

# Spatial working memory in rats: no differences between the sexes

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In a number of mammalian species, males appear to have superior spatial abilities to females. The favoured explanations for this cognitive difference are hormonal, with higher testosterone levels in males than females leading to better spatial performance, and evolutionary, where sexual selection has favoured males with increased spatial abilities for either better navigational skills in hunting or to enable an increased territory size. However, an alternative explanation for this sex difference focuses on the role of varying levels of oestrogen in females in spatial cognition (the 'fertility and parental care' hypothesis). One possibility is that varying oestrogen levels result in variation in spatial learning and memory so that, when tested across the oestrous cycle, females perform as well as males on days of low oestrogen but more poorly on days of high oestrogen. If day in the oestrous cycle is not taken into account then, across an experiment, any sex differences found would always produce male superiority. We used a spatial working memory task in a Morris water maze to test the spatial learning and memory abilities of male and female rats. The rats were tested across a number of consecutive days during which the females went through four oestrous cycles. We found no overall sex differences in latencies to reach a submerged platform in a Morris water maze but, on the day of oestrus (low oestrogen), females took an extra swim to learn the platform's location (a 100% increase over the other days in the cycle). Female swim speed also varied across the oestrous cycle but females were no less active on the day of oestrus. These results oppose the predictions of the fertility and parental care hypothesis.

**Keywords:** memory; spatial ability; oestrus; sex differences

## 1. INTRODUCTION

Human males and male rats have something in common: they both outperform females on spatial problems (e.g. Williams & Meck 1991; Voyer *et al.* 1995). Neural data appear to underlie these sex differences. In small mammals, for example voles (Jacobs *et al.* 1990), there is sexual dimorphism in the hippocampus, the part of the brain implicated in processing spatial information (no conclusive data for humans). Polygynous male rats and voles have larger hippocampal volumes than do females. Males also have higher levels of many hippocampal neurotransmitters and their associated receptor sites (Loy 1986). Manipulation of gonadal hormones during development provides some evidence as to how these sex differences might arise, for example administering testosterone to young female rats produces maze-solving performances akin to those of untreated males (e.g. Roof 1993) and higher pre-natal gonadal hormone levels result in girls with better spatial abilities later in life (e.g. Grimshaw *et al.* 1995).

Sexual selection is commonly suggested to have selected for increased spatial ability in males, either because males compete for females by increasing their home range size or because females select males on the basis of their hunting success (Gaulin & FitzGerald

1986; Jacobs *et al.* 1990). An alternative theory for sexual dimorphism in spatial ability (but still predicting male advantage) is based on variation in foraging behaviour: the further males forage from home, the more those with better long-distance navigational skills will be favoured (e.g. Gray & Buffery 1971; Kolakowski & Malina 1974).

More recently, two other evolutionary scenarios to account for sex differences in spatial ability have been proposed, both of which focus on the female's spatial ability rather than the male's. One predicts better spatial abilities in women rather than men and the other that selection has produced variation in females' spatial abilities rather than the enhancement of spatial abilities in males. The 'female foraging' hypothesis proposes that females should be better than males at remembering the location and identity of objects (Eals & Silverman 1997; James & Kimura 1997) while the 'fertility and parental care' hypothesis proposes that at times of high oestrogen (pro-oestrus), it is beneficial for females to reduce their mobility (Sherry & Hampson 1997). Such reduced mobility lowers the risks of predation or accident to mothers with newly born offspring as well as reducing the probability of encounters with males from other groups. Therefore, sexual dimorphism in spatial ability in mammals may have arisen through selection acting on variation in females and not, as is currently favoured, through favouring an increase in male spatial abilities.

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The female foraging hypothesis does not address the data supporting male superiority in at least some spatial tasks but the fertility and parental care hypothesis might: if spatial abilities in females were to vary across the oestrous cycle, the average performance levels of females would tend to be less than those of males. It is with this possibility that we are concerned here.

