

HOUSING AND WELFARE IN LABORATORY RATS: THE WELFARE IMPLICATIONS OF SOCIAL ISOLATION AND SOCIAL CONTACT AMONG FEMALES

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Abstract

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Female laboratory rats (Rattus norvegicus: Wistar, Alderley Park) were housed as singletons or groups of three in units of two cages. Units were divided by different types of barrier which allowed varying degrees of social contact across the barrier. Singletons were established either with another singleton on the other side of the barrier or with a group of three as neighbours. Single-housing among females had markedly less effect on time budgeting and pathophysiological measures than among males in a similar, earlier study. In particular, singletons showed a less marked increase in self-directed behaviours, particularly tail chasing, and a smaller reduction in undirected movement around the cage. The smaller reduction in mobility may reflect a greater tendency for singly housed females to attempt escape. Females generally showed much higher levels of escape-oriented behaviours than males and up to a threefold increase in such behaviours when housed singly. Differences in time budgeting and in the apparent significance of social separation between the sexes can be interpreted in terms of differences in socio-sexual strategy and potential mating opportunity, with singleton males responding to their cage as a territory, but singleton females seeking to re-establish social contact. Such an interpretation is consistent with the effects of barrier type on behaviour in singleton females, in which time spent in escape-oriented behaviours reflected the extent to which the barrier facilitated, or frustrated, contact with neighbours.

Keywords: *animal welfare, female, isolation, pathophysiology, rat, Rattus norvegicus, time budget*

Introduction

In a recent review, Barnard & Hurst (1996) have pointed to the ambiguity inherent in the use of stress-related measures (eg elevated glucocorticoid levels, immunodepression, behavioural changes) in drawing conclusions about animal welfare. Their central point is that such measures may reflect adaptive life history trade-offs rather than impositions on the

animal (see also Ots & Horak 1996), and thus have little implication for welfare. This difference in interpretation may be resolvable by measuring the impact of circumstances on the animals' decision rules relating to time budgeting and their responses to environmental contingencies (Barnard & Hurst 1996). Hurst *et al* (1997) used this approach to assess the welfare implications of housing conditions on laboratory rats (*Rattus norvegicus*) and, in particular, to examine the consequences of social isolation among caged males.

In Hurst *et al*'s (1997) study, singly housed males showed reduced activity and a greater incidence of self-directed behaviours, especially tail manipulation (chasing and close olfactory attention or manipulation; see also Baenninger 1967) and self-grooming, than males housed in groups. They also spent time performing behaviours apparently related to attempting to escape or seeking social information and had lower post-experimental serum concentrations of corticosterone and organ pathology scores. A negative correlation between tail manipulation and organ pathologies was consistent with Baenninger's (1967) suggestion that tail manipulation in singletons is a surrogate social response. This was also consistent with observations of an overall increase in self-directed activity, reflecting elasticity in time budgeting (Hurst *et al* 1997). Variation in the degree of increase in self-directed activity among singletons, and the negative correlation between self-directed activity and organ pathology among male rats, may have reflected differences in the ability of individuals to avoid an activity limbo (McFarland 1989; Barnard & Hurst 1996).

However, while reduced levels of corticosterone and reduced organ pathology scores among singletons imply that separation removed social stress, the responses of males given different degrees of social contact with neighbours (through different types of barrier) suggested that singletons actively sought olfactory social information and social interaction despite the apparently stressful consequences of the latter. Overall differences in stress responses between singly housed males and those in groups thus appeared to be an inadequate indicator of the animals' welfare.

An additional finding of Hurst *et al* (1997) was that exposure to neighbours through a barrier reduced the aggressiveness of singly housed males when they were eventually introduced into an unfamiliar group. This suggests that a degree of social contact (ie separation but not isolation) may have some welfare benefits for caged rats, depending on procedures.

Hurst *et al* (1996) pointed to the widespread assumption that aggression is greater in male, as compared with female, groups (Ziporyn & McClintock 1991), and that social stress, and thus the consequences of grouping for welfare, will be greater among males. However, in single sex groups housed in enclosures, Hurst *et al* (1996) found that females maintained aggressiveness over time (in contrast to males which initially showed greater aggressiveness when groups were established that declined to lower levels after a few weeks). All females in a group were likely to experience greater social stress as a result of the persistent aggression of dominants against subordinates that fled but were unable (because of confining walls) to leave the vicinity.

Differences between the sexes in aggression, and relationships between aggression and social class in Hurst *et al*'s (1996) study were associated with a number of time budgeting and pathophysiological differences. In particular, females spent less time sleeping and feeding and more time exploring their enclosure and moving around without directed attention than males. Females, especially subordinates, were more likely to spend time

investigating and climbing the walls of their enclosure in an apparent attempt to escape. In conjunction with these behavioural differences, females showed higher serum corticosterone concentrations and a greater prevalence of certain organ pathologies (particularly those of the adrenal glands). Together, these findings suggest that the behavioural and pathophysiological consequences of being housed singly rather than in a group (thus removing social stress) may be different for females. If so, different welfare considerations may apply to single-housing in the two sexes. In terms of specific differences, we might expect single females to be less likely to show increased self-directed behaviour (regardless of whether this reflected a surrogate social response or elasticity in time budgeting among males) if social stress is greater in female groups, since the incentive to seek social stimuli is likely to be reduced. By the same token, single females should show a greater reduction in undirected movement around the cage relative to grouped females (if undirected movement in groups reflects responses to persistent aggression) and less behaviour associated with escape and seeking social information than among males. To test these predictions, we repeated Hurst *et al*'s (1997) experiment using singly housed and group-housed females.

