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Animal Welfare 2010, 19: 235-241
ISSN 0962-7286

A cage without a view increases stress and impairs cognitive performance in rats

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Abstract

*Single housing is believed to be chronically stressful and to have a negative impact on welfare and cognition in rats (*Rattus norvegicus*). However, single housing does not consistently evoke stress-like responses nor does it consistently impair cognitive performance. In an experiment in which all cages were separated by an opaque barrier, single- and pair-housed pigmented (dark-eyed) rats performed equally in a cognitive test and displayed similar levels of anxiety during testing. Additionally, bar biting in the home cage did not differ between the two groups. Stress levels both during cognitive testing and in the home cage were higher than those we have previously reported when rats were housed without opaque barriers between the cages. We conclude that visual interactions between rats in different cages may be of sufficient significance that single housing in a cage with a view to neighbouring rats and to the rest of the laboratory holding room may be preferable to pair housing in a cage without this view.*

Keywords: *animal welfare, rats, spatial cognition, stress, thigmotaxis, visual isolation*

Introduction

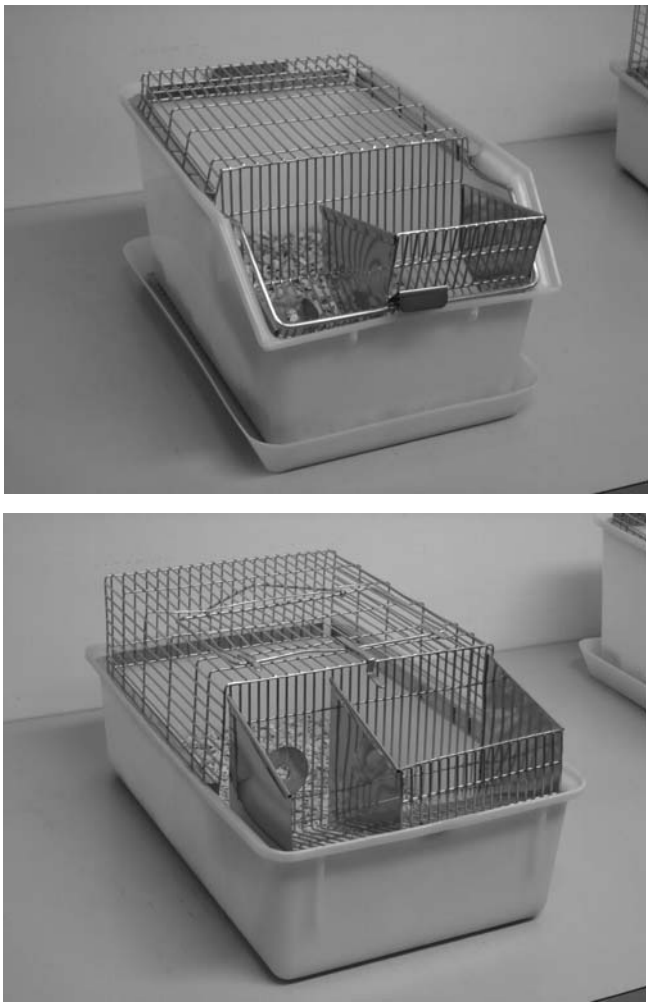
Single-housed rats develop 'odd' behaviours (bar biting, tail chasing), eat more, put on more weight, are more aggressive, have heavier adrenal glands and under-perform in cognitive tests relative to socially housed conspecifics (Hatch *et al* 1963; Baenninger 1967; Hurst *et al* 1998; Patterson-Kane *et al* 1999, 2002; Sandstrom & Hart 2005). As a consequence of these findings, many major animal science regulatory bodies (for example, UK Home Office, The Council of Europe, Australia's National Health and Medical Research Council and the Canadian Council on Animal Care) strongly discourage single housing of rodents in animal research (access to these documents through the Association for Assessment and Accreditation of Laboratory Animal Care International's website: http://www.aaalac.org/). Nevertheless, single housing is still used worldwide for logistical and ethical reasons, for example, to reduce the number of animals used, to avoid pseudoreplication, following surgery, or paradoxically to remove social stress (eg Nyska *et al* 2002; Verwer *et al* 2007).