It is certainly the case that variation in gonadal hormone levels during development affects performance in both radial and Morris water mazes in rodents, both tasks requiring spatial learning (e.g. Roof & Havens 1992). Gonadal hormones also appear to have striking effects on hippocampal structure and function over a single oestrous cycle: the number of dendritic spines and synapses in the CA1 region of the hippocampus decreases by more than 30% between the pro-oestrus (high oestrogen) and oestrus (low oestrogen) phases of the cycle in rats (Gould *et al.* 1990; Woolley & McEwen 1992) and long-term potentiation (LTP), a long-term change in synaptic plasticity thought to be a model for memory, is greatest during pro-oestrus (Warren *et al.* 1995). It should be noted that these data directly conflict with the predictions of the fertility and parental care hypothesis. The evidence for spatial performance by females closely correlating with these neurophysiological and neuroanatomical changes is not very convincing. Chronic oestrogen treatment of ovariectomized rats appears to enhance spatial memory in rats (e.g. Luine *et al.* 1998; Rissanen *et al.* 1999) but it is not clear from these data whether or not there are also short-term effects of oestrogen levels on spatial memory. Frye (1995) found some evidence for fluctuations in female performance with oestrous cycle and Warren & Juraska (1997), who tested females at a single point in their oestrous cycle, showed that females in oestrus performed significantly better than females in pro-oestrus in a Morris water maze task. On the other hand, Bucci *et al.* (1995) and Berry *et al.* (1997) found no differences in performance by females across the oestrous cycle.

In this experiment, we wanted to determine whether or not there was any evidence for changes in spatial learning and memory or in activity level within females across several oestrous cycles which might provide behavioural support for the fertility and parental care hypothesis. We tested rats in a Morris water maze with a working spatial memory task. The animals had four swims a day and the location of the platform was changed each day. Females were tested across several oestrous cycles and males tested for the same number of days. We predicted that, across the oestrous cycle, there would be no sex differences in performance but that females in oestrus would perform better than when they were in all other parts of the cycle. Variation in activity levels should correlate with these cognitive changes.

## 2. METHODS

### (a) *Subjects*

Eight male and seven female Lister hooded rats (200–250 g and aged 70+ days), obtained from Harlan UK, Ltd, were the subjects tested in this experiment. They were housed in same-sex pairs with *ad libitum* pellet food and water and maintained under a 12 L:12 D cycle (lights on at 05.30) at 23–24 °C.

### (b) *Apparatus*

The experiments were carried out using a Morris water maze. This consisted of a circular, fibreglass tank (diameter 195 cm and depth 75 cm) filled to a depth of 32 cm with water (20–23 °C) to which was added 500 ml white flooring latex. This caused the water to become opaque. An escape platform (white PVC of diameter 11 cm and depth 31 cm) was hidden 1 cm below the surface of the water 30 cm from the edge of the pool in one of four possible locations (the four main compass points, designated N, S, E and W). For each of the platform locations there were four start locations at the edge of the pool, two at 140 cm and two at 110 cm from the platform. A video camera (4 mm and wide angle lens) was mounted on the ceiling above the pool and was connected to a black and white video monitor and video recorder. A number of visual cues surrounded the pool, including black and white cardboard shapes on the walls of the room as well as shelving and a wall fan heater. Geometric cues were also available as the pool was not positioned in the centre of the room. Rather it was situated 33 cm from the nearest wall and 194 cm from the furthest.

### (c) *Protocol*

The day of the oestrous cycle was determined daily for each female by taking vaginal smears and assessing the proportions of cell types present in the smear (Turner & Bagnara 1976). The four stages of the oestrous cycle are oestrus, met-oestrus, di-oestrus and pro-oestrus, with a day for each stage. Smears were taken at 13.30 each afternoon, 1 h prior to experimentation. Males were also handled at this time for a similar period (1–2 min). Handling and smear testing began one month prior to the start of the experiment and continued throughout. Subject females were required to have exhibited at least five, consecutive, four-day oestrous cycles prior to the start of the experiment. As a result one female was excluded from the study and, thus, the experiment was carried out using seven females and eight males.

