

# Sex differences in object location memory and spatial navigation in Long-Evans rats

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**Abstract** In both humans and rodents, males typically excel on a number of tasks requiring spatial ability. However, human females exhibit advantages in memory for the spatial location of objects. This study investigated whether rats would exhibit similar sex differences on a task of object location memory (OLM) and on the watermaze (WM). We predicted that females should outperform males on the OLM task and that males should outperform females on the WM. To control for possible effects of housing environment, rats were housed in either complex environments or in standard shoebox housing. Eighty Long-Evans rats (40 males and 40 females) were housed in either complex (Complex rats) or standard shoebox housing (Control rats). Results indicated that males had superior performance on the WM, whereas females outperformed males on the OLM task, regardless of housing environment. As these sex differences cannot be easily attributed to differences in cognitive style related to linguistic processing of environmental features or to selection

pressures related to the hunting gathering evolutionary pre-history of humans, these data suggest that sex differences in spatial ability may be related to traits selected for by polygynous mating strategies.

**Keywords** Object location memory · Sex differences · Watermaze · Spatial ability

## Introduction

Sex differences in spatial ability are observed in numerous species (e.g. for review see Jones et al. 2003). In navigational ability, males typically outperform females in numerous mammalian species, including: voles (Kavaliers et al. 1998), deer mice (Kavaliers et al. 1996), c57BL mice (Berger-Sweeney et al. 1995), rats (Blokland et al. 2006) and humans (Saucier et al. 2002). However, not all spatial tasks produce a male advantage. For instance, in humans, one form of spatial ability, performance of object location memory (OLM) tasks often result in a female advantage (Silverman and Eals 1992). This task typically requires participants to indicate in some way that they have encoded the positions of a number of objects (McBurney et al. 1997; Silverman and Eals 1992).

As is the case for navigation, OLM can be investigated in non-human animals. Memory for the location(s) of food has been investigated in numerous species, including rats (Beck and Luine 2002), dogs (Tapp et al. 2003), pigs (Held et al. 2005), great tits (Hodgson and Healy 2005), chickadees (Petersen and Sherry 1996) and 8-day-old chicks (Vallortigara 1996). Unlike navigation, sex differences in OLM observed in other species exhibit less consistent biases. For instance, a male advantage was observed for rats (Beck and Luine 2002) and chicks (Vallortigara 1996), whereas

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Petersen and Sherry (1996) failed to observe any sex difference in chickadees. Finally, Ennaceur et al. (2005) noted mixed results, with some measures in their task producing biases consistent with a female advantage and others a male advantage.

Of the studies that have examined sex differences in OLM with non-human animals, all utilized a procedure in which subjects were presented with an initial stimulus array followed by a second trial in which the stimulus array now contained objects that had moved from their original position into a novel and previously unoccupied position (e.g. Beck and Luine 2002; Vallortigara 1996). This is an important procedural difference from the original report of Silverman and Eals (1992), who utilized an array in which some of the objects switched places with each other. In fact, when objects are placed into novel positions, the sex difference favouring women on OLM is no longer observed (James and Kimura 1997). Other variations in procedure can result in male advantages; for instance, men outperform women on OLM when they are required to replace objects back to their originally viewed locations, (e.g. Iachini et al. 2005). Thus, inconsistencies in the observed sex difference for OLM in non-human animals may reflect important differences in task demands from the version that produces a female advantage in women.

Rats can integrate object with place, without necessarily introducing novel locations (e.g. Eacott and Norman 2004; Dix and Aggleton 1999). In these investigations, rats investigate three-dimensional objects that are paired with locations. During the test phase of the experiments, a subset of the objects exchange locations with each other; rats typically increase their interactions with objects that have exchanged location with each other (e.g. Dix and Aggleton 1999). Thus, the location-exchange paradigm is much more consistent with the version of the OLM task that produces the female advantage in humans. To date, no one has investigated the effects of sex on this location-exchange form of an OLM task.

