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New Software dedicated to Virtual Mazes for Human NeuroCognitive investigations

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Abstract

Background : compared to previous neuropsychological investigations with standard paper-pen tests limited to test complex spatial learning and memory processes, 3-D virtual immersive technology might offer new tools for research purposes and for diagnosis in patients suffering from mild cognitive impairment or dementia.

Comparison with existing methods : Current software proposes a customizable VR environment combined with an analyser module based on regions of interest and some parameters of analysis or pre-calibrated VR mazes with raw data.

New Method : We attempted to create the Human Virtual Maze (HVM) software offering either turnkey mazes with automatic tracking and analysis, or more complex and specific virtual mazes for human brain-behavioural research adaptable to all desired settings and parameters of analysis. The software combines 3D pre-calibrated VR tests or free customizable VR tests with digitized neuropsychological 2D standard and validated tests or tasks.

Results: We have tested an ERAM, a MWM and a reverse T-maze on 44 healthy subjects, showing gender differences in terms of navigation strategy. We have observed that the choice of benchmarks, instructions, and experimental parameters influence the performances.

Conclusion : HVM software offers a translational approach for research units that wish to combine animal models and patient evaluations as well as complex 3D tasks and standardized neuropsychological tests combined with an automatic analysis opening a large perspective in the neurosciences to investigate cognitive functions. A clinical module with preconfigured 2- and 3-D tasks should offer clinicians an easy way to evaluate their patients routinely.

26

27 Introduction

28 Exploring our environment and wayfinding have shaped spatial orientation, memory
29 and navigation abilities in mammals from visual (Hufner et al., 2011), vestibular and auditory
30 inputs (Besnard et al., 2012; Brandt et al., 2005; Ding et al., 2016; Jacob et al., 2014; Poucet
31 et al., 2014). Brain sensory integration activates the vestibular and multi-sensory cortical areas
32 for body perception (Viaud-Delmon and Warusfel, 2014). This is combined with the
33 modulation of head direction, place and grid cells within the thalamus, hippocampus and
34 entorhinal cortex, respectively (Hafting et al., 2008; Hartley et al., 2014; O'Keefe and
35 Burgess, 2005; Poucet et al., 2014) in order to map and to memorize our environment and
36 then permit wayfinding (Poucet et al., 2015). Some of the neuropsychological tests currently
37 used to evaluate spatial learning and memory involve the Rey Figure, cubes and paper-pen
38 spatial tasks, although larger-scale navigation tasks, in virtual and physical environments,
39 have been developed (e.g., Astur et al., 2004; et al., 2005; Astur et al., 2005; Canovas et al.,
40 2011; Peter et al., 2018). The assessment of spatial learning and memory in humans in
41 relation to navigating in an environment, may require still more complex tasks and scenarios.
42 However, the evaluation of spatial memory and learning performance, ideally performed in a
43 natural environment, makes it impossible to control all parameters, and leads to the
44 development of standardized real mazes. The Morris Water Maze and numerous radial arm
45 mazes were then developed for this purpose in rodents (Morris, 1981; Morris et al., 1982;
46 Machado et al., 2012; Fajnerová et al., 2014; Buckley and Bast, 2018), while similar physical
47 mazes devoted to the evaluation of navigation and spatial memory performance in humans
48 remain difficult to build and require large areas in research laboratories.

49 Since the last decade, computational and immersive technology advances have
50 provided new tools offering specific virtual environments for diagnosis, therapy or

51 rehabilitation, like the Virtual Classroom (hyperactivity), the Vepsy updated program (Riva et
52 al., 2003)(social phobia, telemedicine), or Neuro VR (therapy), for instance. Cognitive maps
53 formed in a virtual space were ecologically validated (van der Ham et al., 2015) and simulated
54 navigation in a real environment (Claessen et al., 2016), while their efficiency compared to a
55 natural environment remains debated (Richardson et al., 1999; Sun et al., 2004). The
56 identification and the use of landmarks themselves were similarly reported in animal models
57 and humans (Ruddle et al., 2011): i) the use of distal rather than proximal cues; ii) alterations
58 in building a topographic map induced by a topographical displacement of distal landmarks
59 more than an increase in their numbers (Bures et al., 1997). A virtual maze combined with
60 fMRI also demonstrated how the human brain worked during a wayfinding task combining
61 both the frontal cortex and hippocampal activities (Astur et al., 2005).

62 In the past, virtual mazes presented in the scientific literature were homemade from
63 complex computer language, inconveniently time consuming, displayed at a low resolution
64 and depended on the ability of the scientists to program. To the best of our knowledge, only a
65 few software packages available on the market offer virtual mazes for navigation and spatial
66 memory investigations in humans, sometimes derived from videogames (Bohbot et al., 2012).
67 However, all of them remain limited in terms of protocol conception, tools of analysis, and
68 appear difficult to adapt by researchers as well as by clinicians to their patients or healthy
69 volunteers. Moreover, the creation of gold-standard mazes similar to those used in animal
70 models, would offer the scientific community a translational approach to their research. The
71 most recent advanced software solutions propose a fully customizable VR environment
72 combined with an analyser module based on regions of interest (ROIs) with four main
73 parameters of analysis (time, path length, rewards/access, velocity) (Mazesuite) (Ajaz et al.,
74 2008,), and another one proposes pre-calibrated VR mazes (ERAM, MWM, T-maze,etc) with
75 raw data (Schenider et al. 2017) or the possibility to realize a customizable scenario on

76 request by the customer (Simian labs). Another option offers a virtual projection of a VR
77 environment in an area of about a 2 m diameter where motion of the participant is recorded
78 with an infrared surface and post-analysed (Hort et al., 2007; Fajnerova et al., 2014).

79 We sought to develop multi-modal software for public and private companies, clinical
80 and research purposes, offering either turnkey mazes with automatic tracking and analysis, or
81 more complex and specific virtual mazes for human brain-behavioural research adaptable to
82 all desired settings and parameters of analysis. The software (Human Virtual Maze) that we
83 have developed may now combine 3D pre-calibrated VR tests or free customizable VR tests
84 with digitized neuropsychological 2D standard and validated tests or tasks like questionnaires,
85 psychomotor vigilance tasks, decision taking tasks, Stroop tests, etc... offering a solution to
86 design one neuropsychological and cognitive evaluation fully adapted and customizable to the
87 purpose sought by the user. In addition, the software proposes the possibility to display the
88 2D and 3D neurocognitive evaluation on standard or large screens, as well as on VR headsets
89 and immersive rooms, an option not yet reached with commercial or free tools currently
90 available in the market.

91

92 **Human Virtual Maze software - 3D tasks:** This HVM software was elaborated in such a
93 way that the experimenter can easily configure their 3D tasks combining them with new or
94 standard 2D tasks and questionnaires (see next paragraph), providing a specific protocol
95 adapted to their objectives on the same computer/support. Moreover, the software creates a
96 database for a patient and provides an automatic and specific analysis of each task that would
97 save time and provide ease of use. HVM enables the researcher to design a complete protocol
98 (number of trials, control conditions, etc...), to test it and to adjust it very easily and quickly.

99 Before designing a protocol, the user chooses the duration time of each experiment,
100 the type of success (all, one and all but one), if necessary the latency before help is available

101 and the projection mode of the test: screen (computer screen, by a projector,...) or in virtual
102 reality headset (Oculus or HTC for instance).

103 A complete protocol is defined according to an environment, a maze and types of trials
104 (**Figure 1A**) and for the convenience of users, the design of the protocols is visible in real
105 time:

106 • The environment is composed of the room that could be cubic, spherical or conical, on
107 which we can place a picture of the environment. The users define room dimensions also
108 (position, orientation and size). Experimenters can use simple pictures or use a cube map
109 picture that will automatically adapt to the room. In this room, users can add 2D elements,
110 lights (position, intensity, radiance, color), timers, etc ... all elements added for this section
111 will be visible in the room throughout the experiment.

112 • Three pre-built mazes can be chosen: i) the Morris Water Maze, ii) the Star Maze, and
113 iii) the X-maze. Users may change the diameter and the height of the pool and material
114 (texture and colour) may be added on the pool wall. The star maze is composed of a central
115 platform and a maximum of eight radial arms. Parameter settings of this maze are reduced so
116 that users can choose: from 0 to 8 arms, the length of each arm and to remove or not the little
117 wall at the end to hide the reward. The last maze available is the X-maze. Differences with the
118 star maze are that all arms will be similar and more settings are available: the inner and outer
119 radius (maze, central platform and little wall diameter), length, thickness and width of the
120 maze walls and the little wall to hide a reward, the number of arms and the angle between
121 arms. Trials are composed of initials settings:

- 122 1. Users can insert text or auditory instructions.
- 123 2. The environment can be modified and can be specific for each trial.
- 124 3. Subject information (starting position: x, y, z position and orientation) and motion
125 (lateral forward and back walking speed, vertical and lateral rotation speed).

126 4. Target settings: users can choose the number of rewards and custom geometry target
127 (coin, platform, 3D objects).

128 Users can choose the settings for each trial, the number of trials and add another
129 experiment with a different environment, maze and trials. The sequence of trials and
130 experiments are automatically set up.

131 Data recording is performed locally on the computer, the recorded product an xml file
132 that will be reviewed by the software and the analysis may be automatically performed by the
133 software or manually done by users. The path is visible for each trial. By default all raw data
134 are recorded (latency, distance, orientation, and success/failure) and users then select filters to
135 customize their analysis.

136

137

138 Figs. 1 about there

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142 Parameters of Analysis:

- 143 1. Total Time Filter: the latency to reach the criterion
- 144 2. Total Length Filter: the distance travel to reach the criterion
- 145 3. Area Filters (radial, angular or rectangle) (**Figure 1B**): the user can apply a geometric
146 surface on the maze and analyse only this surface. Dimensions and position of the area
147 can be customized by the user.

148 All filters can be superimposed; users can choose a circular area located at the centre
149 of the pool and analyse distance and latency only in this area. Others filter are available such

150 as an entry/exit filter for the X-maze or star maze. Two additional filters are available with the
151 Morris water maze:

- 152 1. The corridor filter: a virtual corridor can be applied to simulate the direct trajectory
153 between the start position and the platform, the latency and the distance travelled in
154 this area could be analysed.
- 155 2. The heading error: angles between the heading orientation and the platform are
156 determined all along the way.

157 Finally, the position and motion of the virtual subject may be chosen too: starting position,
158 first or third person-view, lateral and backward motion.

