

# Virtual reality evaluation of the spatial learning strategies in gamers

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## Abstract

Psychological memory tests have traditionally relied on paper-based methods. However, the emergence of virtual reality tools, including adaptations of animal lab tests, has opened up new possibilities for evaluating and enhancing attention and memory processes. The impact of virtual environments on spatial memory remains a topic of ongoing debate. To contribute to this discussion, we conducted a comprehensive study with two primary objectives. Firstly, we aimed to develop a virtual reality tool that could effectively assess search strategies in gamers within virtual environments. To achieve this, we designed a virtual water maze inspired by those used in animal research. Secondly, we conducted a cross-sectional study involving participants to analyze how spatial memory strategies evolve in a virtual environment with increasing gaming experience. The results revealed that participants with more gaming experience exhibited faster and more precise learning in the virtual water maze, along with improved search strategies. Additionally, our study allowed for the evaluation of our software and enabled us to track changes in the efficacy of learning strategies. Overall, this study emphasizes the potential of virtual environments for both evaluation and cognitive stimulation purposes.

Keywords Virtual reality learning  $\cdot$  Virtual reality evaluation  $\cdot$  Spatial memory  $\cdot$  Water maze

# 1 Introduction

Cognitive assessment using virtual reality involves the utilization of interactive virtual environments to evaluate mental abilities and problem-solving skills. This approach offers the advantage of simulating realistic situations [1], providing a means to monitor changes in cognitive processes over time, and enabling the design of personalized interventions based on individual results [2]. The application of virtual reality in cognitive assessment has gained prominence in both clinical practice and research, serving as practical tools for

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evaluating attention in the younger population [3-8]. Many of these virtual reality games are adaptations of traditional paper-and-pencil tests [9-12] or established laboratory tests [13-15]. However, as the potential of these tools continues to grow, there is a need to consider the associated risks of their use and the ecological validity of these assessments [16, 17].

Virtual reality offers another valuable application in the form of cognitive stimulation through interactive games [18]. This utility stems from research conducted on animal models, which has shown that exposure to complex spatial environments is associated with increased grey matter volume in the hippocampus, a region closely linked to cognitive improvements [19, 20]. Similarly to natural environments, immersion in interactive game environments, particularly those developed in three dimensions, has been demonstrated to enhance cognitive abilities and contribute to overall cognitive development [21–24]. A notable example of these findings is the observed cognitive enhancement among gamers [21–25]. Engaging in video games, particularly in three-dimensional environments, leads to an increase in grey matter volume in various brain regions, including the hippocampus, the right dorsolateral prefrontal cortex, and the cerebellum, all of which play critical roles in spatial navigation, behavior planning, working memory, and motor performance [23]. While these findings have shown improvements in cognitive, sensory, and perceptual functions [24], the mechanisms and changes that occur throughout gameplay in players remain to be fully understood.

Hence, virtual reality presents a remarkable opportunity to facilitate cognitive improvements and evaluate these changes [26]. Moreover, considering the rapid expansion of the game production industry [27], virtual reality is poised to have a substantial impact on the future of neuropsychological assessment and rehabilitation. To advance this field of research, it is crucial to gain a deeper understanding of how individuals acquire knowledge and problem-solve within virtual environments. Such insights will be invaluable in shaping the design of cognitive stimulation-oriented tasks and interventions.

## 2 Literature review

Neuropsychological assessment is an applied science that examines the functioning of the central nervous system through observable behavior. These assessments use standardized instruments known for their reliability and validity. However, they tend to have only moderate ecological validity, meaning that they may not accurately predict real-world performance or impairments [28–30] Therefore, there is a growing need to create tests that simulate real-life situations more closely. This is where neuropsychological assessment through virtual reality finds its ideal application.

Recognizing the importance of ecological validity, virtual reality is gaining popularity in neuropsychological research for studying human cognitive processes. As this technology continues to advance, it has found applications in rehabilitation, cognitive evaluation, and the treatment of various disorders [31–34]. Researchers have used a variety of tasks, including mazes, virtual cities, and driving simulators, tailoring them to specific research objectives [31]. Furthermore, there is ongoing work to adapt and utilize established laboratory tests known for their reliability and validity, such as the Morris water maze, in virtual environments [31]. These tests can play a crucial role in the diagnosis and monitoring of pathologies by assessing basic cognitive processes [28].