There are a number of hypotheses regarding the mechanisms by which sex differences in OLM occur, that are not necessarily exclusive of each other (for review see Jones et al. 2003). For instance, Postma et al. (1998) suggest when encoding spatial information, females using categorical representations that are based on verbalization and males using coordinate representations that rely on spatial visualization. As standard tasks of OLM rely on both verbal and categorical processing (i.e. participants must encode both the name of the object and its position), females perform these tasks more readily than males. Alternately, Silverman and Eals (1992) suggest that selection pressures related to the division of labour between the sexes in our prehistory resulted in women exhibiting enhanced ability to recall the position of objects. Finally,

the polygyny-range size hypothesis suggests that male advantages in spatial abilities occur only in polygynous species, wherein polygynous males traversed large ranges to seek and secure mates, with the selection advantage accruing to males that were able to successfully locate females in large territories (Gaulin 1995; Gaulin and Hoffman 1998). Although initial accounts of the polygyny-range size theory only discussed male advantages in spatial ability, recent modifications suggest that the differential cost that reproduction places on females fosters small range sizes with greater attention focused on nearby spatial features (Dab and Robert 2004).

Importantly, rats are not linguistic and do not divide labour between the sexes, although they are polygynous. As such, rats provide one means to critically test these hypotheses, something that Jones et al. (2003) suggest is currently missing from the literature. Both the verbalization hypothesis and the division of labour hypothesis predict that rats should not exhibit sex differences in OLM. If sex differences in OLM are observed in rats, then we must consider that we have some support for the polygyny hypothesis, although alternate hypotheses regarding the underlying mechanisms of this female advantage may still be a factor. Thus, the primary purpose of this study is to investigate whether female rats exhibit superior performance on an OLM task that involves the exchanging of the position of objects rather than the introduction of new positions of objects. We included the watermaze (WM) in this study as a measure of spatial navigational ability that typically exhibits a male advantage.

Finally, we included two control measures in our study: a number of measures of behaviour associated with stress; and housing environment. Notably, the sex difference observed in the WM has been associated with differential stress responses between male and female rats, with decreases in stress responses resulting in better performance by females (Beiko et al. 2004; Perrot-Sinal et al. 1996). As such, we included the elevated plus maze and the open field as a means to assess potential differences in stress-related behaviours among the groups, and to correlate with the performance of the WM and OLM task. Housing environment is known to affect performance of the WM, with rats that were housed in complex conditions typically outperforming rats that were housed in impoverished conditions (e.g. Pham et al. 1999; Larsson et al. 2002; Teather et al. 2002). Further, as humans live in environments that are more stimulating and complex than those of the standard-housed laboratory rat, housing environment may be an important consideration in understanding sex differences in OLM. Thus, we also investigated the effects that housing environment (standard housing and complex housing) had on the performance of the WM and OLM task.

## Methodology

### Subjects

Upon arrival in the colony, 80 Long-Evans hooded rats (40 males and 40 females; Charles River, QC) were randomly assigned to live for 50 days in either a complex cage (20 males and 20 females; five rats per cage) or a standard shoebox cage (20 males and 20 females; five rats per cage). The complex cages (25 cm wide  $\times$  46 cm long  $\times$  62 cm high) contained a running ball, a climbing apparatus, and 3–5 pipe shelters. Shoebox cages (25 cm wide  $\times$  46 cm long  $\times$  21 cm high) contained no additional materials beyond the woodchip bedding. Regardless of housing condition, food and water were available ad libitum and the colony was maintained with a 12:12 light/dark cycle. All procedures were in accordance with the Canadian Council of Animal Care and approved by the University of Saskatchewan Assurance of Animal Care Committee.

Although rats began the experiment at the same age and weight ( $150 \pm 10$  g), following 50 days in the colony, the rats housed in the complex environment (complex rats) weighed significantly more than the rats housed in the standard shoebox environment (control rats),  $F_{1,76} = 6.905$ ,  $P = 0.010$  (complex  $M = 358.225$ ,  $SD = 87.171$ ; control  $M = 336.300$ ,  $SD = 97.169$ ). As well, male rats ( $M = 430.550$ ,  $SD = 46.713$ ) weighed significantly more than the female rats ( $M = 263.975$ ,  $SD = 29.592$ ;  $F_{1,76} = 398.576$ ,  $P < 0.001$ ). There was no interaction between housing environment and sex,  $F_{1,76} = 2.746$ ,  $P = 0.102$ .

### Object location memory test (OLM task)

For this task, each rat was tested individually within a circular arena (153 cm diameter and 80 cm high walls) that was in an otherwise empty, white room (350  $\times$  350 cm<sup>2</sup>). Behaviour was performed in the light phase of the light/dark cycle and was recorded with an overhead video camera. The OLM task consisted of six sessions: one habituation trial, four training trials and one testing trial. For the habituation session, rats were placed in an empty arena for 5 min. Twenty-four hours following habituation, the four training trials began (two per day). For the training trials, the rat was placed in the arena and allowed to explore the arena for 5 min. However, the arena now contained four objects (a water bottle, a funnel, an irregularly shaped object made of Lego<sup>®</sup> and a metallic oxygen cylinder top), which were always in the same position within the arena. To avoid potential confounds, both the objects and the absolute position of the objects were varied among the rats. The four objects and the arena were cleaned with alcohol between rats.

