Sex differences in spatial cognition are not caused by isolation housing

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Summary

In mammals, males typically have better spatial ability than do females. However, most of the data come from laboratory tests and it is possible that factors impacting on the captive animal cause the observed sex differences in spatial cognition. A common influence on cognitive ability is stress, which may have its effect acutely, in the testing situation, or chronically, due to the housing conditions. We used a spatial working and reference memory task (the Morris water maze) to investigate if isolation housing had a differential impact on spatial cognition in male and female rats. Either as juveniles or as adults, rats were housed in pairs or in isolation. We also manipulated the duration of isolation housing. Regardless of housing condition, we found a sex difference in spatial ability only in the youngest rats. However, we found no evidence that isolated rats were spatially impaired relative to pair-housed rats. We also found no difference in body weight, food intake or bar biting behaviour (indicators of welfare in rodents) between pair and isolated rats. We conclude that isolation housing causes insufficient stress to cause sex differences in spatial cognition.

Keywords: isolation housing, spatial cognition, sex differences, Morris water maze, rats.

Introduction

Males and females differ in morphology, physiology and, seemingly, in cognitive ability. The most consistent demonstration of a cognitive difference between the sexes is that males have better spatial skills than do females (Gaulin & Fitzgerald, 1986; Williams et al., 1990; Lacreuse et al., 1999; As-

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tur et al., 2004; Jones & Healy, 2006). A number of evolutionary hypotheses (at least seven) have been proposed to explain this sexual dimorphism, the most strongly supported of which is the 'range size hypothesis', which proposes that relatively larger territory size selects for superior spatial ability in males (Jones et al., 2003). There are experimental data that conform to this prediction. For example, male meadow voles (*Microtus pennsylvanicus*) have larger home ranges than do conspecific females, superior spatial ability and a larger hippocampus (the area of the brain associated with processing spatial information) (Gaulin & Fitzgerald, 1986; Gaulin & Fitzgerald, 1989). Conversely, the closely related pine vole (*Microtus pinetorum*) has no sexual dimorphism in home range size, spatial ability or size of the hippocampus (Jacobs et al., 1990).

Although there is debate as to the ultimate causes for a sexual dimorphism in spatial cognition, the proximate causes are well characterised. In mammals, at least, both organisational (before or soon after birth) and activational (circulating) levels of sex hormones impact on spatial ability throughout life. For example, girls exposed to high prenatal levels of testosterone have better spatial abilities than girls exposed to normal levels and administration of testosterone to newborn female rats results in the development of a male-like hippocampus and spatial ability akin to that of untreated males (Roof & Havens, 1992; Grimshaw et al., 1995). Conversely, administration of testosterone to newborn male rats impairs adult spatial ability and neonatal castration of males (removal of a major source of testosterone) reduces adult spatial ability, indicating a possible U-shaped response curve between testosterone and spatial ability, with optimal levels in the low male range (Williams et al., 1990; Roof, 1993a).

Stress hormones also influence spatial ability. For example, social isolation of preweanling aged rats increases levels of the stress hormone corticosterone (CORT) and results in poor spatial ability in adulthood (Frisone et al., 2002; Sandstrom & Hart, 2005).

The majority of data for a sex difference in spatial ability come from laboratory experiments with rodents, largely rats. Male rats typically outperform females in the Morris Water Maze (MWM) and in a number of other spatial tests in the laboratory (Williams et al., 1990; Roof, 1993a,b; Perrot-Sinal et al., 1996; Seymoure et al., 1996; Markowska, 1999; Beiko et al., 2004). Although a male advantage is not always found, females rarely outperform males (Juraska et al., 1984; Bucci et al., 1995; Warren & Juraska, 1997;

Healy et al., 1999; Lukoyanov et al., 1999; Roof & Stein, 1999). However, one potential problem with testing spatial cognition in the laboratory is that laboratory conditions may themselves affect the motivational and physiological state of the animal. Perhaps the most obvious example is that stress responses of males and females to their environment may differ and this may differentially affect cognitive performance between the sexes (Beck & Luine, 2002).

Laboratory rats tested in spatial tasks may be exposed to stress from at least two sources. Firstly, the cognitive test itself may be a major source of stress for a laboratory rat. Spatial tasks such as the MWM are considered to cause stress because the animals are required to swim, usually in brightly lit conditions, to a platform hidden below the surface of the water at some distance from the side of the pool. Indeed, the UK Home Office classes the MWM as a licensable procedure due to the stress that it causes. Secondly, housing conditions of laboratory rats could be a potential source of stress. For example, the practice of housing rats alone may cause significant increases in stress for this naturally gregarious species (e.g., Patterson-Kane, 2004). Hatch et al. (1963) reported that after just four weeks of isolation, rats may be more aggressive, difficult to handle and show varying physiological impairments.

