STRAIN-SPECIFIC EFFECTS OF CAGE ENRICHMENT IN MALE LABORATORY MICE (*MUS MUSCULUS*)

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Abstract *Animal Welfare 1999, **8:*** 361-379

'Environmental enrichment' is often considered to improve captive animal welfare. However, some studies using male mice, Mus musculus, *indicate that increasing cage complexity increases aggression. Limited evidence suggests that enrichment differs in its effects on behaviour and physiology between strains; but behaviour also differs between strains in non-enriched environments. Differences in enrichment type, evaluation methods, and strains used, have caused difficulty in interpreting the efficacy of environmental enrichment in improving welfare. Using enrichment suitable for commercial laboratories (nesting material and a Perspex tunnel), we compared within-cage behavioural and physiological responses among males of six strains housed in non-enriched standard polypropylene cages with those housed in 'enriched' cages. Outbred ICR(CD-1) and TO mice, and inbred BALB/c mice were more aggressive than C57BL/6, CBA/Ca and DBA/2 mice, which exhibited low levels of aggression typical of most inbred strains. Enrichment did not significantly affect aggression levels. Animals in enriched cages spent more time investigating the internal cage environment, eating and drinking, and in stereotypic behaviour patterns, although levels differed between strains. The greatest increase in stereotypy levels (bar-related stereotypies) with enrichment was found in DBA/2 mice. Higher testosterone levels were maintained over the study period in mice housed in enriched cages, and in more aggressive strains. IgG levels were also higher in mice housed in enriched cages, and in the outbred strains ICR(CD-1) and TO compared with inbred strains. The relationship between aggression, testosterone and 'enrichment' suggests that increasing complexity in laboratory cages may increase a naturally selected territorial response in some strains. The implications for strain-specific welfare are discussed.*

Keywords: *animal welfare, behaviour, enrichment, mice, physiology, strain differences*

Introduction

Laboratory mice are used worldwide in scientific research (Festing 1979). They are typically housed in caging systems designed primarily for ease and economy of maintenance rather than with a consideration for the behavioural requirements of the animals (Newberry 1995). Recently, considerable effort has been made to find ways of improving the housing of laboratory animals. Objects presumed to be of benefit to the inhabitants are often added to increase complexity in captive environments, a process termed 'environmental enrichment'. Objects likely to appeal to the mouse are, or are functionally analogous to, those that are found in its environment of evolutionary adaptation. To be widely implemented in laboratories, enrichment must be easily incorporated into different husbandry regimes (Scharmann 1991; Sherwin 1997), and the materials selected must be cost-effective (Newberry 1995) and not interfere with the purpose of the studies in which they are employed. Apart from its use in attempts to improve welfare, enrichment can be used explicitly to examine learning and brain development (eg Manosevitz & Pryor [1975]; Wainwright *et al* [1994]).

Implicit in the term 'enrichment', is the assumption that modifications are beneficial from the animals' viewpoint – and do not simply cater for the anthropomorphic whims of the caretakers (Newberry 1995). Testing this assumption can be far from straightforward. For instance, some studies using mice have indicated that increasing the complexity of the environment can alter social relationships and increase aggression (eg McGregor & Ayling [1990]; Haemisch *et al* [1994]; Barnard *et al* [1996b]; and as also suggested by Brain [1988; 1989], but see Van de Weerd *et al* [1994]). Such changes may reflect the opportunity for competitive males to express resource defence, arguably a welfare benefit for these individuals, but they may also reflect the inability of males of lower competitive ability to avoid attacks in confined cages, a potentially serious welfare problem. Newberry (1995) suggested that it was more useful to emphasize the functionality and adaptiveness of behaviour in specific environments than its 'naturalness'. Differences in the choice of enrichment objects and cage size which appear subtle to humans could have a big impact on the motivation of mice to perform certain naturally selected responses, which remain in laboratory mice as an 'evolutionary legacy' (Dawkins 1988). In restrictive cages, mice will typically establish dominance hierarchies based largely on postural aggression rather than on escalated attacks between familiar mice that are forced together (Bisazza 1981). However, if the area available to mice is sufficiently increased, they will distribute themselves and attempt to defend territories, becoming intolerant of intruders (as in commensal habitats [Crowcroft 1955; Crowcroft & Rowe 1963]).

