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Environmental enrichment enhances spatial cognition in rats by reducing thigmotaxis (wall hugging) during testing

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Rats, *Rattus norvegicus*, housed with 'environmental enrichment' do better in tests of spatial cognition than rats housed in barren cages. The leading hypothesis is that exposure to 'social and inanimate complexity' leads to better cognitive-processing abilities, which directly enhances performance in a spatial task. However, enrichment is associated with reduced stress responses and anxiety in novel or acutely stressful situations (cognitive tasks are typically both). Therefore, a plausible alternative hypothesis is that experience of enrichment indirectly enhances performance by reducing a rat's anxiety levels during cognitive testing. We found that, irrespective of sex, enriched rats outperformed barren-housed rats in the Morris water maze. However, after accounting for the effects of thigmotaxis (a behavioural anxiety measure during testing), there was no significant difference in performance between enriched and barren-housed rats. Enriched rats were simply less thigmotactic and this indirectly improved their performance. This was true for both males and females. We conclude that enrichment reduces anxiety outside the home cage, in a cognitive test situation, and, subsequently, the cognitive benefits of enrichment occur because enriched animals are less anxious during cognitive testing.

The standard definition of enrichment for laboratory-housed animals is 'a combination of complex inanimate and social stimulation' (Rosenzweig et al. 1978). Early investigations into the impact of enrichment found that rodents living in a complex environment have heavier brains than conspecifics reared in a barren environment (e.g. Bennett et al. 1964; Diamond et al. 1965). More recently, however, the addition of environmental enrichment to the home cages of laboratory rodents has been recommended as a useful way to improve rodent welfare (Sørensen et al. 2004).

Enrichment of home cages (both socially and physically) is thought to improve the welfare of caged animals by providing the animal with the opportunity to carry out species-specific behaviours that the animal is highly motivated to perform (Johnson et al. 2004), leading to a reduction in stress, anxiety and frustration and, to some extent, possibly 'boredom' (e.g. Würbel et al. 1998). As such, the U.K. Home Office encourages the addition of environmental enrichment to the home cages of laboratory rats and mice (Home Office 1995).

As well as improving welfare, enrichment also appears to improve cognitive ability. Rats that have experienced enrichment (social and physical) tend to outperform single or barren-housed (nonenriched) conspecifics, especially in tests of spatial cognition (Falkenberg et al. 1992; Nilsson et al. 1999; Pham et al. 1999; Larsson et al. 2002; Leggio et al. 2005). The currently favoured hypothesis to explain these results is that interacting with a socially and physically complex environment (i.e. informal learning) directly enhances cognitive ability (reviewed in Rosenzweig & Bennett 1996; van Praag et al. 2000). However, rodents that have had exposure to enrichment also tend to have reduced stress responses to acutely stressful (e.g. novel) situations. For example, enriched rodents habituate to novel objects in a familiar test arena faster and enter an unforced open-field test significantly sooner (Chapillon et al. 1999; Zimmermann et al. 2001). They also spend longer in the open arms of the elevated plus maze (a classic test of anxiety) than nonenriched animals (Roy et al. 2001). Socially and

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physically enriched rats explore more initially and habituate sooner to 'open-field'/novel arenas than nonenriched rats (Falkenberg et al. 1992; Larsson et al. 2002). Enriched rats also have reduced corticosterone, adrenocorticotrophin and adrenaline responses to repeated handling compared to nonenriched rats (Moncek et al. 2004). Thus, an alternative explanation for the superior performance of enriched rodents during cognitive testing is that exposure to enrichment reduces the animal's anxiety levels during testing.

A reduced anxiety response during cognitive testing is likely to improve performance. For example, in the Morris water maze (MWM), a commonly used spatial task, the rodent swims in a brightly lit pool to a hidden escape platform, and, owing to the nature of the MWM (a wet, brightly lit, open-field task), it is widely accepted that this task is acutely stressful (e.g. D'Hooge & De Deyn 2001; Beiko et al. 2004). High levels of anxiety during MWM testing in rodents are manifested behaviourally as thigmotaxis (wall hugging). Thigmotaxis may indirectly impair performance in the MWM because the platform is never located in the outer edge of the tank (Herrero et al. 2006).