Concern about the effects of stress arises because the sexes appear to differ in their response to stress: females seem to cope less well than do males (e.g., Shors & Miesegaes, 2002). Certainly there is evidence that a sexually dimorphic response to stress during MWM testing can cause a sex difference in performance in rodents (Galea et al., 1994; Perrot-Sinal et al., 1996; Beiko et al., 2004). For example, thigmotaxis (swimming close to the sides of the pool) is considered a marker of stress shown by rodents in open-field situations and females tend to engage in more thigmotaxic swimming in the MWM than do males. This will tend to result in impaired performance because the platform is never located at the edge of the pool (Treit & Fundytus, 1989; Perrot-Sinal et al., 1996; Beiko et al., 2004; Herrero et al., 2006). Furthermore, there is also evidence that females respond less well to isolation housing than do males. For example, females show more escape-oriented behaviours (and more 'odd' behaviours such as tail chasing and bar chewing) when isolated (Hurst et al., 1998).

It is not yet clear whether the stress caused by isolation housing, either alone or in concert with the stress of swimming in an MWM task, can bring about the observed sex difference in spatial cognition. To address this ques-

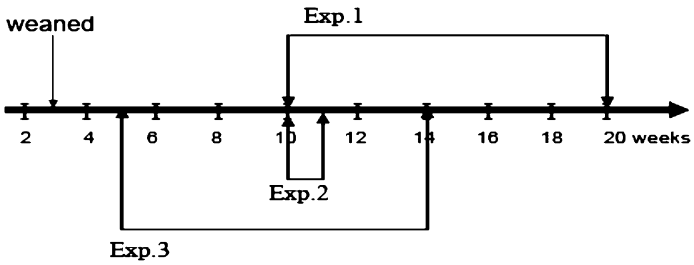

Figure 1. Schematic of experimental time lines. We investigated the effect of isolation housing on spatial cognition in male and female Lister Hooded rats: in Experiment One, rats, aged 10 weeks at onset of the experiment, were tested after 10 weeks of isolation/pair housing; in Experiment Two, 10-week old rats were tested after one week of the housing treatment; in Experiment Three, rats, aged five weeks at the onset of the experiment, were tested after 10 weeks of isolation/pair housing.

tion we housed male and female Lister Hooded rats alone or in pairs and tested their spatial cognition in an MWM. We sought to test whether isolation housing, purported to be stressful, could cause sufficient stress to impact on spatial cognition (possibly to a greater degree in females) and, thus, result in a sex difference in spatial ability. During MWM testing we measured thigmotaxis as an indicator of stress. The MWM protocol we used allowed us to measure two different aspects of spatial memory: working memory (memory for within-trial specific information) and reference memory (memory for between trials). Additionally, we manipulated the timing and duration of isolation housing (see Figure 1). We also measured body weight and food intake, and observed barbiting behaviour, all thought to be indicators of welfare in laboratory rodents (Würbel & Stauffacher, 1996; Hurst et al., 1998). We predicted (1) that the stress brought about by isolation housing would impair performance relative to pair housed controls; and (2) isolated females would perform more poorly than the other groups, such that a sex difference would only be seen in the isolated rats.

Experiment One

Methods

Subjects and housing

The subjects were 18 male and 18 female Lister Hooded rats, age 8–10 weeks obtained from Harlan UK. At time of arrival males weighed 250–

270 g and females 170–190 g. Six rats of each sex were chosen at random and housed in isolation, the remaining 12 were housed in same-sex pairs ($N = 6$ for each treatment group). Rats remained in their respective housing condition throughout the entire experiment. Paired rats were marked with hair dye (Schwarzkopf, R43) to enable identification. One rat from each pair was picked at random (dominance hierarchies, as determined by pinning rate 1, 7, 8 and 9 weeks post arrival, were unstable) to be the focal animal and this rat remained the only source of data from the pair for the duration of the experiment.

All rats were housed in standard plastic bottomed cages, dimensions 45 × 28 × 20 cm (North Kent Plastic Cages, UK). Due to the nature of the cages, visual, olfactory and auditory communication between neighbouring rats was not prevented. Rats were provided with ad libitum pellet food (RM3 diet, Special Diet Services, UK) and tap water, and maintained under a 12L/12D cycle (lights on at 0600 hours) at 21–24°C.

Rats experienced their respective housing condition for 10 weeks before spatial ability was assessed using the MWM. Each isolated and focal rat was tested in the MWM. Rats were killed in a carbon dioxide chamber at the end of the experiment.

Animal treatment and husbandry were approved by the Animals Scientific Procedures Act 1986, UK, and all procedures were performed in accordance with current United Kingdom Home Office regulations.