A review of the published literature reveals that the effect of altering environments has been assessed using a variety of methods. Reactions of mice to intruders inside the cage environment have been used as an indication of the effects of enrichment on increasing the tendency to act in a territorial fashion (Haemisch *et al* 1994; Haemisch & Gärtner 1994). A variety of tests (Hennessy & Foy 1987; Chamove 1989; Van de Weerd *et al* 1994; 1997; Wainwright *et al* 1994) have been used to examine the effect of enrichment on exploratory behaviour, emotionality and fearfulness outside the home cage environment. These methods measure the change in reactivity of mice as a consequence of the enriched treatment but the welfare implications of different degrees of reactivity are hard to assess in this narrow context. Preference testing examines an animal's choice between different options. Associating a cost with each choice (demand function) assesses the motivation to access resources (Dawkins 1988; Sherwin & Nicol 1997). Mice are, however, highly motivated to

explore their environment and may work to access any additional area. The area accessed may not necessarily be beneficial from the point of view of long-term benefits to the individual (Rushen & de Passillé 1992), or reflect long-term preferences (Dawkins 1988). What is perceived as 'choice', could be the satisfaction of escaping the home cage and/or the motivation to search for resources (Sherwin 1997).

Thus, responses may only reflect a 'need' to satisfy immediate motivational priorities rather than an evaluation of the impact of 'enrichment' on a suite of responses, making the results hard to interpret in a wider context. Assessment of time budgets can overcome some of these shortcomings (Dawkins 1988). No studies have looked extensively at the impact of enrichment on behavioural time budgets in the environment which is the focus for improvement and where the study animals spend the vast majority of their time – the home cage.

Assessing behaviour alone cannot provide a true picture of the effects of environmental conditions on welfare. Physiological and behavioural responses are unavoidably interrelated, and their associated costs may be traded off against one another according to the circumstances. For example, Barnard *et al* (1996a) examined the relationships between behaviour, corticosterone (a stress indicator, eg Van de Weerd *et al* [1997]), testosterone (related to competitive ability [Rodgers 1989; Vom Saal 1989]) and total IgG (used as an indicator of peripheral immune responsiveness [Barnard *et al* 1993]) in order to consider the regulation of these potentially immunodepressive hormones as part of an adaptive mechanism of physiological and behavioural decision making. The existence and functional relevance of these adaptive trade-off mechanisms must be considered in the evaluation of welfare. Further, chronic effects of environmental conditions on physiology may be reflected in organ weights measured at termination (Manser 1992). For instance, increased adrenal gland weight is symptomatic of adrenal hypertrophy. Relatively low spleen and thymus weight may indicate chronic immunosuppression, and a relatively large heart may indicate arteriosclerosis caused by overactivation of the sympathetic nervous system (Manser 1992).

Although Dawkins (1988) acknowledged that domesticated mice carry an 'evolutionary legacy' in their behaviour, which must also be the case for their physiological responses, strains differ in both physiology (Haemisch & Gärtner 1994; Van de Weerd *et al* 1997), and behaviour (Southwick and Clark 1968; Van Oortmerssen 1971; Haemisch & Gärtner 1994; Van de Weerd *et al* 1994; 1997). Such differences have led to several authors (Manosevitz & Montemayor 1972; Brain 1989; Van de Weerd *et al* 1994) acknowledging the need to consider strain differences when evaluating the impact of housing conditions.

Thus, in this study, we examined the time budgets, physiology and organ weights of male mice from six commonly used inbred and outbred strains, which previous studies have suggested would be likely to differ in their physiological (Festing 1979) and behavioural (Southwick & Clark 1968; Van Oortmerssen 1971) responses to practical enrichment placed in their home cages.

Methods

Ethical note

All licensed work was carried out under Home Office Project Licence 40/1086. Prior to commencing the study, it was decided that mice would be separated and withdrawn from the study if aggressive interactions involved prolonged bouts of chasing or caused injury. However, this was not required during this study. To induce temporary anaesthesia (for blood sampling), or an overdose (for termination) animals were placed in a dessicator where

cotton wool soaked in an appropriate amount of anaesthetic agent had been placed. Following the (UK) Home Office Code of Practice (Home Office 1989), animals were separated from the anaesthetic by a mesh grill. Although this method of anaesthesia was used in our unit at the time of this study, it has since been discontinued, as it is no longer a recommended method for animal welfare and health and safety reasons (Wolfensohn & Lloyd 1994).