Spatial memory assessment serves as a prime example of the adaptability of such tools [13]. Spatial memory refers to the brain's capacity to encode, store, and retrieve information regarding location and orientation in space. Impaired spatial memory can negatively impact orientation skills, spatial problem-solving abilities, and the utilization of spatial information in everyday activities [35]. This type of memory has been extensively studied across a wide range of species [36–38], highlighting its fundamental role in various living organisms [39–41]. Consequently, comparing performance between species has been made possible, with similar results observed using the Morris water maze in both virtual reality and natural settings among humans and rodents [41].

Originally designed to assess spatial memory in rodents, the Morris water maze test has gained widespread use [42]. It consists of a circular pool, typically filled with dyed water, with a submerged platform serving as the target. The objective for the animal is to locate the hidden platform as efficiently as possible. This test enables the evaluation of spatial learning ability, navigation skills, sex differences, and short- and long-term spatial memory [43–45]. Furthermore, the test has been adapted and utilized in studies examining neurological and neurodegenerative disorders that affect declarative memory [45]. Spatial memory is closely linked to hippocampal activity, a critical region for learning [46]. Consequently, this test could serve as a valuable tool for gaining crucial insights into the proper functioning of this structure.

With the objective of advancing our understanding of spatial memory strategies in a virtual environment, we have developed a virtual reality assessment tool based on the Morris Water Maze test in this study. Our aim was to analyze whether the software was sensitive to record the choice patterns employed during gameplay and describe the progression of spatial memory strategies in a virtual environment with increasing gaming experience. To delve deeper into this matter, we included participants with varying levels of video game experience. As mentioned earlier, experience in video games may contribute to the development of cognitive skills. Thus, participants were divided into groups based on their video game expertise, expecting that those with more experience would exhibit greater proficiency in virtual reality performance. If video games indeed facilitate the acquisition of new cognitive tools for solving virtual problems, experts should demonstrate faster learning compared to non-experts.

#### 3 Methods

#### 3.1 Sample

Nineteen individuals participated in this study, with 14 of them being gamers. The gamers had an average age of 13.36 years (SD = 1.15) and all of them were male. They were selected based on their gameplay in the Fortnite video game, and their accumulated hours of gameplay were obtained from https://fortnitetracker.com/. The average accumulated gameplay time for the group was 297 hours (SD = 170.83). Participants were classified into different categories based on their gameplay time: occasional gamers, comprising 33.3% of the sample, had between 1-190 hours of gameplay (mean = 145.80 h.); moderate gamers, representing the sector comprising between 33.3% and 66.6% of the sample, had 191-329 hours of gameplay (mean = 267.25 h.); and expert gamers (see Table 1), who had more than 330 hours of gameplay (mean = 473.40 h.). Non-players were selected based on their lack of prior experience with video games and their unfamiliarity with Fortnite. This

Groups	Age	SD	Game Average	SD	Interval
Occasional Gamers	13.20	0.64	145.80 h.	28.71	1 – 190 h.
Moderate Gamers	13.25	0.50	267.25 h.	46.86	191 – 329 h.
Expert Gamers	13.60	0.55	473.40 h.	155.04	> 330 h.

Table 1 Age and game times for the gamers' groups

resulted in a sample of adults, consisting of three females and two males, with an average age of 30.4 years (SD = 16.92). It was confirmed that none of the participants had previous exposure to virtual reality technology.

#### 3.2 Materials

#### 3.2.1 Software, Lobato water virtual reality maze

The software was designed as an immersive video game 3D, simulating a virtual water area with a 20 meters diameter and 1-meter scape platform. This pool/platform ratio ensures a low probability, 0.05 %, of accidentally stumbling upon the platform. The software offers various screens for tailoring the evaluation process. Initially, users input participant details, observations, type of scene, and the number of phases. There's also an option to choose between virtual reality equipment or a 2D screen for the test. Once the number of phases has been selected, users can further customize these phases in a separate screen, specifying the number of trials, trial durations, and interval between trials. Within each trial, users have the flexibility to configure the starting location, placement of the escape platform during the test. If the absence of the escape platform is selected, users can designate a specific location to count how many times the participant crosses over it (Fig. 1A-D).

