Sex differences in ICR mice in the Morris water maze task

Jin-Fang Ge[1, 2], Cong-Cong Qi[1], Jin-Ping Qiao[1], Chen-Wei Wang[1], Jiang-Ning Zhou[1*]

1. Key Laboratory of Brain Function and Diseases, School of Life Science, University of Science and Technology of China, Chinese Academy of Sciences, Hefei 230027, Anhui, China.
2. School of Pharmacology, Anhui Medical University, Hefei 230032, Anhui, China.

*Address correspondence to Jiang-Ning Zhou, M.D., Ph.D.,
Department of Neurobiology and Biophysics, School of Life Science, University of Science and Technology of China, 443 Huang-Shan Road, Hefei, Anhui 230027 China;
E-mail: jnzhou@ustc.edu.cn

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Summary

The Morris water maze (MWM) is one of the most common tasks used to assess spatial learning and memory ability in rodents. Genetic strain and gender are two prominent variants that influence spatial performance. Although it was reported that ICR (Institute of Cancer Research) mice exhibited an unchanged baseline performance in the training phase of the MWM task, this outbred strain has been widely used in learning and memory studies, and little is known regarding the effects of sex on behavioural performance. In this study, we demonstrated that both male and female ICR mice could complete the MWM task. Furthermore, a significant sex difference was observed, with females having shorter escape latencies and longer durations in the target quadrant in both the acquisition and test phases. Our findings emphasise the necessity of careful examination of not only the strain effect on behavioural performance but also the sex effect.

Keywords: sex, learning behaviour, memory, mouse, Morris water maze

1. **Introduction**

Spatial learning and memory, which are important skills for adaptation and survival, are based on the ability to encode, store and retrieve mainly visual information regarding route navigation and object locations (Postma *et al.* 2004, Faraji *et al.* 2010). Substantial evidence regarding gender differences in humans suggests that males typically outperform females on tasks requiring spatial ability, such as navigation (Gron *et al.* 2000) and mental rotation (Parsons *et al.* 2004), whereas females perform better on object location memory tasks (Lejbak *et al.* 2009). Moreover, it has been reported that women experience a faster rate of decline in visual-spatial skills with age than men and are three to four times more likely to develop Alzheimer's disease than men (Musicco 2009, Andersen *et al.* 1999). These observations emphasise the importance of understanding the role of gender differences in learning and memory abilities.

Rodent models have been successfully used to study the behaviours associated with sensory, locomotive and cognitive abilities. Generally, spatial performance in rodents is assessed using the Morris water maze (MWM) (Morris 1981, Wang *et al.* 2010), radial arm maze (RAM) (Gresack and Frick 2003), or radial arm water maze (RAWM) (Chen *et al.* 2004, Bimonte *et al.* 2000). The MWM is the primary spatial cognitive task employed by most researchers. In assessments of spatial learning and memory using the MWM, sex differences have either favoured males (Perrot-Sinal *et al.* 1996, Roof and Stein 1999), or no sex differences have been observed (Voikar *et al.* 2001). Similarly, inconsistent findings regarding sex differences have been reported for RAM (Gresack and Frick 2003) and RAWM (Chen *et al.* 2004, Bimonte *et al.* 2000) tests. In addition to the different hippocampal anatomy and function (Madeira *et al.* 1991, Filipek *et al.* 1994, Madeira and Paula-Barbosa 1993) the role of sex hormones (Lowry *et al.* 2010), age (Chen *et al.* 2004), diet type (Valladolid-Acebes *et al.* 2011), maternal experience (Wang *et al.* 2010), and task-dependent procedures (Lejbak *et al.* 2009) have also been proposed to partially explain sex differences. In the MWM specifically, it has been shown that the task parameters (Roof and Stein 1999), genetic strain of the mouse (Upchurch and Wehner 1988, Adams *et al.* 2002), and sex of the mouse (Voikar *et al.* 2001) may influence spatial performance.

The ICR (Institute of Cancer Research) mouse strain is an outbred stock derived from the CD1 strain in 1948 (Adams *et al.* 2002). ICR mice are commonly employed in neuroscience, immunology, and pharmacology studies because of good reproductive performance and a fast growth rate (O'Connor *et al.* 2009, Zhong *et al.* 2009). Although ICR mice are also widely used in MWM tasks (Kim *et al.* 2006, Zhong *et al.* 2009, Muto *et al.* 2010), it was reported that ICR mice displayed no apparent improvement in locating the hidden platform in the MWM task. This result was shown by the absence of a decrease in the cumulative distance from the platform and the absence of an increase in the duration of time in the target quadrant (Adams *et al.* 2002). Moreover, although other sex-related responses have been previously characterised (Komukai *et al.* 1999), little is known regarding the sex differences of ICR mice in behavioural tasks. Therefore, it is of interest to determine whether the ICR strain can serve as a model for spatial learning and memory studies, particularly in the MWM task, and whether sex-specific differences are present.

In this study, we employed a series of behavioural tests, including sensorimotor activity, locomotor activity, and spatial learning and memory tasks, to explore the sex-related differences of ICR mice.

2. Materials and Methods

2.1 Subjects

Thirty-eight male and 35 female 3-month-old ICR mice (Slac: ICR, Shanghai, China) were used in all experimental procedures. The mice were housed in colony cages and maintained under a 12-h light:12-h dark cycle (the lights were on at 7:00 AM) and accessed to food and water ad libitum. The ambient temperature was maintained at 21-22°C with 50±5% relative humidity. All experimental procedures were approved by the Animal Care and Use Committee at the University of Science and Technology and complied with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication no. 8023, revised 1978). The battery of behavioural tasks continued for 9 days, and the mice were weighed prior to the initial task (day 0) and after the last task (day 10). Each task was performed during the light phase and in the following order: beam walking (day 1), open-field locomotor activity (day 2), tightrope (day 3), and the Morris water maze (days 4-9). All tasks were performed in the behaviour test room after a 15-min adaptation to the environment.