Methods

Experimental housing conditions

One hundred and forty-four female Alderley Park rats (a Wistar-derived strain) were housed in paired cages containing either two singly housed neighbours, or a singleton adjoining a group of three females. All neighbours and cage mates were unrelated and previously unfamiliar with each other (see Hurst *et al* 1996) and were established from stock groups of five rats at age 9–10 weeks. Each pair of stainless steel cages (475 x 285 x 200mm high) had a mesh front and floor and was divided in two by one of four types of barrier designed to provide different degrees of contact between neighbours:

- i) solid steel (no contact);
- ii) clear Perspex (visual contact only);
- iii) clear Perspex perforated all over by 6mm holes (olfactory and visual contact);
- iv) double mesh (extensive olfactory, visual and possibly some tactile contact).

Six replicates of each barrier type and combination of stocking densities (1/1 or 1/3 rats) were arranged in four cage racks in a balanced¹ design, though the experiment was run in two batches of 72 rats (the second batch following 1 month after the first) to enable a large number of behaviour samples to be collected to estimate individual time budgets. Adjoining pairs of cages were separated by their solid metal walls, thus rats had no contact with neighbours other than those within their own paired cages. Each cage contained a jar of powdered CT1 diet (Special Diet Services Ltd, UK) and a water spout. The rats were maintained on a 12:12 light:dark schedule with continuous dim red lighting and white lights on between 1200 and 0000h. All rats were given unique ear punch codes at age 3–4 weeks and marked with hair dye (Nice 'n' Easy Natural Black 122 or Burgundy 113A, Bristol Myers Ltd, Uxbridge, UK) 5 days prior to pre-experimental blood sampling (see below) to allow individuals to be identified from a distance during behavioural observations (Hurst *et al* 1996).

¹ Balanced across batches and in terms of cage position on racks with respect to neighbour density and barrier type.

Time budgets

Behaviour samples were spread evenly over the last 4h of the dark phase (the most active period, Hurst unpublished data) and the first 4h of the light phase, over a 5 week period. Each week, instantaneous behaviour samples were collected during three, 4h observation periods in each phase of the light cycle. Each rat within the experimental room (ie one batch of 72 rats) was observed in a predetermined sequence at 4s intervals and its behaviour, posture or movement and location (contact with any of the cage sides, barrier or food pot) at the moment of observation was recorded. Sixty-four behavioural categories were recorded but were assigned to 17 functional categories for analysis (Table 1). To avoid any tendency to focus on the more interesting or obvious behaviours, an audio cue dictated every 4s via headphones (and therefore not audible to subject rats), regulated the timing of each sample. In addition, any aggressive behaviour observed between group-housed rats during the 4h observation period was noted (see Hurst *et al* 1996). A total of 38 instantaneous samples per rat were collected in each 4h light or dark phase observation period, giving a mean \pm SEM total of $1\,129 \pm 2$ observations per rat (excluding missing data) over the 5-week period.

Response to regrouping

After time budget samples had been completed, singly housed rats, (now aged 14-15 weeks), were introduced into the home cage of an unfamiliar group of three for 10min to assess the effect of different degrees of isolation on their social tolerance when rehoused among a group resident in its own home cage. We carried out four treatments (see Table 2), each replicated six times, which varied according to the neighbour contact previously experienced by the introduced singleton and by the resident group over the previous 5 weeks. Each individual and group was used only once. To assess the effect of prior contact with neighbours on singleton aggression, the introduced rats had either experienced: no contact with neighbours (solid-barriers, treatments 1 and 2); olfactory and visual contact through a perforated Perspex barrier with another singly housed neighbour (treatment 3); or olfactory and visual contact with group-housed neighbours (treatment 4) over the previous 5 weeks. To assess the effect of prior neighbour contact on tolerance by the residents, resident groups had either experienced no prior contact with a neighbour (solid barrier, treatment 1); or contact with a singly housed neighbour over the previous 5 weeks (treatments 2-4). Since there were only 4 x 6 caged groups in the study, we used all groups that had had some contact with a neighbour through Perspex or mesh barriers as residents, with two replicates of each barrier type in each of the treatments 2-4.

Singly housed rats were introduced into a resident group during the last 4h of the dark period and the behaviour of all rats was observed continuously for 10min, recording all occurrences of aggressive behaviour (see Table 1 and Hurst *et al* 1996) initiated by each individual. The introduced rat was then removed and returned to its home cage. The observer (CMN) was prepared to retrieve the introduced animal earlier if aggression was likely to result in physical injury or animals showed signs of distress, such as continuous attempts to escape, but this did not occur.

Table 1 Behavioural categories recorded.