Morris water maze apparatus

The MWM consisted of a circular tank made out of glass-fibre approximately 2 m in diameter, 65 cm high, with the bottom of the MWM raised 50 cm above floor level on a custom-built platform. The MWM was situated in an experimental room (dimensions 4.25 × 2.9 m) such that geometric cues (maze was not in the middle of the room) and landmark cues (e.g., light fittings, posters and shelving on walls) were available. The tank was filled to a depth of 32 cm with tap water, which was made opaque by the addition of 500 ml flooring latex, and maintained at 24±1°C. An escape platform (white PVC of diameter 11 cm) was located 2 cm below the surface of the water and 30 cm from the edge of the pool in one of four possible locations (the four main compass points N, E, S or W). For each of the platform locations there were four possible release points into the pool: NE, SE, SW and NW. All

trials were videotaped from above using a camera with a 4-mm wide-angle lens. To reduce both stress and distraction, all trials were observed via a video monitor.

Working memory procedure

Testing occurred during the light phase, to be consistent with the majority of published MWM research, which also uses daytime testing. Each rat received two days of training before testing began. During training each rat was given two swims to the hidden platform each day. The platform location was the same within a day, but changed position each day. Platform location was pseudo-randomly determined so that it was never in the same place two days in a row.

For each swim the rat was gently lowered into the water at its predetermined release point and released facing the side of the tank. A swim started when the rat was released and finished when the rat found and subsequently climbed onto the platform. The time taken by the rat to find the platform was recorded to the nearest second. If the rat failed to find the platform within 120 s it was gently guided to, and allowed to climb onto, the platform. Once on the platform the rat was left for 20 s before being picked up and released from one of the other three possible release points. After the final swim the rat was left on the platform for 20 s and then gently removed from the platform, towel dried, put back in its home cage and placed under a heat lamp for approximately 10 min to dry.

Testing started the day following the two days of training and was exactly as for training with the exception that each rat received four swims each day for 16 consecutive days in total. All MWM procedures were carried out by the same researcher (AH) to reduce stress associated with being handled by different people.

Reference memory procedure

Reference memory was assessed from day two of testing to day five. The percentage of time that a rat spent swimming in each of the four quadrants of the maze in the first swim of each day (days 2–5 only) was recorded. The quadrant that contained the platform was discounted and the proportion of time spent in the remaining three quadrants was calculated to establish if a rat spent more than 33.3% (chance) of its time searching in the target quadrant (the quadrant that contained the platform on the previous day).

Thigmotaxis

The percentage of time that a rat spent swimming within 15 cm of the wall of the maze was recorded for swims one and two on all test days.

Monitoring body weight and food intake

Body weight was measured at least once per week throughout the entire experiment for each isolated rat and each focal rat from each pair. Food intake was measured at least once per week from the second week post arrival to the week prior to MWM testing. To measure food intake, the entire contents of a food hopper (one per cage) were weighed before the food was topped up and re-weighed. Food intake per rat per day was estimated by dividing amount eaten by number of days since last weighed. Where rats were pair housed an average intake was calculated for both of the rats.

Behaviour in home cage

The behaviour of the rats in their home cage was recorded by video camera (Sony mini DV digital handicam). Each cage was filmed for 10 min during the morning in the first and second months post arrival at the animal unit. This footage was then scored for the presence (1) or absence (0) of bar biting during 30-s intervals across the 10 min of footage. Every isolated rat and the focal rat from each pair were observed.

Analysis

All data were analysed using the statistical software package JMP (version 5.1 for Apple MAC). Repeated measures data were analysed using a Repeated Measures Analysis of Variance (RM ANOVA); between-subject factors were sex (male and female) and housing condition (pair and isolated), and within-subject factors were trial (1–4) and day (1–16). All of these factors were included in the analyses, while interactions between main effects that were not significant were removed. The Mauchly-criterion test was used to test for sphericity (the assumption that repeated measures have equal variances and that the correlations between any two measures are the same). When the assumption of sphericity was not met, the Greenhouse–Geisser-adjusted degrees of freedom and the associated p-values were used (this is

why the degrees of freedom reported are not always whole numbers; Quinn & Keough, 2002).

The assumptions of normality of residuals and homogeneity of variance were tested and appropriate transformations applied to the data, where necessary. Tukey's Honestly Significant Difference test ($p < 0.05$) was used to perform post hoc comparisons.

Results

Working memory

There was no significant effect of sex ($F_{1,21} = 0.80$, $p = 0.38$) or housing ($F_{1,21} = 0.25$, $p = 0.62$) on the time taken to find the platform (Figure 2a). The sex by housing interaction was not significant and so was removed from the model. There was a highly significant effect of swim number on the time taken to find the platform: rats learnt the location of the platform during swim one and swam almost directly to it in all three subsequent swims (swim: $F_{1.3,27.4} = 154.20$, $p < 0.0001$; Figure 2a).

There was a significant effect of day on the time taken to reach the platform: as the days progressed the rats took less time to locate the platform ($F_{5.3,110.9} = 9.34$, $p < 0.0001$). No other interactions were significant.