However, despite the widespread belief that single housing impairs welfare, single housing does not consistently evoke greater stress hormone responses (Morinan & Leonard 1980) or result in heavier adrenal gland weights (adrenals secrete the stress hormone corticosterone and enlarge with prolonged activity) than does social housing (Baldwin *et al* 1995). Furthermore, single-housed rats are not always cognitively impaired (Wongwitdecha & Marsden 1996) nor do they always eat and weigh more than socially housed conspecifics (eg Baldwin *et al* 1995). Additionally, in a series of experiments (five in total), we could find no compelling evidence that single housing had a detrimental impact on a range of typical welfare measures (bodyweight, food intake, bar-biting behaviour) or on cognition in either male or female rats (Harris *et al* 2008a,b).

One explanation for these conflicting findings is that single-housing effects vary depending on the severity of the isolation (Krohn *et al* 2006). For example, if visual, olfactory and auditory communication between neighbouring rats is allowed, it is possible that single housing is less stressful than previously considered. Male rats housed alone but with visual, olfactory and auditory contact with neighbours are less aggressive when reintroduced to group housing than are rats without contact with neighbours (Hurst *et al* 1997). Additionally, single-housed rats spend more time investigating a barrier between neighbours the more that barrier allows social contact (Hurst *et al* 1997, 1998). These findings suggest that the degree of social contact among single-housed rats may significantly affect the degree to which single housing is stressful.

https://doi.org/10.1017/S0962728600001615 Published online by Cambridge University Press

Figure 1



Examples of two rat cages which present different levels of social contact between neighbouring rats: (upper) dimensions 380 × 250 × 200 mm (length × breadth × height) with a 20 mm strip of wire mesh at the top of the cage. Rats in these cages have no visual contact with neighbours and a significantly reduced view of the holding room when the cage is in the holding rack and (lower) 450 × 280 × 200 mm with an 80 mm strip of wire mesh at the top of the cage. In this type of cage rats can see their neighbours and out into the rest of the room when the cage is in the holding rack.

Social contact can vary significantly with the type of cage in which the rodents are housed (often laboratory-specific). In many parts of Europe, the USA and Australia 'standard' rat cages are opaque, plastic-bottomed cages with stainless steel wire-mesh lids (see Figure 1 for an example of two cages supplied by North Kent Plastic Cages LTD, Kent, UK). The wire-mesh lids differ in size depending on the type of cage that is used (Figure 1 [upper and lower]) leading to considerable variation in the degree of social contact a rat has with neighbours.

The following experiment had two aims: i) to determine whether preventing visual contact (opaque barriers were placed between the cages) with neighbours induced greater levels of anxiety and 'frustration' in rats housed alone than in pair-housed rats; and ii) to determine whether single housing without visual contact with neighbouring rats is sufficiently stressful to impair spatial cognition in a Morris water maze (MWM).

To measure anxiety and 'frustration' we recorded bar biting in the home cage and we monitored thigmotaxis (swimming in the periphery of the pool) since anxious rodents are reluctant to leave the safety of the edge during cognitive testing in the MWM (Hurst *et al* 1998; Wilcoxon *et al* 2007). Bodyweight and food intake were also monitored as basic indicators of welfare. If visual isolation does induce stress and anxiety, we would expect the isolated rats to display greater levels of bar biting and to perform more poorly in a spatial cognition task than do pair-housed rats.

Materials and methods

Subjects and housing

Eighteen male and eighteen female Lister Hooded rats, aged four to five weeks and obtained from Harlan Ltd, UK were the subjects tested in this experiment. At the time of arrival, the males weighed 160 (± 11) g and the females weighed 120 (± 7) g. Six rats of each sex were chosen at random to be single-housed, the remaining 12 were housed in same-sex pairs (thus n = 6 per treatment group). Rats remained in their housing conditions throughout the entire experiment. Where pair housed, one rat was marked with hair dye (Schwarzkopf, R43, Henkel, Dusseldorf, Germany) to enable identification. To avoid pseudoreplication, 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.

All rats were housed in plastic-bottomed cages (450 × 280 × 200 mm; length × breadth × height) (RB3 cages, North Kent Plastic cages Ltd, Kent, UK) and provided with a 2-cm layer of aspen woodchip bedding. The cages were cleaned out once per week. A barrier made from white plastic and covered with white paper was slotted between each neighbouring cage (the rats could not touch the barrier) within the holding rack. This barrier prevented visual contact between neighbouring rats and reduced visual contact with the rest of the holding room while not impeding olfactory and auditory communication. Rats were fed *ad libitum* food pellets (RM3 diet, Special Diet Services Ltd, Witham, Essex, UK) and tap water and maintained under a 12:12 light:dark cycle (lights on at 0600h) at 21–24ºC. The relative humidity within the room was 50 (± 10)% and the light intensity varied from top to bottom of the rack, but averaged 48 lux in the cages (cages were randomly positioned in the holding rack).