Functional category	Behavioural elements
<i>Sleeping</i>	Lying or sitting not alert, eyes closed
<i>Feeding</i>	Eating powdered diet or faeces
<i>Drinking</i>	Drinking from water bottle
<i>Tail chasing</i>	Circling in pursuit of own tail
<i>Tail attention</i>	Sniffing, manipulating or chewing own tail
<i>Bar chewing</i>	Chewing or scrabbling at cage bars
<i>Non-intake maintenance</i>	Grooming; yawning; stretching; sneezing; urinating; defecating
<i>Stationary</i>	Alert (eyes open) but no directed attention while lying, sitting, standing or leaning against the food pot or cage side
<i>Movement</i>	Alert but no directed attention while walking, stretching up, climbing or running
<i>Investigate barrier</i>	Sniffing or licking barrier between neighbours
<i>Investigate bars</i>	Sniffing the cage bars or sides
<i>Investigate top</i>	Sniffing the roof of the cage
<i>Investigate floor</i>	Sniffing the floor of the cage
<i>Investigate faeces</i>	Sniffing at faeces on the mesh floor or food pot
<i>Investigate air</i>	Sniffing into the air or through the cage bars
<i>Other investigation</i>	Sniffing the food pot or water spout
<i>Social*</i>	
<i>Aggression</i>	Bite; chase; aggressive over (pinning rat on its back); aggressive groom; aggressive sideways; upright; mounting; pull tail
<i>Defence</i>	Defensive over (on back); defensive sideways; flight
<i>Social investigation</i>	Sniffing nose, mouth, head, shoulders, back, flank, anogenital area, belly, tail of cage mate or neighbour
<i>Allogroom</i>	Allogrooming cage mate

* A single category of Social behaviour was used when comparing the time budgets of singly housed and group-housed rats.

Table 2 Experience prior to regrouping.

Treatment	Introduction Singleton	Resident Group
1	Solid metal barrier, no neighbour contact	Solid metal barrier, no neighbour contact
2	Solid metal barrier, no neighbour contact	Perspex or mesh barrier, single neighbour
3	Perforated Perspex barrier, single neighbour	Perspex or mesh barrier, single neighbour
4	Perforated Perspex barrier, group of neighbours	Perspex or mesh barrier, single neighbour

Blood, organ and tissue sampling

All procedures were carried out under Home Office Licence No. 40/00891. Blood samples (up to 1ml) were taken from a caudal vein of each animal 2-6 days prior to introduction into their experimental cages and once again after termination (when aged 14-15 weeks). These were analysed for serum corticosterone, testosterone and total IgG using the following procedures (see also Hurst *et al* 1996).

Rats were removed from their cage and placed in a 'hot box' at 37°C for 5min to increase peripheral circulation and facilitate blood sampling. After sampling, rats were returned immediately to their cage. All blood samples were taken by the same person and between 0800 and 1000h, since pilot tests indicated least variability in hormone levels during the first half of the light period. Blood samples were analysed for serum concentrations of corticosterone, testosterone and total IgG. Concentrations of corticosterone (ng ml⁻¹) and testosterone (ng ml⁻¹) were determined using radioimmunoassay kits (Coat-a-Count™ solid phase ¹²⁵I-corticosterone and ¹²⁵I-testosterone, Diagnostic Products Corporation, Los Angeles). Total serum IgG (mg l⁻¹) was determined by surface plasmon resonance detection, following the method of Fägerstam *et al* (1992). The age and size of the rats in these experiments meant that it was not possible to take a third blood sample prior to the introduction of singletons to resident group cages in the social tolerance tests.

While the handling procedure during blood sampling and the social tolerance tests at the end of the experiment were likely to have had an impact on serum hormone concentrations (Döhler *et al* 1977; Tuli *et al* 1994), especially corticosterone, this was not a problem for our purposes because we were not attempting to measure base levels. Elevations of glucocorticoids, due to challenges such as environmental stressors or administration of adrenocorticotrophic hormone, tend to correlate positively with the severity of pre-existing stressors (Friend *et al* 1977; Restrepo & Armorio 1987; Pitman *et al* 1990). Short-term glucocorticoid responses to such challenges can therefore be used to infer longer-term pre-existing stress, as might occur with inappropriate housing or within established aggressive social relationships (eg Mugford & Nowell 1971; Sapolsky 1983; Manser 1992). Serum concentrations were transformed logarithmically for statistical analysis to meet the assumptions of the parametric tests. Body weight was recorded weekly during routine husbandry procedures.

After euthanasia with Halothane, selected organs (adrenal glands, kidneys, heart, thymus, spleen and testes) were carefully removed from the rats by two experienced prosectors, blotted dry, trimmed and weighed. Organs were then fixed, sectioned and examined for histopathological changes by an experienced veterinary pathologist. Any changes were scored for severity on an arbitrary integer scale from 0 (none) to 5 (severe and extensive); see Hurst *et al* (1997).

Statistical analyses

Scores for each of the behaviour categories in Table 1 were entered as dependent variables in a multivariate analysis of variance (MANOVA) with housing condition (single vs grouped) and barrier type as factors. Additional, repeated measures ANOVAs examined the effects of housing condition, barrier type and phase of the light cycle (light vs dark) on sleeping and general mobility (time spent moving or stretching up in any behaviour). Behavioural measures were averaged across individuals within groups to control for non-independence. Where there were *a priori* reasons for expecting differences or trends in a particular direction, probabilities associated with significance tests are indicated as one-tailed.

As we had no *a priori* reason to expect particular characteristics to be associated with different behaviour or physiological responses, severity scores for each histopathological change were summed to give the total pathology score per organ, and the pathology scores for each organ summed to give the total pathology score per rat for analysis.

