HOUSING AND WELFARE IN LABORATORY RATS: WELFARE IMPLICATIONS OF ISOLATION AND SOCIAL CONTACT AMONG CAGED MALES

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Abstract *Animal Welfare 1997, 6:* 329-347

*Male laboratory rats (*Rattus norvegicus*; Wistar, Alderley Park) were housed as singletons or groups of three in units of two joined, but divided cages. Units were divided by different types of barrier that allowed different degrees of social contact across the barrier. Singletons were established either with another singleton as a neighbour on the other side of the barrier, or with a group of three as neighbours. Relative to group-housed animals, singly-housed rats showed reduced activity and a greater incidence of self-directed behaviours and behaviours apparently related to escape or seeking social information. Pathophysiological evidence was consistent with Baenninger's (1967) suggestion that tail manipulation in singletons is a surrogate social response, but was also consistent with an overall increase in self-directed activity, reflecting elasticity in time budgeting. Variation in the degree of increase in self-directed activity among singletons and the negative correlation between self-directed activity and organ pathology may have reflected differences in the ability of individuals to avoid an activity limbo. While reduced corticosterone concentration and organ pathology compared with grouped rats implied that separation may remove social stress, responses to contact with neighbours, and correlations between behaviours and organ pathology suggested that rats may actively seek social interaction. Broad differences in stress responses between single and grouped housing conditions may therefore be an inadequate yardstick to the animals' welfare. However, exposure to neighbours reduced the aggressiveness of singly-housed males when they were eventually introduced into an unfamiliar group, suggesting that a degree of exposure to neighbours (separation, but not isolation) may have some welfare benefits for laboratory-housed rats, depending on procedures.*

Keywords: *animal welfare, isolation, pathophysiology, rat, social stress, time budget*

Introduction

Laboratory studies of behaviour frequently require animals to be housed singly. However, several species regularly housed singly are naturally social (eg Wolfensohn & Lloyd 1994). Indeed, controlling for differences in social experience during experiments may be the primary reason for housing animals singly in the first place (eg Mormède *et al* 1990; Barnard *et al* 1993). Surprisingly, although the practice of single housing is widespread and its potentially stressful consequences acknowledged (eg Gardiner & Bennett 1977; Brain & Benton 1983; Carlier *et al* 1988; Ehlers *et al* 1993), effects of isolation on measures of stress vary (eg Friedman *et al* 1970; Brain & Benton 1983; Rabin *et al* 1987) and there is little consensus about its implications for welfare in normally social species (Brain & Benton 1983). While much of the variation in apparent stress-related measures is likely to reflect different experimental regimes and the strain, age, previous experience etc of subjects, the debate over welfare implications bears on the deeper problem of interpreting apparent stress responses (eg Mendl 1991; Rushen & de Passillé 1992; Mason & Mendl 1993; Wiepkema & Koolhaas 1993; Barnard & Hurst 1996). Barnard and Hurst (1996) have argued that interpreting the welfare implications of stress-related pathophysiological measures (eg organ pathologies, elevated glucocorticoid levels, immunodepression) requires an appreciation of adaptive trade-offs between life history components reflecting survivorship and reproductive investment. Trade-offs may vary between individuals with different life history strategies so that the same clinical symptoms may reflect adaptive self-expenditure in one case but environmental imposition in another. Barnard and Hurst (1996) have also suggested that such differences in welfare implications can be identified by measuring the impact of circumstances on decision rules relating to time budgeting and responses to environmental contingencies.

Hurst *et al* (1996) used a time-budgeting approach to identify constraints on individual social strategies in free-range groups of rats and relate these to pathophysiological measures of welfare impairment. Here we extend the approach to consider the welfare implications of isolation. Using the same strain of laboratory rat (see Methods) as Hurst *et al* (1996), the aim of this study was to examine the behaviour and pathophysiology of animals housed singly or in small groups to see whether a) single housing could be considered to compromise their welfare, and b) different degrees of contact with neighbours alleviated the effects of separation. As measures of pathophysiological change we chose serum concentrations of total IgG (as a convenient bystander measure of immunocompetence [ie a measure of a general capacity to respond], eg Wahid & Behnke 1993; Barnard *et al* 1996), the potentially immunodepressive/stress-related hormones testosterone and corticosterone (eg Brain & Nowell 1970; Grossman 1985; Barnard *et al* 1996) and early spontaneous organ pathologies (Hurst *et al* 1996) (see Manser 1992 for a general review).

