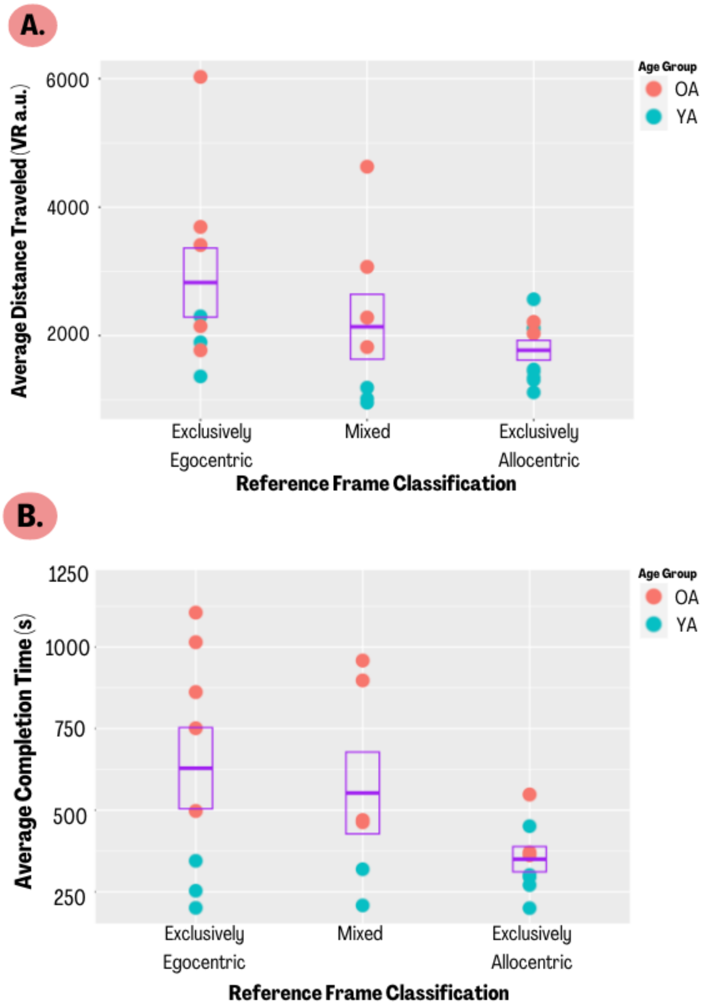


Figure S2. Effects of reference frame classification (exclusively egocentric, mixed, and exclusively allocentric) and naturalistic spatial navigation ability. One-way ANOVA was performed for statistical analysis. **(A)** Average distance traveled was measured in arbitrary VR units (VR a.u.). No significant difference was found for the average distance traveled in the city-like maze between individuals classified as egocentric and those classified as allocentric (f_{24}: 1.89; p = 0.175). Exclusively Egocentric: N = 8; Mixed: N = 7; Exclusively Allocentric: N = 10. (**B**) A significant difference was found for the average completion time between individuals classified as egocentric and allocentric (f_{24}: 3.077; p = 0.066). No outliers were determined in average completion time data Exclusively Egocentric: N = 8; Mixed: N = 7; Exclusively Allocentric: N = 10. Red dots indicate older adult participants (**OAs**); turquoise dots indicate younger adult participants (**YAs**).

Additionally, the probe vs. non-probe ratios were calculated while taking mixed reference frame classification into account. There was no significant difference found in average completion time, distance traveled, or speed (**Figure S3**).