Effects of housing condition, barrier type and neighbour density on pathology scores were analysed nonparametrically. To see whether the effects of housing condition were reflected in associations between pathology scores and those behaviours affected by housing condition, we also carried out a series of Spearman rank correlations.

Results

Effects of housing condition

As expected, univariate tests showed that singly housed females exhibited significantly more tail chasing than group-housed animals ($F_{1,88} = 4.69$, one-tailed $P < 0.02$). Indeed, tail chasing was shown by only 4 per cent ($n = 3$) of group-housed individuals but by 35 per cent of singletons. No significant difference emerged for tail attention. In terms of behaviours related to attempted escape and attention out of the cage, females housed singly showed considerably more bar chewing ($F_{1,88} = 10.15$, one-tailed $P < 0.001$; Figure 1a); sniffing the barrier ($F_{1,88} = 8.35$, one-tailed $P < 0.001$; Figure 2a) and sniffing the top of the cage (mean \pm SEM % time for singles 2.7 ± 0.2 , for grouped 1.3 ± 0.1 ; $F_{1,88} = 32.2$, $P < 0.001$). Pawing was not observed among our animals. Single-housing had no effect on time spent in maintenance-related behaviours (self-grooming, drinking, feeding). No analysis of the effect of housing condition on social behaviours was carried out, since time spent in social behaviours self-evidently differed between singly housed and group-housed rats.

Singly housed females spent less time sleeping over the entire light/dark cycle than grouped females. A repeated measures ANOVA taking light phase into account revealed a significant main effect of both housing condition ($F_{1,88} = 5.36$, $P < 0.05$) and, not surprisingly, phase of the cycle (with most sleep occurring during the light phase, $F_{1,88} = 204.3$, $P < 0.0001$). There was some interaction between housing condition and light phase,

with singly housed females tending to sleep less (5.9% of the time) than group-housed females (12.0% of the time) in the dark phase, but a similar amount in the light phase (26.7% compared with 28.0%; $F_{1,88} = 3.81$, $0.1 < P < 0.05$).

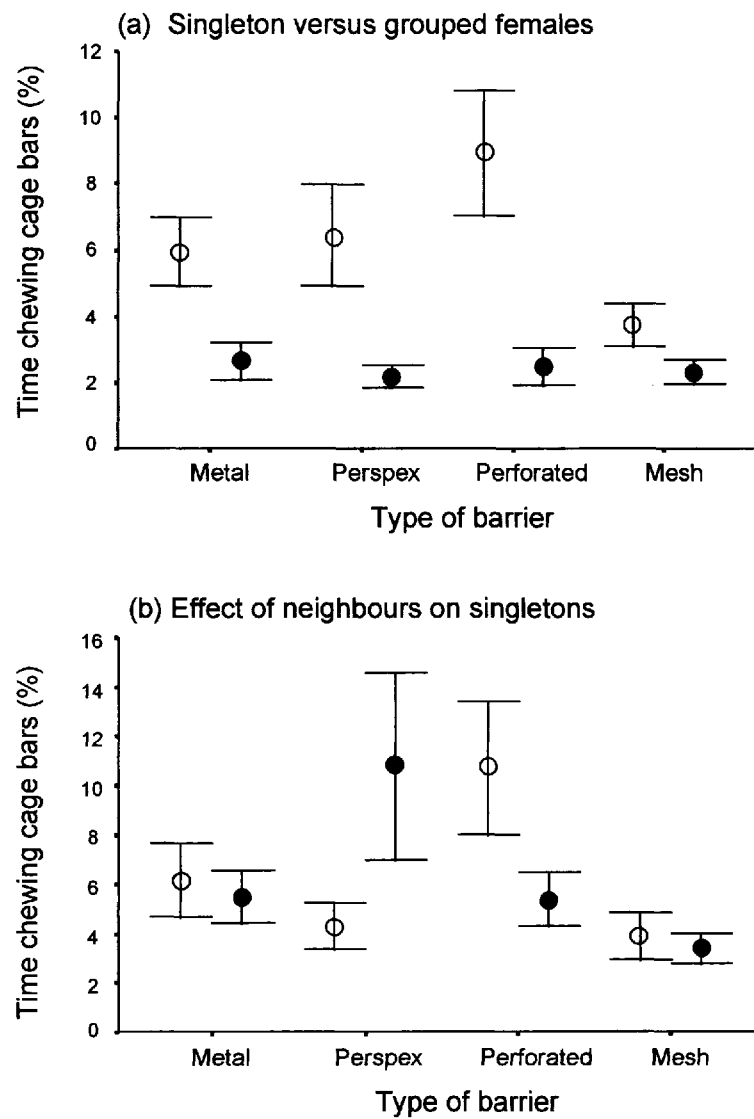


Figure 1 Proportion of time spent chewing cage bars when separated from a neighbouring cage by different types of barrier (mean \pm SEM per female, data for each caged group are the means per group of three). (a) singly housed (open circles) compared with group-housed (solid circles) females. (b) Singly housed females with a singleton neighbour (open circles) or with group-housed neighbours (solid circles).