Rats, in the form of various laboratory strains, are among the most extensively used experimental subjects in commercial, basic and applied research (Wolfensohn & Lloyd 1994). Since they are also highly social (eg Barnett 1975; Lore & Flannelly 1977), the lack of opportunity for interaction imposed by housing singly might be expected to affect them in several ways. An obvious possibility is that, without the opportunity for social behaviour, the overall level of activity of singly-housed rats will become reduced compared with rats housed in groups (but see Garzon & Del Rio 1981 for evidence of hyperactivity in long-term isolates) and more time will be spent in non-social behaviours. As a result, food intake may

increase (Baenninger 1967; Morinan & Leonard 1980) leading to an increase in weight gain (Morgan & Einon 1976; Fiala *et al* 1977; but see Morinan & Leonard 1980) and obesity as a potential welfare problem in the long term. Isolation of adult rats, especially males, can also reduce social tolerance (eg Valzelli & Bernassconi 1976; Brain *et al* 1980), which may be a serious problem if rats are returned to stock or experimental groups following a period of isolation. In addition, early work by Baenninger (1967) has suggested that rats housed singly may develop behaviours not usually observed in grouped animals. Two such behaviours emerging from Baenninger's study were tail manipulation (sniffing, licking, grooming or chasing the tail) and pawing (standing on hind feet making stereotyped alternating paw movements over the surfaces of the cage or water bottle). Baenninger interpreted tail manipulation as a response to a surrogate cage mate. For individuals housed singly, the tail was the only stimulus with apparent spontaneity of movement and responses made to the tail were all social responses normally made towards other rats. Pawing was not a response normally directed towards other rats. It was more rapid than the 'boxing' movements made during aggressive interactions (Grant 1963) and, within individuals, was performed stereotypically in the same part of the cage (Baenninger 1967).

Baenninger's (1967) results thus imply that removing the social component of a rat's environment induces both an adjunctive response (tail manipulation) (see Falk 1971) and a stereotypy (pawing). Even if these are viewed as coping responses (see eg Mason 1991), the welfare implications of single housing are negative. However, caution is needed here. While single-housing may remove an important stimulus and time-budgeting priority, it also removes potentially negative consequences of competition and aggression. Depending on individual competitive ability, therefore, isolation may bring a net welfare advantage. Moreover it is conceivable that under natural conditions rats may sometimes find themselves in situations where social pressure is absent or relaxed and that behavioural changes reflect adaptive plasticity in their repertoire (Mason 1991; Barnard & Hurst 1996). From the opposite viewpoint, however, life history considerations lead to the possibility that rats are designed to compete for social (and thereby ultimately reproductive) opportunities and that removal of these opportunities, costly as they may be in terms of health and survival, frustrates the animal's rules of prioritization and time budgeting (Barnard & Hurst 1996). Thus rats may seek social interaction even though social stress is reduced by remaining alone. Although it is known that olfactory and auditory communication can occur between rats in the same or adjacent rooms (eg Beynen 1992), the most commonly used single housing systems have opaque plastic or metal walls (Wolfensohn & Lloyd 1994) which preclude visual and tactile contact and restrict other channels of communication. From a welfare point of view, therefore, restricted contact with other individuals, allowing some social interaction but precluding aggressive contact leading to social stress, may reduce the need for adjunctive social and stereotypic activities and at the same time indicate the tendency for singly-housed rats to seek social involvement. However, restricted contact may itself induce frustration and stress-related pathophysiology. Pathophysiological changes arising from frustrated attempts to interact socially can reasonably be argued to indicate reduced welfare, while those arising in groups may reflect adaptively tolerated cost. Welfare concern over pathophysiological changes in the latter case should arise where these reflect frustration of individual social strategies, for example where confinement in a cage limits the ability of animals to choose the timing and context of interactions and avoid aggression (see Hurst *et al* 1996).

The experiment reported here was conducted in two parts. First, we examined the behaviour of singly-housed and group-housed male rats given different degrees of contact with neighbours. Second, we tested the degree of social tolerance of rats previously housed singly, but with different opportunities for contact with neighbours, by introducing them briefly into an unfamiliar group. If housing singly reduces social stress, and tail manipulation and pawing are forms of compensatory response to isolation, we should expect a) singly-housed rats to show reduced pathophysiology compared with grouped rats, and b) pathophysiology to decline with increased tail manipulation and pawing. Similarly, if singly-housed rats seek social interaction, we should expect increased opportunity for contact to result in more time being spent at the separating barrier and a reduction in pathophysiology. On the other hand, if contact stimulates singly-housed rats to seek corroborative olfactory social information from their cage floor or elsewhere (eg outside the cage), frustration may lead to an increase in pathophysiology. As well as testing these *a priori* expectations, however, we specifically looked for effects of housing condition and neighbour contact on behaviours (sleep and behaviours associated with attempted escape) shown previously to correlate with frustrated social strategies and pathophysiology in groups of rats (Hurst *et al* 1996) or to differ (sleep and feeding and drinking) between singly-housed and grouped rats (Baenninger 1967; Morinan & Leonard 1980).

Methods

Experimental housing conditions

One hundred and forty-four male Alderley Park (AP) rats (a Wistar derived strain) were housed in paired stainless steel cages (each cage 475x285x200mm high) containing either two singly-housed neighbours or a singleton neighbouring a group of three males. All neighbours and cage mates were unrelated and previously unfamiliar (see Hurst *et al* 1996) and were established from stock groups of five at age 9–10 weeks. Cages had a mesh front and floor and each pair was separated by one of four types of barrier designed to provide different degrees of contact between neighbours:

1) solid steel (no contact);
2) clear Perspex (visual contact only);
3) clear Perspex perforated all over by 6mm holes (olfactory and visual contact);
4) 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 balanced design. The experiment was run in two batches of 72 rats (the second batch following 1 month after the first) so that a large number of behaviour samples could be collected to estimate individual time budgets. Treatments were balanced across batches. 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 water spout. The rats were maintained on a 12h light: 12h 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).