Rats experienced their respective housing condition for 10 weeks before spatial ability was assessed in an MWM. Each single-housed and each focal rat was tested in the MWM.

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MWM apparatus

The MWM consisted of a circular tank (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; length × breadth) with geometric and landmark cues (eg 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]ºC and made opaque with non-toxic white paint (Dulux, ICI, London, UK). 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 equally sized quadrants (labelled as the four main compass points N, E, S and W). For each of the platform locations there were four possible release points into the pool: NE, SE, SW and NW. We videoed all trials from above using a camera (Canon, Tokyo, Japan) with a 4-mm wide-angle lens. To reduce both stress and distraction to the rats during testing, all trials were observed via a video monitor once the rat was placed in the water.

MWM procedure

Each rat received two days of training before testing began. 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 pseudo-randomly determined so that the platform was never in the same place on two consecutive days. A swim started 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 to the nearest second using a stopwatch. If a 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, 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, 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 last day of training and the procedure was exactly as for training with the exception that each rat received four swims each day for 16 consecutive days. All trials were conducted between 1100 and 1500h.

Working and reference memory assessment

The time taken to reach the platform across the four daily swims provided the measure of working memory. To measure reference memory, the percentage of time that a rat spent swimming in each of the four quadrants in Swim one of each day 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 quadrant that contained the platform on the previous day. Reference memory was assessed from Day two of testing to Day five. We restricted the data used to assess reference memory for two reasons: (i) because reference memory cannot be measured on Day one, and (ii) moving the platform every day over 16 days of testing may have led to the rats learning to avoid the previous day's platform location.

Thigmotaxis measurement

The percentage of time that a rat spent swimming within 150 mm of the wall of the maze was recorded for Swims one and two across the 16 days of MWM testing. The footage of each swim was watched on a TV monitor. An acetate sheet, placed over the TV, displayed the outer 150-mm periphery and the time that a rat's head and shoulders spent in this area was recorded with a stopwatch.

Monitoring bodyweight and food intake

Bodyweight was measured once a week until the week that MWM testing began. Food intake was also measured once a 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. The cage floor was checked for food particles before weighing. Food intake per rat per day was estimated 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.

Behaviour in the home cage

The rats were filmed in their home cages for two hours during the dark phase (0300–0500h, pilot work showed this was peak activity time) using a black and white wide-angle (4 mm) lens camera. During filming a 40 W red light bulb was used for illumination. Each cage was filmed prior to MWM testing (approximately 5–8 weeks post arrival). Bar biting was scored for each focal animal in the 1st, 12th, 24th, 36th, 48th, 60th, 72nd, 84th, 96th, 108th and 120th minute of the footage providing an observation period of 11 min per rat. Every five seconds for each of these minutes, we noted the presence or absence of bar biting. The total number of occurrences of bar biting were totalled and multiplied by five (duration of the observation period) to equal total time spent bar biting, which was then converted to a percentage of the observation period. The total number of rats that showed at least one occurrence of bar-biting behaviour was also noted.

Ethical considerations

Animal treatment, husbandry and all experimental procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986, UK, and the associated *Guidelines for the Use of Animals in Scientific Procedures* set out by the Home Office regulations under the Home Office project licence number 60/3531. Although single housing is strongly discouraged by most major animal science regulatory bodies, in this experiment, it was an unavoidable requirement. All experimental procedures were carried out by the same researcher (APH) to keep handling stress to a minimum. To minimise the potential for suffering

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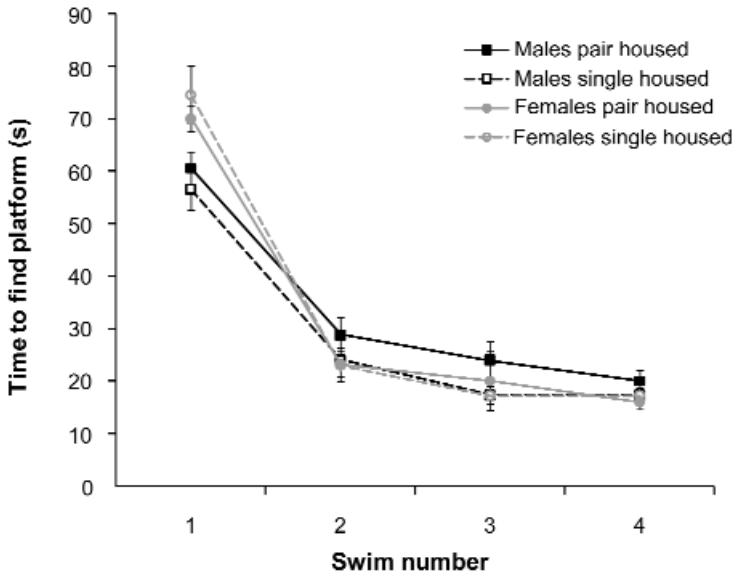
Figure 2