Contrary to expectations (see, *Introduction*), single-housing caused only a slight reduction in mobility (all types of movement regardless of behaviour, including stretching up) among female rats (singletons $2.9 \pm 0.3\%$ time, grouped $3.8 \pm 0.1\%$ time; $F_{1,88} = 3.81$, one-tailed $P < 0.05$). As expected, however, females overall were significantly more mobile during the dark ($3.4 \pm 0.2\%$ time) than the light phase ($2.8 \pm 0.2\%$ time; $F_{1,88} = 22.0$, $P < 0.001$), but there was only a weak interaction between light/dark phase and housing condition ($F_{1,88} = 3.10$, $0.1 > P > 0.05$) with single-housing having a slightly greater effect on mobility during the dark phase.

Effect of barrier type

Females in both housing conditions spent significantly more time sniffing the barrier with increasing contact between neighbours (metal < Perspex < perforated Perspex < mesh, $F_{3,88} = 17.8$, $P < 0.001$; Figure 2a), and increased contact also resulted in significantly more social behaviour (especially social investigation and allogrooming) among grouped females ($F_{3,88} = 4.59$, $P < 0.01$). Moreover, there were significant interactions between housing condition and barrier type both for sniffing the barrier (due to more sniffing by singleton females separated from neighbours by a mesh barrier; $F_{3,88} = 3.09$, $P < 0.05$; Figure 2a) and for social behaviour (due to very little social behaviour of all types among grouped females in Perspex barrier treatments; $F_{3,88} = 4.09$, $P < 0.01$). Barrier type had no significant effects on general mobility, bar chewing or time spent sleeping across the light/dark cycle. However, females in both housing conditions avoided resting near Perspex, but not near solid or mesh, barriers ($F_{3,88} = 8.39$, $P < 0.001$).

Effects of neighbour density and barrier type on behaviour of singly housed females

Before analysing the effects of neighbour density and barrier type on the behaviour of singly housed rats, we checked for differences between neighbour densities when animals were separated from their neighbours by a solid partition. Singleton females spent more time investigating the cage top ($F_{1,16} = 5.60$, $P < 0.05$) when separated from another singleton by a solid partition, despite their apparent lack of contact.

The MANOVA of the behaviour of singly housed females, with neighbour density and barrier type as factors, for Perspex and mesh barriers revealed that neighbour density had a significant effect on investigation of the barrier ($F_{1,48} = 5.20$, $P < 0.05$); singletons housed next to a group showed more interest in the barrier than those not housed next to a group (Figure 2b). There were also marginally non-significant tendencies for reduced investigation of the bars and sides of the cage ($F_{1,48} = 3.57$, $0.1 > P > 0.05$) and tail chasing ($F_{1,48} = 3.29$, $0.1 > P > 0.05$) in animals housed next to a group. Although there was no significant main effect of neighbour density on bar chewing, there was a significant interaction with barrier type ($F_{2,48} = 3.96$, $P < 0.05$).

Singletons separated from neighbours by mesh showed a low incidence of bar chewing, regardless of neighbour density (lower than singletons completely isolated by solid metal barriers, $F_{1,34} = 3.21$, one-tailed $P < 0.05$; Figure 1b). However, bar chewing was much elevated in some Perspex barrier treatments depending on neighbour density, with singletons separated from a group by solid Perspex, or from another singleton by perforated Perspex, showing even more bar chewing than completely isolated singletons (Figure 1b). Barrier type was a significant main effect on time spent stationary ($F_{2,48} = 4.04$, $P < 0.05$) and on

investigation of the barrier by singleton females ($F_{2,48} = 26.30$, $P < 0.001$; Figure 2b) with both being greatest among singletons separated from neighbours by a mesh barrier. Neither neighbour density nor barrier type had a significant effect on time spent feeding or sleeping (even when light/dark phase was taken into account) among singleton females.

