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Sex differences, or not, in spatial cognition in albino rats: acute stress is the key

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Male rats, *Rattus norvegicus*, typically outperform females in tests of spatial cognition. However, as stress affects cognition differently in the two sexes, performance differences may be an artefact of stress. Rats face at least two sources of stress during an experiment: the test situation (acute) and housing conditions (chronic, e.g. isolation). We used a task (the Morris water maze, MWM) that allowed testing of both spatial working and reference memory to investigate whether chronic stress (isolation housing) and/or acute stress (the task) has a differential impact on spatial cognition in male and female albino rats. Irrespective of age at the onset of isolation housing, isolated rats were not spatially impaired relative to pair-housed rats. However, the acute stress of the task led to adult males apparently outperforming adult females: adult females took longer to reach the platform than did males because they spent more time in thigmotaxis (swimming close to the wall) during testing. In juvenile rats, the stress caused by swimming in the MWM resulted in both males and females being highly thigmotactic and no sex difference in performance. We conclude that stress can lead to apparent differences between the sexes in performance on a spatial cognition task.

Keywords: albino rat; Morris water maze; *Rattus norvegicus*; sex differences; spatial cognition; stress; thigmotaxis

Male mammals typically outperform females in tests of spatial cognition (e.g. Galea et al. 1996; Astur et al. 2004; Jonasson 2005) and at least seven evolutionary hypotheses have been proposed to explain the existence of this sex difference (reviewed in Jones & Healy 2006). One reason for the number of hypotheses is that sex differences in spatial cognition have been observed in various experimental paradigms, each of which appears to differ in some important way. However, it is also the case that sex differences are not always observed and it is difficult to compare the predictions of these various evolutionary hypotheses if the supposed difference cannot be reliably produced even when looked for under apparently the same test conditions (e.g. Bucci et al. 1995; Healy et al. 1999).

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This lack of reliable replication has at least two possible hormonal explanations: (1) variation in the sex hormones (known to affect spatial cognition in mammals, e.g. reviewed in Williams & Meck 1991), as a result of either fluctuations in testosterone, causing male performance to go up or down, or variation in oestrogen levels, causing changes in female performance; (2) variation in the stress levels of the animals under test (e.g. Bowman 2005). It is only the second of these that we consider here. The reason for suspecting that stress may explain the lack of replicability in, perhaps even the existence of, sex differences in spatial cognition, is that there are a plethora of data to show not only that stress affects spatial cognition, but also that it does so differently in females and males: females tend to respond more poorly to acute stress, such as is imposed by a test situation, and yet their spatial performance may be unchanged or enhanced by chronic stress. Male cognitive abilities, on the other hand, may be adversely affected under conditions of chronic stress (Luine 2002; Conrad et al. 2003; Beiko et al. 2004; but see Conrad et al. 2004).

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Compounded by the fact that most of the sex difference literature comes from laboratory tests on rodents (often rats, *Rattus norvegicus*), it is conceivable that many of these data are, actually, an artefact of stress caused by one or more laboratory variables. A further significant component is the strain of rat: sex differences in cognition have been more often found using albino rather than pigmented strains (Markowska 1999; Warren & Juraska 2000; Blokland et al. 2006). This apparent strain effect may be because albino strains are more 'anxious' and 'emotional' than pigmented strains (e.g. more likely to freeze) in behavioural tests of anxiety (e.g. light/dark box, open field: Schmitt & Hiemke 1998).

There are at least two potential sources of anxiety or stress that a laboratory rodent may face during an experiment: the test situation and the housing conditions themselves (e.g. isolation housing, reviewed in Patterson-Kane 2001). In cognitive tests a rat often has to venture out into an exposed, brightly lit area to locate a goal or an escape option. For example, during Morris water maze (MWM) testing, rats are required to swim in tepid water to locate a hidden escape platform. Albino strains perform less well than pigmented strains in MWM tasks (Tonkiss et al. 1992; Harker & Whishaw 2002) and while it is possible that poorer vision in albino rats may mean they find it more difficult to see extra maze cues needed to solve the task than do pigmented rats, an alternative explanation is that albino rats find bright open-field tasks, such as the MWM, more aversive than do pigmented rats. If so, they are more likely to spend time being thigmotactic (Andrews 1996; Prusky et al. 2002).

Thigmotaxis (wall hugging) is considered a marker of stress shown by rodents in open-field situations and provides a noninvasive measure of stress during MWM, which is readily quantified. Confirmation that this behaviour is an indicator of stress comes from the fact that both administration of anxiolytics and pretraining in the MWM reduce thigmotaxis (Galea et al. 1994; Beiko et al. 2004). Additionally, thigmotaxis levels are positively correlated with both endogenous and exogenous corticosterone levels during MWM testing (Herrero et al. 2006; Snihur et al. 2008). Furthermore, hippocampal lesions, which impair MWM performance, do not affect thigmotaxis; thus a rodent does not swim near the pool wall simply because it does not know the location of the platform (Morris et al. 1982). In the context of the MWM, high levels of thigmotaxis will lead to longer escape latencies since the platform is usually located at least 30 cm away from the edge of the tank (Treit & Fundytus 1989; Saucier & Cain 1995; Herrero et al. 2006).

Housing conditions constitute a second potential source of stress for laboratory rodents. For example, isolation housing is reported to be stressful for rats, and 'isolation-stress syndrome' (e.g. increased aggression and body weight and hyperactivity) is often reported in albino strains of rat (Hatch et al. 1963; Holson et al. 1991; Shabanov et al. 2004).