159 The Human Virtual Maze software offers customizable data analysis protocols for one
160 experiment, that may be applied to other similar experiences leading to the production of an
161 excel file with a raw and filtered database per protocol.

162

163 **Human Virtual Maze software – 2D tasks and questionnaires:** The Human Virtual Maze
164 allows the researcher to develop directly on the software 2D experiments such as
165 questionnaires (binary, ternary, quaternary, scaled or test questions), reaction and speed
166 experiments (PVT, CPT and MPT), emotional and risk experiments (ERT and BART)
167 (**Figure 1C**) and more. Numerous 2D tasks could be developed on the Human Virtual Maze
168 software, and to facilitate analysis the results of each test are customized.

169 Herein, we present different modules of the software including the development of the
170 3D virtual environment, the module of protocol generation, the module of analysis and the
171 different supports for the display. At the same time we have tested a Morris Water Maze
172 (MWM), an eight-radial arm maze (ERAM), and a Reverse T-maze paradigm (RTM) on 44
173 young healthy subjects. Although distal versus proximal landmarks are usually compared in
174 healthy subjects or in cognitive disorders in patients, we have explored whether the type of

175 distal landmark itself and the starting position, influenced spatial memory performance or
176 strategy in such virtual mazes, respectively.

177

178 **Material and Methods**

179 *Software specifications*

180 Experimentation and analysis modules of the software are written in C#. The program
181 is designed in such a way that the user can configure a large number of experimental
182 parameters and provide the maximum number of possibilities. Indeed, the software allows the
183 specification of variables from ambient light to the subject's walking speed, including the
184 design of the maze, the number of trials and the landmarks used.

185 For this study, the HVM was used to configure the mazes and protocols according to
186 the objectives of the study. The backward movement of the participants was blocked but
187 lateral motion remained free in order to provide a more natural exploration. Moreover,
188 blocking lateral motion prevents heading motion analysis that is an important parameter with
189 the Morris Water Maze for instance. The results were exported and graphics were
190 automatically displayed in an Excel file. The track mode allows re-reading of the video
191 tracking recorded and application of specific algorithms, for instance, specific settings for
192 heading errors or lanes within the Morris Water Maze and/or to manually analyse the raw
193 data. Here we have created three virtual mazes according to those developed in rodent
194 behavioural studies, as explained below.

195

196 *Participants*

197 Forty-four young healthy subjects between 18 to 30 years of age (23 ± 2.27 years), 23
198 females (23.19 ± 2.29 years old) and 21 males (22.8 ± 2.29 years old), were included in the
199 protocol. All participants had an educational level of 4.2 ± 1.6 years after college. Their

200 experience in informatics and an evaluation of the frequency of video game use was scored (1
201 = never, 2 = several times a year, 3 = several times a month, 4 = several time a week, 5 =
202 everyday). It was not the main objective of this study to conduct a systematic comparison of
203 the responses of males and females in these tasks, because this was a feasibility study to test
204 the utility of the software. For this reason, no attempt was made to adequately power this
205 study in terms of males versus females, and therefore it is likely that it was underpowered to
206 detect some sex differences compared to other studies (e.g. Buckley and Bast, 2018). Buckley
207 and Bast's (2018) study suggests that an n of approximately 50-60 subjects of each sex is
208 required to reach a power of 80%, and such sample sizes were beyond the scope of this
209 feasibility study. All subjects had approved the protocol and signed an informed consent letter
210 as required by the French ethical committee rules (CPP: 2016-13; N° ID RCB: 2015-A02014-
211 45).

212

213 *Reverse T-maze paradigm*

214 The reverse T-maze implements the paradigm described by Packard & McGaugh
215 (Packard, 1999; Packard and McGaugh, 1996) and adapted to virtual reality (Levy et al.,
216 2005). The test determines the type of strategy used to solve a spatial task. A reward is located
217 in the same arm (distal part) all through the test. After a period of habituation to find the
218 reward (for example to turn right under the square), a rotation of 180° of the T-maze is
219 performed. The subject has to find the reward either with a spatial strategy, known as an
220 allocentric memory strategy (i.e. navigation using cues exterior to the maze, the square in our
221 example) or with a response strategy, known as a egocentric memory strategy (i.e. navigation
222 using egocentric cues: right turn in our example). After turning right or left, a return to the
223 first or opposite arm is forbidden. Choices in the strategy were expressed as a percentage; the
224 choice of the arm (correct or not) and the time to perform the test were analysed. In our study,

225 subjects had to find a gold coin placed in the distal part of the right arm after four trials (1 to
226 4). After reversal, subjects had to find the same reward during one trial (trial 5th) (both arms
227 were rewarded, not influencing the choice of the arm). If the subject turned right they used a
228 response strategy, if they turned left (the correct arm) they use a spatial strategy (taking into
229 account the spatial cues in the room) (**Figure 2**). Participants were informed that they will be
230 placed at the end of the arm of a T-maze (each arm of the T-maze measure approximately 8
231 virtual m). They had to choose one arm and find a reward that was always placed at the same
232 location. If they found a reward, a black screen appeared during 5 s and the participants
233 performed another trial. If they did not find the reward, they remained in the chosen arm and
234 waited until the end of 60 s before the black screen of 5 s and the new trial.

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238 Fig. 2 about there

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241

242 *Morris Water Maze*

243 This was developed by Morris in 1981 (Morris, 1981; Morris et al., 1982) and is a
244 standard paradigm used in rodents to assess strategies, learning and spatial memory
245 performances in an aversion environment of water, by reaching a submerged rescue platform
246 as fast as possible (Arns et al., 1999). This test is particularly sensitive to mnemonic abilities and
247 spatial representation in the case of central neurological lesions involving the hippocampus
248 (Arns et al., 1999; Gage et al., 1988; Gallagher and Pelleymounter, 1988; Vorhees and
249 Williams, 2006; Zyzak et al., 1995). The starting points of each trial are randomized so that

250 rodents do not have to remember the direction of the platform only, but they have to recognize
251 their location while the platform location remains unchanged.

252 Participants were instructed that they will find themselves in a virtual pool of 15
253 virtual m in diameter. They will have to move with the Xbox remote to find a hidden platform
254 of 1 virtual m in diameter (a ratio pool/platform of 1/15) that will always be placed in the
255 same location. They will have 60 s to find the platform:

- 256 - If they find the platform before the time limit a success sound will be presented and
257 they will not be able to move for 5s.
- 258 - If they do not find the platform before the time limit, a help sound will be presented.

259 They must find the platform to access the following trial.

260 After a black screen of 5 s, participants are returned to the virtual pool.

261 Divided into four quadrants after the tests, parameters of analysis were the time spent
262 in each quadrant, in one of which is the platform (25%), the latency to find the platform, the
263 time spent close to the platform and the strategy to find the platform area from the video
264 tracking of all of the trials. The virtual Morris water maze is a task that assesses these criteria
265 in humans (**Figure 3**).

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269 Fig. 3 about there

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273 **Phase I** (visible and fixed platform, fixed starting position, 4 trials with a 5 sec ITI)
274 consists of measuring the latency and the distance travelled to reach the visible platform. This

275 phase is to ensure the comprehension of the participant and their ability to use the joystick or
276 the software.

277 **Phase II** (5 blocks of 4 trials of 60 secs and 4 different starting positions with an ITI
278 of 5 s) consists of measuring the distance travelled and the time to reach the platform. This
279 configuration is purely a learning phase; the subject must be able to find the submerged
280 platform via spatial cues placed in the room. This test determines the abilities of the subject to
281 create a mental map to find the platform, but not by chance.

282 **Phase III** (only one trial, simulated platform, similar to phase 2, platform location
283 unchanged, 45 sec duration) consists of measuring the percentage of the distance travelled and
284 the percentage of time spent in the target quadrant in relation to the total time allowed.
285 Chance values for the percentage of the distance travelled and the time spent in the target
286 quadrant, were approximately 25%.

287

288 *Eight-Radial Arm Maze*

289 This has been developed by Olton and Samuelson (Olton and Samuelson, 1976) in
290 order to assess reference spatial memory performance (long-term) as well as working spatial
291 memory (short-term). The subject has to explore eight-radial arms from a central platform,
292 looking for four rewards randomly located in the distal part of the arms behind a low wall and
293 always placed at the same spatial location. The ERAM task takes place in a room with the
294 wall and ground with a similar texture and colour, avoiding intra-maze point references
295 (proximal cues). In a room, it displays different sets of 4 distal landmarks on the wall. The
296 tracking module allows us to quantify the latency and the distance travelled to find the 4
297 rewards (here gold coins), the number of entries in each arm (which allows us to determine
298 working and reference memory errors) and to replay the video track of each subject for each
299 trial. The return to an arm already explored is defined as an error of working memory (WME),

300 because the information for the retrieved reward varies from test to test. Exploration of an
301 unrewarded arm is defined as a reference memory error (RME), because it is session-
302 dependent. In our study, the procedure for the ERAM was adapted from animal behavioural
303 tests already developed and tested in our studies (Besnard et al., 2012; Machado et al., 2012).

304 The radial arm maze used in this study was composed of a central platform of 5 virtual
305 m and each arm measured around 12 virtual m (**Figure 4**).

306 The subject was informed that they will be placed in the centre of a platform of an
307 ERAM. The test was to find 4 gold coins as quickly as possible with minimal errors in a time
308 range of 5 min maximal, where 10 successive trials were conducted. If the subject was not
309 able to find the 4 coins over a period of 5 min, the test was automatically finished. Before the
310 next trial a black screen of 5 s appeared and participants were placed back in the centre of the
311 central platform of the ERMA. The positions of the rewarded arms were randomly determined
312 for each subject and the start site was also randomized, avoiding egocentric memory bias
313 (**Figure 4**).

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317 Fig. 4 about there

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321 *Habituation*

322 Before the testing using the virtual tasks, all participants performed a period of
323 habituation of at least 2 trials of 1 min. Subjects were placed in an empty environment
324 (without any landmarks or texture) with 4 rewards (gold coins) easily visible. These trials

325 were used to ensure a good understanding of the instructions and of the Xbox joystick as a
326 remote control.