Mean (± SEM) time to find the platform (s) across the 16 days of testing for male and female rats that were pair or single housed with a visual barrier placed between cages for 10 weeks (n = 6 per group).

due to cold, after the final swim of each day, rats were gently towel-dried and placed in their home cages under a heat lamp for approximately 10 min. At the end of the experiment rats were euthanased by exposure to a gently 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.

Data analysis

Data that included repeated measures on the same subject were analysed using a repeated-measures analysis of variance (RM-ANOVA). The 'between-subject' factors were sex (male/female) and housing condition (pair/single) and the 'within-subject' factors were swim number (one to four) and day (one to 16). All interactions were tested and, if not significant, were removed from the final model. The assumptions of sphericity were tested using the Mauchly-criterion test. If the assumption of sphericity was not met, we used Greenhouse-Geisser adjusted degrees of freedom and the associated *P*-values, which is why the degrees of freedom we report 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.

Results

Working memory

Single- and pair-housed rats with a barrier between their cages performed equally well in the MWM ($F_{1,21}$ = 0.82, P = 0.37; Figure 2). Although the average performance of males and females did not differ significantly (ie averaged over the four swims and across the 16 days of testing; $F_{1,21}$ = 0.56, P = 0.46), females took significantly longer to find the platform in Swim one than the males (swim by sex interaction: $F_{2.1,44.0}$ = 9.47, P = 0.0003; Tukey HSD, P < 0.05; females took a mean of 72 (± 4) s and males took 59 (± 4) s. All of the rats took less time to reach the platform with increasing swim number within a day ($F_{2.1,44.0}$ = 292.98, P < 0.0001; Figure 2) and across days ($F_{7.1,149.1}$ = 8.76, P < 0.0001). No other interactions were significant.

Reference memory

In Swim one, the single- and pair-housed rats did not differ significantly in the amount of time they spent searching for the platform in the target quadrant ($F_{1,21}$ < 0.01, P = 0.95; 40.7 [± 2.1]% for paired rats and 40.6 [± 1.7] for singled rats). Additionally, males and females did not significantly differ in the time they spent in the target quadrant ($F_{1,21}$ = 2.43, P = 0.13; for males 38.5 [± 1.6]% and females 42.6 [± 2.0]%). The amount of time that the rats spent in the target quadrant varied significantly over Days two to five ($F_{2.2,47.0}$ = 4.0, P = 0.02), but this seems to be due to non-directional fluctuations rather than to an increase or decrease across the days (time spent in Day four is longer than on the other days). No other interactions were significant.

Thigmotaxis

Single- and pair-housed rats were equally thigmotactic during testing ($F_{1,21}$ = 0.50, P = 0.49; Figure 3). Also, irrespective of housing conditions, the females were more thigmotactic than males ($F_{1,21}$ = 6.03, P = 0.02) but only in Swim one ($F_{1,21}$ = 8.57, P = 0.008; Figure 3). All of the rats were less thigmotactic in Swim two than they were in Swim one ($F_{1,21}$ = 41.6, P < 0.0001; Figure 3). Thigmotaxis levels changed significantly across testing ($F_{7.3,153.0}$ = 4.12, P = 0.0003), but there was no directional trend (eg thigmotaxis on Day one is significantly higher than on Day eight

