
Volume 193, No. 2, 21 November 2008
ISSN 0166-4328
ELSEVIER
BEHAVIOURAL BRAIN RESEARCH
AN INTERNATIONAL JOURNAL

Behavioural Brain Research 193 (2008) 209–215

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Sex differences in a virtual water maze: An eye tracking and pupillometry study

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ARTICLE INFO

Article history:
Received 25 February 2008
Received in revised form 21 May 2008
Accepted 26 May 2008
Available online 3 July 2008

Keywords:
Spatial navigation
Eye tracking
Pupillometry
Sex differences
Strategy
Allocentric
Egocentric
Water maze

ABSTRACT

Sex differences in human spatial navigation are well known. However, the exact strategies that males and females employ in order to navigate successfully around the environment are unclear. While some researchers propose that males prefer environment-centred (allocentric) and females prefer self-centred (egocentric) navigation, these findings have proved difficult to replicate. In the present study we examined eye movements and physiological measures of memory (pupillometry) in order to compare visual scanning of spatial orientation using a human virtual analogue of the Morris Water Maze task. Twelve women and twelve men (average age = 24 years) were trained on a visible platform and had to locate an invisible platform over a series of trials. On all but the first trial, participants' eye movements were recorded for 3 s and they were asked to orient themselves in the environment. While the behavioural data replicated previous findings of improved spatial performance for males relative to females, distinct sex differences in eye movements were found. Males tended to explore consistently more space early on while females demonstrated initially longer fixation durations and increases in pupil diameter usually associated with memory processing. The eye movement data provides novel insight into differences in navigational strategies between the sexes.

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1. Introduction

During evolution, sex differences in core cognitive abilities may have developed due to distinct requirements in skills and social interactions. Sex differences in spatial navigation tasks that require knowledge of topographical relations of visible, distal cues are robust among species [1,2] and are found in both preadolescent and adult humans [3–5]. Yet, despite consistent findings of sex differences in spatial abilities in humans, the cognitive strategies that underlie this behavioural distinction are virtually unknown.

Studies in human adults commonly report that males find a location in an environment with only distal cues more quickly [6], are more accurate [3] and tend to travel shorter distances [6] than females. Brain imaging studies document that males and females recruit different brain regions in virtual maze [7] and mental rotation tasks [8] suggesting different behavioural strategies. However, despite consistent sex differences in spatial abilities in behaviour [6], neuroanatomy [7], and endocrinological function [9,10], these strategies have not been well defined.

One step towards understanding how men and women navigate is to examine more closely what aspects of the environment men and women utilise during navigation. It has been argued that males favour navigational strategies that involve distal and directional information (allocentric navigation), while females favour landmark-oriented (egocentric) navigation [11,12]. Furthermore, a sex bias has been reported depending on the type of spatial task performed [13–15].

The Water Maze Task [16] requires rodents to find a hidden platform within a pool of water when only distal cues are available. Thus, it restricts the type of navigation strategy that can be used or at least makes an allocentric strategy more efficient than other approaches, which are egocentric. This test has since been routinely used to demonstrate the crucial role of the hippocampus and related brain regions in spatial learning and memory in rodents [17] and humans [18–20]. However, which specific aspects of the spatial cues are being used is unclear. Some researchers have suggested that behavioural differences in spatial memory may be attributable to perceptual or attentional factors [5] possibly founded on distinct navigational strategies [13,14] and evolutionary sex specific roles [15]. It has been suggested that given the presence of sex differences on this task, it is conceivable that males and females are encoding different stimuli or utilise the same stimuli in different ways when navigating around an environment [5].

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0166-4328/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.bbr.2008.05.017

Eye tracking enables the examination of scan paths of visual scenes, as measured by vertical and horizontal saccades, and the points of interest, as measured by fixations [21,22]. For instance, horizontal visual span (or spread) has been related to impaired visual processing in depression [23] and has been found to be increased for expert viewers relative to laymen when viewing chess configurations [24]. Conversely, fixation durations, which are indicative of attentional dwell time on particular locations, are diminished for expert viewers relative to laymen when viewing known pictures or configurations [25] and reduced for high memory span subjects relative to low memory span subjects [26]. Although lower level processes such as stimulus salience play a role in directing eye movements, studies have shown that top-down control, as required in memory tasks, can alter eye-scanning behaviour [27–29].