Animals and housing conditions

Outbred strains ICR(CD-1) and TO, and the inbred strains BALB/c, C57BL/6, CBA/Ca and DBA/2 mice were our subjects. Thirty-two male mice of each strain were purchased from Harlan UK Ltd (Bicester, UK) aged 5 weeks, supplied as four animals per box. As we could not ascertain the relationship or familiarity of mice prior to arrival at our animal house, animals were held in their supply groups for 5 days to standardize their immediate pre-study experience. Following this period, mice were rearranged into strain-specific groups of four mice that had not been housed in the same stock group (eight groups per strain). Prior to and throughout the study, mice were housed in typical, opaque, polypropylene cages, 12.5x45x14cm (Type MB1; North Kent Plastics, Erith, UK), incorporating a metal grill lid with a food-hopper and water bottle. Mice were maintained on a 12:12 h reversed white-/red-light cycle (red-light 0800h to 2000h). Temperature was maintained between 19 and 21°C. Cages were supplied with *ad libitum* food (Harlan-Teklad™ TRM 9607 Rat and Mouse Diet; (Harlan-Teklad™, Bicester, UK) and water. They were provided with a sawdust substrate, and were cleaned twice weekly. Half the cages used for each strain (the enriched treatment) were provided with a clear Perspex tunnel (diameter 40mm, length 120mm) and a small handful (approximately 1g) of shredded tissue nesting material (Biotech Ltd, Finedon, UK) placed in the open end of each cage.

Laboratory mice appear to have a strong motivation to construct nests (Van Oortmerssen 1971; Sherwin 1997; Van de Weerd *et al* 1997), as do mice in commensal environments (Estep *et al* 1975). Nests provide cover and also appear to play a role in thermoregulation (Lynch 1978). Nesting material is often used in laboratories, and its provision is a recommendation of the (UK) Home Office Code of Practice (Home Office 1989). Tunnels mimic the type of structures likely to be used as cover in many commensal and feral habitats (Crowcroft 1966; Berry 1987).

There were eight cages of four mice for each strain; four containing the enrichment, and four maintained in a standard way (the experimental treatments). All cages were suspended on standard cage racks, leaving gaps between rows for behavioural observation. Cages of each strain of mice and treatment were evenly distributed on the racks to avoid any positional or neighbour strain bias.

Behavioural observations

Animals were marked for individual identification during the week prior to placement in their experimental groups. Albino mice (ICR[CD-1], TO and BALB/c) were marked with Clairol Nice n' Easy™ Natural Black hair colour (Shade 122: Bristol-Myers Co Ltd, Uxbridge, UK), and non-albino mice were marked using a bleach and 40 per cent cream peroxide solution (Jerome Russell Cosmetics Ltd, Essex, UK). In previous studies we had established that these solutions had no irritant effects.

Behaviour was recorded during the red-light phase, when mice were most active. Behavioural elements were allocated to nine functional categories for analysis, modified

from those used for rats by Hurst *et al* (1997) and allowing for differences in cage type between this and Hurst *et al's* study. Behavioural category definitions are outlined in Table 1. Animals were observed in sets of six cages (incorporating both cage types [standard and enriched] and a mix of strains); each group was observed for an equivalent period each day (approximately 45min). Instantaneous time budget samples of behaviour (Martin & Bateson 1986) were taken in rotation with a sample taken every 4s, and with approximately 192s between successive samples of the same mouse. Mice within the same cage were observed in numerical order (each dye mark corresponding to a numbered sequence).

Table 1 Categories of behaviour recorded, and behavioural definitions.

Behaviour category	Definition
Aggression	Behaviour of an aggressive nature directed at another individual. Elicits an aggressive or defensive response from the other individual. Includes biting, chasing, pinning, mounting and threat postures.
Social investigation	Non-aggressive investigation of another individual, sniffing of body parts, defensive behaviour.
Sleep	Lying with eyes closed.
Eat/drink	Intake of food or water.
Non-bar-related exploration	Sniffing the components of the cage not related to the external environment eg sawdust substrate, cage walls, airborne odours.
Bar-related behaviour	Investigating the environment external to the cage by sniffing at or through the cage bars, bar gnawing (of less than 10s duration, see 'stereotypic behaviour').
Stereotypic behaviour	Persistent bouts of bar gnawing (of more than 10s duration, after Würbel *et al* [1996]).
	Bar circling: repeatedly tracing a circle on the cage bars with forepaws and body.
	Bar wheeling: repeated movement from the cage bars to the cage floor.
Grooming	Self-maintenance of pelage.
Stationary alert	The individual remains immobile but is not asleep or investigating the environment.
Other non-social behaviour	Bucket category: non-exploration, non-social movement.