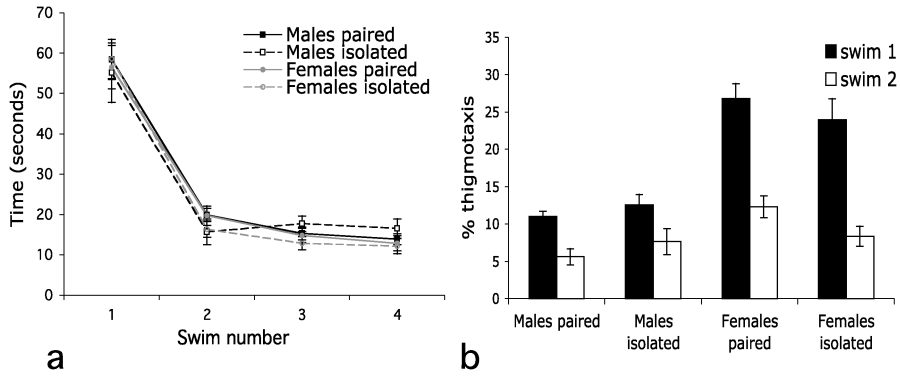

Figure 2. (a) Time taken to find the platform (mean ± SE) in swims one to four for male and female rats that were either pair or isolate housed. Swim times are averages across the 16 days of testing, analyses were conducted on daily swim data ($N = 6$ per treatment group). (b) Proportion of swim one and two (mean ± SE) spent swimming thigmotaxically. Means were calculated for each rat on each day and then averaged over the 16 days of testing ($N = 6$ per treatment group).

Reference memory

The data were arcsine square-root transformed to comply with assumptions of normality and homogeneity of variance. There was no effect of sex or housing condition on the proportion of time spent in the target quadrant (sex: $F_{1,21} = 0.21$, $p = 0.65$; housing: $F_{1,21} = 1.94$, $p = 0.18$). The sex by housing interaction was not significant. There was no effect of day ($F_{1.5,32.8} = 1.87$, $p = 0.18$) and all interactions with day were not significant.

The proportion of time spent in the target quadrant by each rat was averaged across days 2–5, and pooled across sex and housing condition (since there was no significant effect of these factors) and tested against chance 33.3% (the quadrant that contained the platform was ignored) using a two tailed one sample t-test. Rats spent an average of 40% of swim one in the target quadrant; significantly longer than expected by chance ($t_{23} = 2.15$, $p = 0.042$).

Thigmotaxis

There was a significant effect of swim number on thigmotaxis: thigmotaxis was significantly lower in swim two than in swim one ($F_{1,20} = 71.63$, $p < 0.0001$; Figure 2b). There was no effect of housing on thigmotaxis ($F_{1,20} = 0.47$, $p = 0.50$). There was a significant effect of sex ($F_{1,20} = 55.54$, $p < 0.0001$), which was dependent on housing condition ($F_{1,20} = 4.99$, $p = 0.034$). Paired males were significantly less thigmotaxic than paired females (Tukey HSD, $p < 0.05$) but isolated males and females were equally thigmotaxic (Figure 2b). The effect of sex was also dependent on swim number ($F_{1,20} = 17.13$, $p = 0.0005$). Males were less thigmotaxic than females in swim one (Tukey HSD, $p < 0.05$) but not in swim two.

Behaviour in home cage

Males and females, irrespective of housing condition, were equally likely to bite the cage bars ($p > 0.05$, Fisher's exact test). During the observation period in the first month post arrival, four males and seven females spent approximately 5–25% of the time bar biting. During the observation period in the second month post arrival, only one paired female did any bar biting for 5% of the 10-min observation period, suggesting a decrease in this behaviour

that was independent of sex and housing condition. Overall, regardless of sex or housing condition, the occurrence of bar biting was extremely low, with the majority of bar-biting occupying not more than 5–10% of the observation period.

Body weight and food intake

Males weighed more than the females ($F_{1,21} = 513.50$, $p < 0.0001$). Housing condition had no impact on body weight ($F_{1,21} = 2.22$, $p = 0.15$). All rats gained weight as the weeks progressed and males appeared to gain weight at a faster rate than females (week post arrival: $F_{2.9,55.6} = 621.04$, $p < 0.0001$); sex by week interaction: $F_{2.9,55.6} = 133.02$, $p < 0.0001$). The housing by week interaction was not significant.

Males ate more than the females at all time points ($F_{1,21} = 387.93$, $p < 0.0001$). On average, males ate 24 $\pm$ 2 g and females ate 16 $\pm$ 1 g ($N = 12$). Housing condition had no impact on food intake ($F_{1,21} = 2.41$, $p = 0.14$). There was a highly significant effect of week on food intake ($F_{6.0,126.3} = 7.97$, $p < 0.0001$). This effect appears to be due to natural fluctuations in the amount of food eaten as the weeks progress, and not a directional trend, e.g., for the rats to eat more as they age. No other interactions were significant.