Twenty-four hours following the last training trial, rats were again placed in the arena for one 5-min session (testing trial). However, in the testing trial, the positions of two of the four objects were exchanged with each other. Thus, the relative positions of the objects differed from the training trials, although no novel positions were introduced. A coder, blind to the groups and the hypotheses, coded the videotapes and recorded the number of interactions with each object and the total time spent interacting with each object. Interactions were operationally defined as contacts with the object made with either a paw or vibrissa. For the training session, the number of interactions/total time spent with the two objects that were going to be exchanged in the testing session was averaged (exchanged objects), as was the time spent/interactions with the two objects that were going to remain in the same position (unmoved objects) in the testing session. For the testing session, the number of interactions/total time spent with the two objects that were exchanged was averaged (exchanged objects), as was the time spent/interactions with the two objects that remained in the same position (unmoved objects).

### Watermaze (WM)

The WM was a 203 cm diameter pool filled with water (22 C) that had polypropylene beads floating on top (Cain et al. 1993). Behaviour was recorded with an overhead video camera. For the first trial (probe 1), rats were placed in the pool for 60 s. Following this, a circular hidden platform (12 cm diameter) was placed in the pool (the surface of which was  $\sim 3$  cm below the surface of the water). The location of the platform varied among the rats, although it was always at the centre of one of the four quadrants of the pool. The rats received ten training trials, in which they were allowed to swim in the maze for 60 s or until they reached the platform. Rats that failed to reach the platform within the 60-s trial were guided to the platform by the experimenter by having the experimenter place one hand on the hidden platform to act as a visual cue. Rats remained on the platform for 15 s and then placed in a heated drying chamber.

Each trial commenced from a different location at the circumference of the pool (five different start positions roughly corresponding to 0°, 70°, 140°, 210° and 280° around the edge of the pool that were pseudo-randomly ordered among rats). As this resulted in start locations that varied in distance from the hidden platform, the time to reach the platform was averaged for the first block of five trials (block 1) and for the second block of five trials (block 2). Following the ten training trials, the platform was removed and the rats were placed in the pool for 60 s (probe 2). The intertrial interval for the ten training trials and the two probe trials was not less than 5 min, and was

not more than 10 min; all were completed within 1 day of testing. For the probe trials, the time the rat spent in the quadrant that had previously contained the platform was recorded.

#### Elevated plus maze

The elevated plus maze was composed of two arms intersecting at a 90° angle and was raised 51 cm above the floor. Two opposing arms (each arm was 55 cm long and 12 cm wide) were shielded from the outside environment (closed arm) by 18 in. high walls while the other two opposing arms were open to the room (open arms). Rats were placed in the centre of the four arms facing an open arm and allowed to explore the maze for 5 min. An overhead camera recorded all trials. Following testing, the videotape was scored and the amount of time spent in each arm was recorded. As time spent in the open arm is elevated in animals that exhibit greater stress-associated behaviours, a percentage score was calculated for the time spent in the open arm: time in the open arm/[time in the open arm + time closed arm] (Zhu et al. 2006; Steimer and Driscoll 2003).

#### Open field

Rats were individually placed into the centre of the open field (153 cm diameter, surrounded by 71 cm tall walls) and allowed to remain there for 10 min. Behaviour was recorded with an overhead video camera. The open-field was washed with a 25% ethanol solution following each trial. Tapes were digitized and a coder blind to the hypotheses and groups coded the following dependent measures: the total distance travelled in the open field (pixels); time in the perimeter of the open field (defined as the rat placing both front feet within the outermost 17 cm of the field); number of grooming episodes (grooming), number of times the rat froze for more than 1 s (freezing). These dependent measures were chosen as they have been demonstrated to correlate with physiological indicators of stress in the rat (e.g. Steimer and Driscoll 2003).

#### Procedure

All rats were weighed daily. Fifty days after being assigned to live in either the complex or control environment, rats participated in the four tests: open field, elevated plus maze, OLM task and WM. The order of testing was counterbalanced among rats, with rats performing one of four pseudo-random orders. Each testing order began with a different task and had the rest of the tasks ordered differently (e.g. open field, elevated plus maze, OLM task and WM versus WM, open field, elevated plus maze, OLM task).