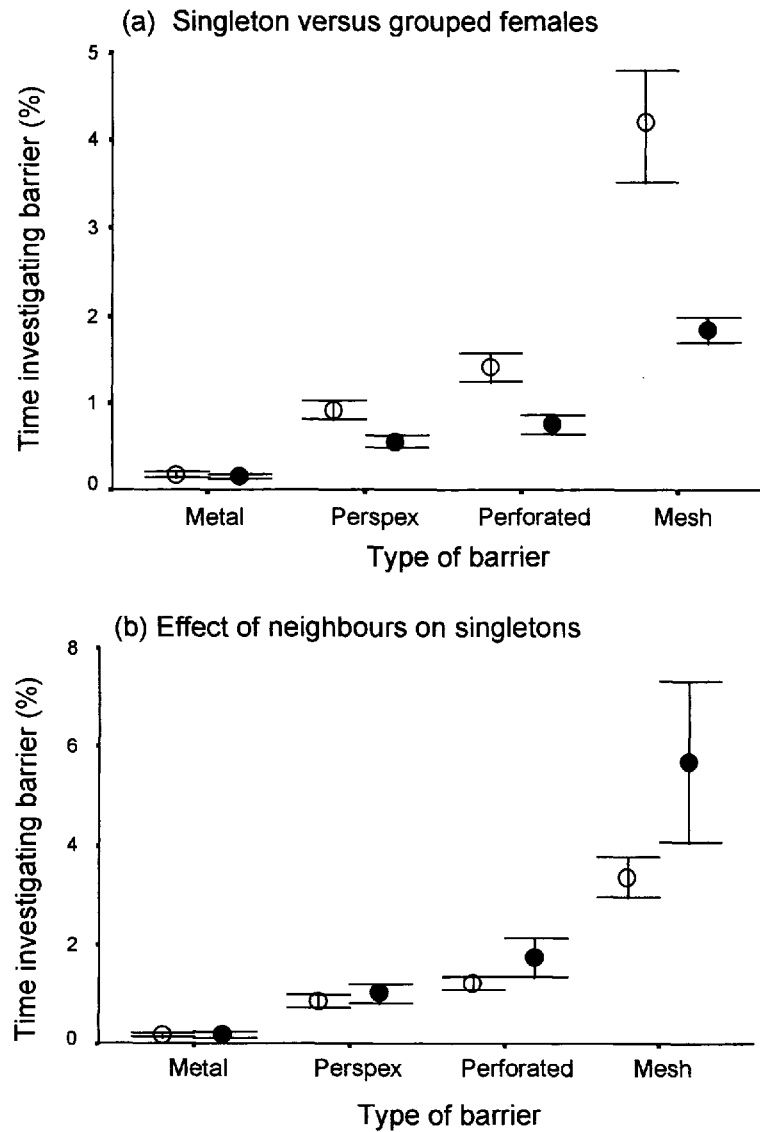


Figure 2 Proportion of time females spent sniffing at the barrier when separated from a neighbour cage by barriers of different types (mean \pm SEM per female, data for each caged group are the means per group of three). (a) Singly housed (open circles) compared with group-housed (solid circles) females. (b) Singly housed females with a singleton neighbour (open circles) or with a group-housed neighbours (solid circles).

Pathophysiological responses*Effects of treatment on organ pathology*

Combining treatments, a very high proportion of females (92.3%) showed evidence of organ pathology. Specific pathologies conformed to those found by Hurst *et al* (1996, see Table III of that paper for detailed descriptions). Mann-Whitney *U*-tests, comparing scores for singly housed females with mean scores for grouped animals, revealed a significantly lower thymus pathology score (characterized by congestion/haemorrhage and slight inflammation) among singletons ($z = 2.26$, $P < 0.05$). As found by Hurst *et al* (1996), the number of animals showing evidence of thymus pathology was low (9.9%). No significant effects of housing condition were found in pathology scores for any other organ or in the total pathology scores (all organs combined). Comparisons for single females exposed to different neighbour densities (Perspex and mesh barriers only) failed to reveal any significant effects of neighbour density on pathology scores. There were no significant effects of barrier type.

Although neither housing condition nor barrier type had significant effects on organ weights, there was an effect of neighbour density on organ weights among singleton females. MANOVA (with terminal body weight as a covariate) revealed a significant increase in adrenal weight in singletons housed next to another singleton ($F_{1,51} = 6.00$, $P < 0.05$).

There were no significant effects of housing condition or barrier type on measures of corticosterone or total IgG concentration (Table 3).

Table 3 Mean (\pm SEM) pre- and post-treatment serum concentrations of corticosterone and total IgG in single housed rats with single (S/S) or grouped (S/G) neighbours and in grouped rats (G/S). (Sample sizes in parentheses).

		S/S	S/G	G/S
<i>Corticosterone</i> (ng ml ⁻¹)	pre	504.1 \pm 6.3 (46)	451.4 \pm 38.8 (24)	473.4 \pm 27.7 (24)
	post	137.3 \pm 17.9 (46)	153.8 \pm 38.8 (22)	138.5 \pm 13.6 (24)
<i>Total IgG</i> (mg l ⁻¹)	pre	2143 \pm 106 (36)	2800 \pm 337 (16)	2414 \pm 158 (22)
	post	3897 \pm 296 (50)	3875 \pm 239 (24)	3798 \pm 166 (22)

Correlations between behaviour and pathology score

The last section reported effects of housing on organ pathology. Here, we present the results of tests of association between behaviours affected by housing conditions, and pathology scores.

Among singly housed females, there was a significant negative correlation between total pathology score (all organs combined) and time spent tail chasing ($r_s = -0.21$, $n = 70$, one-tailed $P < 0.05$; Figure 3). The difference in total pathology score was largely attributable to a significant relationship with kidney pathology (mainly medullary mineralization and cortical tubular basophilia in females, see Hurst *et al* 1996; $r_s = -0.23$, $n = 70$, one-tailed $P < 0.05$). Singletons showing tail chasing had lower kidney pathology scores than those that did not ($z = -1.70$, $n = 72$, one-tailed $P < 0.05$), leading to lower overall pathology scores ($z = -1.61$, $n = 72$, one-tailed $P < 0.05$). It also appears (Figure 3) that singletons which spent more time tail chasing, with associated low pathology scores, were those which had

experienced greatest contact with neighbours through mesh and perforated Perspex barriers; while those with limited or no neighbour contact (through solid Perspex or metal barriers) spent little time tail chasing and many had high pathology scores.

Singleton females showed a significant positive relationship between time spent in undirected movement around the cage and their total pathology score ($r_s=0.36$, $n=70$, $P<0.005$), attributable mainly to significant correlations with kidney ($r_s=0.35$, $n=70$, $P<0.005$) and thymus ($r_s=0.32$, $n=70$, $P<0.01$) scores. Repeating the correlations for all behaviours involving some form of movement around the cage combined, reduced the coefficients, although they were still significant (total pathology $r_s=0.26$, $n=70$, $P<0.03$; kidney pathology $r_s=0.27$, $n=70$, $P<0.03$), suggesting undirected movement as the most important contributor to the associations with pathology.