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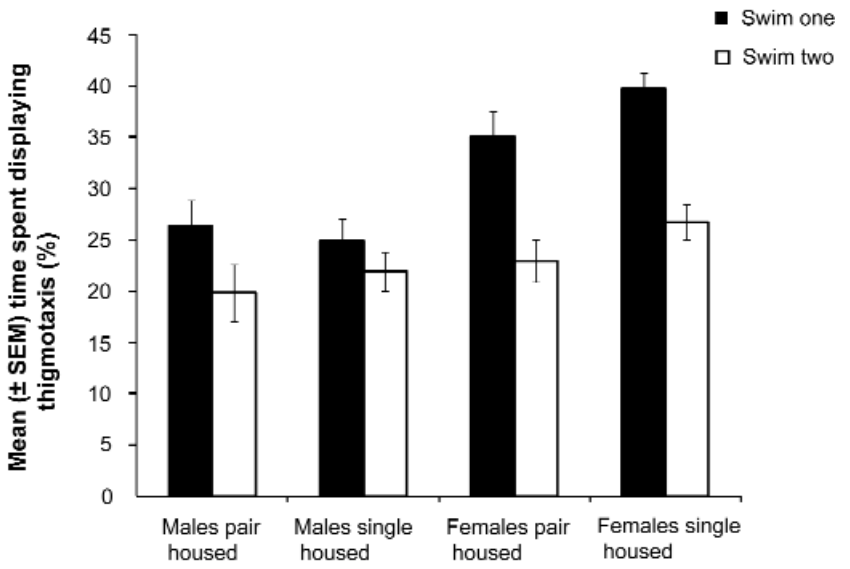
https://doi.org/10.1017/S0962728600001615 Published online by Cambridge University Press

Figure 3

Mean (± SEM) percentage of thigmotaxis in Swim one and two for male and female rats that were pair or single housed for ten weeks (n = 6 per group).



but not Day 16; Tukey HSD, $P < 0.05$). No other interactions were significant.

Bodyweight and food intake

Regardless of sex, single-housed rats did not weigh more than the pair-housed rats: average bodyweight for females 186 (± 30) vs 179 (± 3) g; and males 310 (± 7) vs 296 (± 8) g, for isolated and pair-housed rats, respectively ($F_{1,21}$ = 4.28, P = 0.051). Irrespective of sex, the single-housed rats ate significantly more than pair-housed rats: mean daily intakes for females 19 (± 0.4) vs 17 (± 0.4) g; and males 25 (± 0.4) vs 23 (± 0.3) g, for single- and pair-housed rats, respectively ($F_{1,21}$ = 418.55, $P < 0.0001$).

Bar biting

Single-housed rats did not bite the bars more than pair-housed rats during the observation period ($F_{1,19}$ = 1.83, P = 0.19; mean proportion of time bar biting for paired males: 5 [± 1.6]%; singled males 9 [± 3.7]%; paired females 2 [± 1]%; singled females 6 [± 3.2]%) but not all rats were observed to bite the bars. Analysing only the data from the seven paired and eight singled rats that were observed to bite the bars, there was still no significant difference in bar-biting levels between the two groups ($F_{1,13}$ = 2.96, P = 0.11). Male and female rats also did not differ in the amount of time they spent bar biting ($F_{1,19}$ = 2.04, P = 0.17; n = 22; data from two cages were lost due to human error).

Discussion

Removing visual contact between rats by the insertion of an opaque barrier between neighbouring cages was expected to have a bigger effect on the behaviour of animals housed alone than those housed in pairs (Prediction one). This prediction was not met: although we found that isolated animals ate more (by approximately 10%) than pair-housed animals, bar biting did not differ between the groups. Additionally, there were no group differences in cognitive performance in the MWM or in the amount of time spent in thigmotaxis (our behavioural measure of anxiety during testing). A hasty conclusion may be that single housing is not more stressful than pair housing, even when visual contact with both neighbouring rats and the holding room is removed. However, stress levels both during cognitive testing and in the home cage appear to be higher than we have found previously, in rats that were housed without barriers between their cages, irrespective of whether the rats were housed alone or with a cage-mate (Harris *et al* 2008a). However, although because the experiments were conducted at different times, a formal quantitative comparison between data from the current experiment and from that previous experiment cannot be carried out, a qualitative comparison reveals that the animals in the barrier experiment engaged in considerably more bar-biting behaviour (15 rats) than rats housed without the opaque barrier (6 rats: see Experiment 3, Harris *et al* 2008a). This outcome was observed despite half of the animals being housed in pairs. Additionally, rats housed without barriers seem to display lower levels of thigmotaxis during Swim two in the MWM than rats housed with barriers between their cages (7 [± 1] vs 23 [± 1]% thigmotaxis; Harris *et al* 2008a). As thigmotaxis correlates positively with anxiety levels, increases in this behaviour suggest impaired welfare during testing (Treit & Fundytus 1989; Snihur *et al* 2008). Thigmotaxis also impairs performance in the MWM because the platform is never located in the outer periphery of the tank (eg Beiko *et al* 2004; Harris *et al* 2009). Consistent with previous research, we found a sex difference in favour of males only when sex differences in thigmotaxis were present (Perrot-Sinal *et al* 1996; Beiko *et al* 2004; Harris *et al* 2008b). Additionally, we found better levels of performance in Swim two by rats that were housed without a barrier between their cages than by rats that were housed with a barrier (16 [± 1] vs 25 [± 1] s to find the platform).

https://doi.org/10.1017/S0962728600001615 Published online by Cambridge University Press

Our data highlight how variation in visual contact between rats and their neighbours and the holding room may inadvertently affect the outcome of a cognitive experiment.