2.2 Apparatus and testing procedures

2.2.1 Beam walking

Fine motor coordination can be assessed by the beam walking or beam balance task (Luong *et al.* 2011). In the present study, a 51 cm long wooden beam (1 cm wide) with either end attached to a platform (14×10 cm^2) was fixed by two vertical supports and elevated 50 cm above a flat surface (Chen *et al.* 2004). Each mouse performed three successive trials and was placed perpendicular to the beam at the centre. Each trial continued for a maximum of 60 s. The balance time, during which the mouse did not fall from the beam, was recorded for each of the three trials. If the mouse remained on the beam for the duration of the trial or moved to either of the platforms, the balance time was recorded as 60 s. The mean time recorded for the three trials was used in the statistical analyses.

2.2.2 Open-field task

An open-field procedure was designed according to our previous work to detect spontaneous motor activity and anxiety (Chen *et al.* 2004). An open, black wooden box (81×81 cm2 interior area) with 28-cm high walls was used. The box floor was painted with white lines (3 mm wide) to form 16 equal-sized squares (20×20 cm^2 each). For each trial, a mouse was placed in one of the four corners facing the wall and was permitted to explore the environment for 5 min ad libitum. A video camera was placed above the box, and the path length, moving velocity, the time spend in the center (the centre 4 squares of the box), and the frequency with which the mouse went to the centre were recorded and analysed. The area was cleaned with water before the next mouse was tested.

2.2.3 Tightrope task

The tightrope task is another sensorimotor task that requires greater muscular strength, motor coordination and maintenance of equilibrium relative to the beam walking (Karl *et al.* 2003, Chen *et al.* 2004). In this task, each mouse was forced to grasp and suspend from a tightrope. A taut, small cotton rope (2 mm in diameter with ink marks at 5 cm intervals along its length) was stretched across a tank (100 cm in diameter, 30 cm in height) that was half-filled with water (23°C). Before the test began, each mouse was placed in the water for 5 s. During a 60-s trial, the mouse was raised from the water to grasp the centre of the rope with its forepaws and was then slowly released to support its own weight using its grip. The suspension time and the number of markers crossed by the forepaws of the mouse, which was considered as the amount of horizontal movement, were recorded separately. If the mouse fell into the water or stayed on the rope for 60 s, it was immediately placed in a holding cage and allowed to rest for 30 s before the next trial (three subsequent trials were performed on a single day). The mean suspension time and horizontal movement scores for the three trials were calculated for each mouse. Among the mice with identical suspension time averages, some only suspended motionless on the tightrope, whereas others suspended on the tightrope and also moved horizontally. We used a transformed score to distinguish between these two groups of mice: [(the average suspension time) +10 × (the average number of markers crossed)] (Chen *et al.* 2004).

2.2.4 The Morris water maze

The spatial learning and memory performance of the mice was measured using the MWM task. A circular plastic pool (height: 35 cm, diameter: 120 cm) was filled with water maintained at 23°C. The light intensity, external cues in the room, and water opacity (obtained by the suspension of black food colouring) were rigorously reproduced. The pool was divided into 4 quadrants, and the escape platform (diameter: 4.5 cm) was located in the southwest (SW) quadrant and submerged 1.2-1.3 cm below the surface of the water at a fixed position. The task consisted of a 5-day acquisition phase with 4 massed trials administered each day and a 1-day test phase. The mice were placed on the platform for 30 s preceding the start of each session and then semi-randomly placed in the water facing the wall of one of four predetermined locations, which were located in the northwest, north, east, and southeast quadrants (Vorhees and Williams 2006). The mice were allowed to swim freely for a maximum of 60 sec or until the platform was located. After the mouse reached the platform, it was required to remain there for 30 sec. If the platform was not located during the 60 sec, the mouse was gently guided to the platform and was also allowed to remain there for 30 sec. The escape latency during the 60 s, the percentage of time the mouse was in the platform quadrant, the path length, and the time that the mouse voluntarily stayed on the platform during the 30-s period were recorded. After the completion of four successive trials, the mice were returned to their home cage. On the 6th day (the test phase) following the 5-day acquisition phase, memory retention was determined using a single 60-s probe trial. The underwater platform was removed. The mice were placed into the water facing the wall in the quadrant opposite of the platform and were permitted to explore the environment for 60-s ad libitum. The performance parameters of each mouse, including the swimming distance, swimming velocity, the duration in each quadrant, and latency to the target quadrant that previously contained the platform during the acquisition phase, were monitored and recorded by a digital camera placed above the centre of the pool. The camera was interfaced to a computer, and Noldus EthoVision (Noldus Technologies, Wageningen, Netherlands) video imaging software was used.

2.3 Statistical analyses

The data in the figures are expressed as mean values ± the standard error of the mean values (S.E.M.). The statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) 13.0 for Windows (SPSS, Inc., Chicago, IL). A Student’s *t*-test was used to detect the sex effect on body weight and performance in the beam balance test, open-field test, tightrope test, and test phase of the MWM task.

Between-sex effects during the acquisition phase of the MWM task were analysed by a repeated measures ANOVA followed by a least significant difference (LSD) test with sex and days as the factors. Within-subject effects for escape latency, the percentage of time in the target quadrant, path length, and the accurate time on the platform during the adaptation time of the acquisition phase were analysed using an ANOVA with days as a repeated-measures factor. A correlation analysis was performed using a Pearson test.

3. Results

3.1 Body weight

The mean body weight of the male ICR mice (38.14±0.66 g) was greater than that of the female group (32.33±0.47 g) prior to the behavioural tests (*t*[71] =7.178, *P*<0.001, *t*-test); however, the weight gain during the tests did not show sex differences (*t*[71] =0.063, *P*=0.950, *t*-test).

3.2 Beam walking

Sex had no effect on the balance time (*t*[71] =0.998, *P*=0.322, *t*-test); the mean balance times were 57.75±0.79 s and 56.37±1.16 s for the male and female ICR mice, respectively.

3.3 Open field

The 5-min performance results for the open field task are shown in Figure 1. The path lengths of the male and female ICR mice were 2601.56±137.86 cm and 2604.49±144.93 cm, respectively. The moving velocities of the male and female ICR mice were 9.60±3.09 cm/s and 9.78±3.33 cm per sec, respectively. The time spend in the arena centre for the male and female ICR mice were 7.24±1.23 s and 9.25±2.13 s, respectively. The frequencies that the male and female ICR mice went to the centre were 4.84±0.72 and 4.57±0.83, respectively. There were no significant differences between sexes with respect to the path length (*t*[71] =0.015, *P*=0.988, *t*-test), moving velocity (*t*[71] =0.231, *P*=0.818, *t*-test), time spend in the centre (*t*[71] =0.848, *P*=0.400, *t*-test), or frequency of moving to the centre ($t[71]=0.249$, $P=0.804$, t-test).