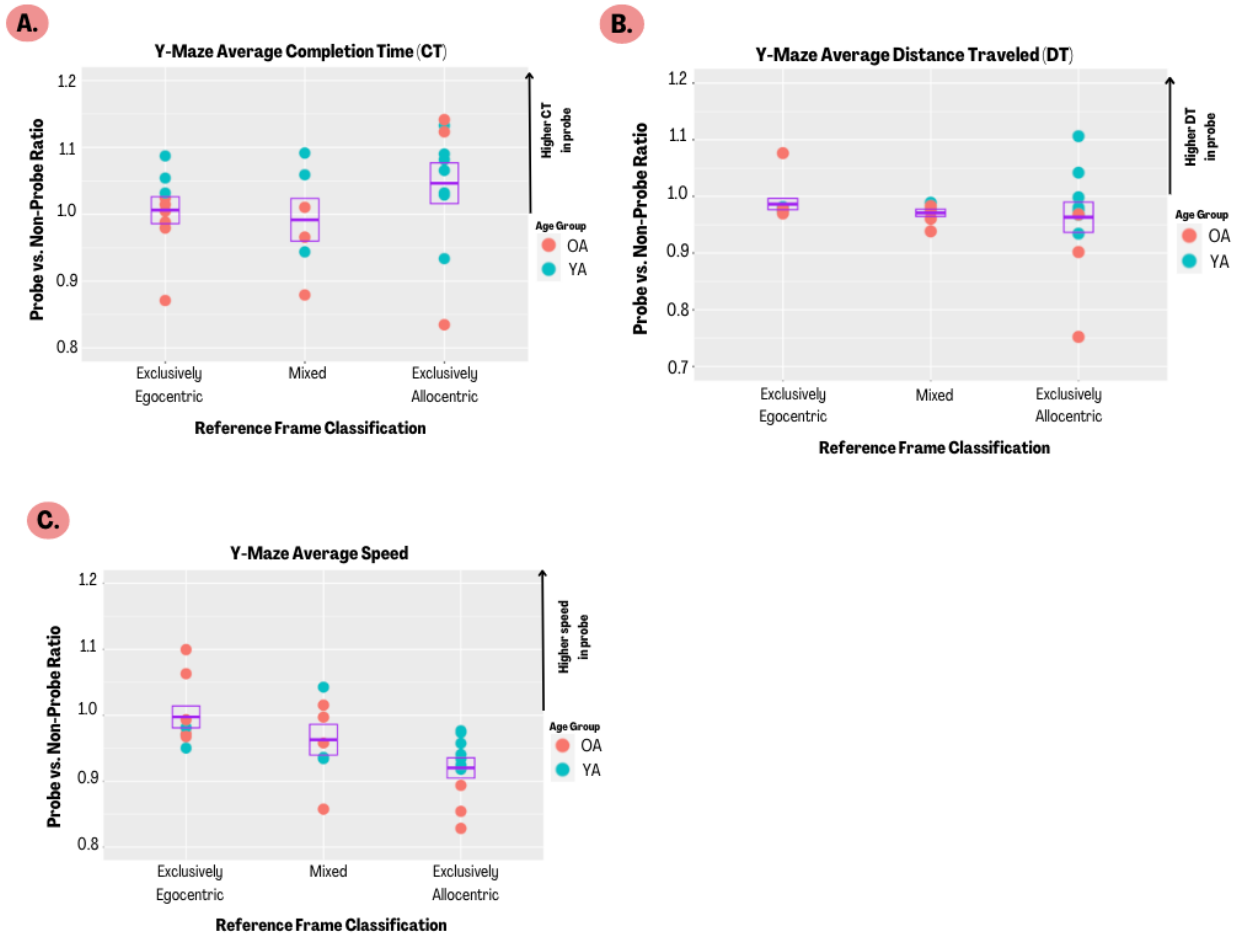


Figure S3. Y-maze probe vs. non-probe trial ratios for individuals classified as exclusively egocentric, mixed, or exclusively allocentric. (A) Probe vs. non-probe ratio for average completion time in the y-maze across all five environments (f_{24}: 0.537; p = 0.591). One individual classified as exclusively allocentric, one individual classified as mixed, and one individual classified as exclusively egocentric were removed due to being outliers (see Materials and Methods: Statistical Analyses for how outliers were determined). Exclusively Egocentric: N = 9; Mixed = 6; Exclusively Allocentric: N = 10. (**B**) Probe vs. non-probe ratio for average distance traveled in the y-maze across all five environments (f_{25}: 0.404; p = 0.672). One individual classified as exclusively allocentric (same outlier as in A.) and one individual classified as exclusively egocentric were removed due to being outliers. Exclusively Egocentric: N = 9; Mixed = 7; Exclusively Allocentric: N = 10. (**C**) Probe vs. non-probe ratio for average speed in the y-maze across all five environments. None of the probe vs. non-probe ratios were significantly different between individuals classified as egocentric and allocentric (f_{25}: 0.972; p = 0.392). One individual classified as exclusively allocentric (same outlier as in A. and B.) and one individual classified as exclusively