If stress plays a significant role in the production of sex differences in spatial cognition in laboratory rats, a large proportion of the data used to support evolutionary hypotheses for those sex differences may be questionable. To determine the degree to which stress affects spatial cognition, we manipulated stress chronically (isolation housing) and tested rats under an acutely stressful situation (MWM testing). We examined the effects of these stressors on the animals' performance in both a working and a reference memory task. We measured thigmotaxis during MWM testing to determine stress behaviourally as well as measuring body weight and food intake as physiological markers of chronic stress, since these are reported to increase in isolated rats (Würbel & Stauffacher 1996; Hurst et al. 1998).

If chronic stress has an impact on performance in the MWM, we would predict that isolated males would respond more poorly than females and there would be no sex difference between these animals, that is, apparently removing a sex difference. If, on the other hand, acute stress impacts on MWM performance in a sex-dependent manner, all females, irrespective of housing condition, will have a greater stress response (higher thigmotaxis) during MWM testing and perform more poorly than males. If stress plays no role in producing sex differences in spatial ability, then we predict a sex difference in performance, regardless of housing, and no differences in stress levels during MWM testing.

EXPERIMENT 1

Methods

Subjects and housing

We used 18 male and 18 female Wistar rats (an albino strain), aged 8–10 weeks obtained from Harlan UK, Ltd. (Bicester, U.K.). At the time of arrival males weighed 280 ± 11 g and females 185 ± 5 g. Six rats of each sex were chosen at random and housed in isolation; the remaining 12 were housed in same-sex pairs ($N = 6$ per housing and sex treatment group). One rat from each pair was chosen at random and marked with hair dye (R43, Schwarzkopf, Hamburg, Germany) for identification. To prevent hair dye odour or the marking procedure affecting behaviour, all of the rats were handled in a similar manner and all of the marking was done 1 week prior to any data collection (e.g. Hurst et al. 1997). To avoid pseudoreplication, and since dominance hierarchies are unstable at this age (Adams & Boice 1989), one rat from each pair was picked at random to be the focal animal and this rat remained the only source of data from the pair for the duration of the experiment. Rats remained in their respective housing condition throughout the experiment.

All rats were housed in plastic-bottomed cages (45×28 cm and 20 cm high; North Kent Plastic Cages Ltd., Erith, U.K.). Visual, olfactory and auditory communication between neighbouring rats was not prevented. Rats were provided with ad libitum pellet food (RM3 diet, Special Diet Services, Ltd., Witham, Essex, U.K.) and tap water and maintained under a 12:12 h light: dark cycle (lights on at 0600 hours) at 21–24 °C.

After 10 weeks of the housing treatment, each isolated and focal rat was tested in the MWM.

Morris water maze apparatus

The MWM consisted of a circular tank made 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 positioned in an experimental room (4.25 × 2.9 m) with geometric and landmark cues (e.g. room corners, posters and shelving on walls) visible from the inside of the tank. The tank was filled to a depth of 32 cm with tap water (24 ± 1 °C) and made opaque with approximately 500 ml nontoxic white paint (Dulux). 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 tank in the centre of one of four imaginary quadrants (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. We videotaped all trials from above using a camera with a 4 mm wide-angle lens, and all trials were observed via a video monitor once the rat was placed in the water; this was to reduce both stress and distraction to the rats during testing.

Working memory

Each rat received 2 days of training before testing began. To reduce stress in the MWM to a degree sufficient to remove sex differences, training typically occurs for at least 10 days (e.g. Healy et al. 1999; Beiko et al. 2004). Two days of training is not considered sufficient to reduce stress; it merely provides the animal with knowledge of the platform's existence and as an escape possibility (indeed, the only one). On a training day each rat received two consecutive swims to the hidden platform. The platform location was the same within each day, but its position was changed from day to day. Platform location was pseudorandomly determined so that the platform was never in the same place on 2 consecutive days.

Each swim began after the rat was gently lowered into the water and released facing the side of the tank, and ended when the rat found and subsequently climbed onto the platform. The time taken by the rat to find the platform was recorded (±1 s) using a stopwatch. Rats that failed to find the platform within 120 s were gently guided to, and allowed to climb onto, the platform. Once on the platform a 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 a rat was left on the platform for 20 s and then gently removed from the platform and returned to its home cage.

Testing started the day following the last day of training and the procedure was exactly as for training with the exception that each rat received four swims (referred to as Swim 1, 2, 3 and 4) each day for 16 consecutive days in total. All trials were conducted between 1100 and 1500 hours.

Reference memory

Reference memory was assessed from Day 2 (because memory cannot be assessed on Day 1) of testing to Day 5 (because moving the platform every day may, over the course of 16 days, lead to the rats learning to avoid the specific location occupied by the platform on the previous day). We recorded the percentage of time that a rat spent swimming in each of the four quadrants of the maze in swim 1 on Days 2–5. The proportion of time spent in the three quadrants other than the quadrant containing the platform was calculated to establish whether 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). The chance level was set at 33.3% because the quadrant that contained the platform on that day was discounted, because the presence of the platform may increase search time in this quadrant, for example, if a rat was to brush against but not climb onto the platform.

Thigmotaxis

The percentage of time that a rat spent swimming within 15 cm of the wall of the maze was recorded for Swims 1 and 2 on all test days. The videotapes were watched on a TV monitor, over which an acetate sheet was attached. Marked on the acetate sheet were the circumference of the MWM and 15 cm from the edge of the MWM. All the time the rat spent in this outer perimeter was recorded.