Time budgets

Behaviour samples (recorded by observer CMN) were spread evenly over the last 4 hours of the dark phase (the most active period, Hurst unpublished data) and the first 4 hours 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 recorded. Sixty-four behaviour categories were recorded but were assigned to 17 functional categories for analysis (Table 1). Note that we have divided Baenninger's (1967) inclusive 'tail manipulation' category into separate categories of 'tail chasing' and 'tail attention', restricting the latter to close attention to, and manipulative contact with, the tail. To avoid any tendency to focus on the more interesting or obvious behaviours, an audio cue dictated every 4s via headphones (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 was collected in each 4h light or dark phase observation period, giving a mean ± SEM total of 1,083 ± 6 observations per rat (excluding missing data) over the 5-week period.

Table 1 Behavioural categories recorded.

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

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

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 in 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 had no contact with neighbours (solid barriers, treatments 1 and 2) or had had olfactory and visual contact through a perforated Perspex barrier with another single-housed neighbour (treatment 3) or 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 had no prior contact with a neighbour (solid barrier, treatment 1) or had had contact with a singly-housed neighbour over the previous 5 weeks (treatments 2–4). Since there were only 24 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.

Table 2 Experience prior to regrouping.

Treatment	Introduced 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

Singly-housed rats were introduced into a resident group during the last 4 hours 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.

Blood, organ and tissue sampling

A pre-treatment blood sample (up to 1ml) was taken from a caudal vein of each animal 2–6 days prior to introduction into their experimental cages and after termination (rats aged 14–15 weeks) and analysed for serum corticosterone, testosterone and total IgG following the procedures of 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 1400 and 1600h 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 and testosterone (ng ml^{-1}) were determined using radioimmunoassay kits (Coat-a-Count solid phase ^{125}I-corticosterone and ^{125}I total testosterone, Diagnostic Products Corporation, Los Angeles, USA). Total serum IgG (mg l^{-1}) was determined by surface plasmon resonance detection following the method of Fägerstam *et al* (1992). The size of the rats at this age 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 parametric tests. Body-weight was recorded weekly during routine husbandry procedures.

At termination, selected organs (adrenal glands, kidneys, heart, thymus, spleen and testes) were carefully removed after euthanasia 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* 1996). As we had no *a priori* reason to expect particular characteristics to be associated with different behaviour or physiological responses, severity scores for each change were summed to give the total pathology score per organ, and the pathology scores of each organ summed to give the total pathology score per rat for analysis.

Results

Differences in behaviour between single and group-housed rats

To test for effects of housing condition and barrier type on behaviour, the 18 behaviour categories in Table 1 were entered as dependent variables into a multivariate analysis of variance (MANOVA) with housing condition (single vs group) and barrier type as factors. In addition, 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). Behaviour measures were averaged across individuals within groups to control for non-independence.

Effects of housing condition

Did housing singly have the expected effects on the amount of tail manipulation, general activity and the tendency for rats to perform behaviours related to escape (bar chewing) or

attention to sources of potential social information (the barrier, the substrate, the environment outside the cage)? Rats housed singly showed significantly more tail chasing ($F_{1,88}$ = 13.30, one-tailed $P < 0.001$, Figure 1) than group-housed animals. Indeed, apart from a single recorded instance among group-housed rats, tail chasing was evident only among single rats, being recorded in 57 per cent of animals. There was a smaller, but still significant difference in tail attention ($F_{1,88}$ = 3.20, one-tailed $P < 0.05$) which occurred in 92 per cent of singly housed rats but only 58 per cent of those housed in groups and accounted for 0.53 ± 0.10 per cent and 0.22 ± 0.05 per cent of time respectively in the two housing conditions. In terms of behaviours related to escape and attention out of the cage, rats housed singly showed significantly more bar chewing ($F_{1,88}$ = 8.95, one-tailed $P < 0.004$) and sniffing the barrier ($F_{1,88}$ = 17.21, one-tailed $P < 0.001$) and bars and sides of the cage ($F_{1,88}$ = 162.1, one-tailed $P < 0.001$) than group-housed animals. Apart from social behaviours, which differed trivially between single and group-housed rats and are not of interest here, the only other behaviours showing significant differences between housing conditions were drinking and self-grooming, both of which were performed more by rats housed singly ($F_{1,88}$ = 4.71, $P < 0.05$ and 5.41, $P < 0.05$ respectively). Pawing (Baenninger 1967) was not observed in this experiment.