### (d) *Training*

During training all rats made four swims to the hidden platform each day (Stewart & Morris 1993). All swims were made during the afternoon in an attempt to reduce variation in their activity levels. The location of the platform was the same for the four swims for all rats on each day and it was moved each day to one of the four possible locations. The location was pseudo-randomly assigned such that the platform did not occupy the same place as used in the previous two days running.

For each swim the rat was placed in the water at the start location facing the side wall. A swim began as soon as the rat was released and stopped when the rat touched (and subsequently climbed onto) the platform. The time taken by the rat to swim to the platform was recorded to the nearest second. Swims were observed via the video monitor in an attempt to reduce distractions to the rat. If the rat failed to find the platform within 120 s it was guided to the platform. Once on the platform the rat was allowed to remain there undisturbed for 30 s before being removed and towel dried. The drying took 30 s and the second trial followed immediately. Once all four swims had been completed the rat was dried off and placed in a holding cage under a heat lamp for further drying for *ca.* 15 min before being returned to its home cage. Training took place over 12 days.

### (e) *Experimental testing*

The procedure for testing was the same as in training with each rat completing four consecutive swims a day with the exception that there was no drying carried out between swims. The intertrial interval consisted solely of the 30 s the rat spent on the platform at the end of each swim. Testing was carried out over 16 days during which the females each completed four, full oestrous cycles. The time (s) the rats took to reach the platform was measured, as was swimming speed, taken from the distance swum in the first 15 s on the first swim of each day.

## 3. RESULTS

Analyses of variance (ANOVA) with repeated measures were used to compare the performances of the different groups of rats.

### (a) *Effect of test day*

While the main purpose of this experiment was to compare the performance of males with that of females on different days in their oestrous cycle, it was first necessary to examine the effect of day through the experiment owing to the lack of synchronization of oestrous cycling amongst the females. From the ANOVA (main effects of sex, day and swim number within day), there was no significant overall effect of day ( $F_{15,195} = 1.53$  and  $p = 0.10$ ), no effect of sex ( $F_{1,13} = 2.88$  and  $p = 0.11$ ) and a highly significant effect of swim number ( $F_{3,39} = 64.85$  and  $p < 0.0001$ ). There was only one significant interaction, between day and swim number ( $F_{45,585} = 2.01$  and  $p < 0.005$ ). Examination of the data showed that there was no trend across days for a change in swim times for either sex.

### (b) *Effects of sex and oestrous cycle on time taken to reach the platform*

There were significant effects on performance of day in the oestrous cycle (oestrus, met-oestrus, di-oestrus and pro-oestrus) and swim number within day (swims 1–4) (day in the oestrous cycle  $F_{3,39} = 4.13$  and  $p = 0.012$ , and swim number  $F_{3,39} = 67.67$  and  $p < 0.0001$ ) but no significant effect of sex or number of oestrous cycle (cycles 1–4) (sex  $F_{1,13} = 3.12$  and  $p = 0.10$ , and number of oestrous cycles  $F_{3,39} = 2.18$  and  $p = 0.11$ ; see figures 1 and 2). There were significant interactions between sex and day in the oestrous cycle ( $F_{3,39} = 4.54$  and  $p = 0.008$ ) and between day in the oestrous cycle and swim number ( $F_{9,117} = 3.39$  and  $p = 0.001$ ).

The interaction between day in the oestrous cycle and swim number appeared to be due to the females requiring an extra swim on oestrus days before reaching asymptotic performance (figure 3). The female data from swim 2 of each day of the cycle were analysed using a repeated-measures ANOVA. There was a significant difference in the time taken to reach the platform on swim 2 across the oestrous cycle ( $F_{6,18} = 4.50$  and  $p = 0.016$ ). This was due to female rats taking a significantly longer time to reach the platform on swim 2 on oestrus days than on any of the other three days of the cycle (linear contrast  $F = 13.39$ , d.f. = 1 and  $p = 0.002$ ).