Thigmotaxis is believed to have evolved as a natural antipredator response, since avoiding open spaces reduces a rodent's exposure to (aerial) predators (Treit & Fundytus 1989; Bonsignore et al. 2008), and there are several lines of evidence to suggest that thigmotaxis is a reliable noninvasive indicator of anxiety during MWM testing. First, thigmotaxis is suppressed by anxiolytics and increases with corticosterone administration (Treit & Fundytus 1989; Snihur et al. 2008); second, thigmotaxis levels are positively correlated with corticosterone levels after MWM testing and pretraining reduces thigmotaxis (Beiko et al. 2004; Herrero et al. 2006).

Females generally express greater levels of thigmotaxis during MWM testing and this can lead to apparent sex differences in performance (Perrot-Sinal et al. 1996; Beiko et al. 2004; Harris et al. 2008a, b). Sex differences in performance appear to be reduced or even eliminated following pretraining or administration of anxiolytics prior to testing in an MWM (Galea et al. 1994; Perrot-Sinal et al. 1996). If enrichment enhances performance by reducing anxiety levels during testing, it might be that sex differences in performance diminish as a result of this kind of housing.

Enrichment may directly enhance MWM performance independently of anxiety, or it may enhance MWM performance indirectly by reducing anxiety-related behaviour (i.e. thigmotaxis) during testing. To determine whether variation in anxiety levels could help to explain the cognitive benefits of enrichment, we tested the impact of enrichment on both spatial cognition and thigmotaxis in the MWM in male and female rats, *Rattus norvegicus*. We also tested anxiety levels in a light/dark box. The light/dark box consists of two compartments, one of which is dark and the other brightly lit from above, joined by a wall containing a small opening. The rodent is placed in the dark compartment and can pass freely into the light compartment. The time the animal takes to enter fully the light side positively correlates with the animal's anxiety level, as demonstrated by dose-dependent effects of anxiolytics (Crawley & Goodwin 1980; Augustsson et al. 2003). If enrichment directly enhances cognition, then we predicted that: (1) enriched rats would outperform the nonenriched rats in the MWM; (2) MWM performance would not be correlated with variation in thigmotaxis between housing conditions; (3) enriched and nonenriched rats would not differ in time to enter the light compartment in the light/dark box. However, if enrichment indirectly enhances cognition by reducing anxiety levels during testing, we predicted that: (1) enriched rats would outperform nonenriched rats; (2) males would outperform females but only under nonenriched conditions; (3) enhanced MWM performance in enriched rats would be accompanied by a decrease in thigmotaxis in these rats; (4) enriched rats would enter the light compartment in the light/dark box faster than nonenriched rats.

METHODS

Subjects and Housing

The subjects were 30 male and 30 female Wistar rats, aged between 4 and 5 weeks, obtained from Harlan (Bicester, U.K.). On arrival, males weighed a mean $\pm$ SD of 84 ± 7 g and females 81 ± 5 g. Six rats of each sex were chosen at random (the rats were numbered and marked with nontoxic marker pen and random numbers were generated using Excel) and housed in isolation in 'nonenriched' (NE) conditions, while the remaining 24 rats were housed in six same-sex groups of four in 'environmentally enriched' (EE) conditions ($N = 6$ per housing and sex treatment). The NE rats were housed in plastic-bottomed cages (45×28 cm and 20 cm high; NKP Cages, Erith, U.K.), and the EE rats were housed in larger plastic-bottomed cages (56×38 cm and 20 cm high; NKP Cages Ltd.). Both groups were provided with a 2 cm layer of woodchip bedding. Additionally, each EE cage was provided with tissue-paper nesting material (Paper Wool, Datesand, Manchester, U.K.), a transparent red tunnel (rat red tunnel, size 9×15 cm, Datesand) on the floor of the cage and an opaque plastic tube (30×10 cm) suspended by chains from the cage top. The EE rats were also given various novel objects (newspaper strips, small cardboard tubes and boxes, wood blocks, empty yogurt pots, etc.) that were changed every 3–4 days. All rats had access to ad libitum pellet food (RM3 diet, Special Diet Services, Ltd, Witham, Essex, U.K.) and tap water and were maintained under a 12:12 h light:dark cycle (lights on at 0700 hours) at 21–24 °C. All cages were cleaned out and each rat was weighed once per week.