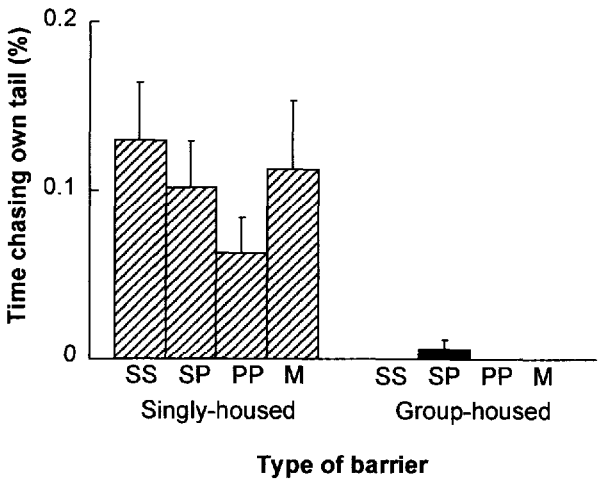

Figure 1 **The mean ± SEM per cent time spent tail chasing by rats housed singly or in groups when separated from neighbours by different types of barrier. Barrier type: SS – solid steel, SP – solid Perspex, PP – perforated Perspex, M – mesh.**

Hurst *et al* (1996) found that time spent sleeping correlated with pathophysiological measures of welfare in AP rats housed in open rooms, reduced sleep being associated with higher organ pathology scores. Baenninger (1967) also showed that rats housed singly spent less time sleeping than group-housed animals, but rats in her study were observed only during the dark phase. Observations during the present experiment suggested a difference between single and group-housed rats in the distribution of sleep and activity over the

light/dark cycle. A repeated measures ANOVA of time spent sleeping, not surprisingly, showed a significant effect of phase of the cycle ($F_{1,88}$ = 718.04, $P < 0.0001$), with most sleep occurring in the light phase (Figure 2a). Housing condition had no effect on the total time spent sleeping ($F_{1,88}$ = 0.02 not significant [ns]) but there was a significant interaction between housing condition and phase of the light cycle ($F_{1,88}$ = 6.19, $P < 0.05$). This was because singly-housed rats slept less than group-housed in the dark, but more than group-housed in the light (Figure 2a). Despite having no effect on the total time spent sleeping, housing condition had a considerable effect on the general mobility of the rats ($F_{1,88}$ = 493.5, $P < 0.0001$), with singly-housed individuals being considerably less mobile than grouped animals (Figure 2b). This difference was apparent during the light phase but was much stronger in the dark (interaction between housing condition and light phase: $F_{1,88}$ = 109.4, $P < 0.0001$) since singly-housed rats showed very little increase in mobility during the active dark phase (see Figure 2b). The tendency for some singletons to show tail chasing (see above) did not significantly increase their time spent mobile relative to those that did not chase their tail (t_{70} = 1.51, ns).

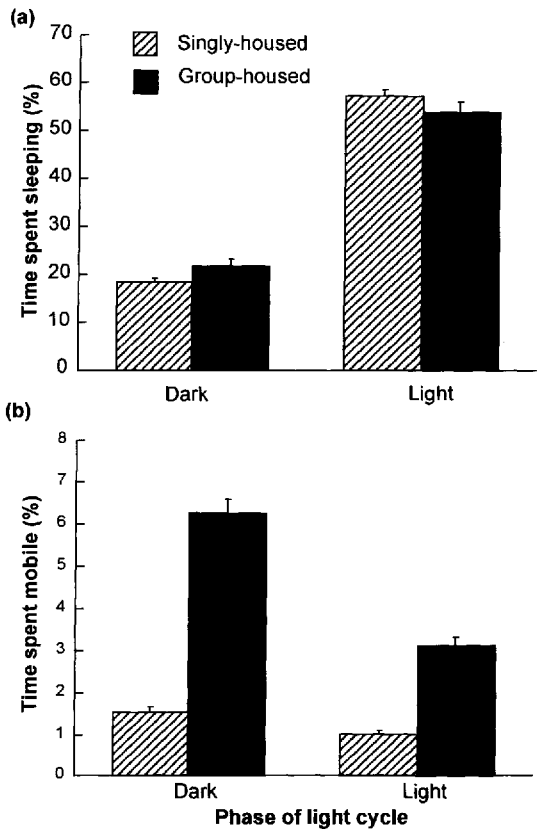

Figure 2 **The mean ± SEM per cent time spent a) sleeping and b) mobile by rats housed singly or in groups in different phases of the light/dark cycle.**

Effects of barrier type

The only significant effect of barrier type on behaviour across all rats was an increase in time spent sniffing the barrier with increasing degree of contact between neighbours (solid < Perspex < perforated Perspex < mesh) ($F_{3,88}$ = 24.98, $P < 0.001$, Figure 3). Barrier type had no effect on general mobility ($F_{1,88}$ = 0.75, ns) or on the distribution of sleep across the light cycle ($F_{2,88}$ = 0.26, ns). There was no significant interaction between housing condition and barrier type for any behaviour.