#### Analyses

Alpha was set at 0.05 for all analyses, and where appropriate, post hoc were performed using Bonferroni *t*-tests, corrected for multiple comparisons.

#### Results

##### Object location memory: time spent interacting with the objects

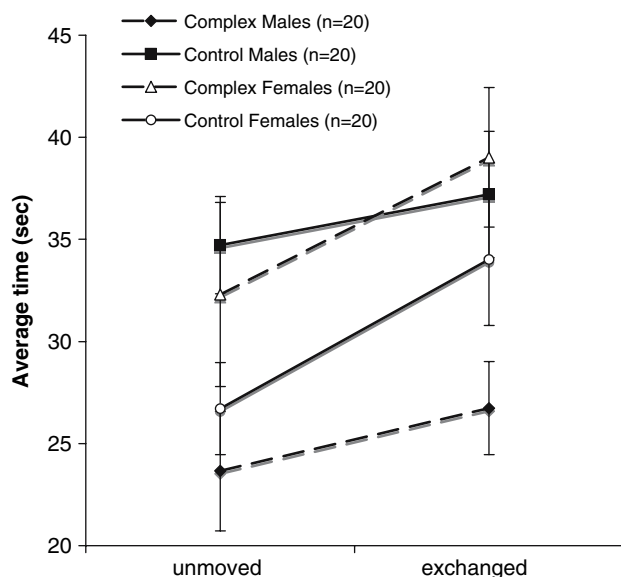
For the time spent interacting with the objects, a  $2 \times 2 \times 2 \times 2$  mixed measures analysis of variance (ANOVA) was performed using sex (male and female) and housing condition (complex and control) as between-subjects measures and session (training and testing) and stimulus type (exchanged and unmoved) as within-subject measures (Table 1; Fig. 1). Significant main effects were observed for stimulus type,  $F_{1,76} = 33.55$ ,  $P < 0.001$  and housing environment,  $F_{1,76} = 9.36$ ,  $P = 0.002$ . There was no significant main effect of session,  $F_{1,76} = 0.18$ ,  $P = 0.63$ , nor was there a significant main effect of sex,  $F_{1,76} = 2.01$ ,  $P = 0.07$ . However, these effects were mediated by three significant interactions.

A significant interaction between stimulus type and session was observed,  $F_{1,76} = 11.29$ ,  $P < 0.001$ . Post hoc comparisons indicated that during the training phase there were

**Table 1** Time (s) spent interacting with objects and the number of interactions with the objects in the OLM task in the training phase

|                             | Complex males | Complex females | Control males | Control females |
|-----------------------------|---------------|-----------------|---------------|-----------------|
| Training phase time         |               |                 |               |                 |
| Exchanged                   | 30.23 (11.56) | 35.17 (14.94)   | 30.06 (7.33)  | 29.07 (7.13)    |
| Unmoved                     | 26.70 (9.98)  | 35.35 (11.66)   | 34.56 (12.88) | 29.63 (11.71)   |
| Training phase interactions |               |                 |               |                 |
| Exchanged                   | 11.00 (2.10)  | 12.68 (3.09)    | 12.99 (2.12)  | 12.83 (2.22)    |
| Unmoved                     | 9.09 (2.66)   | 11.91 (3.23)    | 12.18 (2.70)  | 11.40 (2.42)    |

Note that in the training phase of the OLM task, exchanged and unmoved indicate items that will be either exchanged or unmoved during the testing phase. Numbers represent means (SD)



**Fig. 1** The average time (s) spent interacting with the objects in the OLM TASK in the testing phase. Symbols represent means, error bars represent the standard error of the mean

no significant differences between the exchanged and unmoved,  $t_{79} = 0.21$ ,  $P = 0.82$ , consistent with a lack of pre-existing bias towards any of the objects. However, during the testing phase the exchanged objects received more attention when compared to the unmoved objects,  $t_{79} = 2.28$ ,  $P = 0.02$ . There was also a significant increase in the time spent interacting with the exchanged objects from the training phase to the testing phase,  $t_{79} = 2.30$ ,  $P = 0.01$ , that was not observed for the unmoved objects,  $t_{79} = 1.77$ ,  $P = 0.09$ .