The software was developed using the Unity cross-platform graphics engine from Unity Technologies (https://unity.com/). Unity, based on the C# language, offers compatibility with numerous platforms and allows for easy exportation. Moreover, a free version of Unity is available. The user interface was developed by seamlessly integrating Visual Studio C# with Unity Editor version 2019.1. Visual Studio C# is a Microsoft integrated development environment (IDE) tool. Our software was built on the foundation of default Unity packages, which are conveniently managed using Unity Package Manager. These packages offer customizable, preselected configurations, delivering significant time and cost savings. The software was integrated with the Windows operating system and generates output data in the form of an XLS file, a text document, and a PNG image of the test route, which can be used for further analysis. The software was tested on a laptop Acer Predator Helios 300 computer with an Intel Core i7, 8th Gen processor, and GeForce GTX 1060 graphics. Participants interacted with the software using an HP Windows Mixed Reality VR headset and an HP Controller Mixed Reality controller.

To ensure accurate results even with limited sample sizes, the software evaluates spatial memory in four different scenarios. This approach enables obtaining four repeated measurements from each participant under varying conditions (Fig. 2A-D). Each scene provides specific cues in different environments, such as trees, mountains, elements of a room, or a neutral background with geometric figures.

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Fig. 2 Screen captions from the different scenarios designed by the software: It is a recreation of a classic water maze procedure with rodents in which discrete clues with geometric shapes are used

Furthermore, the software provides flexibility in adapting the experimental settings to meet specific requirements. Researchers can customize various parameters, such as the exploration times, available cues, starting location of the test, visibility or placement of the escape platform, as well as the number of phases and quizzes. This adaptability allows for greater control and precision in conducting experiments and tailoring them to specific research needs.

In terms of user control, the software has been designed to be user-friendly, particularly for individuals without prior digital experience. The control of direction and advancement through gaze has been intuitively implemented, ensuring ease of use for participants. This design consideration enables a seamless user experience and reduces potential barriers or difficulties associated with operating the software.

## 3.3 Evaluation

The evaluation process consisted of two identical sessions per participant, conducted with a test-retest design and a 48-hour interval between sessions. Each participant was assessed in all four software scenarios, which were presented in a counterbalanced manner to mitigate any potential order effects.

The evaluation process encompassed three phases: capacity, learning, and evaluation. The goal was to reach a not visible escape platform located at a geographical point within a pool in the shortest possible time, orienting himself only by environmental cues.

# 3.3.1 First. Capacity phase

The capacity phase aimed to assess the participant's ability to locate and reach a visible platform. The participant started from the western side, and the platform was clearly visible in the northeast. They had 75 seconds to complete the task and familiarize themselves with the environment. This phase also provided an opportunity for participants to become accustomed to the virtual reality experience and gain proficiency in handling the equipment.

## 3.3.2 Second. Learning phase

The learning phase consisted of five trials by scenario, with the platform hidden in a different location compared to the capacity phase. This time, the platform was placed in the southeast. The participant initiated the first trial from the north, followed by subsequent trials from the south, east, west, and finally from a random starting position. Each trial had a maximum duration of 60 seconds to locate the platform, and once found, the participant had an additional 5 seconds for orientation. If the participant failed to find the escape platform within the given time, they were placed on the platform, and the subsequent trial commenced after the 5 seconds reorientation period elapsed.

## 3.3.3 Third. Evaluation phase

The evaluation phase aimed to assess the participants' learning by conducting a trial without a platform. The participant started from the north and had 35 seconds to find the zone where the goal was placed in previous trials. This trial marked the end of the evaluation process. The primary variable of interest during this phase was the time spent searching in the southeast quadrant, where the platform was hidden during the learning phase.

#### 3.4 Study variables

In terms of study variables, the main dependent variable was the time spent searching for the target or target area during the trials. Additionally, the total time spent playing the Fortnite video game was included as an independent variable.

## 4 Results

Participants underwent a preliminary driving test in virtual reality to assess their ability to navigate. All participants completed the phase correctly passing the next phase. Following the test phase, we examined the strategies employed by the participants. We analyzed the time spent searching for the goal in each scene per participant. To evaluate performance across different backgrounds and groups, we conducted an ANOVA with repeated measures. The results showed no significant difference in performance between the scenarios, with all groups spending a similar amount of time to find the platform ( $F_{3.75} = 0.30$ ; p = 0.828, Fig. 3). The participants' scores were calculated as the mean of these four measurements.