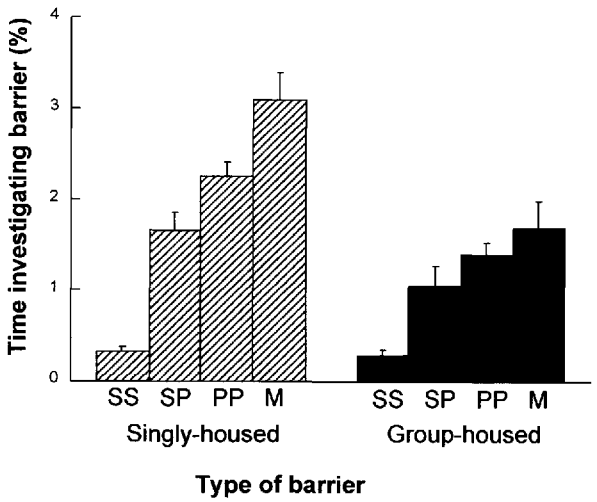

Figure 3 **The mean ± SEM per cent time spent investigating the barrier in relation to housing condition and barrier type. Barrier type as Figure 1.**

The only general effect of barrier type on behaviour thus appeared to be on behaviour directed towards the barrier itself. If time at the barrier reflected motivation to contact or avoid neighbours, we might expect this to influence general use of space by rats. As an index of this, we analysed the effects of barrier type and neighbour density on time spent by singly-housed rats in contact with the barrier. To control for contact with the barrier simply reflecting wall-seeking (Fredericson 1953), we analysed the difference in time spent in contact with the barrier and in contact with the opposite (solid metal) wall of the cage. Two-way ANOVA revealed a significant effect of barrier type ($F_{3,64}$ = 12.30, $P < 0.001$), due to a strong tendency for singly-housed rats to rest away from Perspex barriers (a significant tendency to rest away from Perspex barriers was also present in group-housed animals [$F_{3,20}$ = 11.53, $P < 0.001$]).

Effects of neighbour density and barrier type on behaviour of singly-housed rats

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 (and were thus assumed to have no contact). MANOVA revealed no significant differences between neighbour densities for any behaviour category.

MANOVA of the behaviour of singly-housed rats by neighbour density and barrier type for Perspex and mesh barriers revealed a significant effect of neighbour density on time spent feeding. Time spent feeding was reduced with grouped neighbours (mean ± SEM per cent time = 8.5 ± 0.5 per cent) compared with another singly-housed animal (10.2 ± 0.5 per cent) ($F_{1,48}$ = 4.79, $P < 0.05$) with the reduction tending to be greatest in the mesh barrier treatment. There was no significant main effect of neighbour density but rats spent more time sniffing the barrier as the amount of contact permitted increased (Perspex < perforated Perspex < mesh) ($F_{2,48}$ = 12.81, $P < 0.001$). There was also a significant neighbour density x barrier type interaction ($F_{2,48}$ = 6.21, $P < 0.005$) with degree of contact affecting sniffing the barrier only when the neighbour was another single individual.

A repeated measures ANOVA of time spent sleeping, taking light phase into account, also showed a significant interaction between neighbour density and barrier type ($F_{2,48}$ = 4.34, $P < 0.05$) though there was no main effect of neighbour density ($F_{1,48}$ = 0.65, ns) or barrier type ($F_{2,48}$ = 1.00, ns). Single rats spent more time sleeping when separated from a group of neighbours by a mesh barrier, ie when given the greatest degree of contact with neighbours, spending a similar amount of time sleeping as group-housed animals even in the dark (mean ± SEM per cent time spent sleeping by single rats with a mesh barrier and grouped neighbours in the light = 58.5 ± 6.1, in the dark = 28.8 ± 3.5; by grouped rats [barriers combined] in the light = 54.1 ± 1.8, in the dark = 21.9 ± 1.1). Increasing the amount of contact with neighbours did not significantly increase the general mobility of single rats as there were no significant effects of barrier type, neighbour density or interaction between these factors. However, barrier type and neighbour density did influence the distribution of mobility across the light cycle (interaction between neighbour density, barrier type and light phase: $F_{2,48}$ = 8.17, $P < 0.005$). Neighbour density had no significant effects on their avoidance of resting in contact with Perspex barriers (see above).

Pathophysiological responses

Effects of treatment on organ pathology

A high proportion (70.8 per cent, housing conditions combined) of rats showed evidence of early organ pathology. Specific pathologies conformed to those found by Hurst *et al* (1996 and detailed in Table III of that paper). Effects of barrier type, housing condition and neighbour densities on organ pathology scores (see Methods) were analysed non-parametrically. Mann-Whitney *U* tests comparing singly-housed with mean scores per group revealed significant differences between single and group-housed rats for heart (degenerative cardiomyopathy and mononuclear cell infiltration Z = 2.46, $P < 0.02$) and thymus (congestion/haemorrhage and slight inflammation Z = 2.28, $P < 0.05$) scores, with singly-housed rats showing less evidence of pathology in both cases. However, it is important to note that the prevalence of detectable pathologies was very low in both organs (1.4 per cent [two animals] and 14.6 per cent of individuals respectively). No significant differences emerged for any other organ or for total pathology score.

Similar comparisons for single rats exposed to different neighbour densities (Perspex and mesh barriers only, see above) also revealed a significant effect on thymus score (Z = 1.98, $P < 0.05$), those animals exposed to grouped neighbours showing no evidence of thymus pathology (compared with a mean score of 0.22 ± 0.08 for rats with singleton neighbours).