Groups were sampled in a different order each day to ensure an even spread of behavioural samples for each mouse over the study duration. Eighteen time budget samples were taken per mouse for 4 days each week throughout the 4 weeks of study, yielding 288 time budget samples for each mouse. Bar-related behaviour patterns (see Table 1) were scored as stereotypic (generally defined as repetitive and invariant with no obvious goal or function) if they were over 10s in duration, following Würbel *et al* (1996). As each cage was observed for 16s (with the behaviour an individual mouse recorded every 4s), the observer (C M N) could easily track repeated behaviour patterns by an individual during this period. In addition, 9 x 4min periods of continuous observation were spread evenly throughout each

day to record aggressive interactions only (monitoring two observational groups at the same time). These were used as additional measurements for determining social status within cages since aggression was relatively infrequent. The aggression initiated by each individual was totalled over the study. The mouse initiating most aggression within each group was classified as dominant if it was found to be significantly more aggressive (at $P < 0.05$) than any other mouse (which was then classified as subordinate) within the cage (Bisazza 1981; Mackintosh 1989). Dominance was clearly shown in ICR(CD-1), TO and BALB/c mice, except in one cage of aggressive TO mice where no dominant emerged. The other strains showed very low levels of aggression and, therefore, were not classified in this way.

Body weights

Animals were weighed on arrival and at weekly intervals throughout the study for standard health monitoring. Body weight was used as a covariate in the analysis of physiological parameters.

Blood sampling

In the week prior to experimental grouping, animals were lightly anaesthetized with trichloroethylene (BDH-Merck, Lutterworth, UK) in the room adjacent to where they were housed and a retro-orbital blood sample (88µl) was collected in a heparinized capillary tube by licensed, experienced personnel (see, *Ethical note*). Samples were taken within 2min of an animal being removed from its stock cage. A second sample of blood was taken post-mortem from each individual by severing a major artery. All blood samples were centrifuged for 8min in a haematocrit centrifuge, and the serum frozen at –20°C for later physiological analysis.

Serum assays

We assayed each blood sample for serum concentrations of corticosterone, testosterone and total serum IgG following the procedures used by Barnard *et al* (1994a, b; 1996a, b).

The concentration of corticosterone (ng ml^{-1}) was measured using 6µl samples of undiluted serum and a Gamma-B ^{125}I-Corticosterone kit (Immunodiagnostic Systems Ltd, Tyne and Wear, UK) based on a double antibody radioimmunoassay as advised by the manufacturers. Corticosterone concentrations were calculated by reference to standards provided with the kit.

The concentration of testosterone (ng ml^{-1}) was measured using a Coat-a-Count® Solid-phase ^{125}I-Total Testosterone kit (Diagnostic Products Corporation, Los Angeles, USA) using 50µl samples of undiluted serum for post-mortem assays, and 25µl samples for pre-grouping assays (after consultation with the manufacturers, due to the small serum volumes obtained). Testosterone concentrations were calculated by reference to the calibration curves for each volume.

Serum total IgG concentration (mg l^{-1}) was determined by the method of Mancini *et al* (1965) using radial immunodiffusion kits (The Binding Site, Birmingham, UK). Ring diameters were measured in two directions at an angle of 90° and the mean was used to calculate the concentration of immunoglobulins from a calibration curve obtained using appropriate standards.

In certain cases, limited serum volumes meant that it was not possible to obtain a reliable estimate of all three serum factors from a particular sample. As a result, sample sizes in some analyses varied.

Termination procedures

At the end of the study animals were terminated (see, *Ethical note*) using chloroform (BDH-Merck, Bicester, UK). Order of termination was balanced with respect to strain. The adrenal glands, kidneys, spleen, testes, seminal vesicles, preputial gland, heart (exsanguinated) and thymus of each subject were carefully dissected and weighed (in pairs where appropriate) so that potential relationships between organ weight, physiology and behaviour could be examined.

Statistical analyses

Data were analysed using the SPSS© statistical package (Version 6.0; Statistical Package for the Social Sciences© Inc, Chertsey, UK). One-sample Kolmogorov-Smirnov tests confirmed that the distributions of all variables did not differ significantly from normal, thus parametric statistics were used throughout. We analysed both behavioural and physiological data to compare the overall responses of strains, and the effects of environment and social status on responses. A multivariate analysis of variance (MANOVA; Wilk's lambda) assessed overall differences between strains in behavioural and physiological parameters, examining univariate *F*-ratios to identify which variables contributed most strongly to the overall effect. Body weight was entered as a covariate in analyses involving physiological parameters and organ weights, since inbred strains are generally much smaller than outbred animals.