When the female data were combined across day in the oestrous cycle and compared to the male swim performances there was no sex difference. For both sexes there

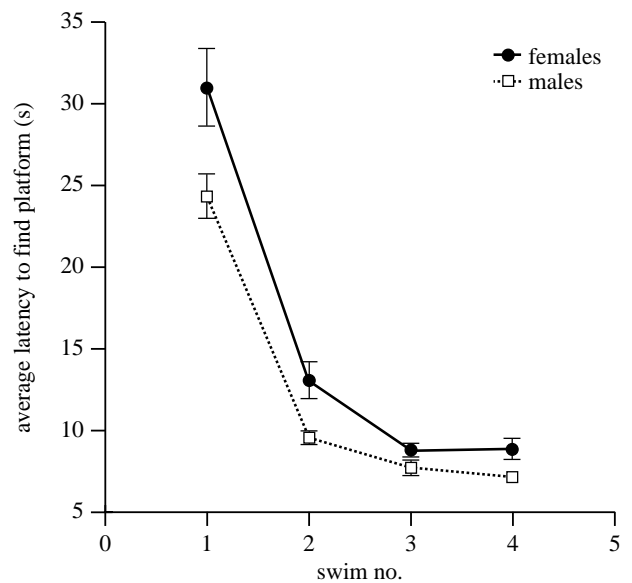


Figure 1. Mean times ( $\pm$  s.e.) taken to find the platform (s) for male and female rats for the four daily swims.

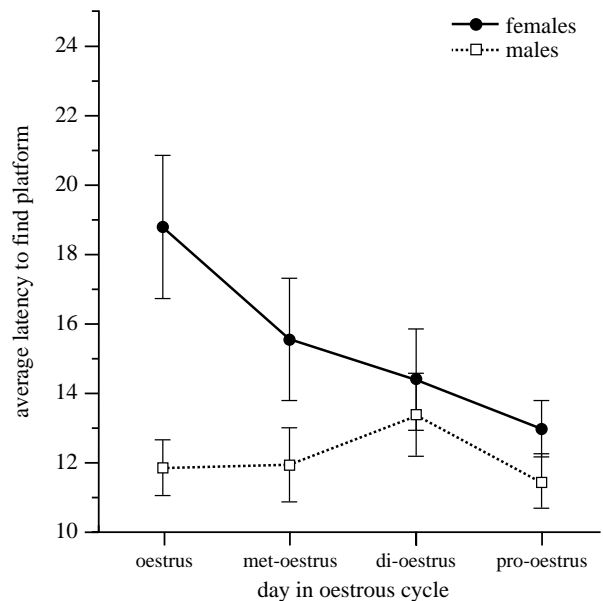


Figure 2. Mean times ( $\pm$  s.e.) taken to find the platform (s) for male and female rats for the four days in the oestrous cycle.

was a significant effect of swim number: the time taken to reach the platform declined markedly between swims 1 and 2 and appeared to have reached a plateau by swim 3 (figure 1). However, when the female data were divided into the four phases of the oestrous cycle and compared with the male data recorded on the same days, there was a significant interaction between the time taken to reach the platform and sex. Males varied little in the time taken to reach the platform while the females' performances were correlated with day in the oestrous cycle and took the longest to reach the platform on the days they were in oestrus (figure 2). When the female data were further subdivided into the four swims within the different days in the oestrous cycle, there was significant variation. On oestrus days females required two swims before reaching a plateau in performance, a requirement not exhibited by