A directly related physiological indicator of memory and cognitive resource requirements provided by eye tracking is pupil dilation [30,31]. Pupillary dilation has been hypothesised to reflect recruitment of cognitive resources to meet task demands [30,32] and has recently been used to inform interpretation of functional imaging data [33]. Studies show consistent increase in pupil dilation as resource and memory requirements increase in memory [30], visual memory [34,35] and sustained attention tasks [32]. Memory-related peak pupillary dilation usually occurs around the first several hundred milliseconds after stimulus presentation and is reflected in the latency to peak dilation [31].

The aim of the current study was to specify the visual correlates underlying sex differences in spatial navigation using eye tracking methodology. If females exhibit an advantage for spatial location memory for objects, as has been shown previously [15], then one would expect a strategy based on memory encoding of specific locations (landmark-oriented) evident in larger pupil dilations and longer fixation durations (indirect indicators of hippocampal and memory systems). By contrast, some studies have shown that visual spread is enhanced when trying to understand interpiece relations among objects [24], which would be indicative of an environment-oriented strategy and thus should be favoured by males.

2. Methods

2.1. Participants

Twelve women ($M = 24$ years, S.D. = 2.4) and 12 men ($M = 24$ years, S.D. = 3.1) from the University of Nottingham participated in the study for £3 compensation. All had normal or corrected-to-normal vision. The University of Victoria Ethics Committee reviewed and approved the protocol. The ethical guidelines of the School of Psychology of the University of Nottingham were followed.

2.2. Apparatus

Virtual Environment The Arena Maze [6] was constructed using the Unreal® Editor and run using the Unreal® game engine (Epic games, www.unreal.com) on a Pentium M computer (1.6 GHz) with 512 MB VRAM. It was presented on a 17 in. CRT monitor 48 cm away from the participant. The Arena Maze was designed as a virtual analogue of the Morris Water Maze and therefore it was intended to provide a configuration of distal cues with no proximal cues to indicate target locations. In overview, the environment consisted of a large circular arena contained within a very large square room with windows, a door, and landscape outside (see Fig. 1A). From the participant's perspective, the circular arena appeared to be about 40 m in diameter, bounded by a low (1 m high) wall that restricted movement but not view of the walls or windows. The very large room outside the arena appeared to be 75 m × 75 m × 17.5 m high. Two facing walls each had 3 windows which provided views of an outside world having green hills sloping to a beach. Another facing wall had a large window providing a view of a large body of water with a mountainous island. The fourth wall had a large door. Although all of these features were visible from the participant's eye-level perspective within the arena, they were only fully viewed from near the windows, thereby requiring participants to use their memory when navigating using these stimuli. The dimensions of the room were scaled up to human size from typical rodent laboratory situations (cf. [36] and large windows and distant landscapes were added for frames of reference. For reference purposes, each of the four walls is assigned a cardinal direction (North—N, East—E, South—S, and West—W). Participants moved in the virtual environment using a joystick, modified to allow forward, left and right movements, but not backing up (in order to simulate movements used by rats in the water maze, and humans in daily life).

2.3. Procedure

At the beginning of each session, participants signed a consent form detailing the nature of the experiment. They then filled out a demographics questionnaire that included questions about their experience with video games and joysticks. Participants rated their experience with a joystick as a child, how often they played computer games in the previous 12 months and their experience with 2D and 3D computer games on a scale from 0 ("never") to 6 ("daily"). Following this they were seated at the head-mount for the eye tracker and their gaze position was calibrated using nine circular stimuli evenly distributed across the corners and centre positions of the screen.

2.4. Behavioural testing

Prior to testing, participants were placed into the virtual room outside of the arena and encouraged to explore the space for as long as they wished, familiarizing themselves with using the joystick and with the features of the room and landscape outside the windows. Once the participants said they were comfortable with the joystick and the environment, behavioural testing began. There were three types of trials: visible platform, invisible platform, and probe (Table 1). Testing began with 4 visible platform trials, in which the platform was visible on the floor from each of the 4 different starting positions. Prior to the trials, participants were instructed to go to the platform as quickly and directly as possible. Testing continued with 10 invisible platform trials in which the platform location was moved from the previous 2 visible trials but constant during the invisible trials. Participants were placed in varying positions at cardinal points in pseudorandom order and required to walk around the arena until they stepped on the platform, at which point the platform would rise with a sound and become visible. The invisible platform was in a constant location within the arena: the centre of the SE quadrant. All start positions were at cardinal points (N, E, S, or W) adjacent to the wall, and were oriented to provide a view towards the centre of the arena and the opposite wall. The final trial was the probe trial, in which the platform remained invisible for the duration of the 20-s trial. Prior to the invisible platform trials, participants were told that the platform would always be in the same place and were reminded to go to it as quickly and as directly as possible. They were also told that on one trial (the final probe trial) the platform would be "very hard to find" and if they thought they were on this trial that they should continue to search for it nevertheless. Once the participants found the platform, they were allowed to remain on it as long as they wanted, and on the first 3 invisible platform trials they were encouraged to look around the room before the next trial ("Look around the room so you can find your way back to the platform. Have you looked enough? Are you feeling OK?"). The next trial was initiated by the experimenter once the subjects said they were ready.