3.4 Tightrope

Figure 2 shows that the suspension time of the female ICR mice (55.70±1.26 s) was significantly longer than that of the males (45.17±2.10 s) ($t[71]=4.213$, $P<0.001$, t-test). The transformed scores of the female group (130.56±6.60) were slightly but not significantly lower than the male group (145.52±8.18) ($t[71]=1.408$, $P=0.164$, t-test).

3.5 The Morris water maze

3.5.1 Acquisition phase

The performance of the mice in the acquisition phase of the MWM task is shown in Figure 3. For all of the mice in this experiment, the escape latency declined during the acquisition phase (Figure 3A), and the escape latency of the ICR female group was shorter than that of the male group. The analysis of the latency using a repeated measures ANOVA (2 sexes ×5 days with repeated measures on days) showed that both the sex and training days affected the learning ability (sex effect: ($F[1,71]=4.771$, $P=0.032$), days effect: ($F[4,68]=47.799$, $P<0.001$), and interaction: ($F[4,68]=1.855$, $P=0.123$)). The results of the pairwise comparisons showed that the male ICR mice reached a learning plateau from day 4 to 5 ($P=0.446$), whereas the females decreased in latency throughout the study ($P=0.008$). The net decrease in the latency between days 1 and 5 in the males and females were 19.83 ± 2.54 s and 24.03 ± 2.30 s, respectively. These results indicated that the 3-month-old female ICR mice may have a greater learning capacity during the consecutive 5-day training phase of the MWM task than their male counterparts.

During the 5-day acquisition phase of the MWM task, all the mice spent more time in the platform quadrant gradually (Figure 3B). A repeated measures ANOVA (2 sexes ×5 days with repeated measures on days) showed that both the sex and number of training days affected the learning ability (sex effect: *F*[1,71] =16.270, *P*<0.001; days effect: *F*[4,68] =24.258, *P*<0.001; and interaction: *F*[4,68]=3.757, *P*=0.008). Consistent with the escape latency result, the percentage of time exploring the target quadrant for the male mice was not significantly different between days 4 and 5 (*P*=0.815) but was markedly higher in the female group (*P*=0.005). The path length on each day of the acquisition phase was also assessed using an identical analysis method (Figure 3C). However, the results showed that an effect of days was presented (*F* [4,68] =25.431, *P*<0.001), but not a sex effect (*F*[1,71]=0.199, *P*=0.657) and interaction (*F*[4,68]=2.306, *P*=0.099) .

In the present study, each mouse was allowed a 30-s adaptation time on the platform prior to the start of each session and was required to remain on the platform for 30-s after each trial. Because the 4 trials were successive, we also considered the post-trial 30 s as an adaptation time for the subsequent trial. However, not all of the mice remained on the platform for the entire 30 s. We recorded the actual time that each mouse voluntarily remained on the platform during the 30 s. The results of the repeated measures ANOVA (2 sexes×5 days with repeated measures on each day) showed that an effect of days (F[4,68] =25.944, P<0.001) and interaction (F[4,68] =8.593, P<0.001), but not a sex effect (F[1,71]=0.226, P=0.636), was present.

3.5.2 Test phase

The mean values of the swimming distance, swimming velocity, duration in each quadrant, and latency to the target quadrant that contained the platform during the acquisition phase are presented in Figure 4. There was no significant sex effect on the swimming distance (Figure 4A) (t[71] =0.258, P=0.797, t-test) or swimming velocity (Figure 4B) (t[71] =0.261, P=0.795, t-test). However, the female ICR mice explored the target quadrant for a longer period than did the males. The longer time (t[71]=4.147, P<0.001, t-test) and shorter latency values (t[71]=2.588, P=0.012, t-test) are shown in Figures 4C and 4D, respectively. Additionally, the female ICR mice had shorter durations in the opposite quadrant than did the males (t[71]=3.538, P=0.001, t-test), which partly resulted from the negative correlation between the time in the target quadrant and the time in the opposite quadrant (r=-0.781**,** P<0.001). The typical swim orbits in the test phase are shown in Figure 5.

4. **Discussion**

This study showed sex differences in the MWM task, which indicated superior performances of the females in both the acquisition and test phases.

The wide application of mice in behavioural tasks has emphasised two important matters. The first concern is the genetic strain of the mouse (Voikar *et al.* 2001). Due to their high genetic homogeneity, many inbred strains have been developed and used for behavioural tests (Voikar *et al.* 2001). However, outbred mice with genetically variable compositions, vigorous physiques, and economical prices have also been considered for behavioural tests. Little is known about the behavioural characteristics of outbred mice (Chen *et al.* 2004). The second concern is the role of sex in behavioural tasks (Bimonte *et al.* 2000, Voikar *et al.* 2001, Chen *et al.* 2004, Benice *et al.* 2006). Evidence concerning sex differences in behaviour are present in both human (Parsons *et al.* 2004, Lejbak *et al.* 2009) and animal studies (Roof and Stein 1999, Saucier *et al.* 2008, Faraji *et al.* 2010).

Beam walking, open-field testing, and tightrope tasks were used in this study to exclude the disadvantageous effects of dysfunctions in physical movement on behaviour performance. The results showed that ICR mice could perform well in these tasks, and no sex-related differences were observed in the beam walking or open field tasks. However, in the tightrope task, the suspension time of the females was significantly longer than that of the males, whereas the transformed score, which accounted for the moving frequency , was not significantly different with that of the males. We ascribed the sex difference in suspension time to the effect of body weight based on the negative correlation between body weight and suspension time (r=-0.318, P=0.006) but not between body weight and the transformed score (r=0.062, P=0.602).