There were no significant main effects of housing condition, neighbour density or barrier type on measures of body-weight or the weight of any organ, and there were no significant interactions.

Correlations between behaviour and organ pathology

Were the above differences in pathology scores reflected in associations between pathology scores and those behaviours affected by housing condition (see above)? Spearman rank correlation analysis suggested they were (Table 3).

Table 3 **Significant Spearman rank correlation coefficients for relationships between behaviours and organ pathology scores in a) individually and b) group-housed rats (n = 72 in all cases). TPS – total pathology score, KS – kidney score, AS – adrenal gland score, ThS – thymus gland score.**

Behaviour	TPS	KS	AS	ThS
a) Individually-housed				
Move around cage	-0.24*	-	-	-
Tail chase	-0.32***	-0.30**	-	-
Self-groom	-	-	-0.29**	-
Sniff faeces	-0.37***	-0.36***	-	-
Sniff substrate	0.32***	0.26*	-	-
Sniff out of cage	0.32***	0.28**	-	-
Sniff food/water pots	-	-	0.27**	-
b) Group-housed				
Feed	-0.32**	-0.38***	-	-
Allogroom	-	-	-	-0.35***
Sniff substrate	-	-	-	0.24*
Sniff bars	-	-	-	-0.27*
Sniff food/water pots	-0.29**	-0.25*	-	0.24*

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Among rats housed singly, there were significant negative correlations between total pathology score and time spent moving around the cage, the amount of tail chasing recorded and time spent sniffing faeces. Separate analyses of each organ (Table 3, section a) suggested these trends were due to kidney (mainly tubular hyaline droplet formation) and adrenal gland (congestion) pathologies: negative correlations emerged between kidney score and both the amount of tail chasing and time spent sniffing faeces, and between adrenal gland score and time spent grooming self. The negative correlations with tail chasing resulted in those singly-housed individuals showing the behaviour having significantly lower total pathology scores than those that did not (Mann-Whitney U test, $Z = 1.74$, one-tailed $P < 0.05$), though there was no difference in kidney scores ($Z = 1.44$, ns). While tail attention did not correlate with the degree of organ pathology, there was a similar difference between singly-housed rats that did and did not show the response ($Z = 1.69$, one-tailed $P < 0.05$), though again there was no difference in kidney scores ($Z = 1.23$, ns). No differences in organ pathology scores with respect to tail attention emerged among grouped rats. Positive

correlations emerged between total pathology score and time spent sniffing the cage floor and sniffing through the bars of the cage. Again, these relationships were reflected by kidney scores (Table 3, section a), while scores for adrenal glands increased with time spent sniffing food and water pots (Table 3, section a).

Very different associations emerged among grouped rats (Table 3, section b, now correlating behaviour and pathology scores for individuals rather than group averages). Total pathology score correlated negatively with time spent feeding and sniffing food and water pots; these were due mostly to correlations with kidney pathology (Table 3, section b). Negative correlations also emerged between thymus gland scores and time spent grooming other individuals and sniffing the bars of the cage. In addition, however, thymus scores correlated positively with time spent investigating food and water pots and the cage floor.

Effects of treatment on serum hormone and total IgG concentrations

Was the tendency for singly-housed animals to show reduced pathology relative to grouped rats in those organs for which differences emerged (see above) reflected by differences in serum concentrations of total IgG and immunodepressive hormones?

Mean pre- and post-treatment concentrations of total IgG, testosterone and corticosterone are presented in Table 4. Analysis of pre-experimental concentrations revealed a chance significant bias in pre-experimental testosterone concentration with respect to housing condition, with animals subsequently housed singly with a single neighbour having lower concentrations than other rats (Table 4, $F_{1,90} = 5.70$, $P < 0.05$, pre-experimental body-weight entered as a co-variate). Both pre-experimental testosterone and post-experimental body-weight were thus entered as co-variates in analyses of post-experimental hormone and IgG levels. There were no significant biases with respect to housing condition or barrier type in pre-experimental corticosterone or total IgG concentrations (controlling for pre-experimental body-weight). As in Hurst *et al*'s (1996) earlier study, corticosterone concentration declined in all groups across the period of the experiment (repeated measures $F_{1,75} = 255.06$, $P < 0.0001$) and total IgG concentration correspondingly increased ($F_{1,88} = 45.06$, $P < 0.001$). There was no significant change in testosterone concentration ($F_{1,76} = 1.39$, ns) (see Table 4).

Two-way ANOVA revealed a significant difference between single and group-housed rats (mean concentrations per group) in post-experimental corticosterone concentration, single rats having lower concentrations of corticosterone than grouped rats ($F_{1,70} = 3.98$, $P < 0.05$). This is in keeping with the differences in pathology scores, and also with the general reduction in activity and social interaction among rats housed singly (see Hurst *et al* 1996). There was no significant effect of barrier type or interaction between housing condition and barrier type. No significant main effects or interactions emerged for post-experimental testosterone or total IgG concentrations.