egocentric (same outlier as one of the outliers in A.) were removed due to being outliers. Exclusively Egocentric: N = 9; Mixed = 7; Exclusively Allocentric: N = 10. Red dots indicate older adult participants (**OAs**); turquoise dots indicate younger adult participants (**YAs**).

Finally, associations between reference frame classification and aging were analyzed. Intriguingly, YAs classified as exhibiting mixed reference frame utilization traveled the shortest distance in the city-like maze.

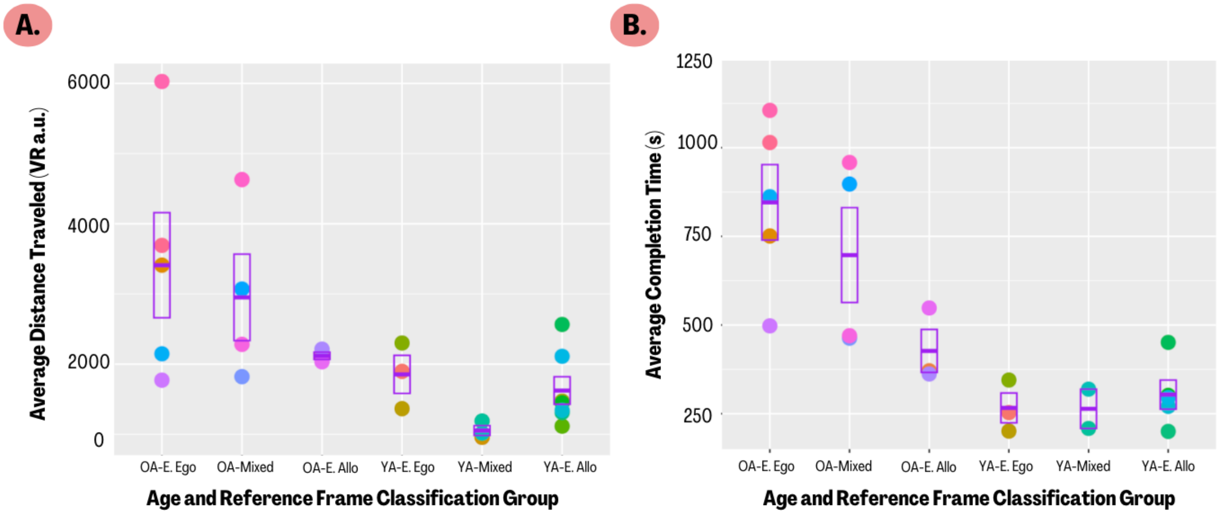


Figure S4. Preliminary data on associations between reference frame classification (exclusively egocentric, mixed, and exclusively allocentric) and aging with naturalistic spatial navigation ability. (**A**) Average distance traveled and (**B**) Average completion time for the 6 age and reference frame classification groups. Average distance traveled was measured in arbitrary VR units (VR a.u.). Each colored dot represents a participant. OAs classified as exclusively egocentric are abbreviated "**OA-E. Ego**," OAs classified as mixed are abbreviated "**OA-Mixed**", OAs classified as exclusively allocentric are abbreviated "**OA-E. Allo**," YAs classified as exclusively egocentric are abbreviated "**YA-E. Ego**," YAs classified as mixed are abbreviated "**YA-Mixed**," and YAs classified as exclusively allocentric are abbreviated "**YA-E. Allo**."