Since the presence of a cage-mate in the current experiment did not influence stress-related behaviour or MWM performance, this suggests that the presence of a cage-mate does not necessarily ameliorate stress. It appears that the barrier increased stress levels not simply because it removed social contact but because it reduced how much the rats could see out of the home cage and into the holding room. For example, it is possible that rats housed with a barrier between the cages were less aware of people in the holding room and therefore had less opportunity to habituate to people. An alternative explanation is that the barriers induce stress because unidentified noises from behind the barrier provoke fear of predation in the rats. It is currently unclear if auditory contact between neighbouring cages is valued or unpleasant to a caged rat (eg Wells 2009).

If the degree of visual contact that a rat has with the holding room is important, then the material from which the walls of the rat's cage are constructed also becomes pertinent. Opaque-sided cages prevent visual contact (see Figure 1) but it is also possible that other types of caging inadvertently prevent visual contact. For example, it is possible that rats cannot see through 'transparent' polycarbonate cages to the same extent that humans can. It is also unclear how important auditory and olfactory contact between a rat and the holding room is (Wells 2009). For example, individually ventilated cages (used routinely to prevent the spread of airborne particles; Renstrom *et al* 2001) totally preclude olfactory contact, significantly reduce auditory contact and may also prevent visual contact, since the cage sides are 'transparent plastic' (eg Krohn *et al* 2003).

Single-housed rats tend to bar bite more than socially housed rats, apparently in frustrated attempts to leave the cage and seek social contact (eg Hurst *et al* 1997, 1998; Nevison *et al* 1999). Although we did not see a difference in bar biting between the single- and pair-housed animals used in this experiment, the levels of bar-biting behaviour that we saw in this experiment seem to be considerably higher than in our previous experiments, in which no barriers were present (6 vs 15 rats performed bar-biting behaviour: Harris *et al* 2008a). An increase in this 'escape-related' behaviour suggests that reduced visual contact with neighbouring rats/the holding room potentially impairs welfare through an increase in frustration levels, which rise even when the rat has a cage-mate (eg Lewis & Hurst 2004).

Animal welfare implications and conclusion

The loss of visual interactions between caged rats and the holding room led to a significant increase in stress-related behaviours, both within the cage and in a spatial cognition task. Males and females were equally affected and the effect did not seem to be ameliorated by the presence of a cage-mate. This effect of the physical, rather than the social, attributes of housing is currently relatively unappreciated, with far more emphasis on within-cage enrichment (Home Office 1995; Patterson-Kane 2004; Balcombe 2006).

Based on the data we present here, the welfare of rats housed alone, but with visual contact with neighbours, may be better than that of pair-housed rats in cages that prevent visual contact with neighbouring rats/the holding room. Explicit demonstrations of the effect of a visual barrier are required to confirm that such a barrier does increase stress in rats. Although barriers are not a feature of rat housing, many of the cages deemed suitable for housing rats preclude visual contact to a significant degree (knowingly or unwittingly). A corollary of demonstrating that visual contact may lead to better welfare than pair housing is that the welfare costs of housing rats alone may be traded off against the reduction in numbers of animals required to deal with pseudoreplication (although group housed, only one animal per cage can be used in any one experiment: Hurlbert 1984; Festing *et al* 2002). Reducing the numbers of animals used in scientific research is one of the aims of the 3Rs (reduction, replacement and refinement), which form the basic principles of humane research (Russell & Burch 1959). Although single-housing rodents is a rather heretical suggestion, based on the results we present here, we would encourage further investigation (using additional measures of welfare to those employed here and using different strains of rat, eg albino) into the role of visual contact allowed by different cages on the welfare of laboratory rats so that pair housing is not used to no welfare benefit.

Acknowledgements

This research was funded by UFAW 3Rs Liaison Group. We thank the MARCH staff for excellent care of the animals and Alex Brudenell for help with data collection and two anonymous reviewers for comments on an earlier version of the manuscript.

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