To determine dominance hierarchies within a cage, we measured the amount of pinning (when a rat uses its forelimbs to pin another rat on its back) by each rat 8–9 weeks after arrival. The animals in each cage were observed for 10 min between 1400 and 1700 hours and we recorded which animal did the pinning, the recipient and the number of pinning incidences (data are not shown). After finding no evidence of stable dominance hierarchies, as is consistent for rats at this age (e.g. Adams & Boice 1989), to avoid pseudoreplication we picked one rat at random (using random numbers generated using Excel) from each EE cage to be tested in the MWM (all NE rats were the comparison test group; Festing et al. 2002). At 10 weeks after arrival these EE rats and all the NE rats were then tested for 22 days in a reference memory (memory across days for the same location) MWM task in addition to the two probe trials, followed by light/dark box testing 13 days later.

Morris Water Maze Apparatus

The MWM consisted of a circular fibre-glass tank (2 m diameter, 65 cm deep) raised 50 cm above floor level on a custom-built platform situated in a room (dimensions 4.25×2.9 m) with geometric and landmark cues (e.g. room corners, light fittings, 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, made opaque by the addition of approximately 500 ml of nontoxic white paint (Dulux) and maintained at 24 ± 1 °C. An escape platform (white PVC of diameter 11 cm) was placed in the centre of one of four imaginary quadrants (the four main compass points N, E, S or W) and was positioned 2 cm below the surface of the water and 30 cm from the edge of the tank. For each of the platform locations there were four possible release points into the pool: NE, SE, SW and NW. An overhead 4 mm wide-angle lens camera was used to record the test sessions for behavioural analysis. To reduce both stress and distraction to the rats, all trials were observed via a video monitor once the rat was placed in the water.

Morris Water Maze Procedure

Probe 1

To assess how much time each rat spent in the four quadrants prior to training, the very first swim each rat received was a probe trial, during which the platform was removed from the MWM. This also allowed observation of thigmotaxis levels before the rat knew the platform's location. Each rat was gently released from a predetermined release point into the MWM close to, and facing, the side of the tank and allowed to swim for 60 s. After 60 s the rat was lifted out of the tank, towel dried and placed into its home cage under a heat lamp for approximately 10 min. All probe trials were recorded from above so that the percentage of time spent in each of the predetermined quadrants (N, E, S or W) could be determined.

Training

Training proper began the day after Probe 1. During spatial training the platform was located in either the east or the west quadrant but for each rat the platform remained in the same location across the test days. Each rat received one swim per day and was released from a different release point each day (NE, NW, SE, SW). All swims took place between 1100 and 1500 hours. The rat was gently lowered into the water close to and facing the wall of the tank. The time taken for the rat to find and subsequently climb onto the platform was recorded. If a rat failed to find the platform within 120 s it was gently guided to, and allowed to climb onto, the platform. Rats were left on the platform for 20 s before being removed from the platform, towel dried and returned to their home cage under a heat lamp for approximately 10 min. Rats had one swim per day for 22 consecutive days.

Probe 2

On the day after the last day of spatial training, each rat received a second probe trial, for which the platform was removed. The procedure for Probe 2 followed that for Probe 1.

Thigmotaxis

To measure thigmotaxis we recorded the percentage of time that a rat spent swimming within 15 cm of the wall of the maze during both probes and throughout training. The videotapes were watched on a TV monitor with an acetate sheet attached over the screen. The circumference of the MWM and 15 cm from the edge of the MWM were marked on the acetate sheet. The total time that the rat's head and shoulders spent in this outer perimeter was recorded by the same observer throughout the experiment (A.H.). The observer was blind to housing conditions of the rats at the time of data collection (it was possible to determine sex on the basis of body size).

Light/dark Box

Eleven days after Probe 2 we tested each rat once in a light/dark box. The light/dark box consisted of an open-top rectangular cardboard box divided into two equal-sized compartments by a cardboard divider. A 10 × 10 cm hole in the divider provided free access between the compartments. Black card lined the dark compartment and white card lined the light compartment; both compartments measured 40 × 25 cm and 25 cm high. A 40 W light positioned directly over the centre of the light compartment provided the only illumination in the test room. Testing took place between 1100 and 1600 hours. Rats were tested in a predetermined random order (numbers were pulled out of a hat). Each rat was placed in the middle of the dark compartment facing away from the opening to the light side and its behaviour was monitored for 5 min. We recorded the time each rat took to cross over into the light compartment completely (latency in seconds). The box was cleaned between rats (faeces removed and box surfaces wiped with soapy water then sprayed with 70% ethanol).

