Every day many different species, including the human being, have to navigate in an environment that is in constant change. This requires an ability called spatial memory, which differs according to gender. Hence, navigation and place learning are among the best documented dimorphisms. Data in both rodents and humans demonstrated that males outperformed females, as they solved the mazes sooner and/or committing lower number of errors (Astur, Ortiz, & Sutherland, 1998; Cánovas, Espínola, Iribarne, & Cimadevilla, 2008; Cimadevilla, Conejo, Miranda, & Arias, 2004; Driscoll, Hamilton, Yeo, Brooks, & Sutherland, 2005; Méndez-López, Méndez, López, & Arias, 2009a; Méndez-López, Méndez, López, & Arias, 2009b; Perrot-Sinal, Kostenuik, Ossenkopp, & Kavaliers, 1996).

Moreover, a growing body of research has demonstrated that not only gender but also sexual orientation influences the performance in spatial learning tasks. Hence, Rahman and Koerting (2008) found that heterosexual men (HeM) performed more accurately than homosexual men (HoM) and homosexual women (HoW) on a virtual reality version of the Morris Water Maze, whereas an analogous of the Radial Arm Maze only showed an advantage of men over women. In addition, a previous study by Rahman, Andersson and Govier (2005) using a map test demonstrated that homosexual men used a significantly greater landmark-based navigation strategy compared with heterosexual men, and performed similar to heterosexual women. Neither revealed differences between heterosexual (HeW) and homosexual women (HoW).

Previous research has suggested that reference and working memory components of spatial memory rely on different anatomical regions. Hence, the reference memory component has been demonstrated to be mediated by the hippocampus and other temporal structures, whereas the working memory component appears to be more related to anatomical regions other than the hippocampus, including frontal and parietal cortices (Astur, Taylor, Mamela, Philpott, & Sutherland, 2002; Owen, 1997). The use of the same task for assessing both spatial reference memory and spatial working memory in subjects with different gender and sexual orientation could help to disclose the neurobiological bases that underlay sexual-dimorphism.
In our study, the influence of sexual orientation on navigation and place learning was measured using «The Boxes Room» (Cánovas et al., 2008), a virtual reality human analogue of the Holeboard, a spatial task traditionally used in rodents (Kuc, Gregersen, Gannon, & Dodart, 2006; Oades & Isaacson, 1978). We conducted two experiments. In Experiment I we studied reference memory abilities in heterosexual and homosexual men and women. The difficulty of the task, i.e. number of rewards, was modified in different groups to prevent ceiling and floor effects (Cánovas et al., 2008; Cánovas, García, & Cimadevilla, 2011). In Experiment II we examined the working memory abilities in the same groups. The relevant parameters were identical in both experiments. We demonstrated that sexual orientation exerts an influence mostly in men and limited to the processes of reference memory, which depends more on the function of the hippocampal system.

Method

Participants

One hundred and twenty participants (30 HeM, 30 HeW, 30 HoM, 30 HoW) were recruited. All subjects participated in Experiment I, and 98 of them (15 HeM, 24 HeF, 30 HoM, 29 HoF) also participated in Experiment II. None of them had impaired vision, neurological disorders or were under medication that could potentially affect their cognitive performance. Participants were recruited from university campus and social networks, like gay and lesbian organisations. Sexual orientation was assessed using the Kinsey scale (0= exclusively heterosexual, 6= exclusively homosexual). Intermediate scores, 2, 3 and 4 were not included in the study.

Demographic information was acquired including age, gender, education level (1: Elementary education; 2: Junior High or Vocational Training I (VT); 3: High School or VT II; 4: Junior college or VT III; and 5: Degree), occupation, prior videogame experience (0, never; 1, rarely; 2, occasionally; 3, frequently), and joystick handling (Yes/No). In addition, homosexual participants also participated in interviews for registering the visibility and age of sexual orientation consciousness. See Tables 1 and 2 for further information.

The study was conducted in accordance with the European Communities Council Directive 2001/20/EC and Helsinki Declaration for biomedical research involving humans. The participants were informed in advance that they would be included in a study examining spatial memory and were fully free to leave the experiment at any time. The research hypotheses were not revealed.

Instruments

«The Boxes Room» task was administered on an Acer 533-MHz notebook equipped with 1 GB of RAM and a 15.4 XGA TFT colour screen (1024x768). Participants navigated through the maze by manipulating a Logitech joystick and received auditory feedback from the computer speaker.