Y-Maze Performance: Probe vs. Non-Probe Ratios by Age Group

In addition to the probe vs. non-probe ratios that compare reference frame classification group, additional analyses were performed to compare y-maze performance by age group (**Figure S5**). None of the ratios were found to be significantly different.

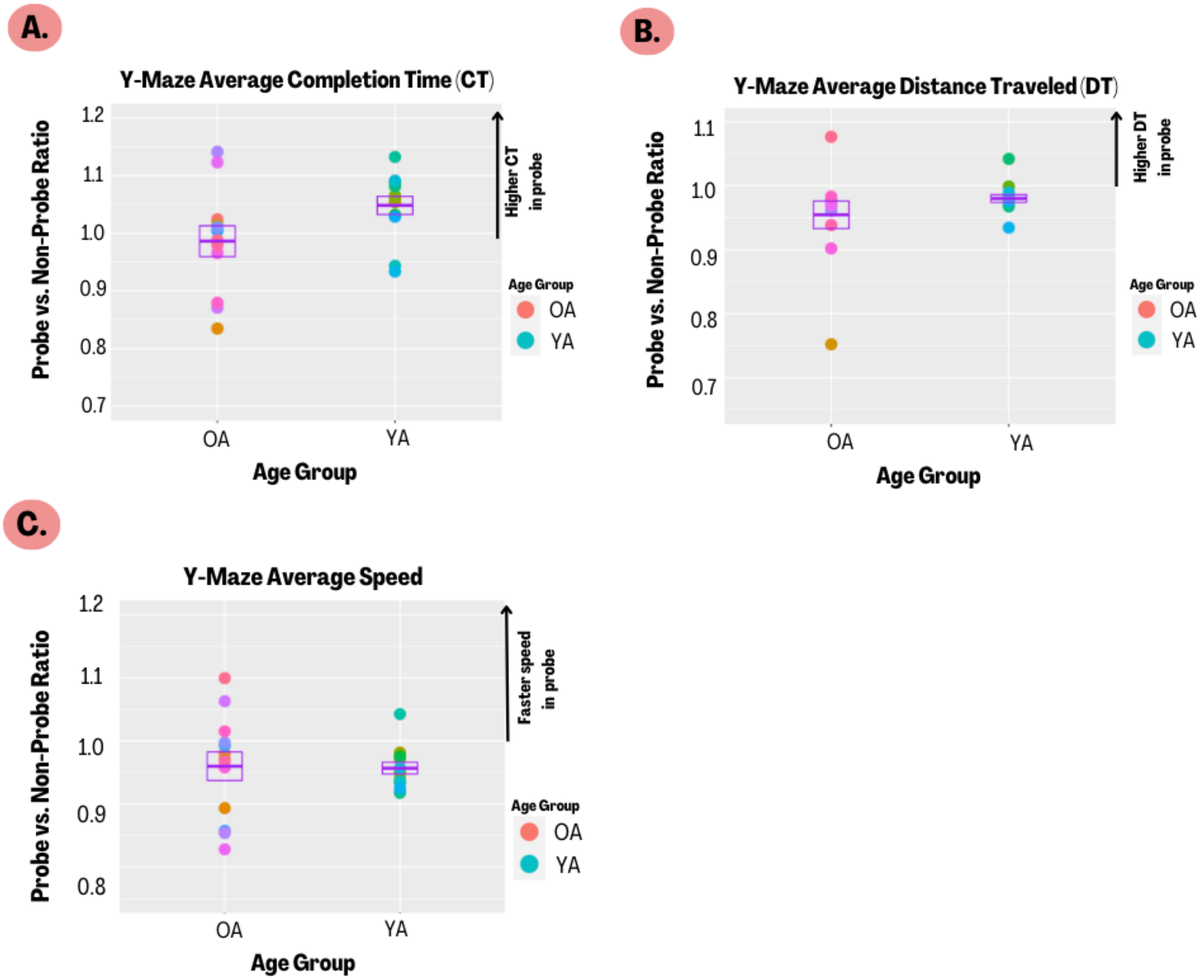


Figure S5. Y-maze probe vs. non-probe trial ratios for OA and YA individuals. (A) Probe vs. non-probe ratio for average completion time in the y-maze across all five environments (t_{24}: 2.004; p = 0.060). One OA individual and two YA individuals were removed due to being outliers (see Materials and Methods: Statistical Analyses for how outliers were determined). OA: N = 12; YA: N = 13. (**B**) Probe vs. non-probe ratio for average distance traveled in the y-maze across all five environments (t_{25}: 1.086; p = 0.297). One YA individual (same outlier as in A.) and one OA individual removed due to being outliers. OA: N = 12; YA: N = 14. (**C**) Probe vs. non-probe ratio for average speed in the y-maze across all five environments (t_{25}: -0.4731; p = 0.643). Two YA individuals (same outliers as in A.) were removed due to being outliers. OA: N = 12; YA: N = 14. Each colored dot represents an individual.

Y-Maze Performance: Probe vs. Non-Probe Ratios by Age Group and Reference Frame Classification (Egocentric or Allocentric)

In addition to the probe vs. non-probe ratios that compare reference frame classification group, additional analyses were performed to compare y-maze performance of age group and reference frame classification (**Figure S6)**. Statistical analyses were not appropriate due to insufficient sample size of OAs classified as allocentric and unequal group distributions. However, the average distance traveled, completion time, and speed ratios of OA classified as egocentric are all below 1; thus, OAs classified as egocentric seem to have had decreased completion time, distance traveled, and slower speed in their probe trials compared to their non-probe trials.