327 *Protocols*

328 All virtual environments were displayed with a video projector on a 2 x 2 m screen in
329 a black room ensuring a good immersion. Virtual exploration within each maze was
330 performed with an Xbox joystick. Moving backwards was not permitted in this study in order
331 to reproduce the natural behaviour of exploration. During the experiment, the software
332 displayed a timer and a counter of remaining targets to find, so that subjects were allowed to
333 monitor their own performance. Before the start of each maze task, an audio file provided
334 standardized instructions to the subjects. The choice of walk and rotation speeds was chosen
335 through the computerized interface. Here, the time required to perform a full rotation of 360°
336 was calibrated at 10 sec and the speed of walking was calibrated at 1.7 m / sec (equivalent to a
337 dynamic walk of 6.1 km / h) while the visual field was calibrated at 90°. Two types of
338 landmarks were tested in the eight-radial arm maze (ERAM) and the Morris Water Maze
339 (MWM), while two starting positions were tested in the virtual reverse T-maze (**Fig. 4**).
340 Landmarks created in the ERAM and MWM originated from a mountain landscape
341 (mountain) or Ancient Rome symbolism such as statue or column (Roma) (**Fig. 5 and 6**). The
342 reverse T-maze paradigm was always performed first, while the orders of the other tests
343 (ERAM and MWM), the positions of the rewards (ERAM) and the platform (MWM), were
344 randomized. Two groups of male and female subjects were tested in the 3 virtual mazes
345 according to two different protocols.

346

347 *Statistical Analysis*

348 A two sample independent t-test was performed to test for gender, age and video game
349 experience effects. A χ^2 using Fisher's Exact Test was used to test for the homogeneity

350 between each group (females and males) in the RTM. For this, the statistical analysis was
351 performed on 18 males (9 started the task with their front to the first arm of the maze and 9
352 started with their back to the maze) and 19 females, (10 started the task with their front to the
353 maze and 9 with their back to the maze).

354 An analysis of the variance with three-way repeated measures (RM ANOVA) was
355 applied to test significance ($p < 0.05$) with a Scheffé post-hoc test for the results obtained in the
356 ERAM and MWM. A three-way RM ANOVA was applied to the ERAM to assess working
357 and reference learning memory (WME and RME) and test for significant differences in the
358 distance travelled and the time spent to complete the test. Statistical analyses were performed
359 on 39 subjects (20 males and 19 females) for the ERAM. Three women and one man were
360 excluded because they did not notice that the rewards were always in the same place and that
361 the start site changed.

362 Another three-way ANOVA was applied to test the significance of the latency and
363 distance travelled to find the platform (phase I and II). A factorial ANOVA was applied to the
364 percentage of the latency to, and the distance travelled in, the target quadrant (phase III) of the
365 MWM task. The percentage of the latency spent in the target quadrant was tested to evaluate
366 if each group performed significantly above the approximate chance level (25%). Analyses
367 were performed on 43 subjects (23 females and 20 males) in this case. One woman did not
368 notice that the platform remained at the same position during the session and was excluded
369 from the analysis. All statistical analyses were performed with Statistica[®] software.

370

371 **Results**

372 *Participants*

373 A t-test revealed no effect of gender on age ($p = 0.42$) but showed a gender effect for
374 the video game frequency, with a higher frequency for men than for women ($p = 0.001$)
375 (**Table 1**).

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Table 1 about there

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385 *Reverse T-maze paradigm*

386 When the starting position faced the first arm, all women adopted an egocentric
387 memory strategy to find the reward after the 180° rotation, while men chose an egocentric
388 (50%) or allocentric (50%) memory strategy. When subjects were positioned with their back
389 to the first arm, women used the egocentric or allocentric memory strategy in a similar
390 manner (55.56% and 44.44%, respectively) and the distribution of the strategy was 66.67%
391 for the egocentric strategy and 33.33% for the allocentric strategy (**Figure 5**).

392 The χ^2 test using the Fisher's exact test was performed on each group (women and
393 men) and no significant difference was reported regardless of the starting position in men ($p =$
394 0.6372). However, a significant difference in strategy was used by women depending upon
395 whether they started with their front or back to the first arm ($p = 0.0325$). This analysis
396 allowed us to show that men used both strategies in the two conditions, whereas women did
397 not.

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Fig. 5 about there

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405 *Morris water maze*

406 Visible platform trials (phase I): During the first phase, no significant differences were
407 revealed regarding the distance travelled. However, analyses showed differences for the
408 latency to find the visible platform with a gender effect ($F_{[1,28]} = 11.53, p = 0.002$): the latency
409 was higher for women than for men. (**Figure 6A**).

410 Hidden phase (phase II): During the second phase, the latency to perform the task
411 significantly decreased with the repetition of the task ($F_{[4,156]} = 2.48; p = 0.04$). The three way
412 RM ANOVA of the distance revealed significant gender and landmark effects ($F_{[1,39]} = 17.42;$
413 $p < 0.001$ and $F_{[1,39]} = 114.65; p < 0.001$, respectively) with a longer distance travelled for
414 women and with the Roma landmarks. (**Figures 6 B1 and B2**).

415 Probe phase (phase III): factorial ANOVA of the percentage of the time and distance
416 travelled in the region of interest, with two factors (gender and landmarks), revealed no effect
417 for the percentage of the latency spent in the target quadrant. A gender x landmark interaction
418 was revealed for the percentage of the distance travelled in target quadrant ($F_{[1,39]} = 4.769, p =$
419 0.035) (**Figure 6 C1 and C2**). For women, the percentage of the distance travelled in the
420 target quadrant was higher with a mountain landmark than a Roma landmark. Conversely, the
421 percentage of the distance travelled in the target quadrant for men was higher with the Roma
422 landmark than the mountain landmark.

423 The percentage of the latency spent in the quadrant of interest was significantly
424 different than chance ($p < 0.001$).

425

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427

428 Fig. 6 about there

429

430 *Eight radial arm maze*

431 The performance of participants significantly improved over the repetition of the trials
432 (**Figure 7 A to D**). The distance travelled, the latency to perform the task, the working
433 memory error and reference memory were significantly decreased over trials ($p < 0.001$ for
434 all).

435 A significant gender difference effect was revealed only for the latency and the
436 distance travelled ($F_{[1,35]} = 19.57, p < 0.001$; $F_{[1,35]} = 5.5792, p = 0.024$; respectively), with the
437 latency and distance travelled for men lower than women (**Figure 8**). The distance travelled
438 was significantly different ($F_{[9,315]} = 21.1749, p < 0.001$), which the Scheffé post-hoc test
439 indicated was due to the distance travelled in the 1st trial being significantly longer than the
440 2nd trial ($p = 0.0025$) and the others ($p < 0.001$). The 2nd trial was significantly different than
441 the 6th and 7th trials ($p = 0.014, p = 0.04$); then higher than the 8th to the 10th trials ($p < 0.001$).

442 A latency effect was revealed ($F_{[9,315]} = 26.1535, p < 0.001$) and the Scheffé post-hoc
443 analyses revealed that the latency significantly decreased from the 1st to the last trial (1st vs
444 2nd, $p = 0.002$, and 1st vs 3rd to 10th, $p < 0.001$). The 2nd trial was also significantly longer than
445 the 5th trial ($p = 0.048$) and then the 6th to the 10th ($p < 0.001$). A latency x gender effect was
446 shown ($F_{[9,315]} = 4.9436, p < 0.001$). The Scheffé post-hoc test demonstrated that the latency
447 of women and men was significantly different only for the first trial ($p < 0.001$), with the

448 womens' latency longer than the men. The first trial for the women was also significantly
449 different from the other trials performed (the 1st vs. the 3rd to the 10th trials, $p < 0.001$).

450 Regarding the learning processes involved in spatial memory, a three way RM
451 ANOVA for working memory error showed a trial x gender effect ($F_{[9,315]} = 3.12, p = 0.0013$).
452 The Scheffé post-hoc test for the WME effect showed a large and significant difference for
453 the performances between trials 1 vs 8, 9 and 10 ($p = 0.002, p = 0.002$ and $p = 0.009$,
454 respectively). As regards the WME x gender effect, post-hoc analyses revealed no significant
455 difference between women and men ($p = 0.061$). Only women showed a learning curve with a
456 significant difference between the 1st trial and the 3rd ($p = 0.027$), the 5th ($p = 0.04$), the 8th and
457 9th ($p = 0.0015$) and the 10th ($p = 0.0044$).

458 An effect of RME was highlighted according to reference memory ($F_{[9,315]} = 30.2164$,
459 $p < 0.001$), and the Scheffé post-hoc test revealed a significant difference between the first
460 and the others trials ($p < 0.001$, for all). The post-hoc analyses also showed a significant
461 difference between the second trial and the 5th to the 10th trials ($p = 0.036, p = 0.007, p =$
462 $0.005, p < 0.001, p = 0.001$ and $p < 0.001$), and between trial 3 and 8 ($p = 0.019$), trial 3 and
463 10 ($p = 0.048$).

464

465

466

467

Figs. 7 and 8 about there

468

469

470

471 **Discussion**

472 We have replicated rodent behavioural tests and created new software to provide
473 preconfigured virtual mazes or permit researchers to build their own mazes (from easy to
474 complex tasks), evaluating spatial learning performance and spatial memory strategies in
475 humans. The ERAM, MWM and Reverse T-mazes tested here, have shown an effective
476 learning curve with gender differences, validating our tool to investigate spatial learning and
477 memory.

478

479 *Technical considerations*

480 The Human Virtual Maze software has been designed for clinical or research use. The
481 software allows the experimenter to determine all settings and parameters of analysis in their
482 virtual mazes (choice of each protocol environment (square room, cylindrical room or a
483 spherical room), the maze itself (1 to 8 radial arm maze or the swimming pool), the size of the
484 room or the maze, the colour of the wall and also the size and type of landmarks). Compared
485 to other MWMs previously developed, here the landmarks and starting position may be
486 randomly determined, and the software permits a full configuration of the trials with a multi-
487 trial option for a session (number of trials, number of mazes to launch consecutively etc...).
488 Users may then configure external and/or internal landmarks and build a large panel of mazes
489 similar to real rodent mazes. The ergonomics of the mazes (sounds associated with the walk
490 into the ERAM or with the swimming in the MWM, type of landmarks, displaying on a large
491 screen) ensured a good immersion of the subject, a good information flow providing real-time
492 visual and auditory information, and may be improved with vestibular and proprioceptive
493 stimulation, according to where the mazes are projected (a simple screen up to oculus rift and
494 immersive room). The speed of walking may be constant and arbitrarily chosen, limiting
495 inter-individual differences in joystick or keypad control ability, due to past experience with
496 videogames or similar. Head orientation and motion controls may be combined

497 simultaneously by the subject during virtual navigation and can be increasingly improved
498 with the number of trials and can be taken into account in the analysis or may calibrate the
499 duration of the habituation period. The area of counting of an entry into an arm has been more
500 distally placed after preliminary testing in order to avoid counting a false entry when a subject
501 was close to an entry without entering into it, particularly if lateral motion was allowed.