Data Analyses

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 (EE and NE), within-subject factors were Block (block 1–11; each block was an average of 2 days' trials). All of these factors were included in the analyses and interactions between main effects that were not significant were removed. The assumptions of sphericity (that differences between repeated measures have equal variances and that the correlations between any two measures are the same) were tested using the Mauchly criterion test. Corrections were made using the Greenhouse–Geisser terms where appropriate. The assumptions of normality and homogeneity of variance were tested and transformations applied to the data where appropriate. The light/dark box data were normally distributed but did not have equal variances and so were analysed using a Welch ANOVA test. To determine whether thigmotaxis explained any differences in performance between EE and NE rats, the mean time spent in thigmotaxis across testing was calculated and covaried using an analysis of covariance (ANCOVA) with the mean time to find the platform. Housing and sex were included in this analysis.

Ethical Note

All animal treatment and husbandry were carried out in accordance with the Animals Scientific Procedures Act 1986, and all experimental procedures were performed under U.K. Home Office licences. Although isolation housing in barren cages is discouraged by the U.K. Home Office, in this experiment such housing was a necessary requirement and so unavoidable. Furthermore, single housing reduced the total number of animals that were used (to avoid pseudoreplication only one animal per cage should be tested; Festing et al. 2002). To reduce suffering after swimming in the MWM all rats were towel dried and then placed under heat lamps. At the end of the experiment all animals were humanely killed by exposure to a gently rising concentration of carbon dioxide. Although there is some debate as to whether this method of euthanasia is humane (e.g. Conlee et al. 2005), Schedule One of The Animals (Scientific Procedures) Act 1986 continues to list CO_2 as a humane means of euthanasia when it is administered in a rising concentration.

RESULTS

Reference Memory

Males reached the platform significantly sooner than females and NE rats took significantly longer to find the platform than EE rats (sex: $F_{1,21} = 4.39$, $P = 0.049$; housing: $F_{1,21} = 9.45$, $P = 0.006$; Fig. 1a). The mean performance levels of all treatment groups decreased significantly as the experiment progressed (block: $F_{10,210} = 19.02$, $P < 0.0001$). No other interactions were significant.

Thigmotaxis

Females were more thigmotactic than males and NE rats were significantly more thigmotactic than EE rats (sex: $F_{1,21} = 4.17$, $P = 0.05$; housing: $F_{1,21} = 25.93$, $P < 0.0001$; Fig. 1b). Mean levels of thigmotaxis decreased as testing progressed ($F_{10,210} = 24.64$, $P < 0.0001$; Fig. 1b) and thigmotaxis in males tended to decrease

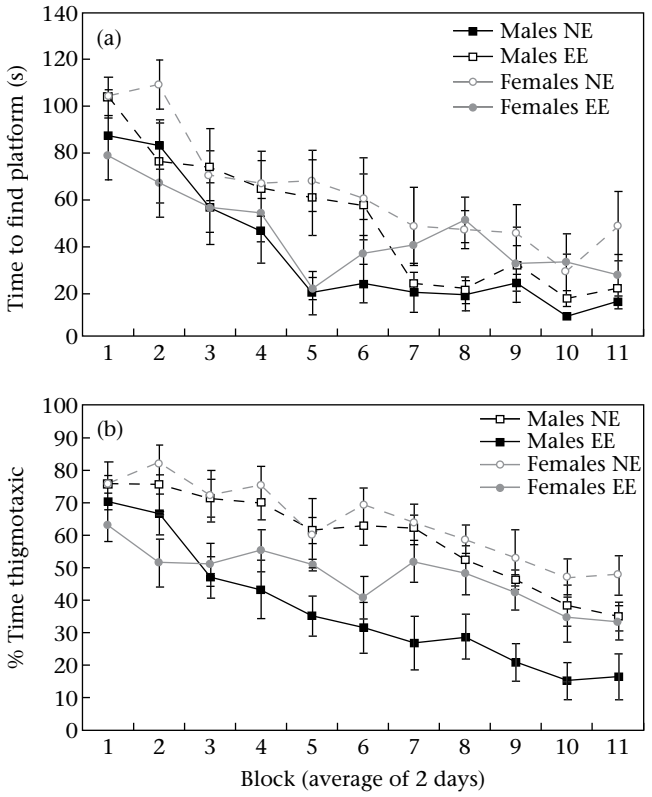

Figure 1. (a) Mean time to find the platform (s) ± SE and (b) Mean % of time spent swimming thigmotactically (within 15 cm of side of tank) ± SE for males and females from either enriched housing (EE) or nonenriched housing (NE). Data are blocked over 2 days. $N = 6$ per group.