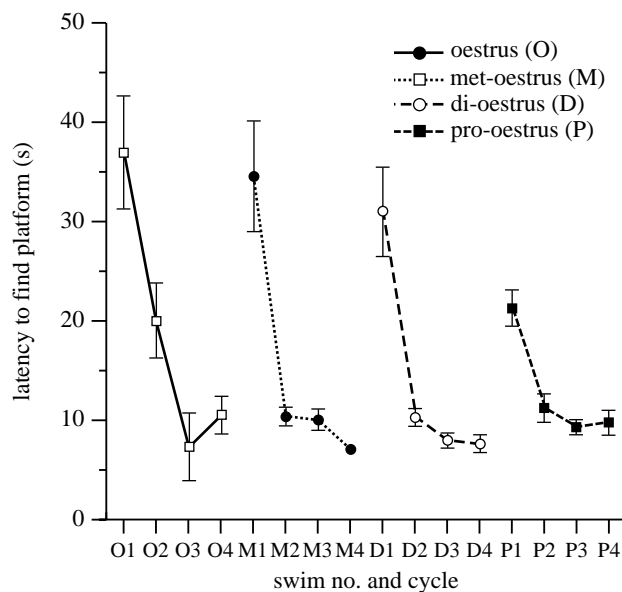


Figure 3. Mean times ( $\pm$  s.e.) taken to find the platform (s) for females for the four daily swims on the four days of the oestrous cycle.

females on any of the other three days in the oestrous cycle (figure 3). On pro-oestrus days the females took less time to reach the platform on the first swim of the day than they took on any of the other three days within the cycle.

#### (c) Effects of sex and oestrous cycle on swim speed

There was no significant difference in swim speeds between males and females ( $F_{1,13}=1.95$  and  $p=0.186$ ) and no effect of cycle number ( $F_{3,39}=0.163$  and  $p=0.921$ ), but there was a significant effect of day in the oestrous cycle ( $F_{3,39}=4.692$  and  $p=0.007$ ). More importantly, there was a significant interaction between sex and day in the oestrous cycle ( $F_{3,39}=4.109$  and  $p=0.013$ ). The male swim speed was constant across the days in the oestrous cycle (as would be expected), whereas there was variation in the swim speeds of the females across the cycle (figure 4). Females swam slowest on met-oestrus and di-oestrus days. There were no other significant interactions.

#### (d) Long-term memory

Although the task we trained and tested the rats on was a relatively short-term one (each trial taking less than 10 min), it was possible to determine whether or not the rats remembered the location of the platform from the previous day. We assessed this by measuring the percentage of time in the first swim of the day that was spent in the quadrant in which the platform had been on the previous day. These data were arcsine square-root transformed before analysis. There was no effect of sex or day in the oestrous cycle on the percentage of time in the first swim spent in the quadrant occupied by the platform on the previous day (sex  $F_{1,13}=0.542$  and  $p=0.475$  and number of oestrous cycles  $F_{3,39}=2.225$  and  $p=0.101$ ). There was a single significant interaction, between sex and day in the oestrous cycle ( $F_{3,39}=3.074$  and  $p=0.039$ ). This would appear to be due to the observation (see figure 5, untransformed data) that, on two days of the cycle, males spent more time in this quadrant and, on two days, females spent more time in this quadrant than did males. On all days of

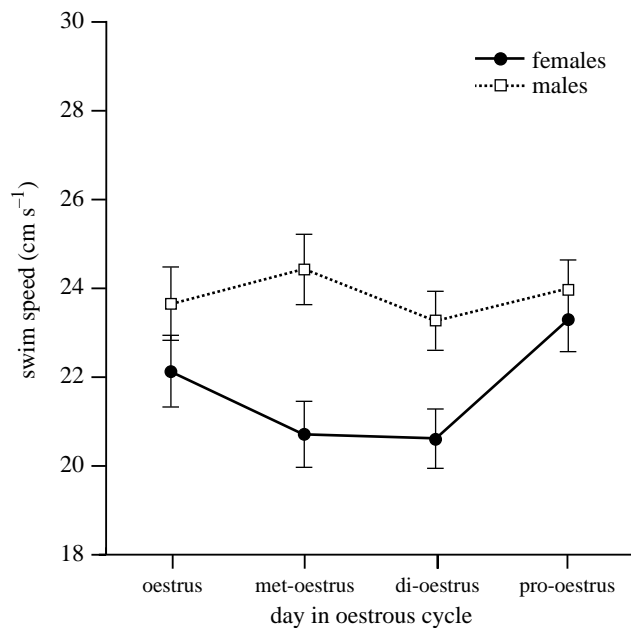


Figure 4. Mean swim speeds ( $\text{cm s}^{-1}$ ) ( $\pm$  s.e.) for males and females for the first 15 s of the first swim of each day.