We used stepwise Discriminant Function Analysis to examine whether there were general differences between aggressive and non-aggressive strains, and to identify which variables other than aggression were important in discriminating between these strains. Variables were entered stepwise into the analysis according to the criterion of minimizing Wilk's lambda (equivalent to the largest multivariate *F*) to provide the most parsimonious discrimination between classes.

Pearson's (product moment) correlations were used to examine specific relationships between particular behavioural and physiological parameters, if suggested by the data.

Results

Behaviour

During observations and husbandry procedures, it was apparent that most mice in the enriched treatment manipulated the nest material and used it as cover. Most, though not all, mice dragged the material under the food-hopper. Mice were frequently observed moving over the tunnel, but were seen to pass through it only infrequently. Tunnels rarely remained where they had been placed, although it is unclear whether the movement was intentional. During husbandry procedures, it was noticed that some tunnels were marked with urine, although observations suggested that this was not a strain characteristic.

The MANOVA of the effects of enrichment and strain on time budgets revealed that enrichment had a highly significant effect on behaviour (multivariate $F_{9,172} = 3.26$, $P < 0.001$) but there were also highly significant differences in behaviour between strains (multivariate $F_{45,772} = 12.54$, $P < 0.001$) and the effects of enrichment varied according to strain (interaction between strain and treatment, multivariate $F_{45,772} = 1.74$, $P < 0.005$).

Inspection of the means confirmed that the two outbred strains (ICR[CD-1] and TO) were more aggressive than three of the inbred strains, while the inbred BALB/c were also relatively aggressive (Figure 1). Enrichment had no effect on time spent in aggressive behaviour ($F_{1,180} = 1.01$, ns) with no interaction between strains and the enriched treatment.

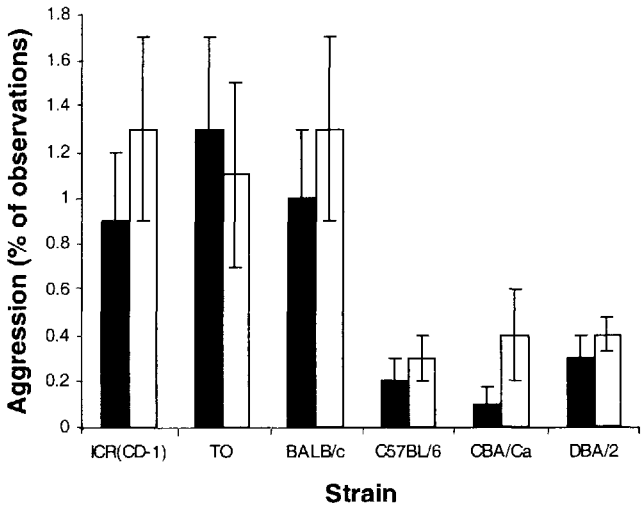

Figure 1 **Mean (± SEM) percentage of observations of aggression recorded over the study period, according to strain and cage type. Dark shading signifies mice in enriched cages, no shading signifies standard-housed mice.**

Although potentially important from a welfare perspective, aggressive behaviour occupied only a small proportion of time budgets even among aggressive strains (< 2%). The major difference in behaviour between strains (see Table 2) was in the time spent sleeping ($F_{5,180}$ = 31.65, $P < 0.001$), although this was not affected by cage enrichment (interaction between strain and cage type $F_{5,180}$ = 0.82, ns). This was due to the very high (mean ± SEM) proportion of time that the outbred TO mice spent asleep (43.3 ± 1.77 %) and the much lower level of sleep exhibited by the inbred BALB/c (16.25 ± 0.91 %) during the red-light recording period. Not surprisingly, these differences were reflected in time spent in other categories of active behaviour such as eating/drinking and exploration, which were particularly low in the TO strain. Each strain differed in the relative time allocated to particular activities as shown in Table 2, with no consistent pattern across strains. However, discriminant function analysis revealed that mice in aggressive strains (ICR[CD-1], TO and BALB/c) generally spent more time in all types of social behaviour and remaining stationary but alert while those in non-aggressive strains spent more time at the cage bars (chi-square = 64.7, $P < 0.001$).