Discussion

We found no significant effect of 10 weeks of isolation housing on working or reference memory in either male or female rats, nor did we find a sex difference in spatial ability. Similarly, body weight, food intake and the amount of time spent bar biting (a behavioural indicator of poor welfare) were not significantly affected by housing condition. Males were less thigmotaxic in the MWM than females, but only if pair housed, and only in swim one.

Thigmotaxis decreased significantly from swim one to swim two in all rats. Male and female rats performed equally well in the MWM task finding the platform equally quickly across the four daily swims. They also appeared to retain information about the platform's location some 24 h later, spending significantly longer than expected by chance in the previous day's quadrant during swim one. These data are consistent with the results from a number of studies in which sex differences have not been reported (Bucci et al., 1995;

Healy et al., 1999; Roof & Stein, 1999; but see Roof & Havens, 1992; Roof, 1993a; Perrot-Sinal et al., 1996).

Although variation in levels of hormone across the oestrus cycle may influence spatial ability in female rats, the findings are inconsistent. For example, performance on spatial cognition tasks may increase during the pro-oestrus (high oestrogen) phase of the cycle (Healy et al., 1999), decrease during the pro-oestrous phase (Frye, 1995; Warren & Juraska, 1997) or, not change with respect to oestrus phase (Berry et al., 1997; Stackman et al., 1997). As our testing protocol involved testing females over four consecutive oestrus cycles, we would have expected to see a sex difference in each comparison, if oestrus did influence performance.

We did find one sex difference: our pair-housed females were more thigmotaxic than were the pair-housed males during swim one in the MWM. Although this finding is consistent with other reports of higher thigmotaxis in females (Perrot-Sinal et al., 1996; Beiko et al., 2004), we did not find the cognitive performance of our females impaired. However, impairments in MWM performance due to thigmotaxis are generally reported when there are much higher levels of thigmotaxis than in our experiment (e.g., $> 60\%$, Perrot-Sinal et al., 1996; Beiko et al., 2004; Herrero et al., 2006). It is not clear why there should be such a disparity in levels of thigmotaxis as most of the methodological features that might lead to differences are similar in all these experiments, but it appears from our data that animals can spend up to 30% of the swim being thigmotaxic without this behaviour affecting performance. While this might seem surprising, it is possible that the less thigmotaxic males search rather inefficiently for the platform (e.g., zig-zagging across the pool). Certainly by swim two there is no sex difference in thigmotaxis so it appears that despite the higher initial stress in the females, one swim is sufficient to reduce stress levels significantly. This might be because they know where the platform is located or the stress in swim one is largely due to getting wet (as is typical, rats were not dried between swims).

Isolation housing did not affect MWM performance. It may be that stress caused by isolation housing was insufficient to impact on cognitive performance, or that stress levels between isolated and paired rats did not differ. We propose the latter as an explanation for our results, since body weight, food intake and bar-biting levels did not differ between the differentially housed rats.

We chose not to measure corticosterone levels (CORT) because our animals showed no behavioural signs of stress and, thus, it is very unclear what CORT elevation, if found, would mean. There are, also, inconsistencies in the literature with respect to the relationship between CORT levels and behavioural measures of stress. In spite of this, it is often considered to be an appropriate physiological confirmation of behavioural indicators of stress and, indeed, sometimes is used entirely alone to demonstrate stress. With respect to isolation housing, there is no agreement that an elevation in CORT is an accurate indicator of stress, in either male or female rats: in isolated, compared to socially housed, rats have been shown to have elevated (Hatch et al., 1963; Gamallo et al., 1986; Perello et al., 2006), depressed (Hurst et al., 1997) or unaffected levels of CORT (Morinan & Leonard, 1980; Brown & Grunberg, 1995; Scaccianoce et al., 2006).

There are several possible explanations for why isolation housing was not stressful: (1) isolation stress has its most significant effect on young animals and these rats were too old to be affected; (2) visual, olfactory and auditory communication between neighbouring cages may have mitigated the effects of physical isolation; (3) the handling and experimental conditions we imposed were sufficient to offset ill-effects of isolation; (4) although high at first, the rats habituate to isolation stress. The last of these hypotheses was tested in our next experiment.

Experiment Two

In Experiment One 10 weeks of isolation did not have a significant impact on cognitive ability. It is possible that housing exerts stressful effects early in the manipulation but animals habituate to their conditions as time goes on. In Experiment Two, therefore, we investigated whether stress experienced during the initial stages of isolation housing affected performance in the MWM. We housed adult male and female Lister Hooded rats in isolation or in pairs and assessed spatial ability in the MWM after one week of exposure to their respective housing conditions.