Body weight and food intake

We measured body weight once per week from week 1 postarrival until the week of MWM testing. Food intake was measured once per week from week 2 postarrival until 1 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 reweighed. We estimated food intake per rat per day by dividing the amount eaten by the number of days since the food was last weighed. Where rats were pair housed an average intake was calculated for both of the rats.

Data analysis

Repeated-measures data were analysed with 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 Swim (1–4) and Day (1–16). We included all of these factors in the analyses, and removed interactions between main effects that were not significant. For within-subject statistics the assumptions of sphericity (that repeated measures have equal variances and that the correlations between any two measures are the same) were tested with the Mauchly–Criterion test. We used Greenhouse–Geisser corrections to account for violations of sphericity (resulting in adjustment of degrees of freedom to nonwhole integers). 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 (HSD, $P < 0.05$) was used for post hoc comparisons of parametric data.

Ethical note

Animal treatment, husbandry and all experimental procedures were carried out in accordance with the Animals Scientific Procedures Act 1986, U.K. and the associated Guidelines for the use of Animals in Scientific Procedures set out by the Home Office regulations. Isolation housing in barren cages is discouraged by the U.K. Home Office; however, this was unavoidable, since isolation housing was a necessary requirement for the experiment. During spatial testing to keep handling stress to a minimum, the same handler (A.H.) carried out all experimental procedures and was aware of the sex and housing conditions of all test subjects at time of testing. The potential for the rats to get cold after swimming was minimized by gently towel drying each rat before placing it in its home cage under a heat lamp for approximately 10 min after the final swim of each day. Rats were killed at the end of the experiment by exposure to a rising concentration of carbon dioxide. To keep suffering to a minimum, care was taken to ensure that carbon dioxide was gradually introduced into the chamber.

Results

Working memory

Males took less time than did females to reach the platform but only in Swim 1 (sex: $F_{1,21} = 4.35$, $P = 0.049$; sex*swim number interaction: $F_{1.4,29.6} = 5.52$, $P = 0.017$; Tukey HSD: $P < 0.05$; Fig. 1). Housing condition had no impact on performance in the MWM ($F_{1,21} = 1.76$, $P = 0.20$). The sex*housing interaction was not significant.

All rats learnt the location of the platform in Swim 1 and swam almost directly to it in all three subsequent swims (swim number: $F_{1.4,29.6} = 315.59$, $P < 0.0001$; Fig. 1). There was a significant effect of day on the time taken to reach the platform: as the experiment progressed the rats took less time to locate it ($F_{6.3,107.5} = 10.2$, $P < 0.0001$). There was a nonsignificant tendency for greater improvement in performance in Swim 1 than in the other swims, suggesting a change in level of thigmotaxis or in search strategy during the experiment (swim number*day interaction: $F_{10.7,181.7} = 1.78$, $P = 0.062$). No other interactions were significant.

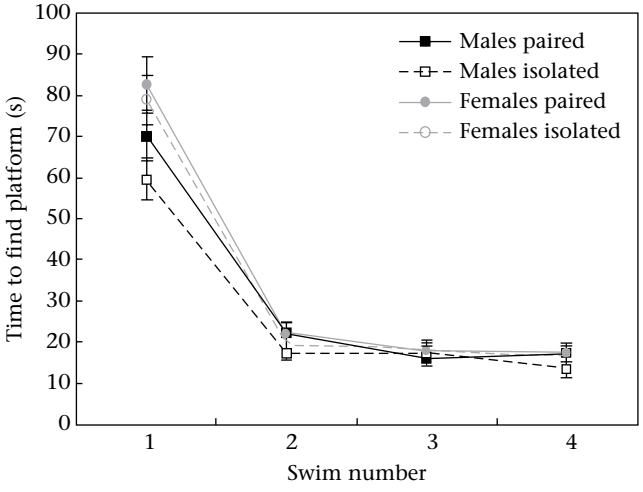

Figure 1. Experiment 1: mean time ±SE taken to find the platform (s) in Swims 1–4 for male and female rats that were either pair-housed or isolated ($N = 6$ rats per treatment group). Swim times for Swims 1–4 are averaged across the 16 days of testing. Analyses were conducted on daily swim data.

Reference memory

Across Days 2–5, males spent longer in Swim 1 searching in the quadrant that had contained the platform on the previous day than did females (RM ANOVA: sex: $F_{1,21} = 4.48$, $P = 0.047$; Fig. 2). There was, however, no impact of housing condition on reference memory and the sex*housing interaction was also not significant (housing: $F_{1,21} = 0.07$, $P = 0.79$). There was nondirectional day to day variation in the amount of time spent searching in the previous day's target quadrant (day: $F_{1.8,37.8} = 3.85$, $P = 0.034$). Days 2 and 4 differed from Days 3 and 5 (Tukey HSD: $P < 0.05$). No other interactions were significant.

To compare reference memory performance with that expected by chance, the proportion of time spent in the target quadrant by each rat was averaged across Days 2–5, pooled across housing condition and tested against chance (33.3%; the quadrant that contained the platform was ignored) using a two-tailed one-sample t test. Both males and females spent significantly longer than expected by chance in the target quadrant (males: $t_{11} = 5.29$, $P = 0.0003$; females: $t_{11} = 2.59$, $P = 0.025$).