502 The search for the platform with a strategy of lateral motion in the MWM increased
503 the performance but skewed the heading error parameter i.e. the subject did not look in the
504 direction of the platform during the test. Consequently, if lateral motion remains allowed by
505 the operator, we have substituted the heading error measurements by the calculation of the
506 optimal direction i.e. the angle between the body and the most direct pathway to reach the
507 platform and to complete the test as previously reported. Additionally, we suggest starting at
508 the front of the swimming pool and asking the subject to adopt the most direct pathway to the
509 platform. The duration of the trials and number of trials per session and the number of
510 rewards, have to be adapted according to the objectives and to the type of people tested and
511 the time to perform the tests.

512 However, if subjects perform the test seated on a comfortable armchair, we deprive the
513 participant of vestibular and proprioceptive information. Our software has been developed for
514 headsets as well as immersive room projections. However, it seems that spatial memory
515 performance or virtual tasks for rehabilitation remain similar regardless of the type of screen
516 used, i.e. laptop versus large screens with or without 3D stereoscopic vision (Dahmani et al.,
517 2012).

518

519 *Validation of the 3D virtual mazes*

520 *Eight Radial Arm Maze*

521 A learning curve and a significant increase in spatial memory performance were
522 observed with the ERAM in accordance with previous similar studies (Astur et al., 2004;
523 Bertholet et al., 2015; Levy et al., 2005) both in men and women. The number of working and
524 reference memory errors decreased rapidly for both men and women. From the eighth trial,
525 the number of errors was constant and the lowest. For working memory, there was no main
526 effect of gender, but a significant interaction of gender with trial, and the post-hoc tests
527 showed a significant difference between women and men in the first trial. The gender
528 difference, only observed on the first trial, might be explained by the greater experience of
529 videogames by men and a possible disorientation in the 3D virtual environment of women at
530 the beginning of the trials and by the remote practice. Indeed the distance travelled was
531 similar for men and women. The interaction reported here (latency to perform the task x
532 gender) was in accordance with the results of Astur *et al.* (Astur et al., 2004) and those of
533 Levy *et al.* (Levy et al., 2005).

534

535 *Morris water maze:*

536 The latency to find the platform was gradually decreased, suggesting a learning
537 process regardless of gender and type of landmark.

538 No gender effect for the results during the learning phase were in accordance with the
539 results obtained by Sneider et al. with teenagers (Sneider et al., 2015). Although, they
540 highlighted an age effect for latency, they did not detect a significant effect of gender for each
541 measure. The study of Astur et al. (Astur et al., 2004) showed a large difference between
542 women and men during the MWM for the latency to find the hidden platform but not the
543 visible platform. The distance travelled was not significantly different for gender. Perhaps the
544 gender effect is only due to practice. Indeed, the difference between males and females to find
545 the hidden platform seems to decrease with the number of trials. The lack of difference due to

546 motor control (visible platform) could be due to the fact that this phase is performed after 21
547 trials.

548 Consistent with Astur et al. (2004), we conclude that spatial navigation in men was
549 faster than women to find the platform or the reward, while the distance travelled was similar.
550 The shorter latency to find the visible platform during the third trial of the test may confirm
551 the greater ability of men regarding joystick control and of spatial navigation in a virtual
552 maze.

553 When the platform was removed in the second part of the test, we did not detect a gender
554 effect regarding the distance travelled and the time spent in the quadrant of interest. Women
555 spent more time and travelled a greater distance in this region than men.

556 During the probe trial, women travelled a greater distance in the region of interest,
557 which suggests a greater persistence of women to find the target. However, Astur et al.
558 (2004), and Sneider et al. (2015) found the opposite for the distance travelled. For the
559 percentage of the time (~90%) and distance (~75%) in the target quadrant for women, our
560 results seem higher than for Astur and other studies such as Sneider et al. (Sneider et al.,
561 2015). However, the results obtained by men were more in agreement with Sneider et al., with
562 a percentage of distance travelled at ~65% for young adult men adult compared to ~60% for
563 our study. Another study performed in healthy young adults, similar to our participants,
564 showed a greater time spent in the target quadrant (between ~60 to ~75 %) (Chae et al., 2019).
565 It is conceivable that because the search preference values were so high, that ceiling effects
566 might be a problem and make the task less sensitive to impairments in spatial learning and
567 memory. Some revision of the parameter settings may therefore be useful.

568 In contrast to Astur et al.(2004), and Sneider et al. (2015), we found that women spent
569 more time to reach the visible platform. Nevertheless, unlike other studies, participants in our

570 study have seen the visible platform at the beginning, this result may be explained by a
571 practice.

572

573 *The reverse T-Maze*

574 According to van Gerven et al. (2012), all men preferred a place strategy while most
575 women preferred a response strategy, requiring fewer attentional resources than with
576 declarative memory (Spataro et al., 2013). Here we reported a starting position effect when
577 the subject started from the front of the first arm: most participants chose a response strategy
578 preferentially as observed by Levy et al. (Levy et al., 2005) and Paleja and Spaniol, (Paleja
579 and Spaniol, 2013).

580 Levy et al. (2005) explained that the response strategy was chosen according to the
581 time point of the probe trial, i.e. sixth and twelfth trials for Levy and similarly fifth and tenth
582 in our protocol. Similar to Levy et al. (2005), we used symbolic cues (table, sofa ... and
583 triangle, circle ...) and we suggest that simple symbolic visual cues facilitate a response
584 strategy, decreasing cognitive resources while complex landmarks like landscapes might
585 foster the adoption of a place strategy. It was reported that an egocentric strategy requires
586 fewer attentional resources than declarative memory (Spataro et al., 2013).

587 We observed that the starting position influenced the choice of the strategy in women.
588 Starting with their backs to the first arm of the T-maze switched the percentage to equal
589 choice of place and response strategies, which may be explained by the fact that the subject
590 had to turn by 180° in order to facilitate the visualization of all visible cues displayed in the
591 virtual room.

592 By comparison, such differences in strategy were not observed with the MWM, where
593 proximal landmarks (e.g., the corner between two arms) did not appear to promote a
594 declarative spatial location. Large inter-individual differences for the time taken to do the task

595 were noticed between women, although the walking distance remained similar, suggesting
596 that women will spend a longer time to observe distal landmarks compared to men. Our
597 results also suggest that the starting strategy was egocentric and then allocentric in women, as
598 mentioned in the literature (Barkley and Gabriel, 2007; Cánovas et al., 2011). However, the
599 strategy adopted in the ERAM was not individually correlated with that used in the T-maze.

600 Despite the differences between the three tests, it appeared that these could be
601 corrected according to a more specific protocol choice. However, a declarative spatial strategy
602 (explicit memory), as it requires more attentional resources, must consider emotional factors,
603 and motivation, as these will impact on the choice of the strategy. The possibility of using
604 both strategies by both sexes indicates that the choice of benchmarks, instructions, and
605 experimental parameters may influence the results.

606

607 **Conclusion**

608 The software proposed herein then offers a translational approach for research units
609 that wish to combine animal models and patient evaluations as well as complex 3D tasks and
610 standardized neuropsychological tests opening a large perspective in the neurosciences to
611 investigate higher cognitive functions. Moreover, the full control of the maze building, setting
612 adjustment of trial and of analysis may offer users the opportunity to better investigate the
613 effect of specific parameters up to virtual reality presence. A clinical module with
614 preconfigured 2- and 3-D tasks should offer clinicians an easy way to evaluate their patients
615 routinely since growing evidence from the literature suggests that virtual mazes such as
616 ERAM or MWM might improve the screening or diagnosis of mild cognitive impairment and
617 dementia, particularly in Alzheimer's disease (Laczó et al., 2012; Lee et al., 2014; Leplow et
618 al., 2002; Tarnanas et al., 2014; Weniger et al., 2011; Zygouris et al., 2015).

619

620

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624

625 Author Contribution Statement

626 Besnard, Machado have designed the experiment, performed the protocol, analyzed the data.
627 Lefevre, Fleury, Madeleine, Machado and Besnard have developed the software. Le Gall and
628 Philoxène were operators for the protocol. Machado and Smith have performed the statistical
629 analysis. Besnard, Machado and Smith have written the paper.

630

631 Conflict of Interest Statement

632 NO

633

634

635

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820 **Legends**

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822 **Table 1: Gender effect on age and video game frequency.** Table representing women and
823 men age (*in years*) and video game frequency score (*1 = never, 2 = several times a year, 3 =*
824 *several times a month, 4 = several time a week, 5 = everyday*). There was no gender effect on
825 age ($p = 0.42$) but there were gender effects on the video game frequency ($p = 0.001$), the
826 score of men was higher than the women's score. (mean \pm SD)

827

828 **Figure 1:** Human Virtual Maze software *A- Parameter setting* -- Screenshot of the part of the
829 software that allows the design of each parameter of the protocol (environment, maze and
830 trial). *B- Analysis area* selected by the user. *C- Continuous Performance Task – Balloon*
831 *Analog Risk Task* -- Example of 2D tasks designed by the Human Virtual Maze software.

832

833 **Figure 2: Reverse T-maze paradigm** with symbolic distal landmarks. During the habituation
 834 trials (left panel), the subject (white square) started back to the arm (grey triangle) and had to
 835 learn the position of the reward turning right at the end of the first arm. During the probe trial,
 836 the T-maze was then rotated by 180°, and the subject who started back to the first arm, had to
 837 find the reward at the same location by turning in the left arm (right panel).

838

839 **Figure 3: Morris Water Maze task** configured with forest and mountain distal landmarks (left
 840 top and bottom panel). The right panels show the tracking of three subjects: the right top and
 841 middle panels illustrate two different strategies to find the platform during the beginning of
 842 the protocol while the right bottom panel shows how a subject quickly achieves the task after
 843 several trials (learning period). The distance and latency to find the platform as well as the
 844 direction and strategy were analysed. The unit of time in the target quadrant is secs.