A significant interaction between sex and housing condition was observed,  $F_{1,76} = 17.77$ ,  $P < 0.001$ . Post hoc comparisons indicated that the complex males spent significantly less time interacting with objects when compared to either the complex females,  $t_{38} = 3.37$ ,  $P < 0.001$  or the control males,  $t_{38} = 3.85$ ,  $P < 0.001$ . The complex females spent significantly more time exploring the objects than the control females,  $t_{38} = 2.33$ ,  $P = 0.02$ . No other significant differences were observed,  $t_{38} < 0.54$ ,  $P_s > 0.60$ .

There was also a three-way interaction between session, sex and housing environment,  $F_{1,76} = 6.62$ ,  $P = 0.01$ . Post hoc comparisons indicated that complex males significantly decreased the time spent interacting with the objects in the testing phase compared to the training phase,  $t_{19} = -2.28$ ,  $P = 0.02$ . Although all of the other groups spent slightly more time interacting with the objects during the testing phase, this was not significant,  $t_{19} < 0.20$ ,  $P_s > 0.86$ . There were no significant differences among the groups for the training phase,  $t_{19} < 0.31$ ,  $P_s > 0.74$ .

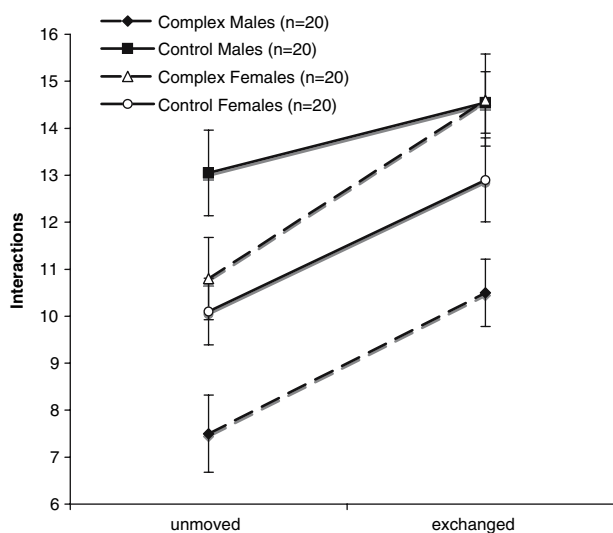
As we did not expect differences in the training phase, nor did we observe them, a separate analysis was conducted

for the time spent interacting with the objects during testing phase, with sex (male and female) and housing environment (complex and control) as between-subjects measures and stimulus type (exchanged and unmoved) as the within-subject measure. ANOVA revealed a significant sex by environment interaction,  $F_{2,76} = 6.59$ ,  $P = 0.01$  (Fig. 1). Both the complex females,  $t_{19} = -2.24$ ,  $P = 0.02$ , and the control females,  $t_{19} = 2.21$ ,  $P = 0.02$ , spent significantly more time interacting with the exchanged objects than the unmoved objects. This was not observed for the males. In fact, the complex males spent the least amount of time interacting with the exchanged objects than either the control males,  $t_{38} = -2.73$ ,  $P = 0.01$  or the complex females  $t_{38} = -2.99$ ,  $P = 0.01$ . For the unmoved objects, the complex males spent significantly less time interacting than the control males,  $t_{38} = -2.92$ ,  $P = 0.01$ .

#### Object location memory: number of interactions with the objects

For the number of interactions with the objects, a  $2 \times 2 \times 2 \times 2$  mixed measures ANOVA was performed using sex (male and female) and housing condition (complex and control) as between-subjects measures and session (training and testing) and stimulus type (exchanged and unmoved) as within-subject measures (Table 1; Fig. 2). Main effects were found for stimulus type,  $F_{1,76} = 33.55$ ,  $P < 0.001$ , and housing condition,  $F_{1,76} = 9.36$ ,  $P < 0.001$ . No significant main effects were observed for session,  $F_{1,76} = 0.01$ ,  $P = 0.97$  or for sex,  $F_{1,76} = 2.57$ ,  $P = 0.10$ .

However, these effects were mediated by a significant interaction between sex and housing condition,  $F_{1,76} = 20.06$ ,



**Fig. 2** The average number of interactions with the objects in the OLM TASK in the testing phase. Symbols represent means, error bars represent the standard error of the mean

$P < 0.001$ , with the complex males interacting less than the complex females,  $t_{38} = -3.67$ ,  $P < 0.001$  or the control males,  $t_{38} = -3.85$ ,  $P < 0.001$ . As well, the complex females interacted more with the objects than the control females,  $t_{38} = 2.33$ ,  $P = 0.02$ . There were no other significant differences observed for this interaction,  $t_{s38} < 1.64$ ,  $P_s > 0.11$ .