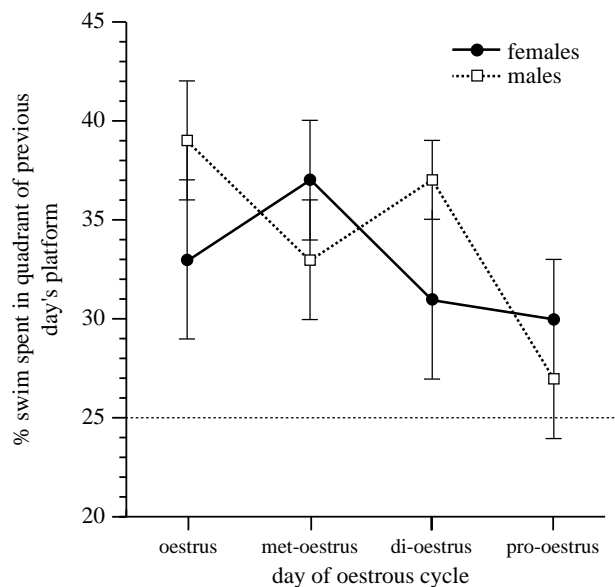


Figure 5. Mean percentages ( $\pm$  s.e.) of the first swim of each day spent in the quadrant containing the platform on the previous day for each of the four days of the oestrous cycle.

the oestrous cycle both males and females spent more time than would be expected by chance in the quadrant occupied by the platform on the previous day (one-sample  $t$ -tests: females  $t=6.943$ – $13.321$ ,  $p<0.0001$ – $0.0004$  and d.f.=6, and males  $t=9.443$ – $12.796$ , all  $p<0.0001$  and d.f.=7).

## 4. DISCUSSION

The results of this experiment are as follows.

- (i) There was no overall sex difference in the time taken to reach a submerged platform in a Morris water maze.
- (ii) The first swim of each day took much longer than the three which followed.

- (iii) On the first swim of the day, irrespective of day in the oestrous cycle, both male and female rats spent longer in the quadrant occupied by the platform on the previous day.
- (iv) Females on the day of oestrus took two swims to reach an asymptote in the time taken to reach the platform.
- (v) Females on the day of pro-oestrus took less time to reach the platform on the first swim than on any of the other three days in the oestrous cycle.
- (vi) Males swam the first swim of each day at a constant rate whereas the female swim speed varied across the oestrous cycle.

As we predicted, we found no sex differences in any of our comparisons. This result is in agreement with those of Berry *et al.* (1997). While Warren & Juraska (1997) did not explicitly compare the sexes (they divided the female data into the different oestrus phases and did not report a main effect of sex), it seems unlikely from viewing their data that statistical analyses would have shown any sex differences. Other tests of spatial memory using a Morris water maze have typically used a reference memory task (e.g. Morris *et al.* 1982; Bucci *et al.* 1995), in which the rats learn to find a hidden platform in the same location over a number of days. These tests have frequently found that males outperform females (e.g. Williams *et al.* 1990; Roof & Havens 1992; Roof 1993). However, they will necessarily have tested females across several oestrous cycles. If females do underperform on some days (specifically oestrus days), then comparisons between males and females on tests across days should show either no differences or a male advantage. The time spent by males and females in the first swim of the day in the quadrant in which the platform had been located the previous day in our experiment gives some indication as to performance on a reference memory task. While the sexes differed across days in the length of time spent in the previous day's quadrant, there was no consistent effect of males outperforming females.