faster than in females (sex*block interaction: $F_{10,210} = 1.84$, $P = 0.056$). No other interactions were significant.

Mean thigmotaxis levels correlated positively with the mean time the rats took to find the platform over the 22 days of testing (Pearson correlation: $r^2_{22} = 0.69$, $P < 0.0001$). Moreover, once thigmotaxis was accounted for, the sex and housing effects were not significant (ANCOVA: sex: $F_{1,20} = 0.65$, $P = 0.43$; housing: $F_{1,20} = 0.30$, $P = 0.59$; Fig. 2).

Probes 1 and 2

The mean time that the rats spent in the target quadrant in Probe 1 was not significantly different to chance (one sample t test: Probe 1: $t_{23} = 1.32$, $P = 0.20$; Fig. 3a). However, in Probe 2, after 22 days of spatial training, the mean time that the rats spent in the target quadrant was significantly longer than expected by chance, indicating that the rats had learnt the platform's location (one sample t test: Probe 2: $t_{23} = 8.23$, $P < 0.0001$). Similarly, the mean time that the rats spent in the target quadrant in Probe 2 was significantly greater than in Probe 1 (RM ANOVA: $F_{1,21} = 65.66$, $P < 0.0001$; Fig. 3a). Males tended to spend longer than females in the target quadrant in Probe 2 (probe trial*sex interaction: $F_{1,21} = 3.23$, $P = 0.087$). Housing had no effect on time in the target quadrant in the probe trials and no other interactions were significant.

Thigmotaxis during Probes 1 and 2

The EE rats were significantly less thigmotactic than NE rats during the probe trials ($F_{1,21} = 13.72$, $P = 0.001$). The mean levels of thigmotaxis for each treatment group decreased significantly from Probe 1 to Probe 2 ($F_{1,21} = 43.94$, $P < 0.0001$; Fig. 3b). Overall, males

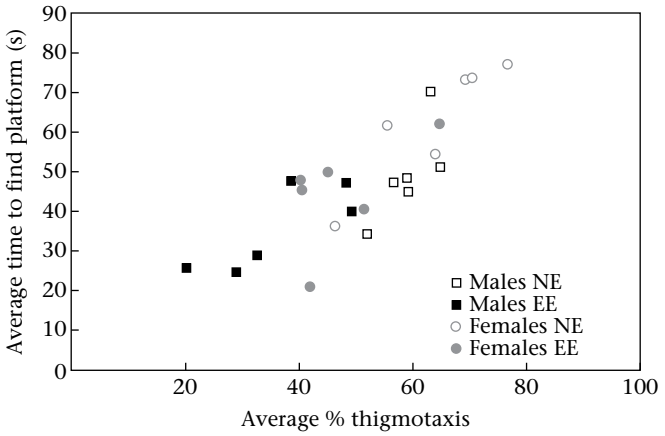

Figure 2. Mean time (s) taken to find the platform (across the 16 test days) in relation to average thigmotaxis (%) across the 16 test days for males and females from either enriched housing (EE) or nonenriched housing (NE). $N = 6$ per group.

and females spent a similar proportion of time engaged in thigmotaxis ($F_{1,21} = 0.43$, $P = 0.517$). However, in Probe 2, males were, on average, significantly less thigmotactic than females (sex*probe interaction: $F_{1,21} = 4.61$, $P = 0.044$).

Light/dark Box

There was a significant difference between the groups with respect to the mean time taken to leave the dark side: irrespective of sex, EE rats were quicker to venture into the light compartment (Welch one-way ANOVA assuming unequal variances: $F_{3,9.7} = 5.3$, $P = 0.021$). The EE rats took a mean ± SE of 32 ± 3.7 s and the NE took a mean of 128 ± 24.8 s to leave the dark side and enter the light compartment.