Thigmotaxis

Males were significantly less thigmotactic than females in Swims 1 and 2 (sex: $F_{1,21} = 19.58$, $P = 0.0002$; Tukey HSD: Ps < 0.05; Fig. 3a, b), and both males and females were less thigmotactic in Swim 2 than they had been in Swim 1 ($F_{1,21} = 268.19$, $P < 0.0001$; Tukey HSD, Ps < 0.05; Fig. 3a). There was a significant interaction between these two factors: females had a greater decrease in thigmotaxis in Swim 2 (sex*swim number interaction: $F_{1,21} = 6.36$, $P = 0.020$). Housing condition was not correlated with variation in thigmotaxis ($F_{1,21} = 2.69$, $P = 0.12$)

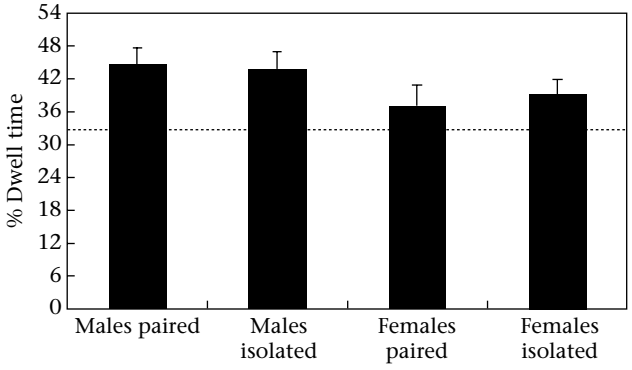

Figure 2. Experiment 1: mean percentage ±SE of Swim 1 spent swimming in the target quadrant (dwell time). For each rat the data were averaged over Days 2–5 ($N = 6$ rats per treatment group). Dotted line represents chance (33.3%).

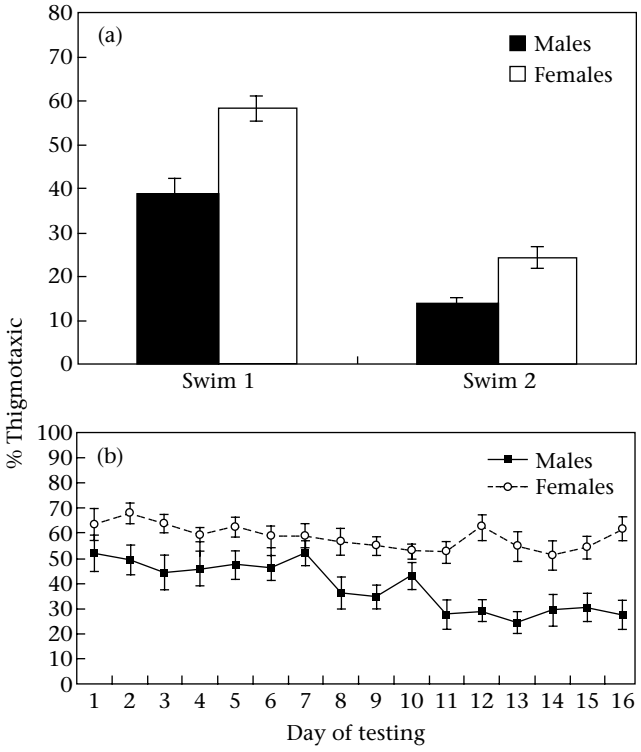

Figure 3. Experiment 1: mean percentage ±SE of (a) Swim 1 and Swim 2 and (b) Swim 1, spent swimming thigmotactically (within 15 cm of the edge of the MWM). For each rat, data are averaged over the 16 days of testing in (a) and given for each day in (b). Data are pooled across housing condition ($N = 12$ per sex).

nor were any other interactions with housing significant. Thigmotaxis in Swim 1 declined significantly across the days of the experiment (day: $F_{15,330} = 4.94$, $P < 0.0001$), but only for males (sex*day interaction: $F_{15,330} = 1.92$, $P = 0.020$; Fig. 3b). No other interactions were significant.

Body weight and food intake

Males weighed more than females (as measured each week from 77 to 133 days old; sex: $F_{1,21} = 555.41$, $P < 0.0001$) and gained weight at a faster rate (sex*week interaction: $F_{2.5,52.5} = 30.3$, $P < 0.0001$). Housing condition had no impact on body weight and the housing*sex interaction was not significant (housing condition: $F_{1,21} = 0.11$, $P = 0.74$). Males ate more than females ($F_{1,20} = 60.6$, $P < 0.0001$; on average, males ate 22 g and females ate 18 g per day). Housing condition had no impact on food intake and the sex*housing interaction was not significant (housing condition: $F_{1,20} = 1.92$, $P = 0.13$).

Discussion

Females appeared to perform more poorly than did males in the MWM: males were both quicker to find the platform each day and spent more time in the first swim of each day in the quadrant that had contained the platform on the previous day. We did not measure path length as latency is the most common measure of performance in the MWM (e.g. Mendez et al. 2008; Saucier et al. 2008) and correlates so closely with path length that authors who do measure both typically report one in detail and mention only that the other measure of performance followed the same pattern (e.g. Roof 1993; Kempermann et al. 1997; Nilsson et al. 1999). Importantly, there is no evidence that male and female swim speeds differ, the reason for wishing to consider distance in addition to latency (Jonasson et al. 2004; Snihur et al. 2008). Therefore, sex differences, both in our work and in the literature, are not explained by differences between latency and distance (as there are none).