845

846 **Figure 4: Eight Radial Arm Maze configured with Roma distal landmarks (left panels).** The
 847 virtual Roma environment has been fully modelled by the CIREVE® from University of
 848 Normandy Caen. The subject was placed at the centre of the test and had to learn to catch
 849 three rewards located in three different arms. The number of entries, the distance travelled and
 850 latency were recorded. This task allowed the evaluation of reference memory and working
 851 memory. Here we show the trace (in orange) of subjects during the learning period looking
 852 for the rewards located in the distal parts of the arms.

853

854 **Figure 5: Strategy to achieve the T-maze paradigm during the probe** according to two
 855 different starting positions (“Front” i.e. front of the first arm, and “back” i.e. started back to
 856 the first arm). Here we show that the starting position influences the type of strategy used in
 857 the reverse T-maze with an egocentric strategy preferred in women when starting in front of
 858 the first arm ($p < 0.05$).

859

860 **Figure 6: Morris Water Maze results** A- Morris Water Maze, Phase I Women spent more
 861 time to reach the visible platform while the distance travelled remained similar compared to
 862 men ($p < 0.01$). B- Morris Water Maze, Phase II we observed a gender difference according
 863 to the distance travelled ($p < 0.001$) (B1) as well as a landmark effect on the distance
 864 travelled, increased with the Roma scene (mountain and Roma, $p < 0.001$) (B2). C- Morris
 865 Water Maze, Phase III The percentage of time spent in the region of interest around the
 866 hidden platform was similar in men and women (C1) regardless the type of landmark,
 867 meanwhile the percentage of distance travelled in the same region depended on the landmarks
 868 and gender (increased in women with mountain scene, $p < 0.05$) (C2). (mean \pm SEM)

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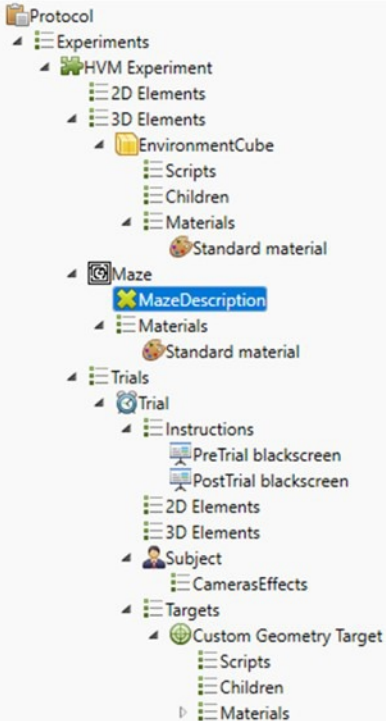
870 **Figure 7: Radial Arm Maze task.** A- Latency and B- Distance travelled to find all rewards:
 871 Trial effect ($p < 0.001$) and gender effect ($p < 0.001$) were obtained for both. C- Working
 872 Memory Errors (WME) and D- Reference Memory Errors (RME). WME and RME effect (p
 873 < 0.001) showed a learning curve. WME and gender effect ($p < 0.01$) was also observed.
 874 (mean \pm SEM)

875

876 **Figure 8:** The latency and the distance travelled to find rewards in the radial arm maze (mean
877 \pm SEM).

878

A



Entity description

Name	MazeDescription
Description	

X-Maze parameters

Inner radius	2.8
Outer radius	9.6
Maze height	1.9
Wall size	0.16
Branch size	1.5
Hide height	1
Hide length	0.5
Hide width	0.5
Hide radius	8.5
Angle	45
Branch count	8

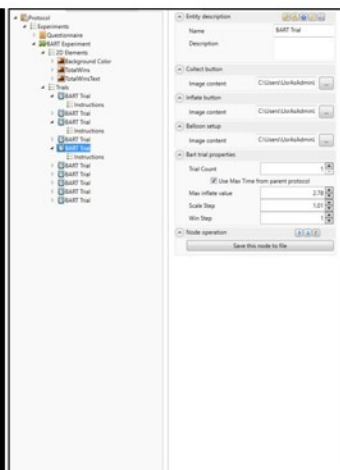
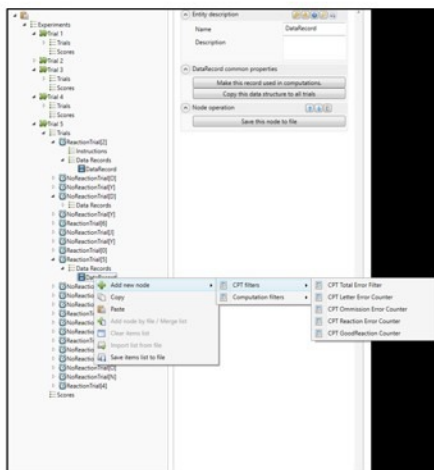
Node operation

Save this node to file

B



C



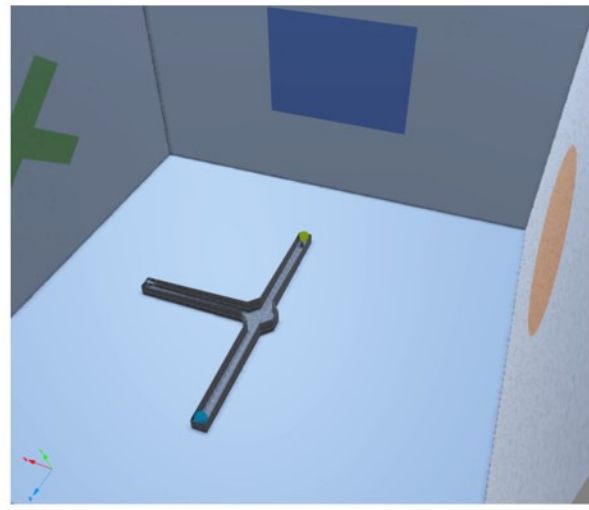
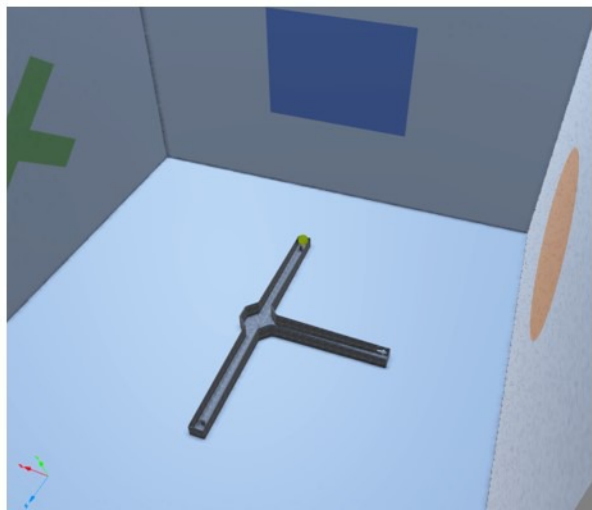
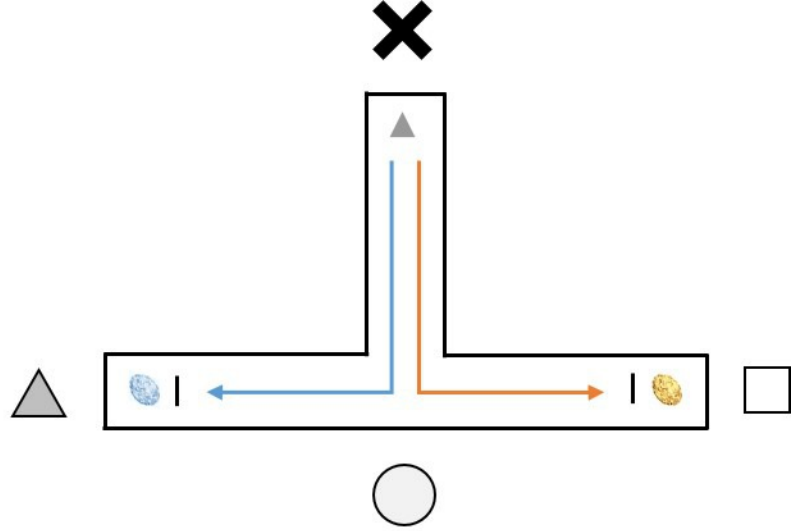
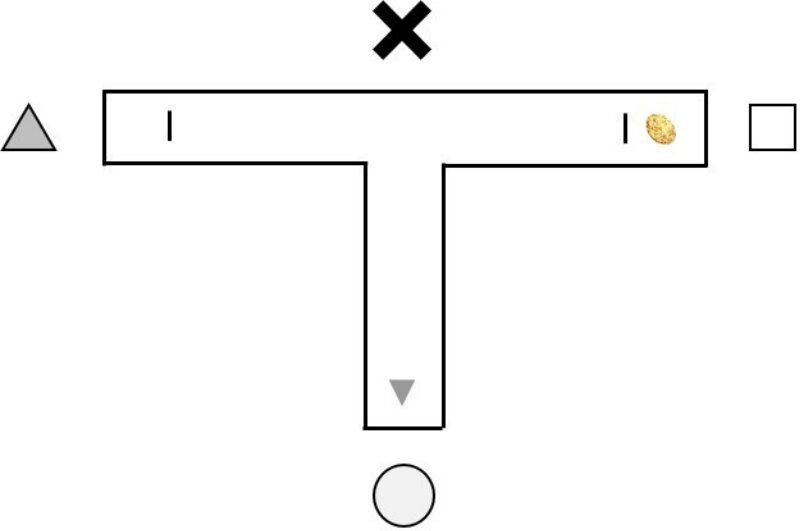
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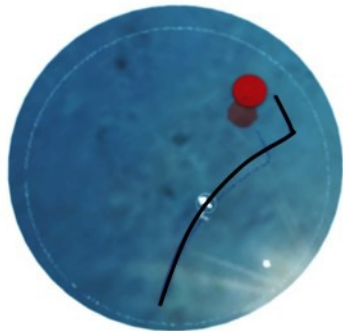
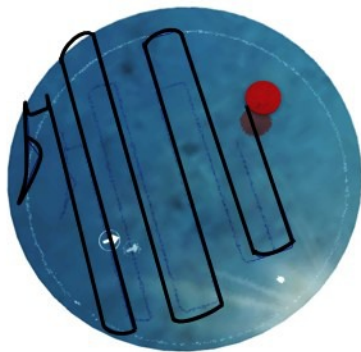
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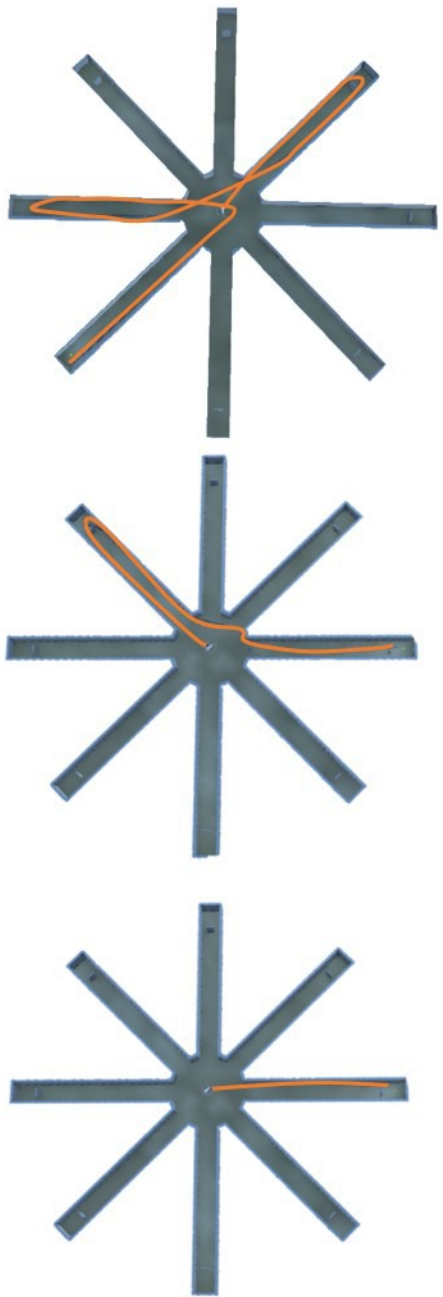
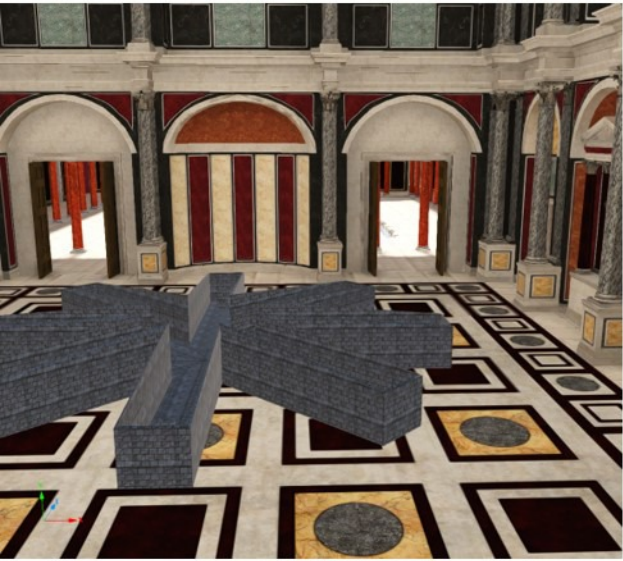
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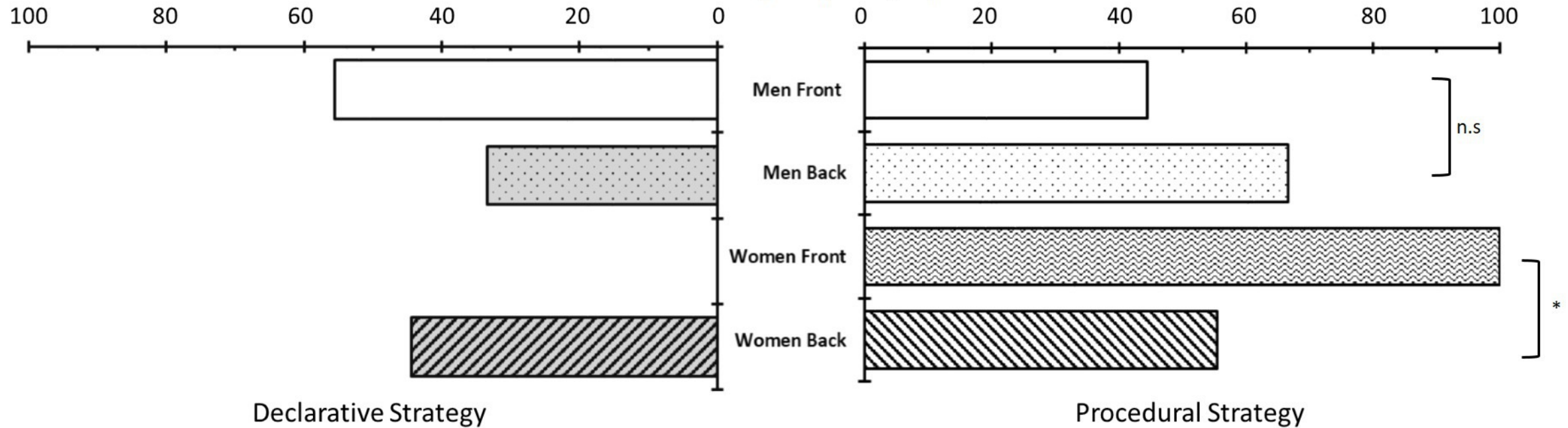