Navigational performance in the virtual environment was assessed using latency to find the platform (measured with a stopwatch), path length (distance in arbitrary units) between the start position and the edge of the platform, and dwell time (percent) in each of the 4 quadrants of the arena (NE, SE, SW, NW) on the probe trial. To ensure consistency between participants, all subjects were run by the same experimenter. The latter two measures were calculated using a custom program (TRAM) written by Ludek Nerad; see [37] for more detail.

2.5. Eye tracking

A 250 Hz Video Eye Tracker (Cambridge Research Systems Ltd.) was used to collect and record eye movement (Fig. 1B) and pupillometry (Fig. 1C) data. To aid eyetracking, the experiment was conducted in a windowless room with the lights off during behavioural testing. At the beginning of each trial, eye movements were recorded for 3 consecutive seconds. However, due to technical constraints, it was not possible to record eye movements on the first trial at the beginning of the experiment.

2.6. Eye-tracker measures

Data from the eye tracker were analysed using Matlab (The Mathworks Inc.). Saccade parameters (visual spread and fixation duration) were calculated using the ILab toolbox [38] for Matlab. An eye movement was considered a saccade if its duration exceeded 35 ms and its velocity exceeded a threshold set at 30°/s. A fixation was defined as an eye movement that lingered for at least 100 ms over a 10 square pixel radius. Resulting fixation durations were calculated. We also wanted to examine what proportion of time males and females spent looking at parts of the maze that could be considered allocentric or egocentric ("visual dwell time").