Methods

Subjects and housing

In this experiment, six rats of each sex were housed in isolation and 12 rats of each sex were housed in same sex pairs from 10 weeks of age (see

Figure 1). Rats remained in their respective housing condition throughout the entire experiment. One week after the beginning of the housing manipulation spatial ability was assessed using the MWM. Body weight and food intake were measured as for Experiment One. The MWM apparatus and testing procedure were identical to Experiment One.

Behaviour in home cage

A black and white wide-angle lens camera was set up to record home cage behaviour for 6 h during the dark phase, recording started immediately after lights went out at 1800 h. Illumination was provided by a 40 W red light bulb. Film footage was scored for the absence (0) or presence (1) of bar biting behaviour every 30 s across 10 consecutive minutes, starting approximately at 2000 h.

Results

Working memory

Males and females did not differ in the time they took to find the platform ($F_{1,21} = 0.61$, $p = 0.44$; Figure 3a). One week of isolation housing also did

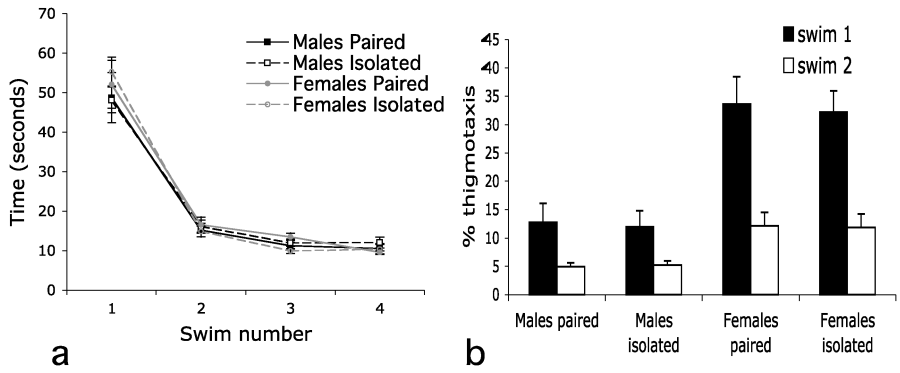

Figure 3. (a) Time taken to find the platform (mean ± SE) in swims one to four for male and female rats that were either pair or isolate housed. Swim times are averages across the 16 days of testing, analyses were conducted on daily swim data ($N = 6$ per treatment group). (b) Proportion of swim one and two (mean ± SE) spent swimming thigmotaxically. Means were calculated for each rat on each day and then averaged over the 16 days of testing ($N = 6$ per treatment group).

not affect the time taken to find the platform ($F_{1,21} = 0.02$, $p = 0.90$). The sex by housing interaction was not significant.

Rats learnt the location of the platform within the four daily swims; the largest decrease in swim time occurred between swims one and two ($F_{1.2,24.4} = 200.4$, $p < 0.0001$; Figure 3a). There was a significant effect of day on performance ($F_{6.1,127.8} = 11.0$, $p < 0.0001$) and a significant interaction between swim number and day ($F_{10.0,210.2} = 3.60$, $p = 0.0002$). The time taken in swim one decreased the most across the experiment (Tukey HSD, $p < 0.05$). No other interactions were significant.

Reference memory

The data were arcsine square-root transformed before analysis. Neither sex nor housing condition had a significant impact on reference memory (sex: $F_{1,21} = 1.76$, $p = 0.20$; housing: $F_{1,21} = 0.04$, $p = 0.85$). The data were averaged across days two to five and pooled across sex and housing condition then tested against chance (33.3%) using a one-sample t-test. The rats spent an average of 43% of swim one in the target quadrant, significantly longer than expected by chance ($t_{23} = 3.65$, $p = 0.013$).

Thigmotaxis

The data were log transformed to meet the assumptions of normality and homogeneity of variance. Thigmotaxis levels decreased significantly by swim two in males and females ($F_{1,20} = 10.67$, $p = 0.0039$). Females had higher levels of thigmotaxis than did males ($F_{1,20} = 51.22$, $p < 0.0001$; Figure 3b). The effect of housing and all other interactions were not significant (p's > 0.05).

Behaviour in home-cage

Bar-biting behaviour did not differ between the sexes ($p = 1$, Fisher's Exact test). However, isolated rats were more likely to bar bite than pair-housed rats ($p = 0.04$, Fisher's Exact test). Bar-biting behaviour was seen in five of the isolated rats for approximately 10% of the observation period but was completely absent in all pair-housed rats.

Body weight and food intake

Males weighed significantly more than females ($F_{1,20} = 774.73$, $p < 0.0001$) and also gained weight at a faster rate (sex by week interaction: $F_{2.3,45.1} = 199.18$, $p < 0.0001$). This was, at least in part, due to males eating more than females ($F_{1,20} = 332.7$, $p < 0.001$). Housing condition had no effect on food intake or on body weight (food intake: $F_{1,20} = 1.5$, $p = 0.24$; body weight: $F_{1,20} = 0.14$, $p = 0.75$). No other interactions were significant.