The MWM is one of the most extensively employed tasks for the measurement of spatial learning and memory abilities in rodents (Morris 1981, D'Hooge and De Deyn 2001, Miyoshi *et al.* 2012). In this test, the animals are required to remember the topographical relationships between distal cues and the pool wall to successfully locate the hidden platform and escape from the water. The escape latency is the routine parameter in the MWM task. Figure 3A shows that the escape latency to find the platform declined progressively during the 5 training days for both the male and female ICR mice in our study, which indicates that all of the mice correctly learned the task based on 5 days of training. The escape latency of the male mice on the 5th day (28.19±2.54 s) was subtle long. However, compared to the latency on the initial day (48.02± 1.73 s), there was a significant improvement (P<0.001), which demonstrated learning in the ICR male mice in the acquisition phase of the MWM task. In other studies, the escape latency of ICR male mice in the control group was approximately 20-25 s on the last day of the MWM acquisition phase (Nagata *et al.* 2009, Zhong *et al.* 2009). This difference may result from the different experimental designs, including differences in the diameters of the maze and platform, the ages of the mice, and the number of training days.

Using a digital camera, we also recorded the time that each mouse explored the platform quadrant and the path length, which are shown in Figures 3B and 3C. The duration in the platform quadrant increased, whereas the path length decreased, in the 5-day acquisition phase, which again indicated that both the male and female ICR mice could accomplish the MWM learning task. However, this result was not consistent with the result reported by Adams (Adams *et al.* 2002). These authors stated that ICR mice were not competent for the MWM task, showing a baseline performance throughout the entire training, and attributed this performance level to visual impairment. The reason for this discrepancy may have resulted from differences in the experimental designs (Roof and Stein 1999). For example, the diameter of the water maze tank in their study was 180 cm (Adams *et al.* 2002) but was 120 cm in our study; the diameter of the platform was 15 cm for the initial 2 days and 10 cm in the subsequent 3 days of the acquisition phase in their study, whereas it remained 4.5 cm for the entire acquisition phase in our study; and their study had two sessions of three trials per day in the 5-day acquisition phase after a 2-day habituation (Adams *et al.* 2002), whereas our study had one session of four trials per day in the 5-day acquisition phase without habituation prior to the trials. Furthermore, there was no mention of sex in their study (Adams *et al.* 2002), whereas obvious sex differences were observed in our study.

In the 5-day acquisition phase of the MWM task, the escape latency of the female ICR mice was shorter than that of the males on each day. However, it is notable that we did not observe any difficulties in the swimming ability of the mice, none of the animals floated, and all were able to climb onto the escape platform. Consistent with the escape latency results, the females had longer durations in the platform quadrant than did the males during the 5 days of training, which indicates that the female ICR mice performed better than the age-matched males in the acquisition phase of the MWM task. Regarding the absence of a sex-linked effect on sensorimotor and locomotor activity, the observation that females tended to be better than males in the acquisition phase of the MWM task cannot be attributed to a difference in the general activity level. In contrast, given the important role of the hippocampus in spatial learning and memory tasks, the gender differences in hippocampal anatomy and function may provide a possible interpretation. An imaging study reported that relative to the brain size, women have greater volumes in the hippocampus (Filipek *et al.* 1994) than do men. Moreover, previous studies have demonstrated that females exhibit a greater number of mossy fibre synapses in the CA3 region (Madeira *et al.* 1991), whereas males have a greater total number of granule neurons in the dentate gyrus and a greater number of mossy fibre synapses in the hilus (Madeira and Paula-Barbosa 1993). Furthermore, it has also been reported that 2- to 3-month-old female rats had more neurogenesis in the hippocampal sub-granular zone (SGZ) than age-matched males (Tanapat *et al.* 1999).

However, inconsistent with the results for the escape latency and the percentage of time exploring in the platform quadrant, our results showed that training days, but not sex, affected the path length and duration on the platform during the 30 s adaptation period in the acquisition phase of the MWM task. This result indicated that the task parameters could influence the valuation in the MWM task (Roof and Stein 1999) and that both sex and training days play significant roles in the MWM task but in different manners.

The swimming distance and velocity were calculated during the test phase performed 1 day after the last trial, and no significant differences between the sexes were observed (Figures 4A and 4B). When observing the performance in the quadrant that contained the platform during the acquisition phase (the target quadrant), we observed that both the male and female mice swam preferentially in the target quadrant during the 60 sec session (Figures 4C and 5). The ratio of the duration in the target quadrant for the male and female ICR mice was 33.72% and 45.85%, respectively, which indicated that both sexes correctly learned and memorised the platform location. Furthermore, the female ICR mice explored the target quadrant for a longer duration than did the males (Figure 4C), which indicated that the female ICR mice learned to a greater extent. This result was consistent with the results shown in the acquisition phase.

Hormonal fluctuations that occur during the oestrous cycle have been accepted as a confounding factor in behavioural studies (van Goethem *et al.* 2012, Gouveia *et al.* 2004). However, no differences in cognitive performance between the phases of the menstrual cycle (oestrous cycle in rodents) have been observed in studies of humans (Gordon and Lee 1993, Epting and Overman 1998) or rats (Stackman *et al.* 1997). Moreover, it has also been advocated that the majority of females housed in one room displayed identical cycle phases, and the behavioural phenotype of female mice can be assessed without the risk of confounding effects of the oestrous cycle (Meziane *et al.* 2007). In the present study, the oestrous cycle was not monitored in the female mice, although the examination of performance on different days by individual females did not exhibit any obvious cycling of the performance.

In conclusion, this study demonstrated sex- and task-specific differences of ICR mice in behavioural tasks. The sex difference of ICR mice was present in the MWM task, and the females showed superior learning and memory abilities than did the males. These data, together with the data of previous studies, indicate that the proper choice of mice in behavioural tests should be based on a suitable knowledge of sex behaviour, genetic strain differences, and the specific goals of the study. Additionally, for the initial screening of mice, well-established behavioural paradigms are required.

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Figure Legends

Figure 1 The performance of male and female ICR mice in an open-field task.

There was no significant difference between the sexes. Each symbol and bar indicates the mean±standard error of the mean (S.E.M.) in the male (n=38) and female groups (n=35).

A, path length(cm); B, moving velocity (cm/s), duration in the centre (s) and frequency to the centre.

Figure 2 The performance of male and female ICR mice in a tightrope task.

The suspension time of the female ICR mice was significantly longer than that of the males, although there was no significant difference in the transformed scores between the sexes.

Each symbol and bar indicates the mean±standard error of the mean (S.E.M.) in the male (n=38) and female groups (n=35).

**P*<0.05, ***P*<0.01 *vs*. the male group.

Figure 3 The performance of male and female ICR mice in the acquisition phase of the MWM task.