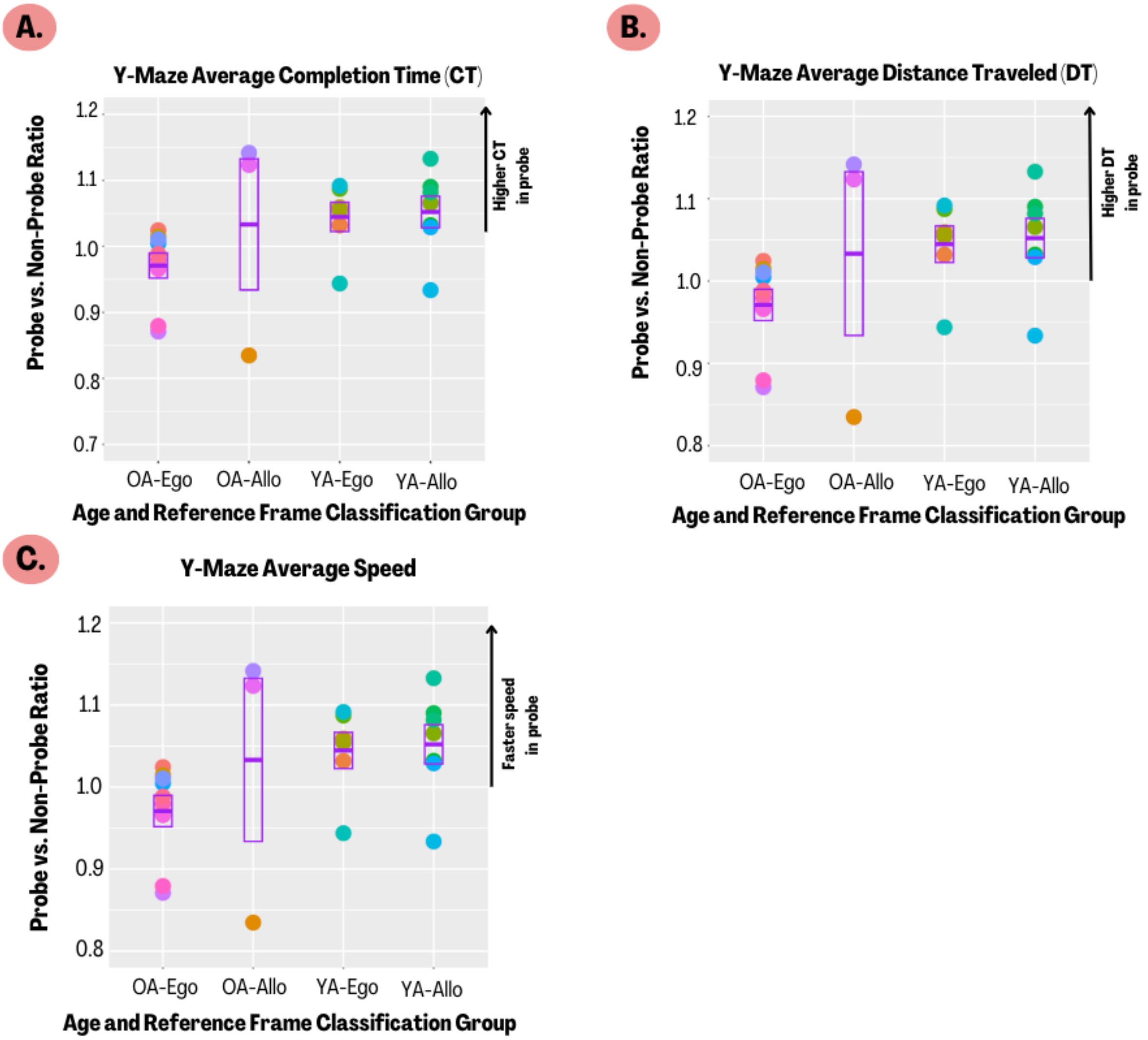


Figure S6. Y-maze probe vs. non-probe trial ratios for OA and YA individuals classified as egocentric or allocentric. No statistical analysis was performed due to insufficient sample size of OAs classified as allocentric. Each colored dot is a participant. OAs classified as egocentric are abbreviated "**OA-Ego**," OAs classified as allocentric are abbreviated "**OA-Allo**," YAs classified as egocentric are abbreviated "**YA-Ego**," and YAs classified as allocentric are abbreviated "**YA-Allo**."

Appendix

Protocol

Objective: To investigate associations between reference frame classification and age with naturalistic spatial navigation ability to answer the larger question of why there is an observed aging-related behavioral difference in spatial navigation ability between younger and older adults.

A. **Consent Form**
A form stating the purpose of the study, participant role, possible risks/discomforts, and equipment description was given to the participant to sign. Confidentiality was guaranteed, and participants were encouraged to ask any questions or concerns they may have.

B. **Study Questionnaire**
A questionnaire with questions pertaining to medical history, mobility status, experience with virtual reality, and lifestyle was given to the participant to complete. Follow-up questions were occasionally asked.

C. **Pittsburgh Sleep Quality Index:**