Across strains, the significant overall effect of enrichment on behaviour (multivariate $F_{45,772}$ = 12.54, $P < 0.001$) arose from differences in only a few categories of behaviour (see Table 2). The time spent feeding and drinking was significantly less in enriched cages ($F_{1,180}$ = 6.17, $P < 0.05$), but stereotypic behaviour (ie bar-related stereotypy) was increased ($F_{1,180}$ = 5.10, $P < 0.05$). There was also a tendency for mice in enriched cages to explore their internal cage environment more than animals in standard housing ($F_{1,180}$ = 3.59, P = 0.06). However, there were large differences in these activities between strains (eating and drinking $F_{5,180}$ = 29.87, $P < 0.0001$; bar-related stereotypies $F_{5,180}$ = 12.11, $P < 0.001$; non-bar exploration, $F_{5,180}$ = 9.29, $P < 0.001$); and enrichment caused a strong reduction in feeding

Table 2 **Mean (± SEM) percentage of the time budget spent in each behaviour category, split by strain and cage type.**

Behaviour	ICR (CD-1)	TO	BALB/c	C57BL/6	CBA/Ca	DBA/2
Aggression						
Enriched	0.9 ± 0.3	1.3 ± 0.4	1.0 ± 0.3	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.1
Standard	1.3 ± 0.4	1.1 ± 0.4	1.3 ± 0.4	0.3 ± 0.1	0.4 ± 0.2	0.4 ± 0.1
Social investigation						
Enriched	4.4 ± 0.7	5.4 ± 0.6	5.3 ± 0.4	5.1 ± 0.4	3.2 ± 0.3	3.3 ± 0.5
Standard	5.3 ± 0.6	5.0 ± 0.7	5.3 ± 0.3	4.8 ± 0.4	3.6 ± 0.3	2.4 ± 0.4
Sleep						
Enriched	27.7 ± 3.2	40.3 ± 2.7	15.4 ± 1.2	31.5 ± 2.2	24.1 ± 2.5	21.3 ± 1.8
Standard	26.6 ± 2.3	46.3 ± 2.1	17.1 ± 1.4	32.1 ± 1.9	23.6 ± 3.0	25.4 ± 2.6
Eat/drink						
Enriched	14.5 ± 1.1	10.1 ± 0.7	19.8 ± 1.3	15.8 ± 0.8	13.2 ± 0.7	21.6 ± 1.2
Standard	15.4 ± 1.3	12.8 ± 0.7	19.0 ± 1.0	17.9 ± 0.8	15.8 ± 0.7	21.3 ± 0.9
Non-bar exploration						
Enriched	15.7 ± 1.4	15.9 ± 1.0	20.1 ± 0.9	17.5 ± 0.8	15.7 ± 0.8	12.3 ± 0.8
Standard	14.9 ± 0.8	12.3 ± 0.9	18.0 ± 1.1	16.6 ± 1.1	14.9 ± 1.2	13.8 ± 1.0
Bar-related behaviour						
Enriched	6.1 ± 2.3	1.9 ± 0.4	10.4 ± 1.7	6.9 ± 1.9	17.7 ± 1.9	17.7 ± 1.9
Standard	8.1 ± 2.8	1.2 ± 0.2	12.0 ± 1.6	5.9 ± 1.4	16.6 ± 2.7	14.5 ± 2.2
Stereotypic behaviour						
Enriched	7.5 ± 1.6	1.5 ± 0.6	4.3 ± 1.3	1.3 ± 0.3	10.2 ± 2.3	10.0 ± 1.4
Standard	5.6 ± 1.0	1.1 ± 0.4	5.2 ± 1.5	0.5 ± 0.2	9.7 ± 2.7	1.7 ± 0.6
Grooming						
Enriched	16.0 ± 1.5	19.8 ± 1.4	16.0 ± 0.7	17.6 ± 0.8	12.0 ± 1.1	9.8 ± 0.7
Standard	14.5 ± 1.0	15.8 ± 1.0	15.6 ± 0.7	18.5 ± 0.7	11.2 ± 0.8	11.1 ± 0.8
Stationary alert						
Enriched	5.0 ± 0.7	2.8 ± 0.4	5.2 ± 1.1	3.0 ± 0.4	2.4 ± 0.4	1.8 ± 0.2
Standard	5.6 ± 0.9	3.6 ± 0.3	4.1 ± 0.8	2.5 ± 0.2	1.9 ± 0.5	6.3 ± 0.7
Other non-social						
Enriched	1.3 ± 0.2	1.1 ± 0.2	2.6 ± 0.3	1.1 ± 0.3	1.5 ± 0.3	1.8 ± 0.3
Standard	2.5 ± 0.7	0.8 ± 0.2	1.6 ± 0.2	0.9 ± 0.2	2.3 ± 0.5	3.0 ± 0.5