Any stress caused by isolation housing had no discernible impact on MWM performance by either sex. However, the sex difference in performance in the MWM was explained by the difference in the proportion of time spent in thigmotaxis. It is possible that thigmotaxis reflects impaired allocentric learning (e.g. search 15 cm away from the edge of the tank) or it may be easier to view landmarks from this area of the maze. However, not only should easier viewing lead to better performance, but there is also an overwhelming body of literature that demonstrates that thigmotaxis correlates positively with anxiety (e.g. Treit & Fundytus 1989; Beiko et al. 2004). Thus, the apparent sex difference in memory in this experiment can be ascribed to the greater stress response of females swimming in the MWM and thus appearing to have a poorer memory for the platform's location.

We assessed the impact of stress on two measures of cognitive performance: reference memory and working memory. Working memory can be investigated by the time taken to find the platform in Swims 2–4, when the animals use information acquired in the swims of that day to locate the platform. As the sexes did not differ on this measure, we interpret the sex difference in performance in Swim 1 to be a result of a difference in the stress response to swimming in an MWM, rather than to a cognitive impairment. Our finding that males and females performed equally well in the working memory component of the task is consistent with other studies that have failed to find a sex difference in working memory in the MWM (Healy et al. 1999; Conejo et al. 2004).

We also attribute the apparently superior reference memory in our male rats to the higher levels of thigmotaxis in the females, since swimming around the edge necessarily precludes searching in the quadrant that contained the platform on the previous day. Furthermore, these data are consistent with the finding that stress can impair retrieval of long-term spatial memory in rats (de Quervain et al. 1998).

Our findings strongly suggest that investigations into sex differences in reference memory in the MWM should include the consideration of thigmotactic behaviour. Since reference memory is typically measured either by giving a single swim per day or by averaging latencies over several swims per day, if females find the first swim of the day more stressful (Fig. 3b) it is possible that this methodology serves to bias the results in favour of the males (e.g. Roof & Havens 1992; Blokland et al. 2006).

Although in our experiment females were significantly more thigmotactic than males in Swim 2, they did not differ in working memory performance from the males. One

explanation is that, to detect a performance difference, the time spent in thigmotaxis relative to the time taken to reach the platform must exceed some threshold. In Swim 1 females spent 60% of their time in thigmotaxis (49 s in real time), whereas in Swim 2 they spent only 24% of their time in thigmotaxis relative to the males' 13%, which was a difference in real time of only 2 s. In a series of previous experiments, we found that the sexes' performance differed significantly only when at least one of the sexes (always females in our experiments) spent at least 35% of the time swimming thigmotactically and the difference was at least 13% between the sexes, a difference in real time of approximately 11 s (Harris et al. 2008). The results of the current experiment coupled with our previous work, in which adult Lister Hooded rats differed in thigmotaxis but not in cognitive performance (Harris et al. 2008), as well as that of others (e.g. Perrot-Sinal et al. 1996; Beiko et al. 2004), suggest that thigmotaxis must reach a threshold level (>35%) before the performance of either sex is impaired. Furthermore, a sex difference (in contrast to simple performance impairment) is seen when the difference between the sexes in thigmotaxis is greater than 13% (or 11 s in absolute time).

Acute stress, then, may be the explanation for at least some of the sex differences in cognitive performance reported for adult laboratory rats. However, a considerable proportion of the data come from rats that were obtained from breeding establishments as juveniles (i.e. exposed to isolation rearing), so it is important to determine whether the effects seen in adults (i.e. only acute stress affecting performance, if at all) are also seen in juveniles. For this reason we carried out experiment 2, in which all manipulations were as in experiment 1, but those manipulations began when the rats were only 4–5 weeks old.

EXPERIMENT 2

Play behaviour in rats increases from 18 days of age, peaks at around 32–40 days of age and then gradually decreases into adulthood (Panksepp 1981). Thus, young rats play more than old rats and since social isolation removes the opportunity for play, it is plausible that social deprivation (i.e. isolation housing) of juvenile rats may cause greater chronic stress than it does in adults. For example, rats isolated from approximately 21 days of age show a variety of behavioural and cognitive changes, such as hyperactivity in an open field (Einon & Morgan 1977; Einon et al. 1978; Parker & Morinan 1986), impaired reversal learning (Schrijver et al. 2004), and impaired spatial learning (Lu et al. 2003; Hellemans et al. 2004). Greater chronic stress in juveniles might lead to differences in cognitive performance compared with that of adults, given that males appear to be more susceptible to chronic stress than do females (e.g. Bowman et al. 2003; Sandstrom & Hart 2005).

We housed rats aged 4–5 weeks in isolation or in pairs for 10 weeks before testing their spatial ability in an MWM. We made the same predictions as for experiment 1: that (1) if chronic stress impacts specifically on males, and if sex differences exist, isolated males should perform more poorly than pair-housed males but there should be no sex differences between isolated males and females; (2) if acute stress impacts specifically on females, females should perform more poorly than males; (3) if stress is not a significant contributory factor to performance, we would predict a sex difference irrespective of housing condition.

Methods

Subjects and housing

We used 18 male and 18 female Wistar rats, aged 4–5 weeks, obtained from Harlan UK, Ltd. At the time of arrival males weighed 146 ± 4 g and females 121 ± 2 g. Six rats of each sex were chosen at random to be housed alone; the remaining 12 were housed in same-sex pairs ($N = 6$ per sex and housing treatment group). Housing and husbandry were the same as for experiment 1.