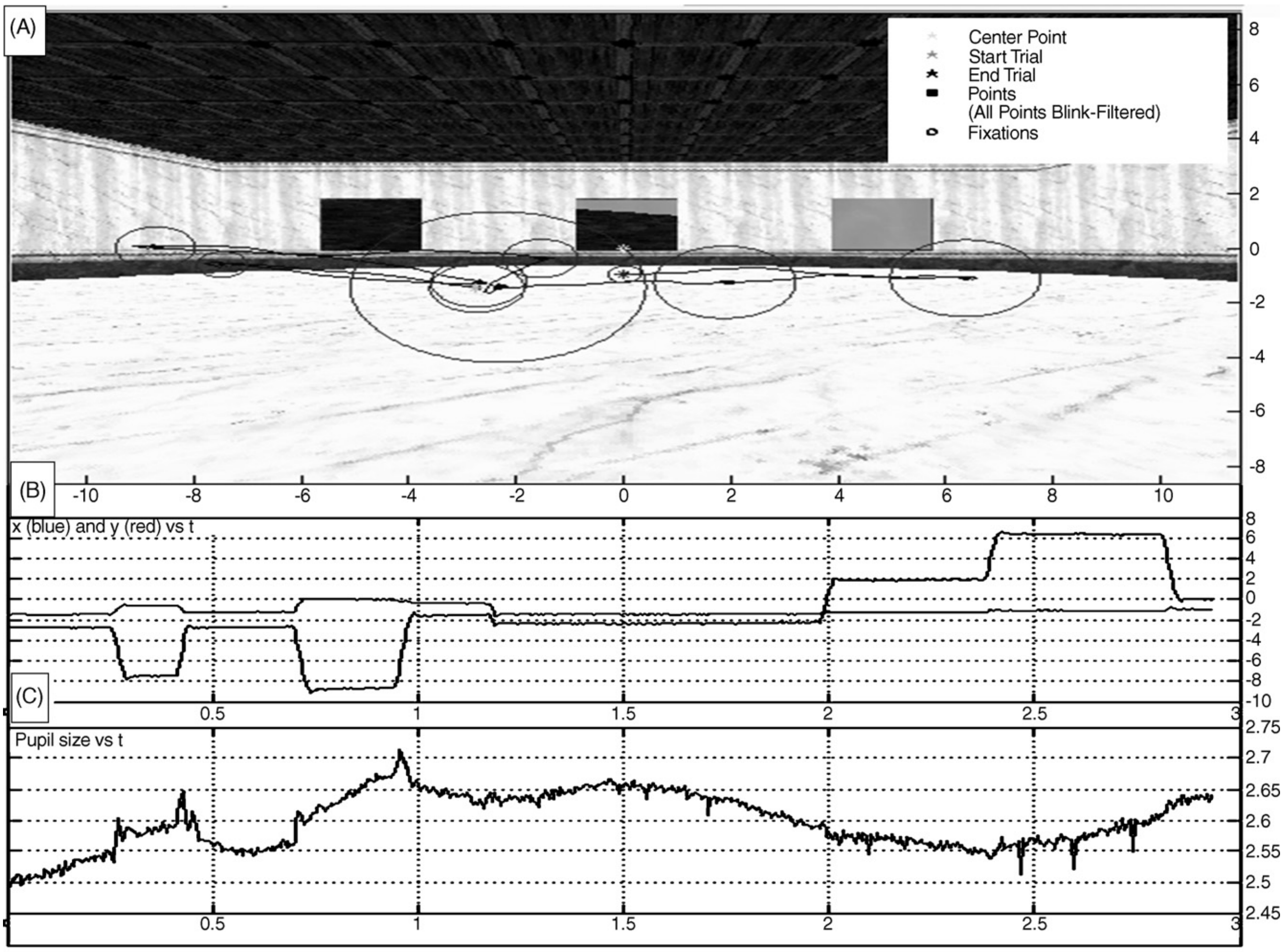

Fig. 1. Annotated participant view and eyetracking data. (A) Ilab screen shot of a sample trial with scan path for one start position illustrating the fixations (indicated by circles), start and end positions (indicated by asterisks). (B) Corresponding horizontal and vertical eye movements for that trial (in mm). (C) Pupil diameter for that trial (in mm).

In order to dissociate whether both sexes might use different parts of the maze to orient themselves the screen was divided into two parts. The egocentric part was defined as anything below the top end of the arena wall (the floor and the arena wall) and the allocentric part was defined as any space above the arena wall, which included the windows, the door and the walls of the room. To calculate pupil dilation, the first 50 ms of each trial were averaged to form a baseline. Each following time point was then subtracted from the baseline value to determine the pupil dilation over the course of a trial. Peak pupillary dilation was calculated based on a least squares method [32], in which the 4 samples prior to and following the peak pupil dilation value were averaged in order to gain a more stable estimate of the peak rather than basing it on a single value. The latency to peak pupil dilation was then calculated as the difference between the time of the mean peak value and the beginning of the trial. Artefacts such as blinks and eye movements that occurred off screen were excluded from analysis. Blinks were filtered based on an algorithm that excluded any data indicating eye movements off screen or a pupil dilation of zero [38]. Data 36 ms prior to and 36 ms after such an artefact were excluded.

2.7. Data analysis

To examine differences between the groups in experience using a joystick, age and handedness independent *t*-tests were used. To additionally examine any potential effects of previous computer experience, Pearson product moment correlations were performed between computer experience and overall performance. Data were subjected to a mixed ANOVA with trial as the within subjects variable and sex as the between subjects variable. Separate ANOVAs were performed for the visible (2 × 4) and invisible (2 × 10) platform trials. Additionally, means for visible and invisible trials were computed and subjected to a 2 (visible vs. invisible) × 2 (male vs. female) ANOVA. To investigate various indicators of memory demands, correlations were conducted on latencies and pupil dilations and fixation durations.