Discussion

We found no effect of sex or of one week of isolation on working or reference memory in the MWM. However, unlike the results in Experiment One, all females spent more time engaged in thigmotaxis in the MWM than did the males. Again, we found no effect of housing condition on body weight or food intake. However, in Experiment Two isolated rats (regardless of sex) were more likely to bite the cage bars than were pair-housed rats. Since bar biting was seen most in the first month post arrival in all rats, regardless of housing condition, in Experiment One, it seemed possible that stress levels were greatest during that period and would result in impaired performance in a cognitive task. This prediction was not met. Despite the higher incidence of bar biting in isolated rats (possibly indicating greater levels of anxiety) this stress was not sufficient to impact on cognitive ability since performance levels in the MWM were not affected by housing condition, and, were comparable between Experiments One and Two. Similarly, levels of thigmotaxis were not affected by housing condition. In this experiment, although females had higher levels of thigmotaxis in swims one and two, MWM performance did not differ between males and females. It appears that thigmotaxis needs to be greater than 35% to impact on performance.

Coupled with the results from Experiment One, it appears that isolation for neither short nor long periods has much impact on cognitive ability in adult rats. We suggest four possible explanations: (1) the major impact of isolation occurs in young, rather than adult, rats; (2) the strain we used is one that is less susceptible to the stress of isolation; (3) that pair and isolation housing cause equal levels of stress; (4) isolation housing does not cause sufficient stress to impair performance in cognitive tests. We tested the first of these hypotheses in the next experiment.

Experiment Three

To investigate if isolation housing has a greater impact on juvenile rats than it does on adults, male and female Lister Hooded rats were housed in isolation or pairs from five weeks of age, and spatial cognition was assessed, after 10 weeks, in the MWM (Figure 1).

Methods

Subjects and housing

Six Lister Hooded rats of each sex were housed in isolation and 12 rats of each sex were housed in same sex pairs from five weeks of age for 10 weeks before spatial ability was assessed using the MWM. Housing and husbandry conditions and the MWM apparatus and testing procedure were identical to Experiment One. Body weight and food intake were measured as in Experiment One, and filming of in-cage behaviour as for Experiment Two.

Results

Working memory

There was a significant effect of sex on performance: males took less time to find the platform than did females ($F_{1,21} = 5.21$, $p = 0.033$; Figure 4a). There was, however, no effect of housing on performance ($F_{1,21} = 2.11$, $p = 0.16$).

As in Experiments One and Two, there was a significant effect of swim number on time taken to find the platform: the largest decline in swim time occurred between swim one and two ($F_{1.9,39.0} = 409.25$, $p < 0.0001$). No other interactions were significant.

The time taken to find the platform decreased across the experiment ($F_{15,315} = 7.13$, $p < 0.0001$). However, this effect was dependent on the sex of the rats: males improved their overall performance faster than females as testing progressed (sex by day interaction: $F_{15,315} = 2.03$, $p = 0.013$). No other interactions were significant.

Reference memory

The data were arcsine transformed before analysis. There was no main effect of sex or housing on the amount of time spent searching in the target quadrant

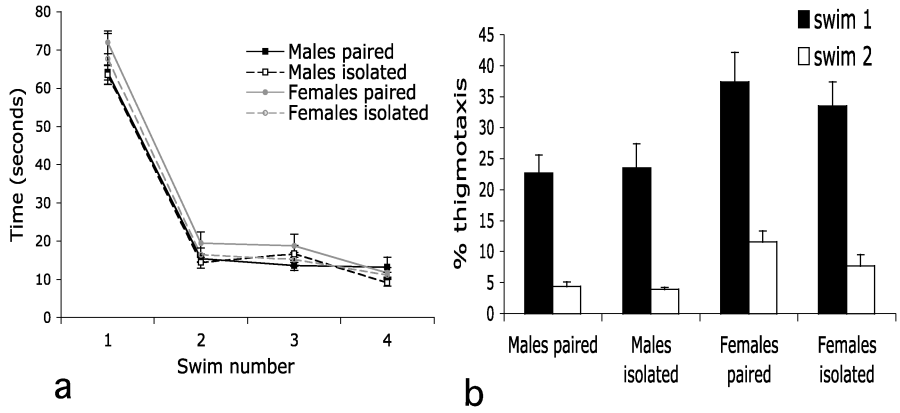

Figure 4. (a) Time taken to find the platform (mean ± SE) in swims one to four for male and female rats that were either pair or isolate housed. Swim times are averages across the 16 days of testing, analyses were conducted on daily swim data ($N = 6$ per treatment group). (b) Proportion of swim one and two (mean ± SE) spent swimming thigmotaxically. Means were calculated for each rat on each day and then averaged over the 16 days of testing ($N = 6$ per treatment group).