Rats experienced their respective housing condition for 10 weeks before spatial ability was assessed using the MWM. MWM testing and apparatus were as for experiment 1. The measurement of thigmotaxis, body weight and food intake, the fate of the rats and the data analyses were also as for Experiment 1.

Results

Working memory

The sexes did not differ in their working memory and the performance of neither sex was affected by housing condition (sex: $F_{1,21} = 0.43$, $P = 0.52$; housing condition: $F_{1,21} = 0.45$, $P = 0.51$; Fig. 4). Additionally, the sex*housing interaction was not significant.

There was a highly significant effect of swim number on performance ($F_{1.7,36.2} = 177.94$, $P < 0.0001$; Fig. 4): all rats took significantly longer to find the platform in Swim 1 than in all other swims (Tukey HSD: $P < 0.05$). There was also an effect of day on performance: as testing progressed, performance improved ($F_{7.2,151.3} = 16.85$, $P < 0.0001$). The day*swim interaction was not significant, indicating that

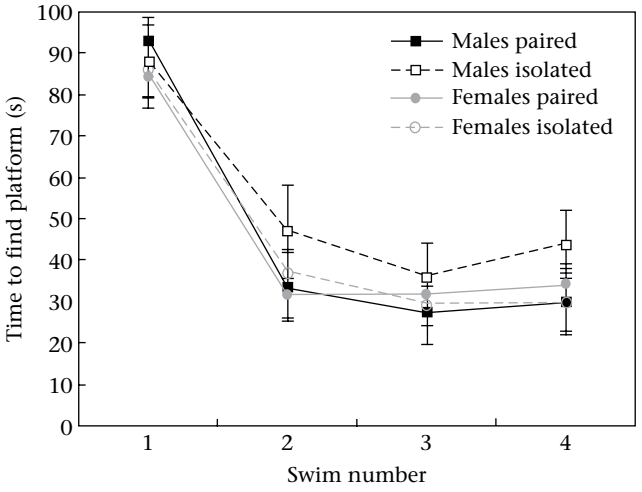

Figure 4. Experiment 2: mean time ±SE taken to find the platform (s) in Swims 1–4 for male and female rats that were either pair-housed or isolated ($N = 6$ rats per treatment group). Swim times for Swims 1–4 are averaged across the 16 days of testing. Analyses were conducted on daily swim data.

the improvement across the days was seen in all four swims. No other interactions were significant.

Reference memory

The sexes did not differ in the amount of time spent during Swim 1 in the target quadrant across days 2–5 (RM ANOVA: sex: $F_{1,21} = 0.47$, $P = 0.50$) and there was no effect of housing ($F_{1,21} = 0.21$, $P = 0.65$). The sex*housing interaction was not significant. There was, however, a significant effect of day on time spent in the target quadrant: as the experiment progressed rats spent less time in this quadrant ($F_{3,60} = 5.10$, $P = 0.003$).

To compare performance with that expected by chance, the proportion of time spent in the target quadrant by each rat was averaged across Days 2–5, pooled across sex and housing condition (thus $N = 24$) and tested against chance (33.3%; data from the quadrant that contained the platform were ignored) using a two-tailed one-sample *t* test. Rats tended to bias their searching in Swim 1, spending longer than expected by chance in the target quadrant (mean % of time: 37.4%; $t_{23} = 2.07$, $P = 0.05$).

Thigmotaxis

The sexes did not differ in the amount of time spent in thigmotaxis ($F_{1,21} = 0.002$, $P = 0.9$; Fig. 5a, b). There was no effect of housing condition on thigmotaxis and the sex*housing interaction was also not significant (housing: $F_{1,21} = 0.003$, $P = 0.95$; sex*housing interaction: $P > 0.1$). However, thigmotaxis decreased significantly between Swims 1 and 2 ($F_{1,21} = 149.39$, $P < 0.0001$; Fig. 5a). Thigmotaxis in Swim 1 changed significantly across the days of the experiment, but there was no directional trend (day: $F_{15,330} = 4.94$, $P < 0.0001$; Fig. 5b), and males and females did not differ significantly over the days (sex*day interaction: $F_{15,330} = 0.99$, $P = 0.46$; Fig. 5b). No other interactions were significant.