Procedure

Participants received written instructions on how to proceed with the task. The Boxes Room consisted of a computer-generated square room which had a door, a window, tins and several pictures on the walls. The room also had sixteen boxes distributed on the floor. Participants were asked to find the position occupied by the rewarded boxes as quickly as possible, by opening the lowest number of boxes necessary. Upon discovering a rewarded box, it turned green and a pleasant melody sounded, whereas when participants opened a wrong box it turned red, and an aversive, discordant tone sounded. The already opened boxes remained green or red during the trial to help the participants to remember their position, whereas non-opened boxes remained brown (Fig. 1). After discovering all the rewarded boxes, or if 150 s elapsed, the maximum trial duration, a congratulatory text message was displayed and it announced that the next trial would proceed. When a new trial began, all the boxes turned back to their original brown color. No information regarding useful strategies, the location of the rewarded boxes or any other features of the experiment was provided.

Experiment-I was aimed to evaluate Reference Memory. The position of the rewarded boxes was constant during the whole session of 10 trials. The starting position changed from one trial to the next and participants began the same number of times from each side of the room. The inter-trial interval was 5 s. The number of rewarded boxes was manipulated to be 3, 5 or 7 in 16 possible positions. Participants were randomly distributed into the three testing conditions (see also Cánovas et al., 2008).

Experiment-II was designed to study Working Memory. All the participants had to discover two rewards through 14 blocks of trials. Each block consisted in two trials (acquisition-retrieval). The position occupied by the rewarded boxes was constant during a block. However, the rewards location was changed pseudorandomly for each block so that the rewards were located in a different position from the immediately preceding block. The starting position changed from one trial to the next and between blocks. Both the inter-trial interval; i.e., the interval between acquisition and retention trials, and the interval between blocks were 4s.

Data analysis

A Student t-test and X² were used for comparing videogames and joystick experience. Age and educational differences were explored using one-way ANOVA and Kolmogorov-Smirnov Z.

Figure 1. Screenshot of a sample trial in the Boxes Room
In Experiment 1, latency and the number of errors (visiting a non-rewarded box) were statistically analysed using ANCOVA (Joystick Handling as covariate), with Gender, Sexual orientation and Rewards as the between-subjects factors and Trial as the repeated measures factor, followed by post hoc Newman–Keuls tests.

In Experiment 2 latency and the number of errors in each retrieval trial (i.e. second trial of a block) were statistically analysed using ANOVA, with Gender and Sexual Orientation as the between-subjects factor and Trial as the repeated measures factor, followed by post hoc Newman–Keuls tests. Significant differences were reported for \( p < 0.05 \).

Results

Experiment 1: Reference Memory

The groups (heterosexuals vs. homosexuals) did not significantly differ in age, handedness, videogame experience or joystick handling \( (p > 0.05) \). However, homosexuals’ education level was lower compared with heterosexuals \( (Z(1) = -4.32, p < 0.05) \). Besides, males were significantly older \( (28.3±7.7 \text{ vs. } 23.6±5.6) \) \( (F(1,118) = 14.34, p < 0.001) \) and had more experience with the joystick \( (X^2(1) = 6.26, p < 0.05) \) than females. Males and females did not differ on any other measure \( (all \ p > 0.05) \) (Table 1).

Statistical analyses of latency discovered a significant main effect of Rewards \( (F(2,107) = 11, p < 0.001) \) and Trial \( (F(9,963) = 64.26, p < 0.001) \), but Gender \( (F(1,107) = 0.55, p > 0.05) \) and Sexual Orientation \( (F(1,107) = 0.48, p > 0.05) \) were not statistically significant. Specifically, participants were significantly faster to look for 3-rewards than for both 5-rewards and 7-rewards \( (\text{Newman-Keuls}, p < 0.05) \) and spent more time to find the rewards in the first five trials that in the last four ones \( (p < 0.05) \), regardless of their gender and sexual orientation. There were also significant Gender \( \times \) Sexual Orientation \( (F(1,107) = 4.72, p < 0.05) \) and Rewards \( \times \) Sexual Orientation \( (F(2,107) = 4.18, p < 0.05) \) interactions. Post hoc Newman-Keuls test of the interaction terms indicated that heterosexual males outperformed heterosexual females and homosexual males by spending less time to find the rewards \( (p < 0.05) \) but did not differ from homosexual women \( (p > 0.05) \). Besides, heterosexual participants spent significantly more time to find 7-rewards than to find 3- and 5-rewards, whereas 5-rewards was the slowest condition in the homosexual group \( (p < 0.05) \). Analysis for the other interactions did not approach significance \( (all \ p > 0.05) \) (Figure 2A).