DISCUSSION

As predicted, the enriched rats reached the platform sooner than the nonenriched rats and males were faster to do so than females. However, both of these effects were entirely explained by the time spent in thigmotaxis. We did not measure the path length that the rodents took to reach the platform because latency is the most commonly reported measure of performance in the MWM (e.g. Santucci et al. 2008; Snihur et al. 2008). Furthermore, authors who measure both latency and path length typically present one parameter in detail and report that the other measure of performance followed the same pattern (e.g. Kempermann et al. 1997; Nilsson et al. 1999). Enriched rodents do not swim faster than barren-housed rodents and males do not swim faster than females (e.g. Kempermann et al. 1997; Snihur et al. 2008).

Living in enriched conditions is associated with enhanced spatial cognition in rodents (e.g. Nilsson et al. 1999; Leggio et al. 2005) and the favoured interpretation is that it is through interactions with a socially and physically complex environment that cognitive abilities are enhanced via 'informal learning' (e.g. Rosenzweig & Bennett 1996). However, as we found in our experiment that the time rats spent in thigmotaxis completely explained both sex and housing differences in performance, we propose that enhanced cognition in enriched rodents is a consequence of a reduced anxiety response during cognitive testing. We did not measure the levels of the stress hormone corticosterone in our rats during MWM testing because this would have meant invasive sampling (e.g. taking blood), which may have affected subsequent anxiety and performance levels. Additionally, corticosterone levels do not necessarily provide a reliable measure of stress or anxiety

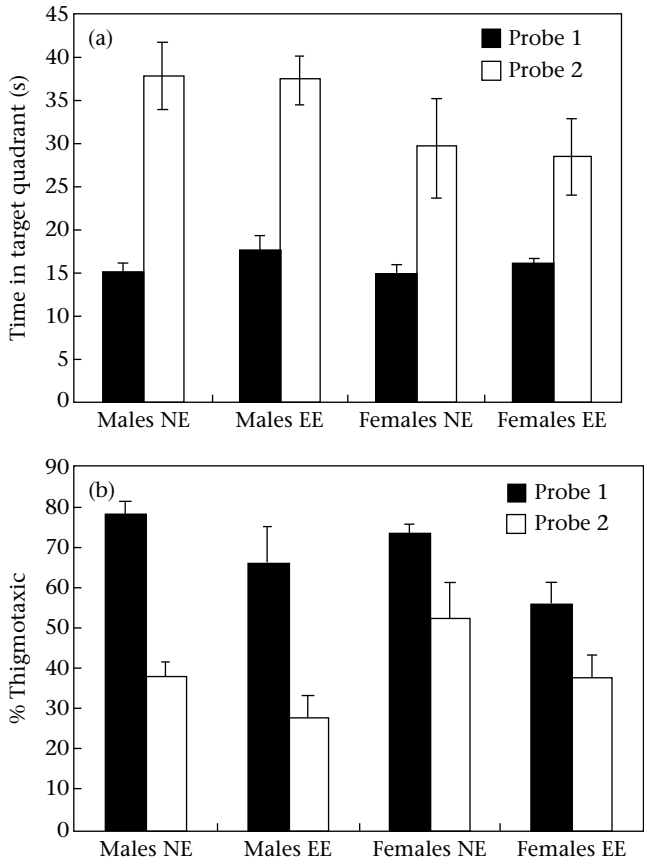

Figure 3. Mean time (s) ± SE spent in the quadrant that contained the platform and (b) Mean time ± SE spent swimming thigmotactically during Probe 1 and 2 for males and females from either enriched housing (EE) or nonenriched housing (NE). $N = 6$ per group.

levels in the home cage since corticosterone levels can return to base levels after periods of chronic stress (e.g. Jensen et al. 1996). Also, corticosterone levels increase with increased activity (e.g. not just stress). There is little consensus, then, on the impact of isolation or barren housing on corticosterone levels in rodents (e.g. depressed: Hurst et al. 1998; increased: Perelló et al. 2006; unaffected: Scaccianoce et al. 2006).