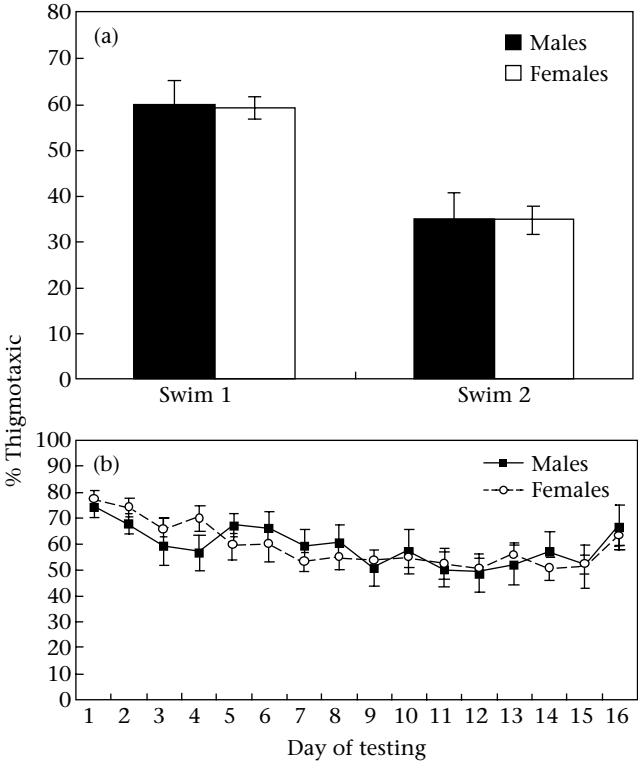

Figure 5. Experiment 2: mean percentage ±SE of (a) Swim 1 and Swim 2 and (b) Swim 1 spent swimming thigmotactically (within 15 cm of the edge of the MWM). For each rat, data are averaged over the 16 days of testing in (a) and given for each day in (b). Data are pooled across housing condition ($N = 12$ per sex).

Body weight and food intake

Males weighed more than females (sex: $F_{1,21} = 200.84$, $P < 0.0001$) and gained weight at a faster rate (sex*week interaction: $F_{2.3,49.5} = 235.87$, $P < 0.0001$). Housing condition had no impact on body weight and the housing*sex interaction was not significant (housing condition: $F_{1,21} = 1.5$, $P = 0.23$). Males ate more per day (23 g) than females (18 g; $F_{1,20} = 89.94$ $P < 0.0001$). Housing condition had no impact on food intake and the housing*sex interaction was not significant (housing condition: $F_{1,21} = 1.81$, $P = 0.19$).

Discussion

We did not find a sex difference in either working or reference memory in the MWM in these juvenile rats. Isolation housing did not impact on cognitive performance or on food intake or body weight. We did, however, see a significant impact of acute stress on performance in the MWM: all of the rats spent about 60% of their first swim in thigmotaxis and, correspondingly, performance was poorer in experiment 2 than in experiment 1. Although the juvenile females spent a similar proportion of Swim 1 in thigmotaxis as did adult females in experiment 1, the difference between the two experiments is due to the much higher proportion of thigmotaxis observed in the juvenile males. Reference memory performance, then, was equally obscured in both sexes in the juveniles. Working memory, too, was equally impacted in both sexes: although thigmotaxis dropped in Swim 2, it remained at 40% for both, a level similar to that of the adult females in experiment 1 (and thus much higher than that of the adult males).

Isolation housing imposed at 3 weeks of age can have an impact on spatial cognition in males after as little as 4–8 weeks (e.g. Wongwitdecha & Marsden 1996; Lu et al. 2003). However, in those studies, the 'control' groups were either housed with enrichments (e.g. cage furniture, toys) or in social groups of four to five rats, which confounds social housing with larger home cages and physical complexity. Additionally, the degree of isolation (auditory, olfactory and visual) was not made explicit in these studies. Despite our rats being young when the housing manipulation was imposed (4–5 weeks) and this exposure lasting 10+ weeks, no discernible impact on cognition or stress (thigmotaxis) was detected in either sex. To our knowledge, previous studies have not investigated thigmotaxis during MWM testing in juvenile rats following isolation housing. However, rats reared in isolation from 35 days of age show more 'stress-related'

behaviours, such as bar biting and tail manipulation in their home cages, than group-reared conspecifics (Baenninger 1967; Hurst et al. 1997, 1998).

As in experiment 1, we attribute the outcome of this experiment to the effects of acute stress, the difference being that in this case, juvenile males were also affected to a degree similar to that of females.

GENERAL DISCUSSION

We proposed that variation in stress might lead to differences between the sexes in performance in spatial cognition, especially as chronic and acute stress seem to impact differentially on male and female spatial cognition. We manipulated chronic stress by housing animals alone or in pairs and acute stress by using the MWM as our memory task. As we could find no effect of isolation housing on performance in the spatial cognition test, we conclude that chronic stress, as incurred by isolation housing, is an unlikely explanation for sex differences in spatial ability in albino rats.

Chronic stress (e.g. 6 h of daily restraint for 21 days) impairs male spatial ability but enhances or has no effect on female spatial ability (e.g. Bowman et al. 2001; Bowman 2005). We did not find that isolation housing impaired spatial ability, in either sex. However, as we saw no conspicuous signs of stress in the isolated rats, since food intake and body weight were indistinguishable between pair-housed and isolated rats, and none of the isolated rats had scaly tails (e.g. Hatch et al. 1963), it is possible that isolation housing did not impact on performance because it was not sufficient to cause chronic stress.

Our finding that isolation had little discernible impact on our rats conflicts with the widespread belief that housing rats alone is detrimental to their wellbeing because it is chronically stressful (Home Office 1995; Patterson-Kane 2004). However, at least two reviews suggest that there is a distinct lack of in-depth, well-controlled studies in this area and more data are needed before concluding that isolation is stressful (Brain & Benton 1979; Krohn et al. 2006). Additionally, it is possible that the routine handling our rats received was sufficiently stimulating to mitigate any deleterious effects of isolation (e.g. see Holson et al. 1991). Alternatively, isolation stress may become increasingly significant as visual, auditory and olfactory communication between neighbouring cages, none of which were prevented in our housing conditions, are reduced. Nevertheless, if rats can be singly housed without detrimental impact on either their welfare or the outcome of the experiment, data could be collected from all of the animals that are used (testing all animals from one cage is pseudoreplication), which would ultimately reduce the number of rats used to study this specific question (as is encouraged by the U.K. Home Office).