(sex: $F_{1,21} = 0.04$, $p = 0.85$; housing: $F_{1,21} = 0.75$, $p = 0.40$). Although there was a significant effect of day on the amount of time spent in the target quadrant ($F_{3,63} = 3.95$, $p = 0.012$), this appeared to be due to rats spending longer in the target quadrant on day four, rather than a progressive change across the days.

The data were averaged across days 2–5 and pooled across sex and housing condition and tested against chance (33.3%). Rats spent a mean of 42% in the target quadrant: significantly longer than expected by chance ($t_{23} = 3.41$, $p = 0.0023$).

Thigmotaxis

The data were log transformed before analysis. Females were more thigmotaxic than were males in swim one and swim two ($F_{1,20} = 21.07$, $p = 0.0002$; Figure 4b). There was no effect of housing and the sex by housing interaction was not significant ($p > 0.05$). Thigmotaxis decreased significantly in swim two in males and females (swim: $F_{1,20} = 264.75$, $p < 0.0001$). No other interactions were significant ($p > 0.05$).

Behaviour in the home cage

Male and female, pair and isolate housed rats were all equally likely to engage in bar biting behaviour ($p > 0.05$, Fisher's Exact test). During the observation period bar biting was absent in isolated males but present in one paired male, three paired females and two isolated females.

Body weight and food intake

Consistent with Experiments One and Two, males weighed significantly more than females (sex: $F_{1,21} = 655.66$, $p < 0.0001$) and also gained weight at a faster rate (sex by week interaction: $F_{1.8,38.3} = 2617.57$, $p < 0.0001$). Housing condition had no impact on body weight ($F_{1,21} = 2.37$, $p = 0.14$). Again, males ate more than females (sex: $F_{1,21} = 329.57$, $p < 0.0001$) irrespective of housing condition ($F_{1,21} = 0.03$, $p = 0.86$).

There was a non directional effect of week post arrival on food intake: this was at least in part due to natural daily fluctuations in intake, rather than any directional increase or decrease (week post arrival: $F_{4.0,83.8} = 11.6$, $p < 0.0001$).

Discussion

Isolation housing did not affect working or reference memory in young male or female rats. However, males outperformed females in the working memory component of the MWM task. While this result is in consistent with the common finding of superior male performance in the MWM (for a review see: Jonasson, 2005) it is at odds with the hypothesis that isolation housing is a sufficient stressor to induce sex differences in cognition. As seen in older rats, none of the other behavioural indicators of stress were affected by isolation housing in these younger animals. These results are also not consistent with the widespread belief that isolation housing should have a negative impact on physiology and behaviour in rats, which are a naturally gregarious species.

General discussion

It is commonly considered that isolation housing is stressful and as females respond more poorly in cognitive testing to stress it seemed plausible that

isolation housing might cause a sex difference in spatial cognition. Although we found a sex difference in the predicted direction in Experiment Three, it was not due to any of the housing manipulations we made. Rather, it occurred only in the rats that had travelled from the breeding establishment to our laboratory when the rats were aged five weeks. It is possible that travelling is stressful and this stress had a greater and longer lasting impact on females such that their performance in the MWM was impaired relative to males. This possibility seems plausible as housing manipulations of younger animals typically have a greater impact on behaviour than those imposed on older animals (e.g., Einon et al., 1981). Additionally, performance levels of the younger rats were slightly poorer than those of the older rats. It is possible, that travelling to or being in an unfamiliar setting immediately post weaning affects performance in both sexes, but to a greater extent in females.

Age at testing seems unlikely to be the explanation. We tested rats at several ages (77, 105 and 130 days) and although as rats age, differences in spatial cognition between the sexes may diminish or disappear completely (Bucci et al., 1995; Lukoyanov et al., 1999; Markowska, 1999), we found a sex difference only in the 'middle-aged' rats. As none of the other proposed causes of sex difference via stress (e.g., water temperature, handling or training) differed among the experiments they cannot be the explanation for the sex difference.

Finally, the acute stress of swimming in the MWM does not appear to be the explanation for the occurrence of a sex difference only in Experiment Three. Although females were more thigmotaxic than males in all three experiments, higher thigmotaxis in females did not lead to poorer performance. The only explanation we have for these data is that thigmotaxis acted as an alternate searching strategy in the female rats (e.g., see McCarthy & Konkle, 2005). For example, it is possible that the females spiralled gradually out from the edge until encountering the platform, although a more plausible explanation is that the females went back and forth from the edge until they encountered the platform.

In summary, although we found a sex difference in spatial cognition it cannot be explained by stress imposed by isolation housing. We conclude that isolation housing is not sufficiently stressful to cause the observed sex differences in spatial cognition found in the literature. Rather, it appears that travel or introduction to a novel environment (i.e., our laboratory) when very young (i.e., five weeks old) has a much more significant impact on cognitive

ability. Experimental manipulations are required to determine which of these two is the more important.

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