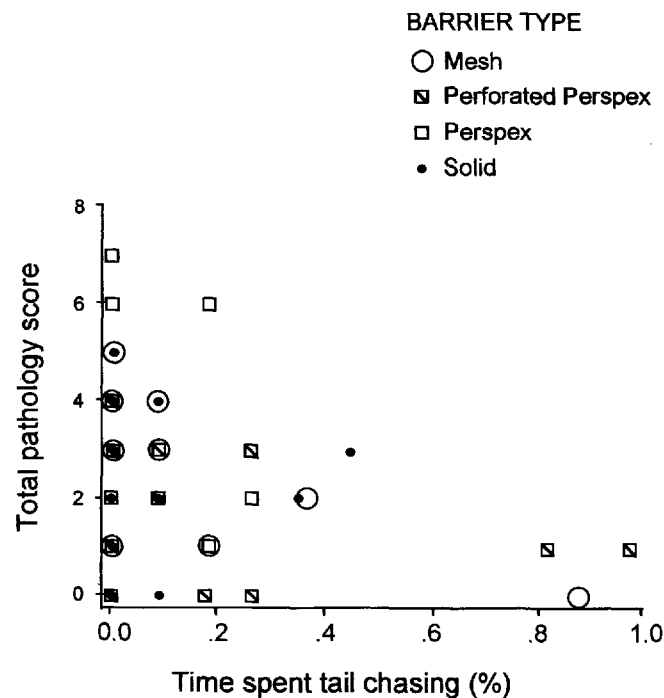


Figure 3 Correlation between the time singly housed females spent chasing their tails and their pathology scores at autopsy totalled across organs. Symbols indicate the type of barrier that separated them from a neighbouring cage.

Effects of neighbour contact on social tolerance of singly housed females

Aggression in groups of females when an unfamiliar singleton was introduced was generally low (Figure 4). Nevertheless, introduced singletons were significantly more aggressive than resident group members ($F_{1,18} = 28.3$, $P<0.001$), but with no main effect or interaction relating to trial type (Figure 4). In treatments where singletons had no prior contact with neighbours (treatments 1 and 2), prior neighbour contact by the resident group had no significant subsequent effect on aggression, with no interaction between a group's prior

experience and its being the initiator of aggression. There was no significant effect of barrier type or prior exposure to grouped rather than singleton neighbours on aggression by introduced singletons, though there was a tendency for reduced aggression after exposure to grouped neighbours (Figure 4).

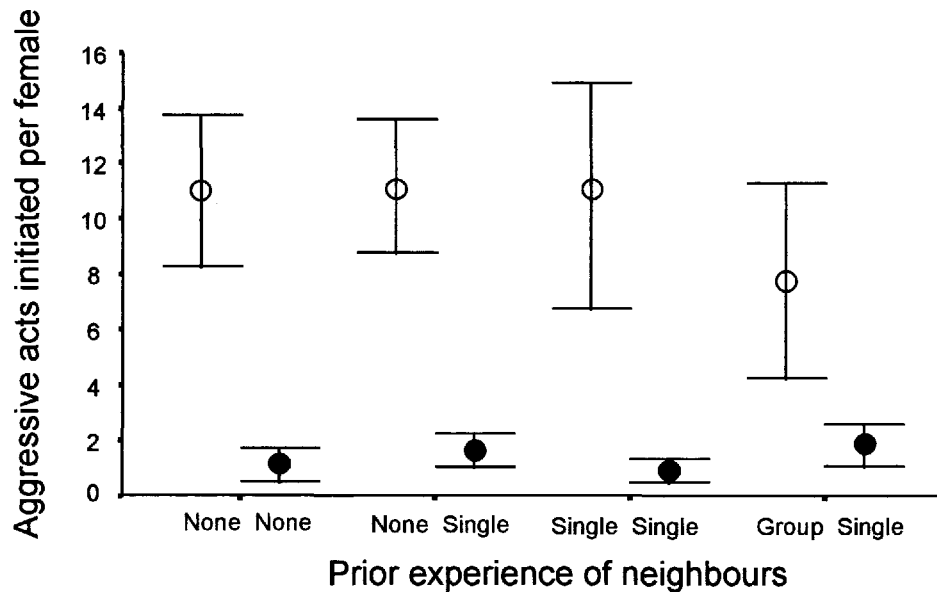


Figure 4 Effect of prior experience of neighbours (through a barrier) on the number of aggressive acts initiated (mean \pm SEM per female) when singly housed females (open circles) were introduced into an unfamiliar resident group of three (solid circles). Data for resident groups are the means per group.

Discussion and animal welfare implications

Taken together, and in comparison with Hurst *et al*'s (1997) findings, the results of these studies suggest that the welfare implications of single-housing may be substantially different for males and females. At first sight, some of the effects seen among females suggest that both the social deprivation and reduced social stress effects of single-housing found in males (Hurst *et al* 1997) were less marked. Singleton females showed only a small increase in tail chasing (35% of singleton females were seen to chase their own tails, compared with 57% of singleton males in Hurst *et al* [1997]) and no increase in other self-directed behaviours. However, the reduction in organ pathology associated with increased tail chasing in males observed in that study, also occurred in females. In addition, singleton females showed less reduction in general mobility than singly housed males (in contrast to our *a priori* expectation that the reduction would be greater). Together, these outcomes might suggest a lesser impact of removing social stimulation on time budgeting in females than in males. At the same time, the lack of any significant reduction in serum corticosterone concentration

and organ pathology scores among singly housed females might imply that separation had little impact on stress relative to group-housing (see Hurst *et al* 1996). However, careful consideration of the results suggests a more cautious interpretation.