Our data are, however, consistent with the hypothesis that performance by females in hippocampal-dependent tasks is affected to a greater degree by acute stress than is that of males (Shors & Miesegaes 2002; but see Conrad et al. 2004). Our data also support the hypothesis that acute stress, associated with the test situation, can explain the presence and absence of sex differences in cognitive tasks (e.g. Perrot-Sinal et al. 1996; Beiko et al. 2004; Harris et al. 2008). A sex difference is caused when the sexes respond to a similar stressor to a different degree. In this case, females in experiment 1 were more stressed than were males, leading them to perform more poorly in the cognitive task as a result. However, when the sexes were equally stressed, there was no sex difference in performance (experiment 2).

Importantly, the MWM is a task in which the effects of stress can be seen as variation in thigmotaxis, which provides a quantitative (noninvasive) measure of stress while the animal is completing the task. More time spent in thigmotaxis in the first swim of the day will result in apparently poorer reference memory performance. Differences in thigmotaxis may also lead to apparent sex differences in cognition in the working memory version of this task: latency to reach the platform will necessarily be longer, the more time is spent in thigmotaxis. If thigmotaxis continues to be high in Swim 2, working memory in such thigmotactic animals will appear to be poorer than in animals spending less time in thigmotaxis in Swim 2 (experiment 1). In neither instance was there evidence for cognitive differences.

Another potential source of variation in spatial ability is hormonal fluctuations caused by the oestrous cycle. However, while fluctuations in hormone levels across the oestrous cycle may influence spatial ability in female rats, the findings are inconsistent. For example, performance in spatial cognition tasks may be enhanced during the pro-oestrus (high oestrogen) phase of the cycle (Healy et al. 1999), impaired during the pro-oestrus phase (e.g. Warren & Juraska 1997) or remain stable across the oestrous cycle (Stackman et al. 1997). Similarly, stress effects in females may also depend on the oestrous cycle phase; for example, greater stress responses are found during the pro-oestrus phase of the cycle (Viau & Meaney 1991; but see Frye et al. 2000). However, we tested our females over several oestrous cycles (there were 16 days of testing) and found no conspicuous cycling in performance or thigmotaxis levels across testing: the females were always more stressed (i.e. thigmotaxic) and underperformed, relative to the males in experiment 1, and equally stressed and performed equally in experiment 2.

It is not clear what caused the increase in stress for the juvenile males in experiment 2 (nor, indeed, why the females in both experiments were more susceptible to acute stress). It is possible that travel to or the change in housing conditions, as occur between the producer (Harlan Ltd) and our animal unit, affect females irrespective of age but males are susceptible only when young and there is some evidence that transport of rodents is stressful (reviewed in Swallow et al. 2005). Whatever the cause, it has a long-term effect on the rats' ability to deal with acute stress.

The effects of acute stress on performance have rarely been considered in typical MWM tasks, that is reference memory tests. In at least two studies, however, in which the effects of acute stress levels (i.e. thigmotaxis) were explicitly investigated, sex differences in MWM performance are also accounted for by thigmotaxis (Perrot-Sinal et al. 1996; Beiko et al. 2004). Additionally, a role for acute stress is implicated in a number of studies in which rats

received extensive pretraining leading to no differences in performance between the sexes (Bucci et al. 1995; Warren & Juraska 1997). Similarly, in working memory tests, there was no sex difference after extensive pretraining in the MWM nor when comparisons of performance were made for Swim 2 only (Healy et al. 1999; Conejo et al. 2004).

As a final note, we did not measure levels of the stress hormone corticosterone in our rats because, in general, levels return to baseline levels after a period of chronic stress, which jeopardizes the value of corticosterone as a measure of chronic stress caused by isolation housing (Jensen et al. 1996). Correspondingly then, there is little consistency in the literature as to how corticosterone levels change in response to isolation housing. For example, there are studies that report that corticosterone levels in isolated rats are elevated (e.g. Perelló et al. 2006), depressed (e.g. Hurst et al. 1998) or unaffected relative to socially housed rats (e.g. Scaccianoce et al. 2006). It would also have been inappropriate to use corticosterone levels as an indicator of acute stress following MWM testing, since the blood sampling required may itself affect subsequent MWM performance (e.g. the next day).

In conclusion, we found that acute but not chronic stress had sufficient impact on the rats to cause apparent sex differences in cognitive performance in the MWM. However, when equally stressed, the sexes did not differ in performance. We suggest that a significant proportion of the sex difference literature that comes from testing laboratory rats may result from an artefact (stress) of the test situation rather than selection for better spatial cognition in males than in females. However, sex differences in spatial cognition have been demonstrated in a number of mammalian species using a range of tasks (Gaulin & Fitzgerald 1986; Galea et al. 1996; Lacreuse et al. 1999; Gresack & Frick 2003; Jones & Healy 2006). Our data do not, then, speak to all previous work demonstrating sex differences in spatial cognition but they may go some way to explain the inconsistencies in the contributions from testing laboratory rats. Our data also raise two concerns: (1) that it is possible during cognitive tests to bias tasks or data analyses inadvertently in such a way as to produce or to exaggerate sex differences in performance; (2) laboratory rats may not be ideal subjects for investigations into sex differences in spatial cognition. For understanding the causes of sex differences (i.e. hormonal), laboratory rats remain very useful. However, if the question of interest concerns the evolution of sex differences in spatial cognition, perhaps species recently taken from the wild, or tested in the wild, would better suit the purpose.

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