The Pittsburgh Sleep Quality Index was developed to measure sleep quality in a clinical setting and has become a standard tool utilized in both clinical practices and research studies (Buysse et al., 1988). It is a self-reported questionnaire that assesses sleeping habits and sleep disturbances within the last month. Participant completed the questionnaire based on the most accurate answer for the majority of days and nights in the past month. The Pittsburgh Sleep Quality index was then completed by the participant.

D. **Stanford Sleepiness Scale (Pre-Study)**
The Stanford Sleepiness Scale is a self-reported questionnaire consisting of a 7-point scale indicating levels of alertness. It aims to track changes in alertness throughout the day and has frequently been used in research and clinical settings (Hoddes et al., 1972). The Stanford Sleepiness Scale was presented to the participant, and they were asked to indicate their current level of sleepiness.

E. **Santa Barbara Sense of Direction Scale**
The Santa Barbara Sense of Direction Scale is a 7-point scale consisting of several statements about spatial and navigational abilities or preferences. It is a standardized self-report method used to collect subjective records of spatial navigation ability (Hegarty et al., 2002). The participant was given the scale and asked to circle a number from 1-7 that reflects the extent they agreed with the statement; "1" if they strongly agree that the statement applies to them, circle "7" if they strongly disagree, "4" if they neither agree nor disagree, or some number in between if their agreement is intermediate.