The smaller reduction in mobility, and the overall reduction in sleeping among singleton females (cf Baenninger 1967; Hurst *et al* 1997) may reflect a greater tendency for singly housed females to attempt escape. Compared with males, females generally showed much higher levels of escape-oriented behaviours such as investigation of the sides and top of the cage and bar chewing. Bar chewing was six times commoner among females in both housing conditions (mean \pm SEM % time for singleton females = 6.30 ± 0.70 ; for singleton males = 1.01 ± 0.11 ; for grouped females = 2.40 ± 0.20 ; and for grouped males = 0.41 ± 0.06). They also showed up to a threefold increase in all these behaviours when housed singly.

Our *a priori* assumption that the level of general mobility was a reflection of social stress in groups was based on the further assumption that it reflected frustrated attempts, particularly by subordinates, to avoid potentially aggressive encounters in an artificially bounded environment (Hurst *et al* 1996). That study also found that undirected movement was associated with low social status among females but not among males. However, rats are social animals whose reproductive opportunities are to be found in groups (Lore & Flannelly 1977). Hurst *et al* (1997) have argued that the social stress costs of being in a group are something that rats may be designed to trade-off (and they may, in any case, be lower than those induced by confinement in a laboratory environment). Moreover, there are good reasons for supposing that the trade-off will be different in the two sexes. Among males, territorial resource defence may well result in periods of social separation, especially in dominants (Calhoun 1962; Barnett 1975; Lore & Flannelly 1977). Although females compete aggressively for resources, this is focused on nest and litter defence (Calhoun 1962; Barnett 1975; Lore & Flannelly 1977) and they do not show the same aggressive social structure as males (Hurst *et al* 1996). Instead, female reproductive strategy is based on attaining dominance (and therefore breeding status) and on mating preferences among available males (Calhoun 1962; Barnett 1975; McClintock *et al* 1982).

The functional significance of the social separation of single-housing may thus differ between the sexes, with singleton males responding to their cage as a territory but females by seeking to re-establish social contact. This would account for the differences in general mobility and self-directed behaviours (ie tail chasing) between singleton males and females and also for the reduced amount of aggression (especially on introduction into an unfamiliar group) by females. It might also account for the generally higher prevalence of organ pathologies among females (92.3% in this study compared with 70.8% of males [in Hurst *et al* 1997]). If self-directed behaviours are responses to an activity limbo, as suggested by Hurst *et al* (1997), they should be more likely among singleton males, which are responding territorially but without the normal time budget component of aggressive defence, than among singleton females – which fill a much greater proportion of their time budget with moving around and trying to escape.

This last argument regarding differences in tail chasing is consistent with the effects of barrier type on behaviour. As in males, singleton females showed more interest in a separating barrier than grouped individuals. However, responses depended strongly on the degree of social contact permitted by the barrier and on neighbour density. Time spent investigating the barriers that allowed some degree of contact (ie all except solid metal ones)

was consistently greater among singletons than among grouped rats, with the difference increasing with the permitted degree of contact between neighbours (Figure 2a). Furthermore, for any given barrier type, interest was always greater for females with grouped as opposed to singleton neighbours (Figure 2b). However, trends in the escape-oriented behaviour of bar chewing (Figure 1a,b) suggest that intermediate degrees of contact (Perspex and perforated Perspex barriers) may have tantalizing effect, stimulating interest in neighbours but frustrating attempts at contact, so that escape responses are enhanced over those of socially isolated (separated by solid metal barriers) animals. The sharp reduction in bar chewing with open mesh barriers suggests that the greater degree of contact permitted removes this frustration. In addition, Figure 1b suggests an interesting interaction between barrier type and neighbour density in determining the amount of bar chewing. With solid Perspex barriers, the visual stimulation of a neighbouring group appears to induce a greater degree of frustration among singleton females than that of another singleton. With perforated Perspex barriers, however, the reverse appears to be true. An obvious explanation for these contrasting effects is that the additional provision of olfactory – and limited tactile – contact offered by the perforated barriers leads to greater frustration when it is limited to a single neighbour rather than a group, especially since rats preferred not to rest next to Perspex barriers thus further restricting the opportunity for contact. In both cases, the combination of apparent opportunity but limited fulfilment heightens attempts to escape. It is when these frustrations are apparently removed, with the open mesh barriers, that the reduction in escape-oriented behaviour (Figure 1a) creates the time budget opportunity for tail chasing (Figure 3).

Although the degree of neighbour contact failed to have a significant effect on the level of aggression shown by singleton females when eventually introduced into an unfamiliar group (cf males, Hurst *et al* 1997), this may well have been due to a floor effect resulting from the generally much lower level of aggression by females compared with males across barrier treatments. Nevertheless, there was a tendency for aggression to be least among singletons that had experienced a group through a mesh barrier.

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