There was also a significant interaction between stimuli and session,  $F_{1,76} = 11.29$ ,  $P < 0.001$ , in which exchanged objects received more interactions when compared to the unmoved objects in testing phase,  $t_{38} = 2.066$ ,  $P = 0.04$ . No other differences were significant,  $t_{s38} < 0.35$ ,  $P_s > 0.72$ .

There was also a significant three-way interaction among session, sex and housing condition,  $F_{1,76} = 6.62$ ,  $P = 0.01$ . Post hoc comparisons revealed that although the complex females and the control males all had slightly more interactions with the objects, only the control males significantly increased their total number of interactions, from the training phase to the testing phase  $t_{19} = -2.82$ ,  $P = 0.01$ . There were no significant differences among the groups for the training phase,  $t_{s19} < 0.36$ ,  $P_s > 0.73$ .

Again, we did not expect differences in the training phase, nor did we observe them. Thus a separate analysis was conducted for the number of interactions with the objects during the testing phase, with sex (male and female) and housing environment (complex and control) as between-subjects measures and stimulus type (exchanged and unmoved) as the within-subject measure. ANOVA revealed a significant sex by environment interaction,  $F_{1,76} = 9.54$ ,  $P < 0.001$  (Fig. 2). Post hoc comparisons revealed that the complex males had the fewest interactions with the objects than any other group (unmoved objects all  $t_{s19} > -2.39$ , all  $P_s < 0.02$ ; exchanged objects,  $t_{s19} > -2.09$ ,  $P_s < 0.04$ ). All groups had significantly more interactions with the exchanged object than the unmoved object,  $t_{s19} > -3.32$ ,  $P_s < 0.01$ , with the exception of the control males that did not significantly differ,  $t_{19} = -1.49$ ,  $P = 0.15$ .

## Watermaze

For the training trials, a  $2 \times 2 \times 2$  mixed measures ANOVA was performed on the time to reach the platform, with sex (male and female) and housing condition (complex and control) as between subjects measures and block (blocks 1 and 2) as within subjects measures. ANOVA revealed a main effects of sex,  $F_{1,76} = 5.05$ ,  $P = 0.01$ , and of block,  $F_{1,76} = 228.59$ ,  $P < 0.001$ . Males ( $M = 19.82$  s, SD 7.50) located the platform faster than females ( $M = 25.44$  s, SD 15.38), and all rats improved in block 2 ( $M = 14.12$  s, SD 13.12) as compared to block 1 ( $M = 31.15$  s, SD 11.98). There was no significant main effect of housing condition,  $F_{1,76} = 0.29$ ,  $P = 0.59$ .

However, these effects were mediated by a significant block by sex interaction,  $F_{1,76} = 5.76$ ,  $P = 0.01$ . Post hocs revealed that in block 2 the males ( $M = 9.96$  s, SD 5.29) outperformed the females ( $M = 18.28$  s, SD 16.89),  $t_{78} = -2.98$ ,  $P = 0.01$ . Although the males outperformed the females in block 1 (male  $M = 29.69$ , SD 9.70; female  $M = 32.61$ , SD 13.87), this difference was not significant,  $t_{78} = -1.09$ ,  $P = 0.28$ . Both males,  $t_{39} = 15.06$ ,  $P < 0.001$  and females  $t_{39} = 7.82$ ,  $P < 0.001$ , significantly improved from blocks 1 to 2.

For the probe trials, a  $2 \times 2 \times 2$  mixed measures ANOVA was performed on the time spent in the quadrant containing the platform during the training trials, with sex (male and female) and housing condition (complex and control) as the between subjects variables and probe trial (probes 1 and 2) as the within subjects variable. There was a main effect of probe trial,  $F_{1,76} = 52.76$ ,  $P < 0.001$ , with all rats increasing the amount of time within the quadrant that contained the platform in the training trials during the post-training probe trial (probe 2,  $M = 26.08$ , SD 6.58) compared to the probe trial completed before training (probe 1,  $M = 13.87$ , SD 6.01). No other main effects or interactions were observed,  $F_{s1,76} < 1.45$ ,  $P_s > 0.23$ . Further, single sample  $t$ -tests confirmed that the first probe trial did not exhibit a significant bias for the quadrant that would contain the platform in the training trials,  $t_{79} = 1.68$ ,  $P = 0.09$  and that once training was complete (probe 2) there was a significant bias for that quadrant,  $t_{79} = 15.06$ ,  $P < 0.001$ .