Next, we performed an ANOVA to compare the results among the four groups. The analysis revealed a significant overall effect in the search time within the target zone during the assessment phase ( $F_{3, 75} = 6.78$ , p < 0.01,  $\eta^2 = 0.19$ ,  $1 - \beta = 0.928$ , Fig. 4). Bonferroni post hoc tests indicated that the differences were mainly between occasional and non-gamers groups and moderate and expert gamers (*all ps* < 0.05; Fig. 4). The performance of occasional and non-gamers was similar (p = 0.896; Fig. 4), as it was the performance of moderate and expert gamers (p = -0.054; Fig. 4). The analysis of regression revealed a significant correlation between search time in the target quadrant and the level of gameplay experience ( $F_{1.75} = 15.80$ , p < 0.001, C = 0.419,  $R^2 = 17.6\%$ ,  $1 - \beta = 0.977$ , Fig. 5).



**Fig. 3** Average search time for first evaluation, measured in seconds. The search time specifically concerns to the time spent in the target quadrant during the evaluation phase. Standard error bars are depicted. The following scenes were included: Standard scene (N = 19, SD = 7.64), Forest scene (N = 19, SD = 8.51), Desert scene (N = 19, SD = 8.71), and Laboratory scene (N = 19, SD = 9.21)



**Fig. 4** The average search time in the four scenarios during the first evaluation. The search time specifically refers to the target quadrant in the evaluation phase, measured in seconds. Standard error bars are depicted. The study included different groups: Non-gamers (N = 20, SD = 9.22), Occasional gamers (N = 20, SD = 9.88), Moderate gamers (N = 16, SD = 4.31), and Expert gamers (N = 20, SD = 4.45). Welch's test yielded a significant result of 6.982, p < 0.001



**Fig. 5** Regression analysis of search time in the target area per group. Search time in seconds. Groups; Non-gamer (N = 20, SD = 9.22), Occasional gamers (N = 20, SD = 9.88), Moderate gamers (N=16, SD = 4.31), and Expert gamers (N = 20, SD = 4.45).

After analyzing the data from the groups across different scenarios, we assessed the test-retest reliability of the software with t Student. For this purpose, we re-evaluated a group consisting of players from the moderate and expert conditions under identical conditions. The results indicated no significant differences between the evaluations ( $t_{71} = -0.40$ , p = 0.689, Fig. 6). In addition to assessing the test-retest performance of the new group,



Fig. 6 Moderate and Expert gamers, evaluation test-retest. Search time in the target quadrant in the evaluation phase in seconds. First evaluation (N = 36, SD 4.32), and Second evaluation (N = 36, SD = 4.35).

we analyzed their performance in the different scenarios. Once again, no significant differences were found among them (ANOVA:  $F_{3, 35} = 0.49$ , p = 0.689, Fig. 7).

We further examined the distribution of total search time between the different scenarios with ANOVA. Our analysis revealed a statistically similar distribution in all assessments (see Figs. 8 and 9). Specifically, the search time distribution between scenarios during the first and second evaluations showed no significant differences ( $F_{3.75}$  =



Fig. 7 Average search time for second evaluation, measured in seconds. The search time specifically pertains to the target quadrant during the evaluation phase. Standard error bars are depicted. Standard scene (N = 9, SD = 4.82), Forest scene (N = 9, SD = 4.88), Desert scene (N = 9, SD = 3.84), Laboratory scene (N = 9, SD = 4.44).



**Fig. 8** Distribution of time by scenes during first evaluation. Percent search time over the target quadrant in the evaluation phase. Standard (N = 19, SD = 7.64), Forest (N = 19, SD = 8.51), Desert (N = 19, SD = 8.71), and Laboratory (N = 19, SD = 9.21).



**Fig. 9** Weight Distribution of time by scenes during second evaluation. Percent search time over the target quadrant in the evaluation phase. Standard (N = 9, SD = 4.82), Forest (N = 9, SD = 4.88), Desert (N = 9, SD = 3.84), and Laboratory (N = 9, SD = 4.44).

0.21, p = 0.887, Fig. 8) and ( $F_{3.35} = 0.40$ , p = 0.751, Fig. 9), respectively. In addition, the intra-group analysis between the first test and the second one (retest) for the moderate and expert gamer samples did not yield significant differences in the distribution of search time across scenarios ( $F_{7.71} = 1.60$ , p = 0.152).

Finally, we found no evidence of differences in performance based on sex; that is, sex did not have an impact on virtual water maze performance [26, 44]. We conducted an t Student analysis to compare the performance of women and men in the non-gamer sample, and no significant differences were observed ( $t_4 = -0.60$ , p = 0.549). Despite the low number of subjects, the scores were similar. In regard to age, we found differences between the non-gamer and gamer groups, as we expected.