3. Results

3.1. Demographics

Males and females were well-matched for age and handedness and neither group differed in terms of experiencing dizziness or experience using a joystick (all $t(22) < -1.77$, ns). Two females in the sample had a previous history of brain injury (one female suffered from a fractured skull (occipital) during childhood and one had experienced a concussion). To ensure that this did not influence the results, the data were analysed with and without these subjects. As there was no substantial change in results, the data were retained in the dataset. Although groups differed in terms of previous computer experience at the outset of the experiment on gaming experience as a child ($t(22) = 5.15$, $p < .05$), in the previous 12 months ($t(13.53) = 3.22$, $p < .05$), in 2D ($t(11) = 2.42$, $p < .05$) and 3D environments ($t(13.26) = 3.72$, $p < .05$), there were no significant correlations between performance (mean speed to find the platform of invisible platform trials) and experience with a joystick as a child (r^2 (24) = −0.18, $p = .40$), gaming experience over the last 12 months (r^2 (24) = −0.14, $p = .53$), 2D (r^2

Table 1
The table illustrates the different types of trials that were utilised during the experiment, the number of occurrence, the order of occurrence, the trial duration and the platform location and visibility

Trial type	Trial duration	Platform
Exploration trial (1×)	Unlimited; subjects could explore for as long as they wished	No platform visible inside the arena
Visible trials (4×)	Subjects could look around the room once on the platform for as long as they wished; they could also jump off the platform if they wished but were not explicitly instructed about this	Platform was visible but changed from trial to trial: Center, SE, NE, SW.
Invisible trials (10×)	Maximum trial duration is 180 s. Participants were allowed to look around the room from the platform until they were ready for the next trial.	Constant platform location (SE quadrant)
Probe trial (1×)	Minimum 20 s	Same location as invisible trials but platform was set up to occur automatically after 20 s from trial beginning and could not be triggered by the participants

(24) = 0.09, $p = .66$) or 3D (r^2 (24) = −0.17, $p = .42$) gaming experiences.

3.2. Behavioural measures

3.2.1. Latencies

On the invisible trials, a significant effect of group showed that males were overall significantly faster ($F(1,22 = 4.85$, $p < .05$) in reaching the platform (mean S.E.M.: 6.75 ± 1.02 s) than females (9.93 ± 1.02 s). Moreover, consistent with a learning effect, a significant main effect of trials ($F(9,198) = 5.51$, $p < .001$) indicated that participants became faster in finding the platform as the experiment progressed ($F(1,22) = 21.83$, $p < .001$, see Fig. 2A). The interaction, however, was not significant ($F(9,198) = 0.66$, ns).

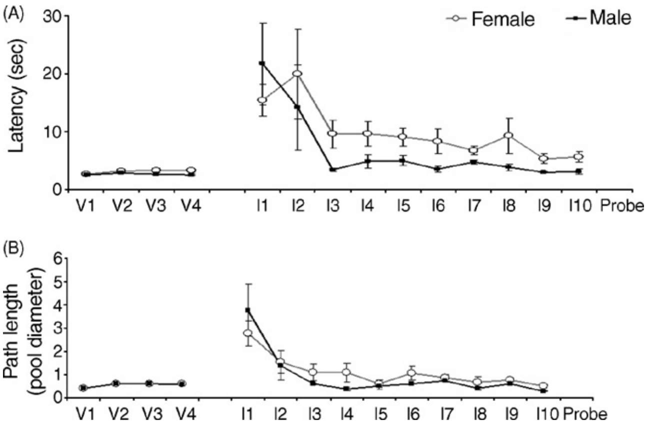

Fig. 2. Graphs for the navigational behavioural data (mean ± S.E.M.) for males (filled black square, black line) and females (open circle, grey line). (A) Latency, i.e. time to reach platform (in seconds). (B) Path length from start point to platform (in arbitrary units of pool diameter).

On the visible trials, there was a main effect of sex ($F(1,22) = 5.00$, $p < .05$, males were on average 0.5 s faster than females). There was no significant learning curve ($F(3,66) = 1.36$, ns) and the interaction between latency and sex was not significant ($F(3,66) = 0.72$, ns).

3.2.2. Path length

Overall, on the invisible trials, males also took more direct paths to the platform (120 ± 8.8 units) compared to females (161 ± 8.8 units) and this difference was significant ($F(1,18) = 10.49$, $p = .005$). As with latency, path length decreased as trials continued ($F(9,162) = 11.55$, $p < .001$) showing a significant learning effect of platform location ($F(1,18) = 52.15$, $p < .001$, see Fig. 2B). There was no significant interaction between sex and trials ($F(9,162) = 0.65$, ns). In the visible platform trials, there was no interaction ($F(3,66) = 0.78$, ns) but a main effect of sex ($F(1,22) = 5.51$, $p < .05$), which showed that males traversed on average 0.003 units less than females (0.564 vs. 0.561).