Our finding that, relative to barren-housed animals, enriched animals are less anxious when challenged with a stressful situation is consistent with other research (Chapillon et al. 2002). However, we are the first to show that this decrease in anxiety-related behaviour can cause an apparent improvement in performance in the MWM, a cognitive task in which thigmotaxis impedes performance. Indeed, we believe the nonenriched rats learnt the platform location as accurately as the enriched rats since in Probe 2 both groups of rats spent similar amounts of time in the training quadrant. However, in Probe 2, the nonenriched rats were still significantly more thigmotactic than the enriched rats, which suggests that, despite knowing the platform's location, the nonenriched rats were more reluctant to leave the maze wall than the enriched rats, which explains why the nonenriched rats took longer to find the platform during training. That thigmotaxis is a useful measure of anxiety is supported by our finding that the nonenriched animals were also slower to emerge from the dark side of the light/dark box than the enriched animals.

One interpretation of these results could be that the nonenriched animals underperformed relative to the enriched animals because isolation housing was stressful. However, this seems unlikely since we previously found that isolated and pair-housed rats were indistinguishable in terms of anxiety levels in the home cage and in the MWM. Additionally, performance levels during MWM testing were also comparable between pair-housed and isolated rats (Harris et al. 2008a, b).

An alternative interpretation of these results could be that the enriched rats had a better memory for the platform's location. However, this also seems unlikely because the enriched rats significantly less thigmotactic than the nonenriched rats in Probe 1, prior to any training, suggesting that the enriched rats were bolder right from the outset.

It may seem counterintuitive that a 'stress-free life', apparently experienced if housed with enrichment, should equip a rodent to deal well with novelty. Instead, we propose that the addition of novel objects into the home cage of a rodent may actually be a mildly stressful experience. However, since no aversive outcome is experienced from interacting with the enrichment objects, the animal learns that novel objects are not coupled with negative outcomes and as a result becomes habituated to novelty. Our light/dark box results would suggest that enriched rats may even seek novelty (even under aversive conditions), since they were four times faster to enter the light side than the nonenriched rats. Similarly, social housing, through the development or constant switching of social hierarchies or inability to escape from cagemates (for whatever reason), results in a changing or novel environment, which may be mildly stressful. Thus, it is possible that habituation to a changing environment (social or physical) explains why living in enriched housing is associated with a reduced anxiety response in acutely stressful situations.

We predicted that a sex difference in performance would be found only in animals housed in nonenriched housing; however, regardless of housing conditions, the males outperformed the females. Males found the platform faster during spatial training and, during the final probe trial when the platform was absent, spent longer in the platform quadrant. This finding is consistent with other studies that have also found a male advantage in reference memory tasks (Cimadevilla et al. 1999; Isgor & Sengelaub 2003; Saucier et al. 2008). However, the females were more thigmotactic during testing than the males, suggesting that the females experienced greater levels of anxiety. Therefore, consistent with other research, the sex difference in reference memory performance may be attributable to different anxiety levels in the males and females during testing (Perrot-Sinal et al. 1996; Beiko et al. 2004; Harris et al. 2008a, b). It is unclear why enrichment did not lead to sufficient reductions in thigmotaxis in females such that sex differences were not seen. Typically, reduction of the acute stress response, either through adrenalectomy, anxiolytic drugs, opioid inhibitors or pretraining, leads to equivalent male and female reference memory performance (Galea et al. 1994; Kavaliers et al. 1996; Perrot-Sinal et al. 1996; Beiko et al. 2004). There are no data on the effects of anxiolytics or pretraining with regard to cognitive differences between nonenriched and enriched rats but our results lead us to predict that either would remove, or substantially diminish, enrichment benefits on cognitive performance.

Surprisingly, given the sex difference in thigmotaxis in the MWM, we found no differences between males and females in the light/dark box. However, it is possible that the light/dark box is either not a very sensitive measure of acute stress or not as stressful as the MWM.

Our results raise several issues. First, they cast doubt on the leading hypothesis that enrichment directly improves cognitive ability by increasing 'brain power' (e.g. Kempermann et al. 1997; Pham et al. 1999). Second, as most measures of performance in the MWM consist of the time or distance to find the platform, both of which are affected by the time spent swimming thigmotactically, there are implications for all work done using the MWM. For

example, changes in performance in the MWM that are affected by age or drug administration may be the result of effects on thigmotaxis and not cognition itself.

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