#### 5.1 Participant analysis

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The proliferation of video game consumption has had a significant impact on our society. As a result, concerns have been raised about the risks associated with excessive use, particularly among younger individuals [15]. However, there is also growing recognition of the benefits that video game consumers could experience, with numerous studies demonstrating cognitive improvements among users [22, 25, 47, 48]. These improvements are attributed to the enriched virtual environments provided by video games during gameplay [21, 23, 49].

The results revealed the presence of two distinct groups: non-gamers and occasional gamers in the first group, and moderate and expert gamers in the second group. A significant difference with a large effect size was observed between these groups. A further regression analysis explained this finding, as we identified a significant model where the increase in target search time followed an exponential pattern. This model demonstrated that rapid growth occurs after approximately 200 hours of gameplay, marking the transition from occasional to moderate gaming. This result aligns with previous research on video games and spatial memory, supporting the notion of a critical event that enhances learning [23, 25]. It is also consistent with structural studies indicating an increase in the volume of the posterior hippocampus in gamers, a region consistently associated with spatial memory [23, 50, 51]. Additionally, comparative studies have linked the proliferation of new neurons in the hippocampus to faster and more direct search strategies, improving spatial navigation in tasks like the Morris water maze [43, 52].

Traditionally, the analysis of spatial behavior has involved observing the routes taken in different environments to identify commonalities [53]. This approach has proven effective in uncovering invariance in scanning patterns, and it has been applied to categorize a typical behavior in the virtual water maze [54]. These kinds of behavior include thigmotaxis, where animals move along the walls of the maze in a circular trajectory; circling, where animals follow an arc-shaped search path while moving away from the walls; enfilading, which involves minor positional adjustments, directional changes, and some straight lines; and visual scanning, where participants remain in a fixed position and turns to advance in straight lines. In our study, the moderate and expert gamer groups predominantly employed visual scanning and enfilading strategies during the trials, while non-players and occasional gamers relied on thigmotaxis and circling strategies, resulting in poorer performance.

Figure 10A-D depict the performance of different participants in the standard scene, selected as representatives from each group. These illustrations highlight the development of search strategies based on participants' gaming experience, demonstrating that greater hours of gameplay correspond to more efficient search approaches. These data supports the idea of a more effective search strategy that is usually dependent of hippocampus activity [24, 52].

An important aspect of our study was the age difference between the non-gamer group and the gamers. Previous research in both animal models and humans has demonstrated that allocentric spatial memory, as assessed in our study, develops at an early age. In fact, studies have shown that by the age of twelve, individuals can exhibit adult-level performance in tasks related to spatial memory [55, 56]. Consistent with these findings, all participants in our study employed the strategies analyzed in the Lobato water maze, regardless of their gaming experience. However, the non-gamer group and the occasional player group performed similarly, and both groups performed worse than the moderate and expert gamer groups. A Non-gamer route.







C Moderate gamer route.







D Expert gamer route.





**Fig. 10** Schematic representation of the trajectories. Each picture represents a path followed by a participant in the standard scene, selected as representative of each group. The first column is represent the Trial 1 Learning, the second is the Trial 3 Learning, and the third is the evaluation trial. The dot represents the escape platform

In conclusion, our study not only demonstrates enhanced spatial memory performance in the most experienced gamers, but also sheds light on the underlying mechanisms behind this improvement by visually correlating search times with search strategies. This sets our research apart from previous studies [13, 14, 57].

#### 5.2 Software analysis

The design of our software was carefully planned to ensure that all participants experienced the same conditions, effectively serving as their own control subjects and minimizing the influence of extraneous variables [58]. To achieve this, we implemented parallel scenes, which is crucial for maximizing the effect size and reducing intra-subject error variance [58]. This feature sets our software apart from others such as NavWell or Machado Virtual Maze [13, 14], as it allows for more reliable measurements and comparisons.

The scenarios in our software were designed to closely resemble real-life spatial navigation, incorporating both natural and artificial cues. These cues play a vital role in spatial navigation, both in real-life environments and in water maze tasks [14, 59]. By adjusting the presence or absence of these cues and varying their difficulty levels, our software offers flexibility in evaluating different populations with diverse spatial memory abilities.