3.2.3. Probe trial

On the probe trial, males spent more time in the quadrant where the platform was located than females (72.67 ± 3.99% vs. 60.36 ± 4.17%), a difference that was significant ($F(1,23) = 4.53$, $p < .05$, $d = 0.93$).

In summary, males found the platform faster, were searching for the platform in the correct quadrant, and traversed less space than females.

3.3. Eye movement data

3.3.1. Fixations

Although the data indicated that females maintained a relatively high and constant mean fixation duration during the visible platform trials whereas males decreased their mean durations over trials (Fig. 3A), this difference was not significant ($F(2,44) = 0.50$, $p = .61$). In contrast, during the invisible trials, initial fixation durations of females were higher than those of males on the first 5 trials, but were comparable after that, resulting in a significant interaction between sex and trial ($F(10,220) = 2.82$, $p = .003$). Thus although fixation durations on the second visible platform trial were the same for males and females, males subsequently

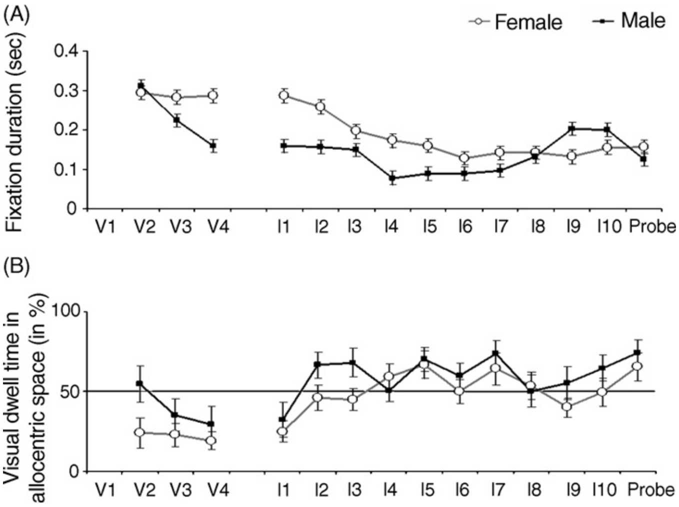

Fig. 3. Eyetracking data for males (filled black square, black line) and females (open circle, grey line). (A) Fixation duration is displayed in seconds (mean ± 1 S.E.M.). (B) Visual dwell time spent in allocentric space (in percent) (mean ± S.E.M.).

decreased their durations faster than females, suggesting that males learned the spatial features of the environment faster than women did.

3.3.2. *Visual dwell time*

During the visible platform trials, both sexes attended mostly to regions of the maze where egocentric stimuli (like the arena wall and the floor) were located (Fig. 3B). By the third trial, gaze was allocated to egocentric space 76% of the time, significantly more than would be expected by chance ($t(23) = 4.23$, $p < .001$). Gaze direction at the start of the first invisible platform trial was much the same as in the last visible platform trial for both sexes, i.e. gaze was directed more into egocentric than allocentric space. From the second invisible to the last trial, males however spent most of their gaze time (63 ± 5.7%) scanning the allocentric portion of the maze; this was significantly greater than 50% ($t(11) = 2.33$, $p < .05$). Females also spent more time looking in allocentric space than egocentric space from the second invisible to the final trial, but this was only 54 ± 5.8% and not significantly different than 50% ($t(11) = 0.67$, $p = .51$). However, the difference between sexes was not significant ($t(22) = 1.14$, $p = .27$). Taken together, males and females spent 59 ± 4.1% of their gaze time in allocentric space, significantly greater than 50% ($t(23) = 2.10$, $p < .05$).

3.3.3. *Visual spread*

The mean visual spread traversed (Fig. 4A) indicates how much of the available spatial information is being used for orientation. While males consistently explored roughly the same amount of space in visible and invisible trials for orientation ($F(1,11) = 0.91$, $p = 0.36$), females explored less widely in the visible than in the invisible trials ($F(1,11) = 48.39$, $p < .001$) resulting in a significant visual spread by sex interaction ($F(1,22) = 4.79$, $p = .04$). Moreover, both sexes explored more space visually in the invisible than the visible trials ($F(1,22) = 15.21$, $p = .001$), which suggests that participants were following instructions and trying to orient themselves in the environment.

In summary: during the invisible trials females exhibited longer fixation durations than males during the early learning trials. In terms of visual dwell time, both sexes spent significantly more time in allocentric space. Finally, although males showed a significantly larger visual spread utilising more of the visual space available early on in the visible trials, women increased their visual spread from the visible to the invisible trials.