<table>
<thead>
<tr>
<th>Variable</th>
<th>HeM, n= 30</th>
<th>HeW, n= 30</th>
<th>HoM, n= 30</th>
<th>HoW, n= 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean( \pm )S.D</td>
<td>27.07±8.19</td>
<td>22.43±6.07</td>
<td>29.53±7.17</td>
<td>24.83±4.89</td>
</tr>
<tr>
<td>Education, mean( \pm )S.D</td>
<td>3.63±0.81</td>
<td>3.86±0.57</td>
<td>3.77±1.22</td>
<td>3.53±1.33</td>
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<td>Right handedness (%)</td>
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<td>93.3</td>
<td>90</td>
<td>93.3</td>
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<tr>
<td>Computer game experience, mean( \pm )S.D</td>
<td>1.9±1.06</td>
<td>0.9±0.80</td>
<td>1.13±1.19</td>
<td>1.16±0.91</td>
</tr>
<tr>
<td>Joystick handling (%)</td>
<td>56</td>
<td>13.33</td>
<td>33.33</td>
<td>33.33</td>
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<tr>
<td>Visibility (%)</td>
<td>86.6</td>
<td>66.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of S.O. consciousness, mean( \pm )S.D</td>
<td>14.6±5.31</td>
<td>13.9±4.37</td>
<td></td>
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</tr>
</tbody>
</table>

![Figure 2](image.jpg)
The analysis of the number of errors disclosed significant main effects of Sexual Orientation \(F(1,107)= 6.14, p<0.05\), Rewards \(F(2,107)= 6.23, p<0.05\) and Trial \(F(9,963)= 133.97, p<0.001\), but not of Gender \(F(1,107)= 0.76, p>0.05\). There were also significant Rewards \(\times\) Sexual Orientation \(F(2,107)= 3.54, p<0.05\), Rewards \(\times\) Gender \(F(2,107)= 3.2, p<0.05\), Trial \(\times\) Rewards \(F(18,963)= 3, p<0.001\), Trial \(\times\) Rewards \(\times\) Sexual Orientation \(F(18,963)= 1.98, p<0.05\) interactions. Newman-Keuls test of the interaction term indicated that heterosexuals committed fewer errors than homosexuals specifically in the first five trials of the 5-rewards condition \(p<0.05\). Analysis for the other interaction did not approach significance \(p>0.05\) (Figure 2B).

Finally, we analyzed at a descriptive level the searching strategies during the five initial trials. Subjects could either navigate directly to the rewarded locations from the starting points (direct strategy) or move to a certain location and/or landmark, and from there, open the boxes following the same sequence (landmark strategy). Those participants who navigated with no apparent strategy or mixing the direct and landmark strategies were considered separately (mixed/unknown). Heterosexual men referred more to a direct strategy and less to a landmark strategy as the number of rewards increased (3 rewards: 60% vs. 40%, 5 rewards: 70% vs. 30%, 7 rewards: 60% vs. 40%). Within the homosexual group, men used both direct and landmark strategy almost equally during the 3- (60% vs. 40%) and 5-rewards (40% vs. 40%) conditions, but referred to a landmark strategy (30% vs. 70%) in the 7-rewards condition, while women referred to a landmark strategy in the 3-rewards condition (30% vs. 70%), but they used direct and landmark strategy almost equally in the 5- (40% vs. 40%) and 7-rewards (40% vs. 50%) conditions (Fig. 4). Note that in several cases the sum of the proportions did not reach 100%. That was due to the presence of subjects that did not follow any apparent strategy.

Experiment II: Working Memory

The groups did not differ significantly in handedness, videogame experience or joystick handling \(p>0.05\). Homosexuals, however, were significantly older \((27.3±6.5 vs. 20.1±3.1)\) \(F(1,96)= 41.56, p<0.001\) and their education level was lower \(Z(1)=-0.4, p<0.05\) compared with heterosexuals. Besides, males were significantly older \((26.9±7.4 vs. 22.3±4.7)\) \(F(1,96)= 13.53, p<0.05\) than females. Males and females did not differ on any other measure \(p>0.05\) (Table 2).