and drinking among TO and CBA/Ca mice, which also spent comparatively little time in these activities in standard cages. BALB/c and DBA/2 spent much more time eating and drinking regardless of enrichment (see Table 2). There was a highly significant difference between strains in time spent in (bar-related) stereotypies ($F_{5,180}$ = 12.11, $P < 0.001$) and, conversely, in time spent simply stationary but alert ($F_{5,180}$ = 8.09, $P < 0.001$). Under standard housing conditions, three strains, the outbred ICR(CD-1), and the inbred BALB/c and particularly CBA/Ca, spent a relatively high proportion of their time in bar-related stereotypies (an average of 5% to 10%) while outbred TO and inbred C57BL/6 and DBA/2 showed little bar-related stereotypic behaviour (< 1.5%). Enrichment had a general effect of increasing stereotypic behaviour across all strains ($F_{1,180}$ = 5.10, $P < 0.025$) but this effect was most apparent among the inbred DBA/2 mice; those housed in non-enriched cages spent less than 2 per cent of their time in bar-related stereotypies, as compared with those in enriched cages which spent over 10 per cent of their time in such behaviour (interaction between cage type and strain $F_{5,180}$ = 2.79, $P < 0.025$; Figure 2). Possibly reflecting this, inbred DBA/2 spent little time sitting stationary but alert in enriched cages. Other strains which showed small increases in stereotypies in enriched cages similarly showed a corresponding decrease in such stationary behaviour.

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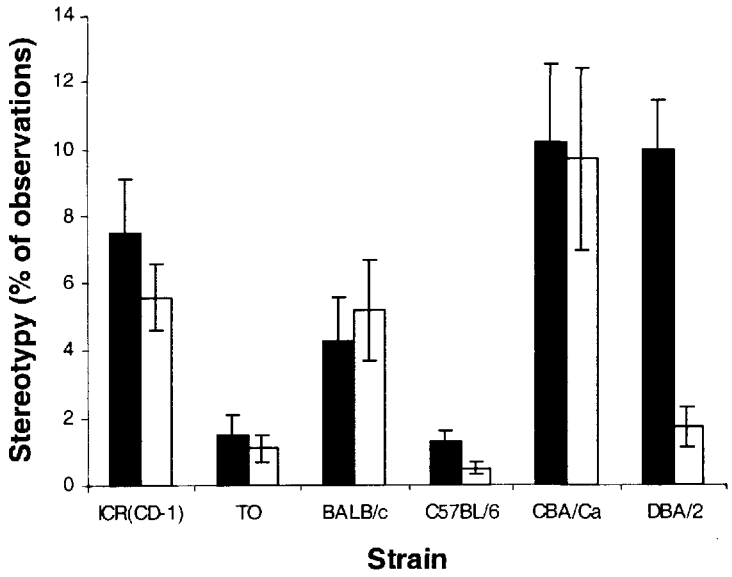

Figure 2 **Mean (± SEM) percentage of observations of (bar-related) stereotypies recorded over the study period, according to strain and cage type. Dark shading signifies mice in enriched cages, no shading signifies standard-housed mice.**

Physiology

The MANOVA confirmed that there were no significant pre-grouping differences in body weight or physiological measures in mice allocated to different cage types in any strain. Also, there were no significant differences in pre-grouping physiological measures between strains ($F_{15,\ 177}$ = 1.48, ns). At this time the mice were approximately 40 days old. All differences were, therefore, due to changes over the study period.