Two-way ANOVAs failed to reveal any significant effects of neighbour density or barrier type on serum concentration measures for singly-housed rats with Perspex or mesh barriers, but those with a single neighbour tended to show higher post-experimental testosterone than those with a group of neighbours ($F_{1,35} = 3.29$, $P < 0.1$). No significant correlations emerged between serum concentration measures and time spent in different behaviour categories among either singly-housed or grouped rats.

Effects of neighbour contact on social tolerance of singly-housed rats

It is well-known that isolation reduces social tolerance in male rodents, including rats (Brain *et al* 1980; Cairns *et al* 1985). Was there any evidence that housing in contact with neighbours offset this effect and increased tolerance when rats previously housed singly were introduced into an unfamiliar group?

Repeated measures ANOVAs examined the effects of prior contact with neighbours (treatments 1–4, see Table 2) on aggression initiated by the resident group (mean per group) and introduced singleton. On introduction to an unfamiliar group, rats previously housed singly were considerably more aggressive than rats in the resident host group across all treatments ($F_{1,19} = 96.7$, $P < 0.0001$), though there was also a significant interaction between the initiator of aggression (singleton or group) and prior contact with neighbours ($F_{3,19} = 8.57$, $P = 0.001$) (see Figure 4). This was due to the introduced singleton's response to previous experience of neighbours rather than any response from the resident host group.

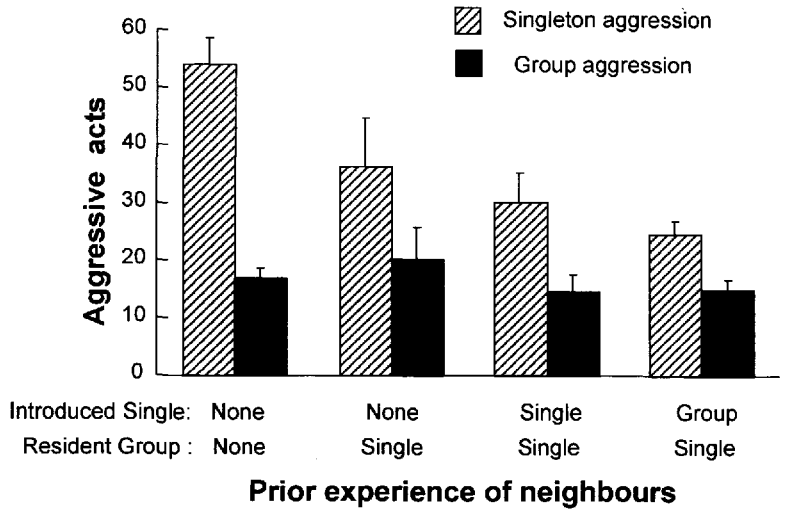

Figure 4 **The effect of prior experience of neighbours on the number of aggressive acts initiated by a singly-housed rat when it was introduced into an unfamiliar resident group. Subjects had either had no prior contact with neighbours (None), or contact with a single neighbour (Single) or group of neighbours (Group) (see Methods and Table 4). Means ± SEM of aggressive acts initiated by the introduced singleton (hatched bars) and per member of the resident group (solid bars). SEM based on the mean per group rather than per individual.**

In treatments where the introduced singleton had no prior contact with neighbours (treatments 1 and 2), prior neighbour contact by the resident group had no subsequent effect on aggression ($F_{1,10} = 0.36$, ns), with no interaction between the group's prior experience and the initiator of aggression ($F_{1,10} = 3.13$, ns). In contrast, prior contact with neighbours

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by the introduced singleton significantly decreased subsequent aggression as expected (effect of singleton's barrier type: $F_{1,21} = 6.38$, $P < 0.05$), though this only affected aggression from the singleton (interaction between initiator and singleton's barrier type: $F_{1,21} = 6.65$, $P < 0.05$). The greater aggression shown by previously isolated singletons thus did not induce greater aggression from the resident group (see Figure 4). Further, prior exposure of singletons to grouped neighbours (treatment 4) reduced their aggression more than exposure to single neighbours (treatment 3) (interaction between treatment and initiator: $F_{1,9} = 8.13$, $P < 0.05$) although, since aggression by the resident group was not influenced by the singleton's prior experience, there was no significant overall reduction in aggression within the cage between these treatments ($F_{1,9} = 0.86$, ns).

Discussion

The results confirm Baenninger's (1967) earlier findings that housing rats individually rather than in groups leads to behavioural changes that are consistent with social deprivation. However, the implications for the welfare of individually-housed rats are not straightforward.