The escape latency of the female ICR mice was shorter than that of the males (A) for each day, whereas the percentage of time exploring the platform quadrant for the female ICR mice was longer than that of the males (B). The training days, but not the sex, affected the path length (C). During the adaptation period, the ICR female mice remained on the platform longer during the initial 2 days, remained for an identical time on the 3rd day and had shorter durations during the last 2 days relative to the males (D).

Each symbol and bar indicates the mean±standard error of the mean values (S.E.M.) in the male (n=38) and female groups (n=35).

A, escape latency(s); B, percentage of time exploring the platform quadrant; C, path length(cm); D, accurate time staying on the platform(s).

Figure 4 The performance of male and female ICR mice in the test phase of the MWM task.

Although there were no sex differences for the swimming distance (A) and swimming velocity (B), the female ICR mice explored the target quadrant for longer time (C) and had a shorter latency (D) relative to the males. The “South-West” quadrant was the target quadrant, and the ‘‘North-East’’ quadrant was the starting quadrant in the test phase; the quadrants were indicated as “SW (target)” and “NE (start)”. The ‘‘North-West’’ and ‘‘South-East’’ quadrants were indicated as “NW” and “SE”.

Each symbol and bar indicates the mean±standard error of the mean

(S.E.M.) in the male (n=38) and female groups (n=35).

A, swimming distance (cm); B, swimming velocity (cm/s); C, duration in each quadrant (s); D, latency to the target quadrant (s).

**$P<0.01$ *vs.* the male group.

Figure 5 The typical swim orbits of male and female ICR mice in the test phase of the MWM task. The directions “North”, “South”, “East”, and “West” were indicated as “N”, “S”, “E”, and “W”, respectively. The “South-West” quadrant was the target quadrant, and the ‘‘North-East’’ quadrant was the starting quadrant.

A, swim orbit of the male ICR mice; B, swim orbit of the female ICR mice.