Analysis of the latency to discover all the rewarded boxes showed a significant main effect of Gender \(F(1,94)= 4.23, p<0.05\) and Block \(F(13,1222)= 10.79, p<0.001\), but Sexual Orientation \(F(1,94)= 0.01, p>0.05\) was not statistically significant. There was also a significant Block \(\times\) Sexual Orientation interaction \(F(13,1222)= 1.84, p<0.05\). Newman-Keuls test indicated that men found the rewards sooner than women \(p<0.05\), and participants spent more time when the two rewards were located in lateral and centre positions, respectively \(2^{nd}\) block). However, they found them faster when rewards were placed both in the centre or corner positions \(10^a, 12^a\) and \(14^a\) blocks) \(p>0.05\). Besides, post-hoc analysis of the interaction term revealed that heterosexuals spent more time to find the rewards in the 2\(^{nd}\) block than homosexuals \(p<0.05\). Analysis for the other interactions did not approach significance \(p>0.05\) (Figure 3A).

Analysis of the number of errors disclosed significant main effects of Block \(F(13,1222)= 8.09, p<0.001\) but not of Gender \(F(1,94)= 1.32, p>0.05\) or Sexual Orientation \(F(1,94)= 0.3, p>0.05\). There was also a significant Block \(\times\) Sexual Orientation interaction \(F(13,1222)= 2.07, p<0.05\). Newman-Keuls test of the interaction term indicated that heterosexuals and homosexuals committed an equivalent number of errors within each block, but

<table>
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<th>Variable</th>
<th>HmM, n= 15</th>
<th>HmW, n= 24</th>
<th>HoM, n= 30</th>
<th>HoW n= 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean±S.D</td>
<td>19.9±3.1</td>
<td>20.6±2.9</td>
<td>20.8±3.2</td>
<td>25.0±4.8</td>
</tr>
<tr>
<td>Education, mean±S.D</td>
<td>3.8±0.5</td>
<td>3.7±0.5</td>
<td>3.8±0.5</td>
<td>4.0±0.5</td>
</tr>
<tr>
<td>Right handedness (%)</td>
<td>0.9±0.3</td>
<td>0.8±0.3</td>
<td>0.8±0.3</td>
<td>0.8±0.3</td>
</tr>
<tr>
<td>Computer game experience, mean±S.D</td>
<td>1.1±0.3</td>
<td>1.1±0.3</td>
<td>1.1±0.3</td>
<td>1.1±0.3</td>
</tr>
<tr>
<td>Joystick handling (%)</td>
<td>0.6±0.3</td>
<td>0.6±0.3</td>
<td>0.6±0.3</td>
<td>0.6±0.3</td>
</tr>
<tr>
<td>Visibility (%)</td>
<td>0.6±0.3</td>
<td>0.6±0.3</td>
<td>0.6±0.3</td>
<td>0.6±0.3</td>
</tr>
<tr>
<td>Age of S.O. consciousness, mean±S.D</td>
<td>14.6±2.1</td>
<td>13.5±3.1</td>
<td>13.7±4.1</td>
<td>13.9±4.1</td>
</tr>
</tbody>
</table>

Table 2
Demographic features of the sample for Experiment-II, a working memory task

Figure 3. Latency (A) and errors (B) to locate 2 rewards in the working memory study. No differences between heterosexual and homosexual participants were found in any of the variables studied. X axes show the block number. Mean+ S.E.M.
heterosexuals committed more errors in the 2nd block than in the other blocks and more errors than homosexuals in their blocks, with the exception of the 2nd and 11th blocks ($p<0.05$) (Figure 3B).

Discussion

We demonstrated that sexual orientation influences the performance of men and women in the Boxes Room, a virtual spatial memory task based on the holeboard. Heterosexual men outperformed homosexual men and heterosexual women in reference memory, but did not differ from homosexual women. They showed shorter searching latencies and fewer errors finding rewards that remained in the same position throughout the ten trials of the task. This finding is consistent with that of Rahman et al. (2008), who used the virtual Morris water maze and showed that homosexual men performed more like heterosexual men than homosexual men in looking for a hidden goal. As with Rahman et al. (2008), we did not find differences between groups in the distance covered (data not included). These results also agree with the well documented dimorphisms on virtual navigation and place learning tasks (Astur et al., 1998; Cánovas et al., 2008; Driscoll et al., 2005).

On the other hand, the Boxes Room allows to easily modifying the level of difficulty, so it can be adapted from homosexual populations, and ceiling and floor effects can be avoided. Increasing the number of rewards lead to higher latencies, distances and errors as demonstrated in previous studies (Cánovas et al., 2008; Cánovas et al., 2011). It is important to note that the advantage of heterosexual men appeared in the 5-rewards condition, but not with 3 or 7 rewards, and differences completely disappeared once the sequence had been learned. This fact indicates that group differences are due to differences in the learning process and only under suitable levels of difficulty. This effect of difficulty was demonstrated before in another population (Cánovas et al., 2008). Moreover, since differences between groups were restricted to certain levels of difficulty, this shows that the differential experience observed in joystick handling have a low effect on the final performance in the task.