The most marked behavioural effect of housing rats singly was a reduction in overall mobility relative to grouped animals. The concern that a chronic lack of activity may lead to obesity in the long term is receiving some support from AP rats housed singly for a much longer period (10 months, Hurst *et al* unpublished data). Other significant effects of single housing (increased amounts of tail chasing, tail attention, self-grooming, investigating and chewing the bars of the cage and investigating the barrier separating companions) are consistent with an interpretation of self-directed behaviour as a surrogate social response (see also Baenninger 1967) and attention to the bars, walls and barrier as a reflection of motivation to escape or join companions. Support for these interpretations emerges from measures of pathophysiology in individually-housed animals. The suggestion that self-directed behaviour reflects a coping response in the absence of social stimulation seems at first sight to be borne out by negative correlations between organ pathology scores and tail chasing, self-grooming and sniffing faeces and the fact that, overall, individually-housed rats had lower post-treatment stress hormone (serum corticosterone) concentrations and some reduction in organ pathology (heart and thymus) compared with grouped rats. The results for cardiac pathology contrast with those of Carlier *et al* (1988), who found evidence of hypertension-induced ventricular hypertrophy in isolated rats, but this may in part reflect the intermittent nature of isolation in that study and the established stressful effect of changing social environments (Edwards *et al* 1980). While a reduction in corticosterone levels in isolated versus grouped animals has also been found in some strains of mice (Rabin *et al* 1987), the difference in corticosterone levels in our experiment may partly reflect the reduced activity among singletons rather than reduced social stress. However, it is noteworthy that, among grouped rats, organ pathology scores correlated negatively with the amount of time spent allogrooming and feeding or investigating food and water dispensers (except for thymus scores in the latter case), behaviours which are likely to correlate negatively with social stress. However, scores for adrenal glands among individually-housed rats also correlated positively with time spent investigating food and water dispensers.

While it is possible that tail chasing and tail attention are substitutes for social interaction, they may instead, along with self-grooming and drinking, reflect the extension of elastic self-maintenance components of the rat's time budget in the face of relaxed social

constraints. In an environment where needs are met easily and demands and opportunities for what would normally be another important activity (social interaction) are reduced, expanding self-maintenance components may provide an alternative to entering a state of decision-making limbo (McFarland 1989; Barnard & Hurst 1996). The fact that time spent in tail chasing and self-grooming correlated negatively with organ pathology and rats showing tail chasing and tail attention had lower overall pathology scores than those that did not may reflect variation in the ability of different individuals to adjust their time budget in this way. In addition, the reduced pathophysiological responses of singly-housed rats cannot be taken as evidence that the welfare of animals was improved by single housing. As suggested in the Introduction, the costs of social interaction may be a price that rats are adaptively prepared to pay and social interaction a high priority component of their time budget which they actively seek. In keeping with this, attention to the barrier increased with the degree of contact permitted with neighbours and there was some evidence that organ pathology among singly-housed rats was reduced when animals had the opportunity to contact neighbours. In contrast, organ pathology scores in individually-housed rats increased with time spent investigating the cage floor and sniffing through the bars, responses consistent with seeking olfactory social information. A similar positive association with floor investigation arose in grouped rats, perhaps because of a mismatch between odours from a neighbouring singleton and substrate odour cues in the home-cage.

Other welfare implications of exposure to neighbours emerged from the interaction effect of barrier type and housing condition on time spent sleeping, the difference between housing conditions being reduced as individually-housed rats spent more time sleeping in the dark when separated from neighbours by an open mesh barrier. Although no direct correlation between sleeping and pathophysiological change emerged in this experiment, earlier work with the same strain in free-range groups suggests that, over a longer period (9 rather than 5 weeks) sleep may be associated with individual differences in immune function and organ pathology (Hurst *et al* 1996). However, other studies have implicated social isolation in changes in neurological patterns of sleep in rats (Ehlers *et al* 1993) which suggest that time budget studies should be combined with measures of the underlying quality of certain behaviours. The tendency for both single and grouped rats to rest away from Perspex barriers merits further investigation from a welfare viewpoint. Whether it was due to eg aversion to the material or a preference for darker areas of the cage may have implications for the use of clear plastic caging in some housing regimes. It is worth emphasizing that the degree of contact with neighbours had no effect on overall mobility, so the provision of neighbours did not appear to stimulate activity.

That exposure to neighbours resulted in some degree of socialization is implied by an increased social tolerance among animals previously housed singly when introduced into an unfamiliar group. This apparent socialization is important from a welfare viewpoint since it suggests that the aggression-inducing effects of temporary isolation can be offset by maintaining singletons in some degree of contact with other individuals, and not necessarily the same individuals as those with which they will later be rehoused.

Overall, therefore, while housing male rats singly produces social deprivation, the removal of social responses from the time budget may be at least partly offset in some individuals by an increase in self-directed behaviours, even though these may not significantly affect the reduction in mobility among singletons. The fact that

individually-housed animals showed reduced corticosterone concentration and organ pathology relative to their grouped counterparts suggests that individual housing also removes social stress. However, behavioural responses to contact with neighbours, and some of the associated pathophysiological changes, suggest that rats seek social interaction and that any concomitant social stress may be an adaptively tolerated cost (though it is important to acknowledge again that the social responses of grouped rats confined in a cage are likely to be artificially constrained and that some degree of social stress will probably have arisen from this [Hurst *et al* 1996]). Thus, while exposure to neighbours separated by a barrier may provide social stimuli without the negative consequences of aggressive social conflict (Hurst *et al* 1996), concluding that this improves the welfare of rats ignores the possibility (and some evidence) that the rats' priorities are geared to social interaction. Nevertheless, because of its apparent ameliorating effects on aggression when previously isolated males are introduced into groups, separation, but not isolation, may have some welfare implications for laboratory-housed rats.

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