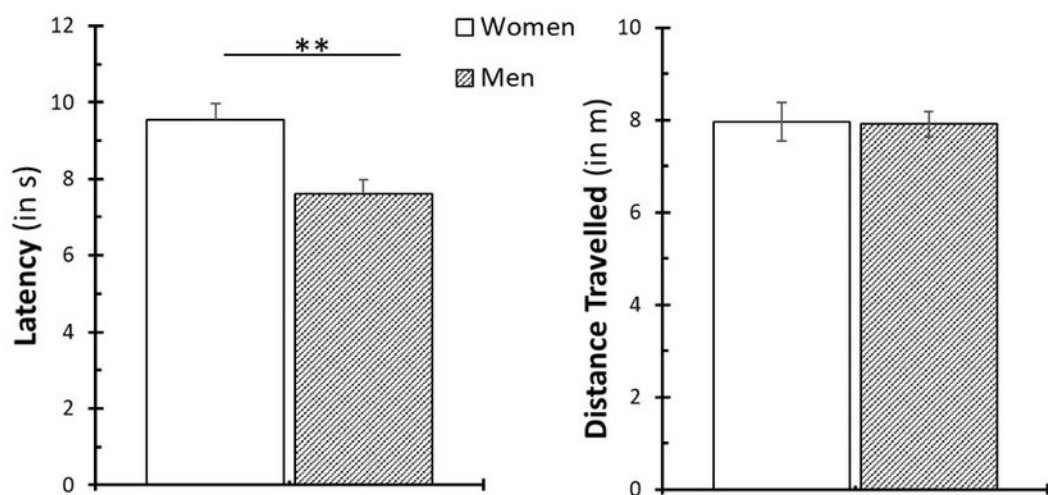
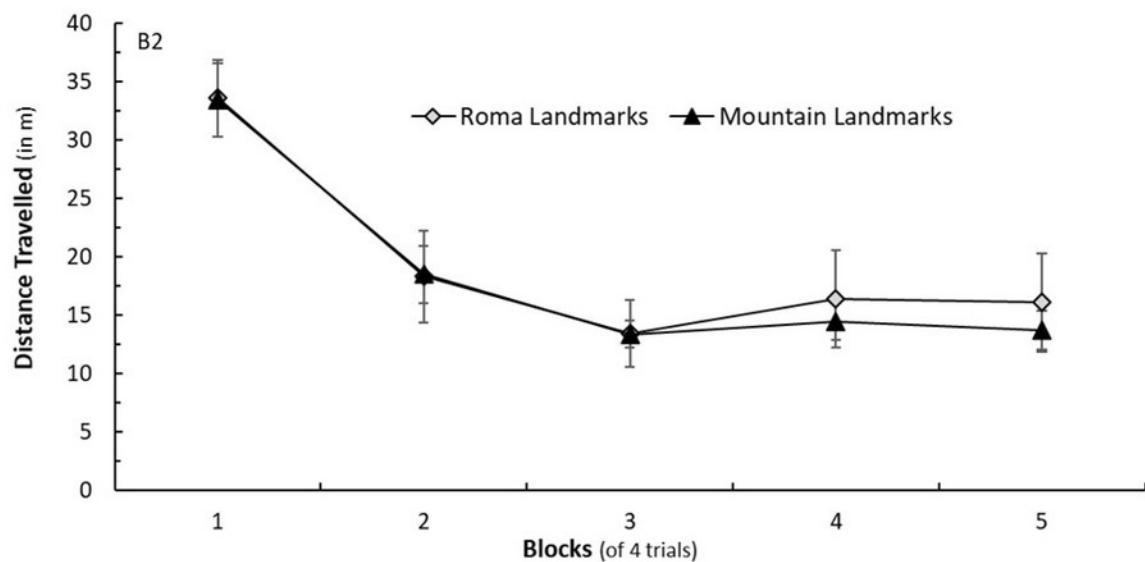
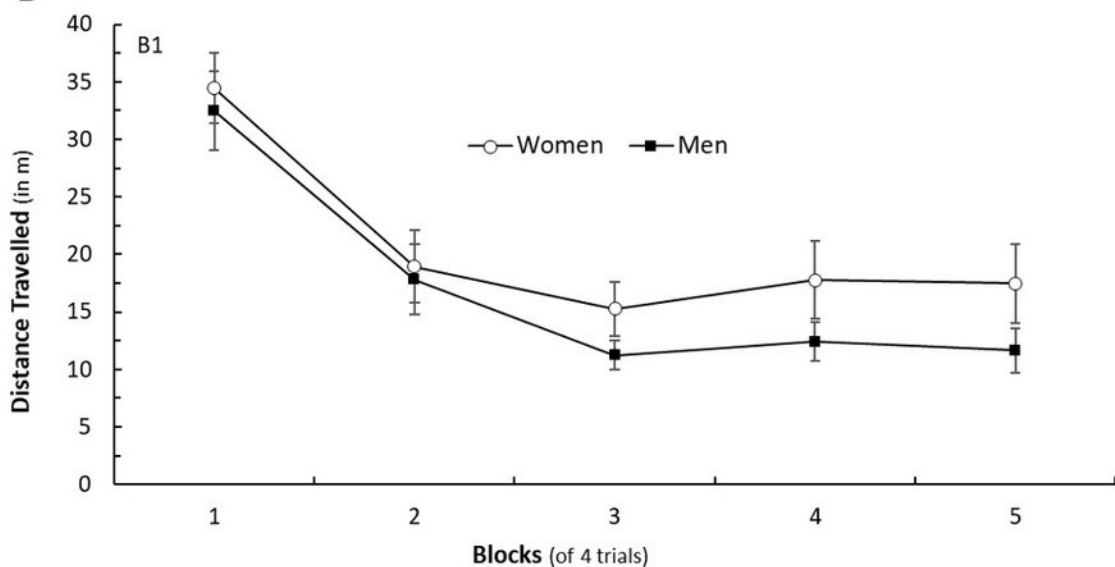
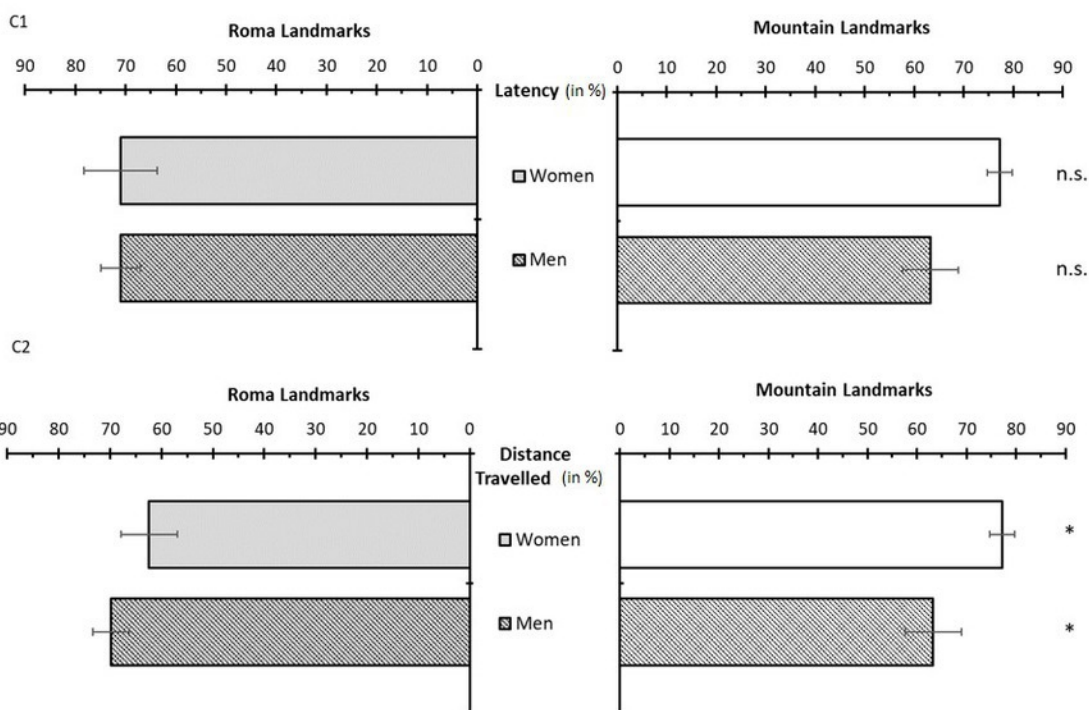