F. **Mini-COG Assessment**
The Mini-COG is a brief cognitive assessment consisting of two parts: three-item word memory and clock drawing. It is used frequently to assess cognitive ability of older adults in clinical and research environments (Borson et al., 2003). The Mini-Cog assessment was administered to the participant. ***If the participant scored 0-2, they were excluded from the study as it suggests a concern for cognitive function.***

G. Simulator Sickness Questionnaire (Pre-VR)

The Simulator Sickness Questionnaire assesses 16 symptoms, including fatigue, nausea, blurred vision, vertigo, and difficulty focusing. The participant's sickness level (none, slight, moderate, severe) was recorded and scored on a scale of 0-60 (Kennedy et al., 1993). If participants scored above a 10 on the questionnaire, they were asked to provide additional consent before participating in the VR maze.

H. Virtual Reality Explanation

After completion of questionnaires, the participant was led to a chair in the middle of the VR cameras and asked to sit while listening to instructions. It was explained that for the Virtual Reality portion, they were to use a headset and 2 hand-held controllers. The headset was explained as what the participant sees the virtual maze through and the controllers were explained as what will help the participant move through the maze. Instructions on how to use the controller were given. Next, it was explained that the participant would be put into a familiarization trial first to help the participant get familiar with the virtual reality world and the controllers. It was emphasized that the familiarization trial was not the actual maze. After the verbal explanation was given, the headset was placed over the participant's head with the help of the research technician and adjusted based on participant feedback. Participants were asked to verify that they could clearly see the contents of the VR screen along with the virtual arrows pointing on the ground. Then, they were helped to a standing position by the research technician.

I. Familiarization Trial

The participant was told that they could turn left and right with their body to look around; look up and down with their head; use the controllers in their hand to move around. They were then asked to locate the starting block on the virtual ground, and it was explained that the starting block would be where they will always start for each mission to a different target building. Then, they were asked to look for target building instructions towards the top of their vision. Participants were told that when they find the building they are looking for, they will navigate to the white square in front of the building and stand on it. After standing on the white square, they will be transported back to the green starting block. When each trial resets, they can use the start block to reorient yourself in the starting direction. Participants were told they should always begin by facing the starting block." The familiarization trial was completed as many times as necessary until the participant was confident in how to use the VR equipment.

J. City-Like VR Maze Task

Following the familiarization trial, the participant was then put in the actual city-like maze. It was explained that they would be completing 3 sessions with 8 missions in each session, which meant that for each session, you will navigate to 8 buildings. Participants were told that the name of the target building will pop up at the top of the screen and they will navigate through the maze to try to find the target building. Each building is labeled and also has visual clues for what participants will be looking for (for example, a fast-food restaurant might have fast food icons on it). Participants were told that once they reach the target building, they would stand on the white box and it would bring them back to the start just like it did in the familiarization trial. Then, once back to the start, participants were told to face the start box again in order to correctly orient themselves.

Participants were informed they were being timed and instructed to complete each trial as efficiently and safely as possible.

K. Simulator Sickness Questionnaire (Post-VR)

The Simulator Sickness Questionnaire was administered after the city-like VR maze.

L. Y-Maze Task

After the city-like VR maze, the participant was guided over to the computer. They were told they would be placed in a 3D environment where their task would be to use the arrow keys to move through a corridor and make a choice where to end. They were told to keep moving down the hallway until they hear a positive (guitar chord) or negative (buzzer sound) auditory cue. Participants were told that when they reached the end, they would automatically be teleported back to where they started, and the maze would restart. Participants were told there would be a total of 5 environments, and that when they completed all the trials for one environment, the next environment would immediately begin.

L. Stanford Sleepiness Scale (Post-Study)

The same Stanford Sleepiness Scale was administered at the end of the study to assess the participant's current level of sleepiness.

M. Closing Statement

Participants were told that it was the end of the research study and were reimbursed for their time.

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