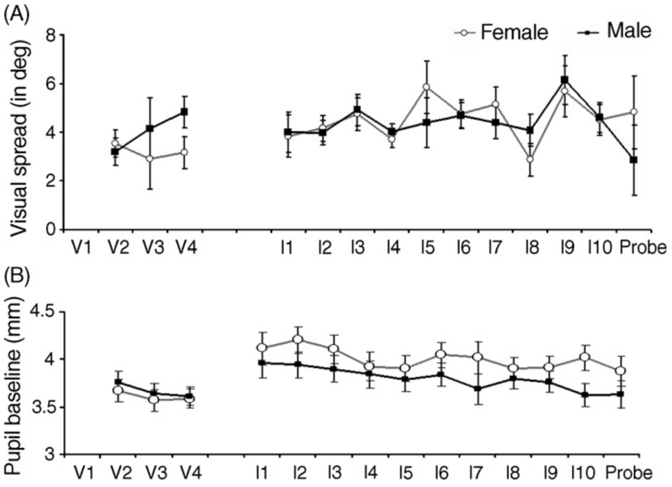

Fig. 4. (A) Eyetracking data (mean ± S.E.M.) for males (filled black square, black line) and females (open circle, grey line) for visual spread (in degrees). (B) Displays the mean (±S.E.M.) for the pupil baseline measure (in mm).

3.4. *Pupillometric measures*

Although males and females did not differ in pupillary measures (Fig. 4B), clear indicators relating to task demands were apparent. Consistent with learning effects, a reduction in resource requirements was indicated by decreasing pupil baseline values over time in the invisible trials ($F(10,130) = 5.03$, $p < .001$) and as seen in a linear trend ($F(1,13) = 16.38$, $p = .001$). Importantly, the data also demonstrated that cognitive demands were greater in the invisible trials relative to the visible trials, as mean pupil baselines in the visible trials were characterised by lower mean baseline values than in invisible trials (3.64 mm vs. 3.76 mm; $t(23) = 3.84$, $p = .001$). The time to peak pupil dilation also differed significantly over the course of the trials ($F(10,220) = 2.39$, $p = .01$) as did the peak pupil dilation value ($F(10,220) = 3.63$, $p < .001$). No other effects were significant.

In summary, both sexes demonstrated reductions in pupillometric measures consistent with learning effects. However, larger pupil baselines during the invisible trials indicated increased task demands relative to the visible trials.

3.5. *Correlations*

A sex difference was apparent on the association between performance and peak pupil dilation. Women but not men showed that faster performance (latency trials 2–10) was associated with increased memory processing as indicated by peak pupil dilation, on the first visible and invisible trials (r^2 $(12) = -0.59$, $p = .04$ and r^2 $(12) = -0.65$, $p = .02$, respectively). Overall performance also correlated with pupil measures. In the invisible trials longer fixation durations were associated with poorer performance (r^2 $(24) = 4.7$, $p = .02$). However, there was no significant correlation between the mean fixation duration of the visible trials and latency (r^2 $(24) = 0.14$, $p = .52$), indicating that during visible platform trials there was only a low cognitive demand for processing of visual stimuli.

In summary, females but not males showed correlations between pupillometric measures and performance efficiency. However, both sexes indicated poorer performance with longer fixation durations.

4. Discussion

The study sought to explore and specify which aspects of the environment are being used during navigation. The behavioural data replicated previous findings of faster and shorter paths to the platform for males relative to females. With regard to eye movements and the hypotheses several findings were apparent. Females demonstrated longer fixation durations than males during early invisible platform trials and significant associations between performance and peak pupil dilation were only found in females. By contrast, males showed a consistently high visual spread in early trials of the experiment while females increased their visual spread from early to later trials. Finally, both sexes overall spent significantly more time in allocentric space as the experiment continued but exhibited reduced pupillary dilation.

In the navigational behavioural data, the finding of shorter latencies and shorter travel paths for males relative to females during maze navigation common to previous studies [6,39] was replicated. It might be argued that the increased performance for males in the virtual maze is unrelated to cognitive factors and instead reflects increased computer experience when navigating with a joystick given that males tend to have more exposure to computer games early in life. This scenario is unlikely for two reasons. First, we did not find any evidence that performance was related to exposure to

3D environments or gaming experience, which is consistent with previous reports [3,39]. Second, and more importantly, both sexes displayed distinct occulometric correlates during the task that can be related to cognitive and memory processes rather than gaming experience per se.