Figure 1

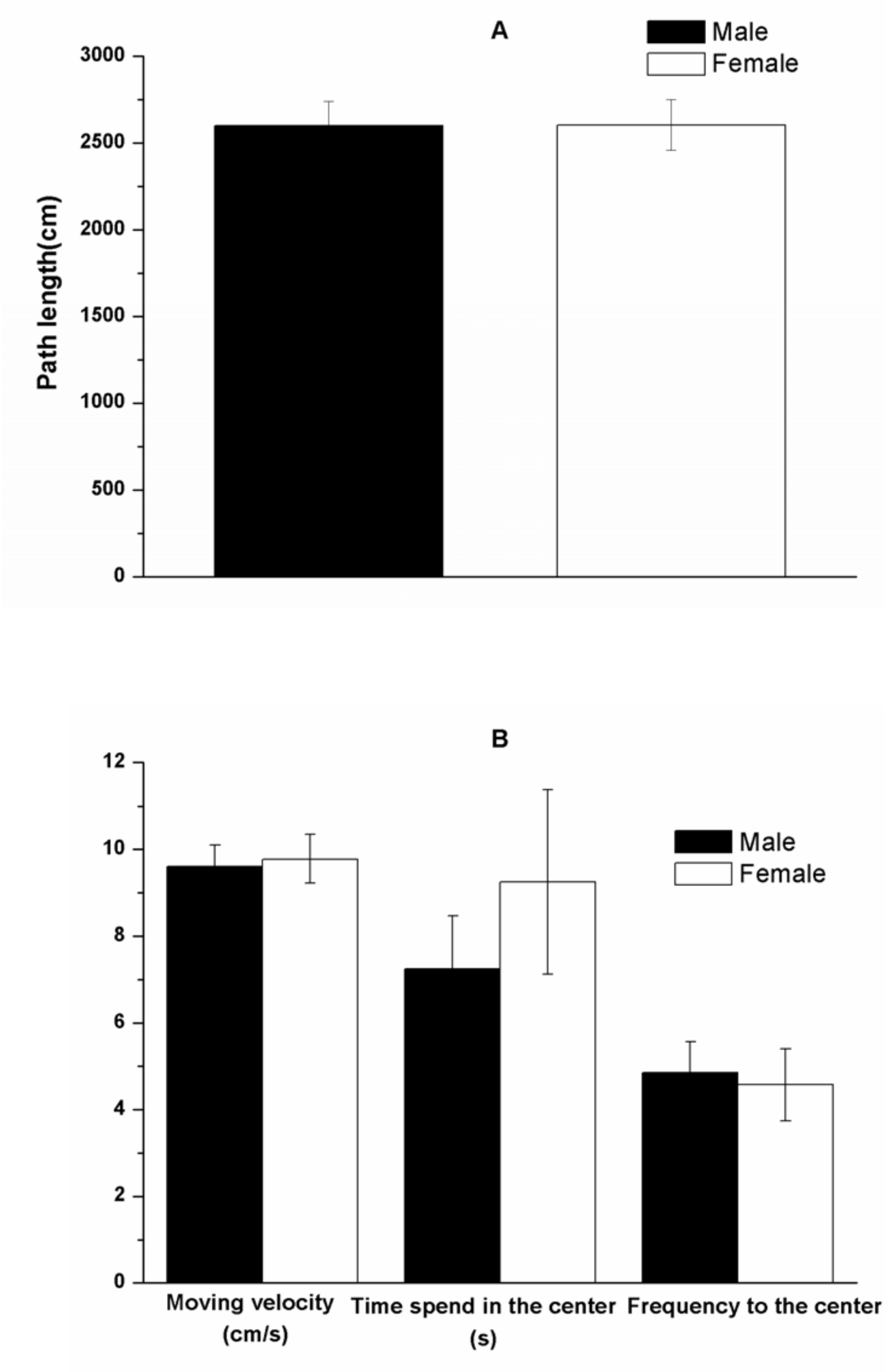
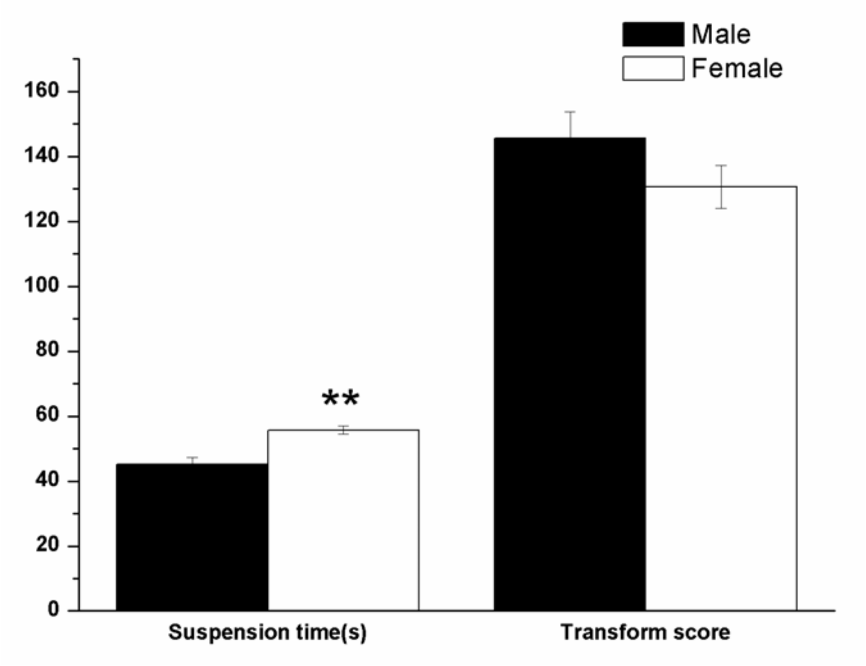
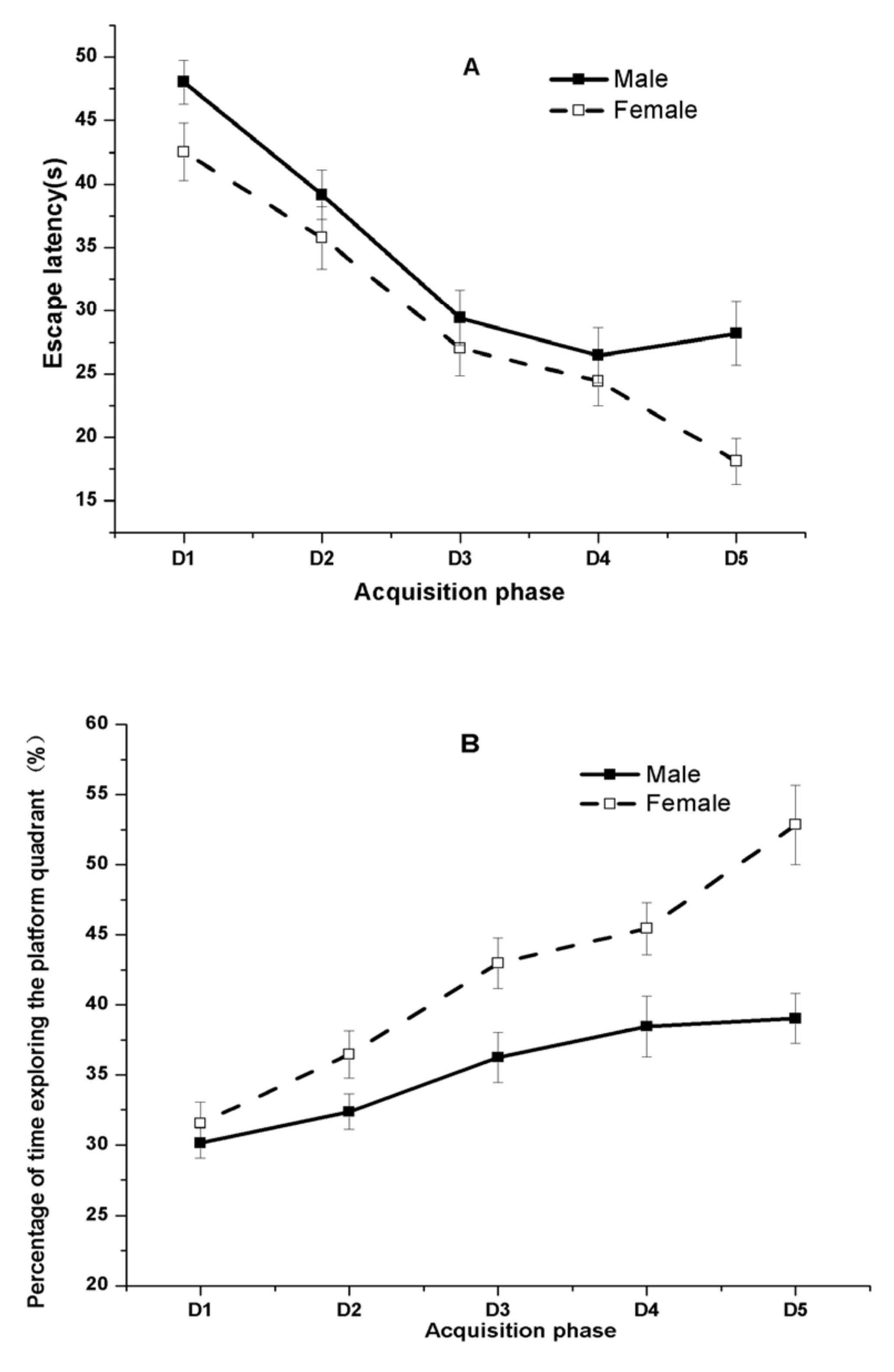

A
■ Male
□ Female
Path length(cm)
3000
2500
2000
1500
1000
500
0
B
12
10
8
6
4
2
0
■ Male
□ Female
Moving velocity (cm/s)
Time spend in the center (s)
Frequency to the center