## Elevated plus maze

For percentage of the time spent in the open arm, a  $2 \times 2$  ANOVA with sex (male and female) and housing condition (control and complex) as between-subjects measures was performed. A significant main effect of sex,  $F_{1,76} = 3.86$ ,  $P = 0.04$  was observed, with females ( $M = 32.79$  and SD 13.80) spending a greater proportion of time on the open arm than males ( $M = 26.02$  and SD 17.89). No significant main effect of housing condition was observed,  $F_{1,76} = 0.56$ ,  $P = 0.46$ . However, there was a significant interaction between sex and housing condition,  $F_{1,76} = 7.33$ ,  $P = 0.01$ . Post hoc comparisons revealed that complex females ( $M = 38.74$  and SD 12.26) spent the greatest proportion of time on the open arm compared to all three groups (complex males,  $M = 22.65$ , SD 20.71; control males,  $M = 29.40$ , SD 14.27; control females,  $M = 26.85$ , SD 12.89),  $t_{s38} > 2.20$ ,  $P_s < 0.03$ . There were no other significant differences observed for this interaction,  $t_{s38} < -1.20$ ,  $P_s > 0.24$ .

## Open-field

Using the total distance travelled, a  $2 \times 2$  ANOVA with sex (male and female) and housing condition (complex and

control), ANOVA revealed a significant main effect of sex,  $F_{1,76} = 44.36$ ,  $P < 0.001$  that was modified by a significant interaction between sex and housing environment,  $F_{1,76} = 6.65$ ,  $P = 0.01$ . Post hoc comparisons revealed that although the complex females ( $M = 6,146.40$ ,  $SD 954.47$ ; control females  $M = 5,468.10$ ,  $SD 1,316.33$ ) travelled greater distances than the other three groups, they were only significantly greater than either group of males (complex  $M = 3,701.95$ ,  $SD 1,300.76$ ; control  $M = 4,388.50$ ,  $SD 1,123.92$ ), both  $t_{s_{19}} > 5.50$ ,  $P_s < 0.001$ .

A  $2 \times 2 \times 2$  mixed measures ANOVA was performed on the time spent in the perimeter of the open field, with sex (male and female) and housing condition (complex and control) as the between subjects measure. ANOVA revealed a main effect of sex,  $F_{1,76} = 7.71$ ,  $P = 0.01$ , with females spending significantly less time in the perimeter ( $M = 245.50$  and  $SD 29.61$ ) than males ( $M = 260.87$  and  $SD 32.06$ ). There was also a main effect of housing condition,  $F_{1,76} = 43.14$ ,  $P < 0.001$ , with complex rats spending less time in the perimeter ( $M = 234.99$  and  $SD 33.39$ ) than controls ( $M = 271.40$  and  $SD 15.21$ ). The interaction was not significant,  $F_{1,76} = 1.75$ ,  $P = 0.19$ .

Separate analyses were conducted for freezing and grooming. Although females ( $M = 0.40$  and  $SD 0.63$ ) froze more often than males ( $M = 0.18$  and  $SD 0.45$ ), this was not significant,  $F_{1,76} = 3.40$ ,  $P = 0.058$ . Similarly, no effect of housing environment,  $F_{1,76} = 2.06$ ,  $P = 0.15$  nor an interaction between sex and housing environment,  $F_{1,76} = 0.38$ ,  $P = 0.53$  was observed. For grooming, there were no significant effects observed,  $F_{s_{1,76}} < 2.24$ ,  $P_s > 0.14$ .

#### Relation of measures of stress-associated behaviours to spatial behaviours

Pearson product-moment correlations were conducted to determine the relation among performance on the OLM task during the testing phase, WM and the production of behaviours associated with stress (proportion of time spent on open arm of the elevated plus maze, the time in the perimeter of the open field, grooming sessions and number of freezing episodes). The number of interactions with the exchanged items in the OLM task during the testing phase was significantly correlated with: the proportion of time spent in the open arm,  $r_{80} = 0.22$ ,  $P = 0.04$ ; and with the total distance travelled in the open field,  $r_{80} = 0.34$ ,  $P = 0.01$ . For the unmoved objects, there were no significant correlations between the number of interactions or time spent interacting with them and the measures of stress-associated behaviour,  $r_{s_{80}} < -0.18$ ,  $P_s > 0.12$ . For the WM, only the correlation between the average time to find the platform for block 2 and the proportion of time spent on the open arm of the elevated plus maze was significant,  $r_{80} = -0.34$ ,  $P = 0.01$ .