A visual analysis of the searching trajectories during the first five trials lighted up two strategies used. The direct strategy involves more spatial processing than the other strategies (Astur, Tropp, Sava, Constable, & Markus, 2004). Previous literature used only one goal and reported that men refer to a direct strategy, while women refer to landmark information (Astur et al., 2004; Saucier et al., 2002). Here, we used several rewards and found that as the number of goals increased, men preferred a direct strategy, while women preferred a landmark strategy. However, homosexual men, contrary to heterosexual men, preferred a landmark strategy as the number of rewards increased (Figure 4). This finding agrees with previous literature reporting a preference for landmark information in homosexual men (Rahman et al., 2005). Interestingly, homosexual women exhibited less strategy preference as rewards increased. This suggests a sex-atypical strategy pattern in homosexual women although this is not directly associated with differences between heterosexual and homosexual women performance.

In Experiment-II, spatial working memory was examined in the same context used before in the reference memory task. Each block consisted of two trials: first trial for acquisition and another for retrieval. The pattern of findings replicates those of Rahman et al. (2008), who did not find any differences between heterosexual and homosexual participants in the Radial Arm Maze, but an advantage of men compared to women. The lack of differences in distance (data not included) and number of committed errors, however, may suggest that differences only prove that men are faster than women and not better navigators. Failure to find sex differences has previously been reported in other studies (Astur et al., 2004; Levy, Astur, & Frick, 2005).

The existence of sex and sexual orientation dimorphisms in spatial reference memory but not in working memory using the same task supports the notion that reference and working memory processes have different neurobiological mechanisms (Morris, Garrud, Rawlins, & O’Keefe, 1982; Owen, 1997).

It is now well established that reference memory depends on the integrity of the hippocampus (Astur et al., 2002; Morris et al., 1982). Furthermore, there is evidence of sexual-dimorphism in the hippocampal volume and the morphology of its cells (Isgor & Sengelaub, 1998; Isgor & Sengelaub, 2003) as well as in men and women hippocampal activation during navigation (Gron, Wunderlich, Spitzer, Tomczak, & Riepe, 2000). It has been hypothesized that gender-related differences may be due to the

![Figure 4. Analysis of the searching trajectories in the initial five trials of the reference memory study. Note that those participants who navigated with no apparent strategy or mixing the direct and landmark strategies were not considered for the analysis](image)
role of sex hormones. In fact, a number of works have shown a preponderance of sex steroid receptors in the hippocampus with a higher density of receptors for androgens than for estrogens (Roof & Havens, 1992). Consistent with this, Hier and Crowley (1982) found that men with androgen deficits had impaired spatial ability compared to control men. Moreover, research on people with congenital adrenal hyperplasia, characterized by prenatal overproduction of adrenal androgens, demonstrated that women with severe congenital adrenal hyperplasia performed better than control women and similarly to both healthy men and men with congenital adrenal hyperplasia on a virtual version of the Morris Water Maze (Mueller et al., 2008). In addition to this, experiments carried out in this study showed that female rats treated prenatally or early postnatally with androgens markedly improved their spatial performance compared to control females (Cimadevilla et al., 1999; Risk, Robertson, & Raber, 2005; Roof & Havens, 1992; Williams, Barnett, & Meck, 1990).

Although, to the best of our knowledge, hippocampal differences between heterosexual and homosexual individuals have never been explored, given the existence of differences not only in performance but also in preference strategy between heterosexual and homosexual men in the reference memory task, it seems reasonable to hypothesize that homosexual men might have a sex-atypical hippocampal organization and/or functioning. Hence, it was reported that heterosexual and homosexual men differ in their patterns of brain activation (Hu et al., 2008). On the other hand, the working memory task did not reveal any difference between these groups, but it has been related with anatomical regions other than the hippocampus, including the inferior prefrontal, premotor and dorsolateral regions of the frontal lobe (Owen, 1997), which are less influenced by hormonal level fluctuations. It would be necessary to study brain activity in heterosexual and homosexual subjects performing, in the same context but separately, a reference memory and a working memory task to clarify any differences between the hippocampal and frontal areas.

Overall, this study supports the notion that sexual orientation represents a critical factor that should be considered when studying human spatial navigation, as well as the fact that sexual orientation affects men and women differently.

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