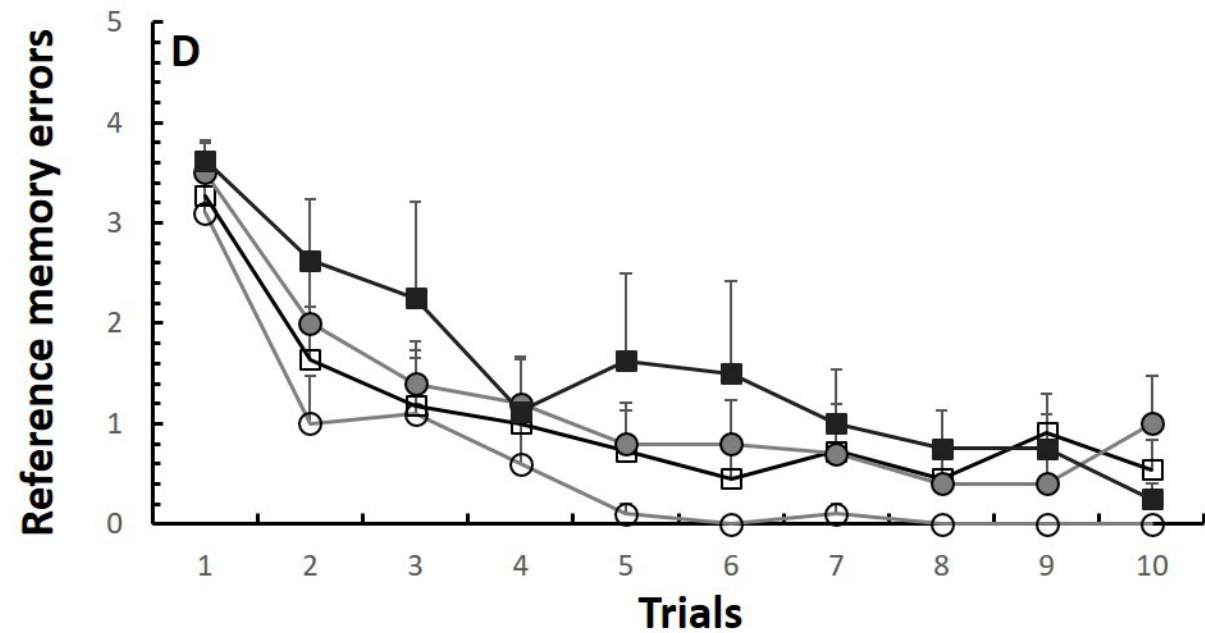
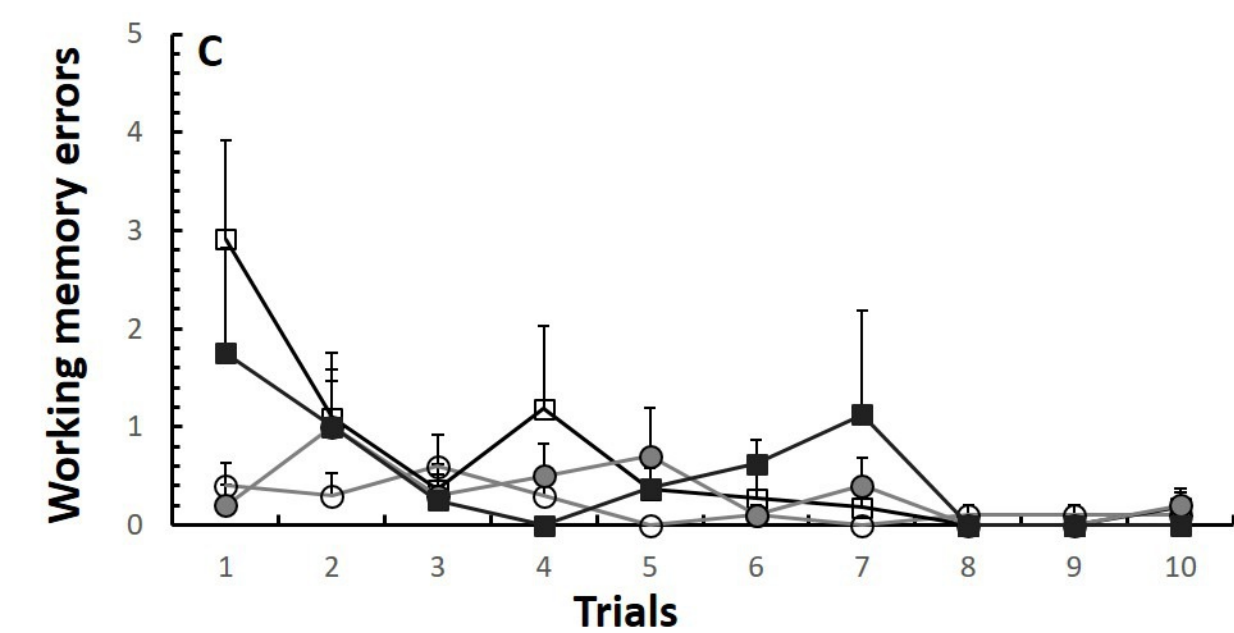
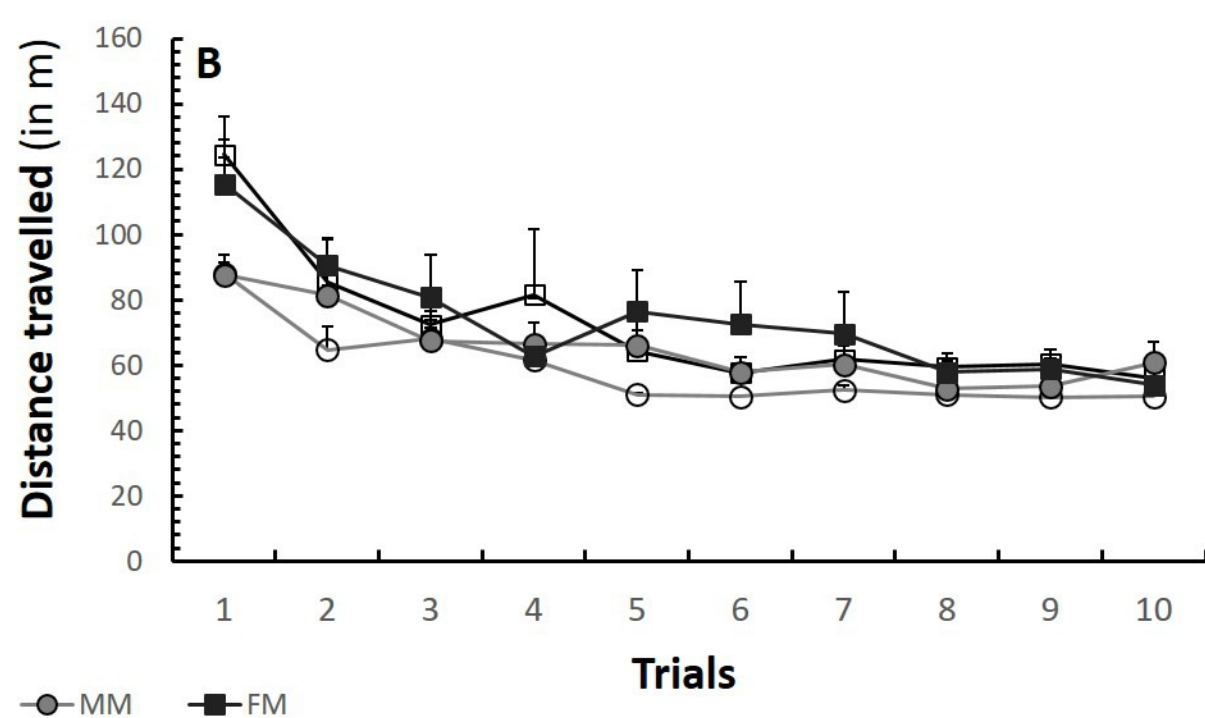
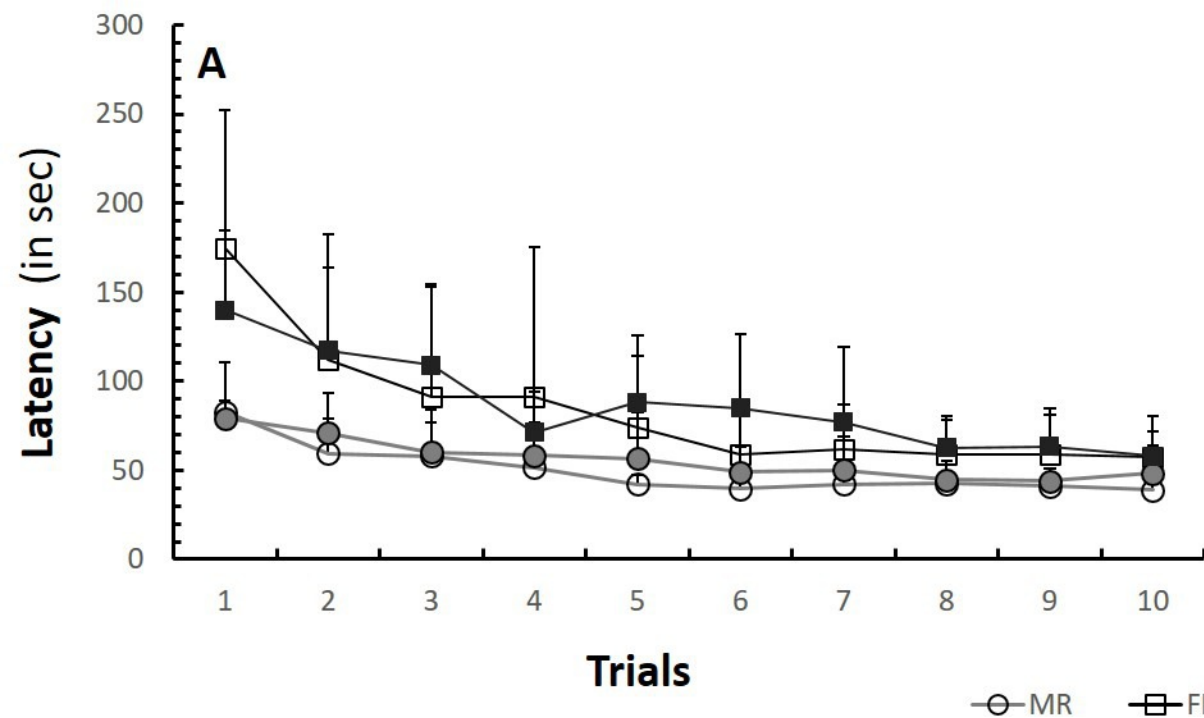