## Discussion

To date, sex differences have not been examined in a rodent model of OLM in which the objects exchange positions with each other rather than occupying novel positions (e.g. Beck and Luine 2002). Consistent with the data from human participants, we observed that the rats interacted more often and for a longer duration with the exchanged objects than the unmoved objects in the testing phase; although the interaction indicated that this was largely due to the behaviour of the complex females and the control females. Similarly, for the number of interactions with the objects, both groups of females and the complex males had significantly more interactions with the exchanged objects compared to the unmoved objects during the testing phase. During the training phase, as there were no significant differences between the objects that were to be either exchanged or unmoved for either dependent variable, the change in behaviour in the testing phase cannot be attributed to some innate preference for one object or position over another. Thus, these data are consistent with the performance of female humans, suggesting that OLM may be better in females than males in at least two species—humans (e.g. Silverman and Eals 1992) and rats.

In the WM, we found as expected (e.g. Perrot-Sinal et al. 1996), both sexes improved over the testing period and that males displayed enhanced spatial navigation by locating the platform faster than the females in the second block (regardless of housing condition). Although previous studies have suggested that complex housing should have positive effects on spatial navigation tasks (e.g. Leggio et al. 2005); this was not the case here. The failure to observe a difference due to housing environment may reflect a ceiling effect for our tasks (i.e. the male rats in block 2 were locating the platform in under 10 s, on average). The ceiling effect may have been a result of the relatively short inter-trial interval, which promotes enhanced learning (Kanit et al. 1998). As the effects of complex housing may be more pronounced in males than in females (e.g. Elliott and Grunberg 2005), our use of a short inter-trial interval may have precluded further improvements by the males.

However, enrichment may have differentially affected the behaviour of male and female rats depending upon which task was examined. For instance, in the OLM, control males interacted most frequently, and for the longest period of time, with the objects regardless of whether they were unmoved or exchanged. The opposite was observed for the complex males which spent the least amount of time and had the fewest numbers of interactions with the objects, regardless of whether they were unmoved or exchanged. The reluctance of complex males to explore and interact with the objects was surprising, as typically complex housed rats are more explorative, at least in an

open field (Hall et al. 2000). Certainly, our data from the open field suggested that the complex housed rats were more likely to explore the open field. Further, it did not appear that the complex male rats exhibited significantly more behaviours associated with stress than the control males; thus, it does not appear likely that these differences in the OLM were due to differential stress responses in the complex male rats.

However, complex housed males differ in their exploration patterns from standard housed rats when exploring objects in a familiar environment (Zimmermann et al. 2001). Zimmermann and colleagues suggest that complex housed rats habituate to the environment more quickly, with exploration ceasing sooner and with fewer interactions following introduction into the arena. Notably, Zimmermann et al. (2001) did not examine the behaviour of female rats. As others have demonstrated that housing environment differentially affects some, but not all, behaviours of male and female rats (e.g. Elliott and Grunberg 2005; Pena et al. 2006), it may be that complex housing environment may result in faster habituation in male rats than females. However, as the Zimmermann et al. (2001) study only utilized novel objects and male rats, this remains speculative and must be confirmed.

Several hypotheses have been put forth in an attempt to explain the existence of sex differences within navigational and OLM spatial abilities. Explanations of sex differences in spatial ability suggest that differences exist due to spatial cognitive style (Postma et al. 1998); division of labour (Silverman and Eals 1992); and polygyny-range size (Gaulin 1995; Gaulin and Hoffman 1998). Of these, only the latter hypothesis is consistent with the current data. As rats are not linguistic nor do they divide labour between hunting and gathering, the female advantage for OLM observed in our study only offers support for the modified polygyny-range size hypothesis (Dab and Robert 2004).

Thus, our data demonstrates that female rats exhibit better performance for a task of OLM and male rats exhibit better performance on the WM. Thus, at least for one task of spatial ability, rats do not exhibit a universal male advantage. These data are consistent with observations from human participants and provide an important test of a few of the hypotheses that purport to explain the female advantage for OLM. Finally, our data provide support for the modified polygyny theory, which suggests that sex differences in spatial navigation and OLM relate to evolutionary selection pressures that relate to mating strategy and parental care.

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