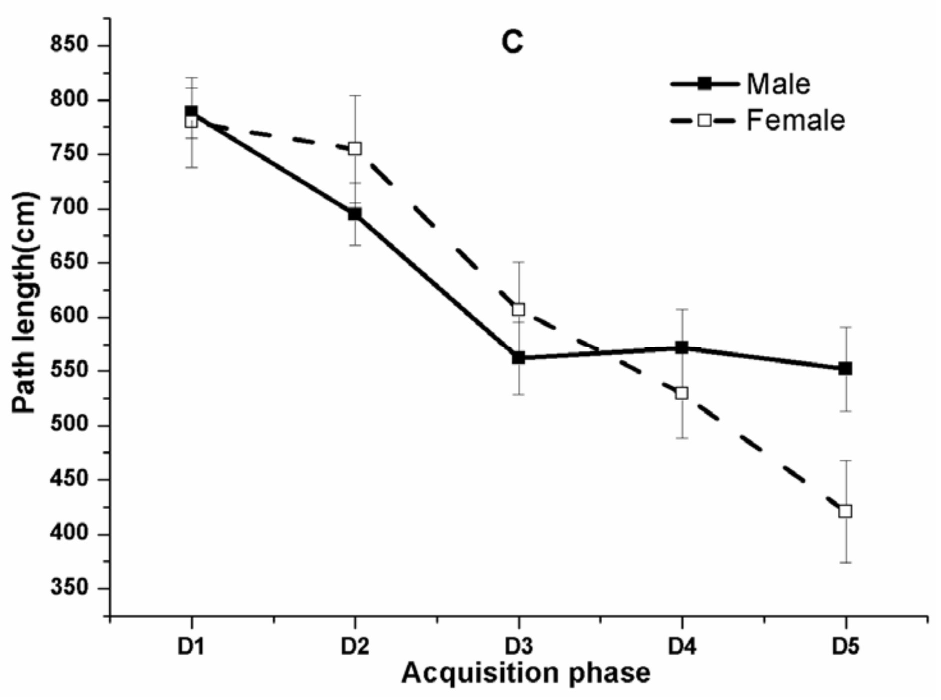
Figure 2



Male
Female
160
140
120
100
80
60
40
20
0
**
Suspension time(s)
Transform score

Figure 3


A
Male
Female
Escape latency(s)
50
45
40
35
30
25
20
15
D1
D2
D3
D4
D5
Acquisition phase
B
Male
Female
Percentage of time exploring the platform quadrant （%）
60
55
50
45
40
35
30
25
20
D1
D2
D3
D4
D5
Acquisition phase


C
Male
Female
Path length(cm)
850
800
750
700
650
600
550
500
450
400
350
D1
D2
D3
D4
D5
Acquisition phase

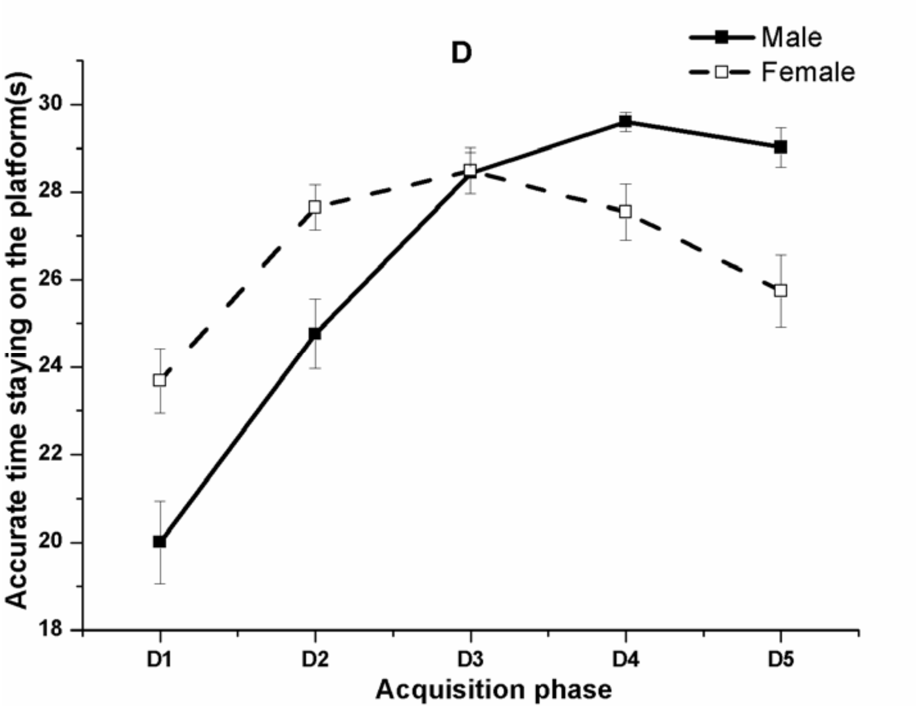
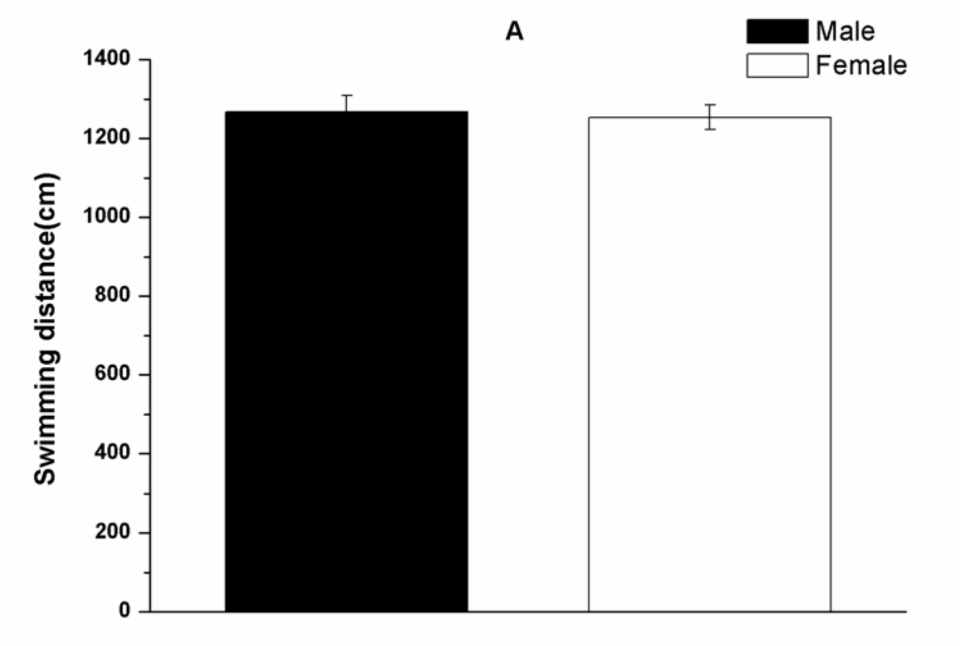
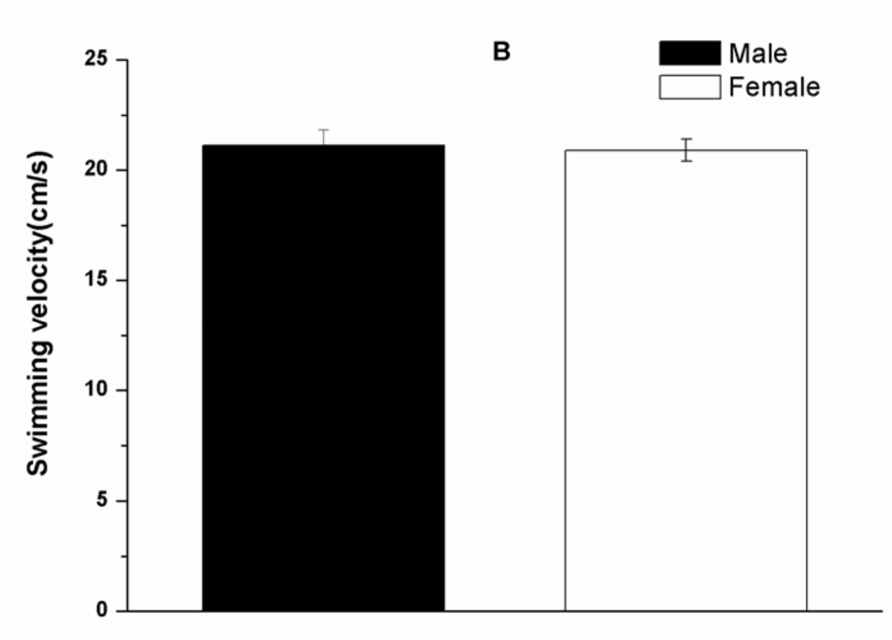
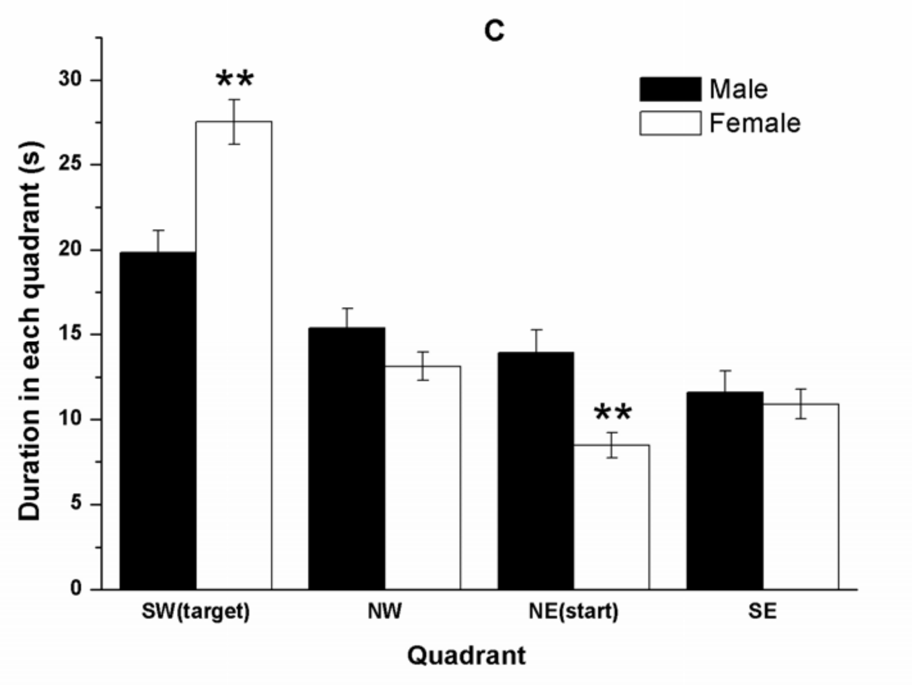