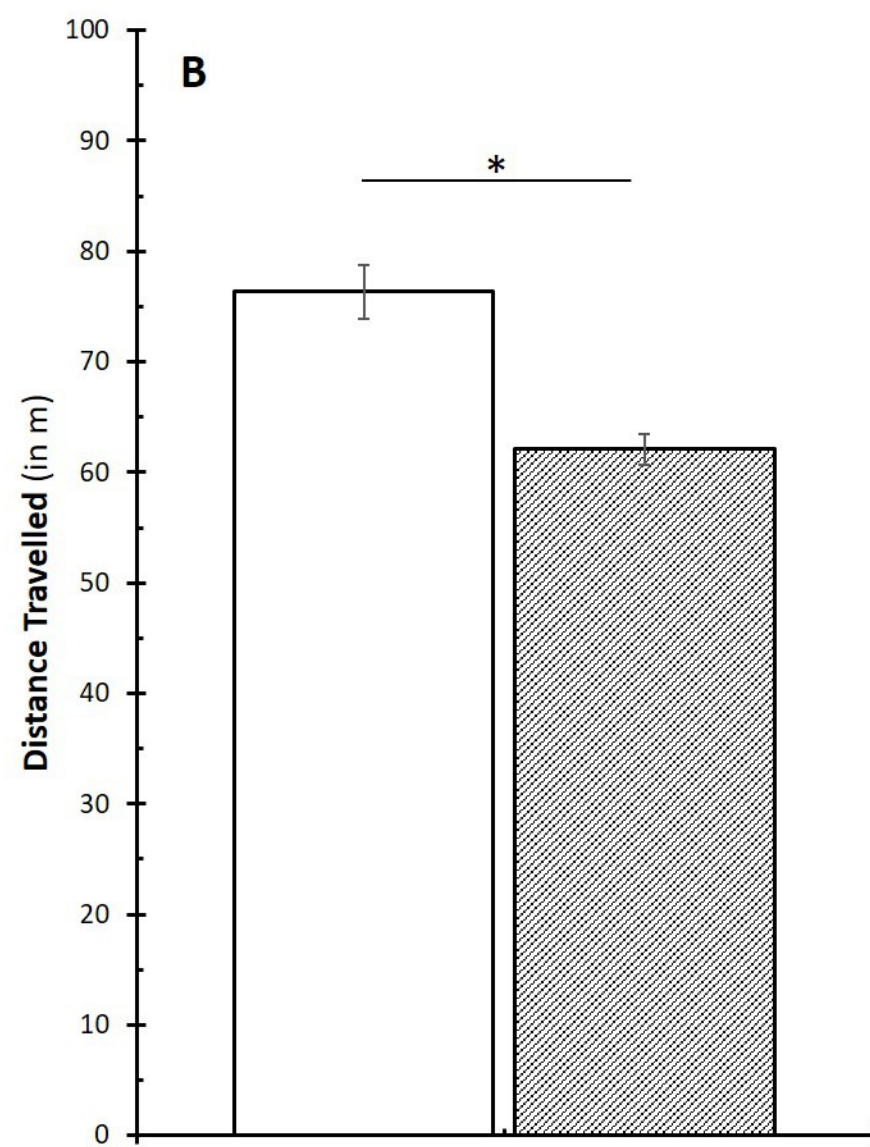
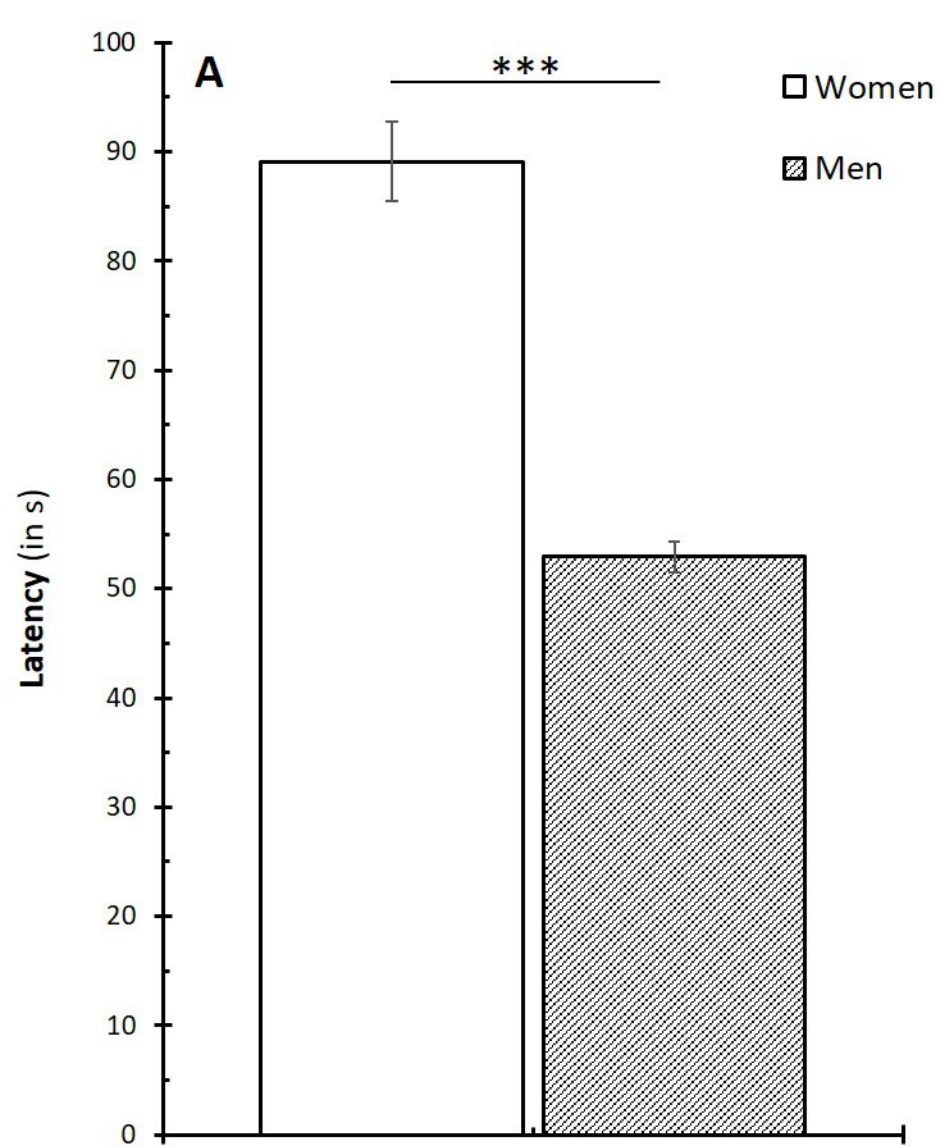
□ Men Front ▨ Men Back ▩ Women Front ▧ Women Back

Percent of participants (*in %*)



A**B****C**





1

1 **Table. 1**

2

	Age in year (mean \pm SD)	Video game frequency Score 1=never to 5=everyday (mean score \pm SD)
Women	23.19 \pm 2.29	1.76 \pm 0.8
Vs.	n.s.	***
Men	22.8 \pm 2.29	2.95 \pm 1.28

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