To assess the similarity of the scenarios, we compared the performance of the entire sample across different designs and found no significant differences. We also examined whether certain types of gamers exhibited preferential performance in specific scenarios but found no interaction between gamer type and scenario type. Furthermore, when comparing the performance of a sample of moderate and expert gamers across different scenarios, no performance differences were observed. Finally, we assessed the impact of each scenario on the trial results and found a comparable influence of the scenarios within each group. This consistent pattern was also observed when analyzing data from the first and second assessments. In sum, these results indicate that the software comprises four parallel tests that evaluate spatial memory using different types of reference signals.

To ensure the stability of the measurements over time, we conducted two evaluations with a selected group of moderate and expert gamers. The test-retest analysis revealed no significant differences between the two assessments. Although there was a slight increase in search time over the target in the retest (that is, more time spent in the quadrant where the goal was placed), this may be attributed to a learning effect, as the second evaluation took place 48 hours later.

Another important aspect of the software design was its adaptability to meet different experimental requirements. A recent review of spatial memory tasks in virtual reality highlighted the popularity of virtual water maze analogues due to their advantages [31]. However, it also pointed out challenges related to standardization, including configuration and procedural issues, which complicate replicating findings and comparing studies [31]. Researchers have employed various designs for assessing spatial memory with the water maze [45, 60], and our software offers flexible configurations that can be tailored to specific experimental needs. This flexibility includes the ability to adjust parameters such as the number of trials, search durations, starting positions, reference cues, visibility of the escape platform, and more.

Understanding the participant's performance and the reference signals or strategies they employ in water mazes can be challenging. Both vestibular and visual systems play a role in navigation, and accurately analyzing behavior requires distinguishing between these systems [61]. The software enables us to work with different reference signals and starting points, allowing for independent analysis of these systems [61]. Previous research has shown that rats in the Morris water maze can rely on vestibular cues for directional responses, but this response can be disrupted if the starting route is rotated [61]. Our software is designed to accommodate this configuration and facilitate such analyses.

In addition, our software not only measures spatial memory, but also provides data on the distance travelled during the test. While we did not consider this variable relevant for this study, the software offers the option to analyze distance travelled, which, when combined with the changing location of the escape platform, can provide insights into working memory.

Overall, our software reliably detected performance differences between groups and offers advantages over other programs by allowing multiple observations of the same variable under different conditions [13, 14]. This feature streamlines the sampling process, reduces evaluation duration, and minimizes associated costs.

## 6 Conclusions

The developed software provides a user-friendly and flexible approach to assess spatial memory associated with the hippocampus. By utilizing a nonverbal test, we can examine the status of this cognitive function in older individuals as well as the learning ability of younger individuals. The software's multiple scenarios allow for customization, enabling researchers to draw meaningful conclusions even with a smaller sample size. The primary objective of this study was to investigate the development of spatial memory in video game players. We observed that spatial memory follows an exponential learning curve, with the rate of learning varying among different types of gamers. The learning progression gradually increases and then transforms into exponential growth at the threshold between occasional and moderate gamers, facilitated by improved search strategies. Consequently, the software is capable of distinguishing populations based on their distinct behavioral patterns. Nevertheless, further research is required to ascertain whether these differences could serve as predictors of susceptibility to behavioral disorders [62].

Furthermore, the development of this software seeks to encourage the creation of novel assessment tools rooted in virtual reality. In particular, those adaptations from traditional animal behavior tests commonly used in laboratory settings. We believe that such tests have the potential to make substantial contributions to the diagnosis and ongoing monitoring of neurodegenerative diseases. Additionally, through the design presented in this study, we aim to promote the standardization of future virtual maze tasks.

Authors' contributions The study was conceived and designed collaboratively by all authors. FJ Lobato-Camacho was responsible for the material preparation, data collection, and analysis. The initial draft of the manuscript was written by FJ Lobato-Camacho under the supervision of JP Vargas and JC López. All authors provided feedback and contributed to subsequent revisions of the manuscript. JC López and JP Vargas oversaw the data acquisition and management. The final manuscript was read, reviewed, and approved by all authors.

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#### Declarations

The datasets generated and analyzed during the present study are available in the Research Repository of the University of Seville (Spain) https://hdl.handle.net/11441/148808 from the corresponding author upon reasonable request.

**Competing interests** All authors certify that they have no affiliations with or involvement in any organization or entity with any financial or nonfinancial interest in the subject matter or materials discussed in this manuscript.

**Institutional Review Board Statement** The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Comité Coordinador de Ética de la Investigación Biomédica de Andalucía, Junta de Andalucía (Spain) with the code (1221-N-17).

Informed Consent Informed consent was obtained from all subjects involved in the study.

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