D
Male
Female
Accurate time staying on the platform(s)
30
28
26
24
22
20
18
D1
D2
D3
D4
D5
Acquisition phase

Figure 4


A
■ Male
□ Female
Swimming distance(cm)
1400
1200
1000
800
600
400
200
0


B
■ Male
□ Female
Swimming velocity(cm/s)
25
20
15
10
5
0


C
Male
Female
**
**
Duration in each quadrant (s)
0
5
10
15
20
25
30
SW(target)
NW
NE(start)
SE
Quadrant

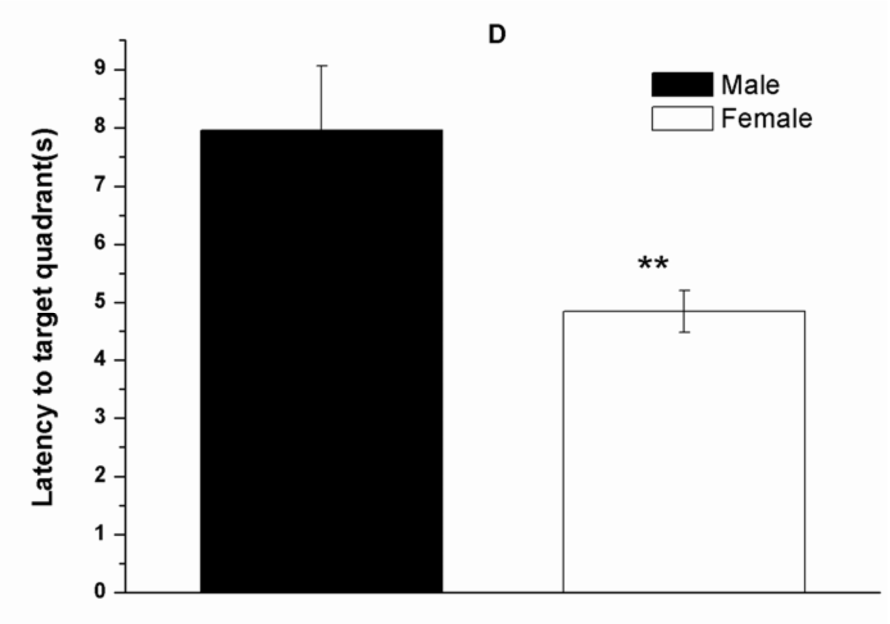

D
Male
Female
**
Latency to target quadrant(s)
0
1
2
3
4
5
6
7
8
9

Figure 5