We also predicted that females would perform better on oestrus days than on other days in the cycle. This was not what we found. On oestrus days, females took longer to learn about the platform than on other days in their cycle. That this was not simply an effect of swim speed can be inferred by two aspects seen in figure 3: the time taken to reach the platform on the first swim was consistent across oestrus, di-oestrus and met-oestrus and the time taken to reach the platform reached the same asymptotic level across all four days of the cycle. It was only on oestrus days that the females required the second swim before reaching asymptotic time levels. In addition, swim speed (measured during the first swim) was not slowest on oestrus days. We interpret these results to mean that it was only on oestrus days that the females needed two swims to be sure of the platform's location. These data do not agree with the results of Warren & Juraska (1997) who found that oestrus females performed better than pro-oestrus females. There are two conspicuous differences in our experimental designs which might explain the difference in experimental outcome. The first concerns the much more extensive training our rats were given before testing: one month of handling and 20 days (with four swims a day) training in the Morris water bath contrasted with Warren & Juraska's (1997) two weeks

handling (at least) and three training swims only before testing. The latter in particular may be a crucial difference. Prior experience in a water bath has been shown to affect subsequent learning performance in males (e.g. Bannerman *et al.* 1995) and Perrot-Sinal *et al.* (1996a) found that prior training in a water maze improved the performance of both males and females but eliminated the male advantage. Perrot-Sinal *et al.*'s (1996a) explanation for this result was that the sex differences seen in maze performance might be due to the differential effects of stress on males and females. Thigmotaxis, a fear response, is highest at the beginning of training in the maze and also higher in females than in males (e.g. Saucier & Cain 1995; Perrot-Sinal *et al.* 1996b). Galea *et al.* (1994) found that administration of naloxone to meadow voles prior to testing in a water maze facilitated maze acquisition in females but not males. Naloxone is an opiate antagonist and, thus, is predicted to facilitate spatial learning under stressful conditions. It seems possible that the lack of sex differences found in our experiment may have been due to the stress reduction brought about by the pre-training which we gave to all of the rats.

The second difference in experimental design between our experiment and that of Warren & Juraska (1997) is that our rats had only four swims a day, one immediately after another, while their rats had 16 swims, each several minutes apart and with 1 h separating the first eight swims from the second eight. It is not clear how these procedural differences might explain the difference in our results but it is striking that their rats, even on their last four out of 16 swims of the day, were not reaching the platform as speedily as our rats on their third swim (in our slightly larger maze).

Our results are also consistent with the explanation that there is simply a rise in exploratory behaviour (presumably accompanied by a change in motivation) in females on oestrus days (Finger 1969; Birke 1979). Indirect evidence that this is not entirely the explanation for our results comes from the data shown in figure 5 in which females do not spend more or less time in the previous day's quadrant on oestrus days relative to other days in the cycle. In addition, female swim speed is no slower on oestrus days than on other days in the cycle.

Although when in oestrus the females took an extra swim to learn the location of the platform, their asymptotic level of performance (reached on swim 3) did not differ from that on other days or from males. It appears then that there is an effect of hormonal status on spatial learning but not on spatial memory (insofar as this test assesses this; see also Daniel *et al.* 1997). The rapid change in spatial behaviour we observed between pro-oestrus and oestrus corresponds to the timing in changes in LTP across the oestrous cycle found by Warren *et al.* (1995) as well as the changes in dendritic spine density (Gould *et al.* 1990). The effects of circulating hormones on spatial abilities during development have been convincingly demonstrated in rats and in other rodent species (e.g. Galea *et al.* 1995, 1996) but this is the first study to show an effect of circulating hormones, in the direction predicted by neurophysiological and neuroanatomical data, on a spatial working memory task in a Morris water maze in female rats. With respect to the current evolutionary scenarios for putative sex differences in spatial ability, we find no support for those which

propose a male advantage (e.g. Gray & Buffery 1971; Gaulin & FitzGerald 1986), no support for a female advantage (e.g. Eals & Silverman 1997), and no support for the prediction from Sherry & Hampson (1997) that high levels of oestrogen should be correlated with low spatial ability. Our data support the proposal of Sherry & Hampson (1997) that spatial abilities in females do differ across the oestrous cycle but that high rather than low levels of oestrogen are correlated with better female spatial learning. Measurement of hormone levels at the time of task performance will be required to confirm that this really is the case as will determination of the temporal relationship between circulating hormone levels and changes in spatial performance.

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