In the present investigation, females exhibited longer fixation durations during the first few invisible trials than males. Moreover, generally speaking, longer fixation durations were associated with poorer overall performance. Such a finding is consistent with visuo-spatial eye movement studies that have documented longer fixation durations for laymen relative to expert viewers [25] or low memory performers relative to high-memory performers [26] suggesting differences in training, aptitude, or cognitive strategy. By contrast, the amount of visual spread is increased in experts relative to laymen, which is hypothesised to reflect enhanced perception of the interpiece relations [24]. Some studies have shown that in real life 3D object location memory tasks, males have an advantage compared to females in recalling distances between objects and the size of the layout [40]. Thus, consistently exploring visually large areas of space could be linked to the encoding of relations between objects rather than the objects themselves. Such a finding would be in line with the idea of an allocentric navigation preference for males [11,12].

However, findings from studies that investigate whether males employ allocentric and females employ egocentric navigation strategies have been mixed [11,12,41,42]. Unfortunately, the current data concerning visual dwell time were ambiguous in this respect. Although males but not females spent significantly larger amounts of visual dwell time in allocentric space after the first invisible trial when compared to a baseline of 50%, the interaction between visual dwell time and sex was not significant possibly due to large inter-subject variance. Given that both sexes initially spent more time in egocentric space and then gradually spent more time in allocentric space suggests either a familiarization with the environment or adaptation to a successful strategy in finding the desired object location.

Within the present context, we interpret differences between males and females in fixation durations and visual spread as reflecting distinct strategies that males and females employ. Navigation in the water maze requires an allocentric strategy as the environment is void of proximate cues. Some researchers have documented enhanced object location memory in females [15]. It is conceivable that women tried to employ an egocentric navigation strategy in the current task as evidenced by changes in fixation durations and pupil dilations, which reflect such memory processing. However, because proximate cues are absent in the water maze task, a strategy based on encoding of individual locations is unsuccessful resulting in decreased performance. Indeed, some studies have shown that the addition of proximate cues such as placing objects on and close to the arena wall allows are more egocentric strategy and improves performance in rodents [43] and in humans with TBI [44]. Consequently, it is possible that performance on this specific task could be improved for females by adding proximate objects to the water maze, which could then be encoded. Future research would then investigate whether such an addition would be accompanied by altered visual cognition.

An indicator of memory demands is provided by changes in pupil size [30,32]. Karatekin et al. [32] reported a decrease in pupil dilation during repeated presentation of an easy task version during an attention and working memory experiment and reduced pupil dilations have been reported when fewer items have to be recalled [30]. An increased pupil baseline and a linear decrease in pupil size during the more demanding invisible platform trials relative to the visible platform trials in the current study are consistent with these previous findings and suggest reduced cognitive resource recruitment during successful learning. Moreover, the fact that only females but not males showed associations between performance and peak pupil dilation further supports the idea that men and women use different navigational strategies.

Such a notion is corroborated by functional imaging studies that indicate that males and females use different brain networks for spatial navigation [7]. For instance, in some studies men show stronger activations in the left hippocampus, while women, by contrast, evidence stronger activations in left superior parietal cortex and prefrontal cortex [7]. Onishi et al. [45] divided subjects into good navigators and poor navigators regardless of sex and found that good navigators showed stronger hippocampal involvement than poor navigators. Poor navigators, on the other hand, exhibited more parietal cortex activations than successful navigators. It has been argued that while the parietal cortex may subserve several types of reference frames consistent with its role in sensorimotor integration [46], allocentric navigation additionally recruits structures of the temporal lobe [47,48]. The current data are consistent with these findings in that the more successful strategy, at least for the water maze task, seems to favour an allocentric frame of reference that is commonly preferred by males.

We have successfully demonstrated that eye tracking can be used to examine strategic preferences in men and women during spatial navigation in a virtual water maze. Distinct occulometric correlates for both sexes were found in fixation durations, visual spread and peak pupil dilations during a task that required processing of distal cues of the environment. Our preliminary findings show that while women employ a strategy based on memory, males seem to use spatial relations in order to navigate. These findings are consistent with previous data that show sex differences between different types of spatial tasks [14]. Future studies will need to investigate the occulometric correlates that underlie differential performance in males and females in other types of spatial tasks.

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