There were significant differences between strains in their changes in physiology over the study (multivariate $F_{15,\ 133}$ = 2.12, $P < 0.05$) largely due to differences in IgG responses. In standard cages, the outbred strains ICR (CD-1) and TO showed a greater change in circulating levels of IgG over the study, with the aggressive BALB/c showing the next greatest change in IgG levels. However, this pattern was not consistent over both cage types, as the inbred strains C57BL/6 and CBA/Ca housed in enriched cages had much higher levels of circulating IgG than did their counterparts held in standard cages. There were no interaction effects between strain and cage type (multivariate $F_{15,\ 133}$ = 1.25, ns). Overall, there were interesting effects of enrichment on physiology (multivariate $F_{3,\ 48}$ = 5.02, $P <$ 0.005), affecting all three parameters measured. Enrichment caused an increase in testosterone concentration ($F_{1,\ 114}$ = 6.125, $P < 0.05$; Figure 3), primarily due to an increase in the aggressive strains ICR (CD-1), TO and BALB/c. IgG titres were higher in enriched cages in all strains ($F_{1,\ 72}$ = 16.31, $P < 0.001$; Table 3), but particularly so in the outbred ICR (CD-1) and in the inbred C57BL/6, CBA/Ca and DBA/2. Corticosterone levels were also generally higher in enriched cages ($F_{1,\ 108}$ = 4.83, $P < 0.05$; Table 3), although mainly in the outbred TO and the inbred CBA/Ca.

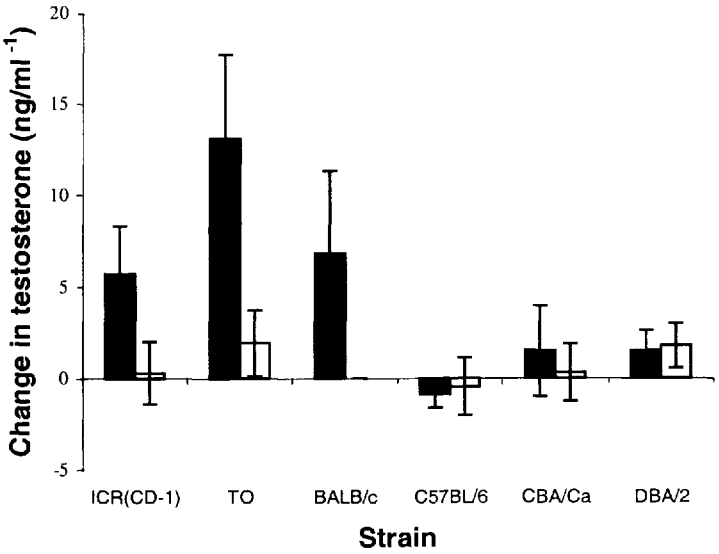

Figure 3 **Mean (± SEM) change in testosterone titre (ng ml^{-1}) over the study period, according to strain and cage type. Dark shading signifies mice in enriched cages, no shading signifies standard-housed mice.**

Table 3 **Change in physiological titres over the study period, split by strain and cage type.**

	Testosterone (ng ml^{-1})		IgG (mg ml^{-1})		Corticosterone (ng ml^{-1})	
Strain	**Enriched**	**Standard**	**Enriched**	**Standard**	**Enriched**	**Standard**
ICR(CD-1)	5.8 ± 2.6	0.3 ± 1.7	6.8 ± 1.2	3.3 ± 0.6	80.0 ± 79.0	40.7 ± 30.5
TO	13.1 ± 4.6	1.9 ± 1.8	3.2 ± 0.3	2.7 ± 1.5	295.4 ± 61.6	82.0 ± 15.8
BALB/c	6.9 ± 4.5	-0.0 ± 0.0	2.3 ± 0.5	1.7 ± 3.3	12.0 ± 14.9	17.3 ± 12.8
C57BL/6	-0.9 ± 0.8	-0.5 ± 1.6	2.4 ± 0.4	0.5 ± 5.1	243.9 ± 110.2	108.5 ± 36.9
CBA/Ca	1.5 ± 2.5	0.3 ± 1.6	3.5 ± 1.3	1.3 ± 0.2	180.5 ± 86.1	-10.4 ± 19.2
DBA/2	1.5 ± 1.1	1.8 ± 1.2	1.9 ± 0.3	0.8 ± 0.2	57.0 ± 40.7	110.8 ± 46.0

As there has been much interest in both corticosterone levels and stereotypic behaviour with regard to the assessment of welfare, and as this study found enrichment elevated levels of both, we checked whether there was a significant correlation between the levels of corticosterone and (bar-related) stereotypy shown by individuals. Interestingly, animals which performed stereotypic behaviour more frequently in standard cages had lower corticosterone titres (r_{49} = -0.32, $P < 0.025$), while in enriched cages there was no correlation (r_{68} = 0.05, ns; Figures 4a and 4b).

We predicted that social status might affect physiological titres, particularly with regards to testosterone. However, there were no differences in testosterone, corticosterone or total IgG measures according to dominance status.

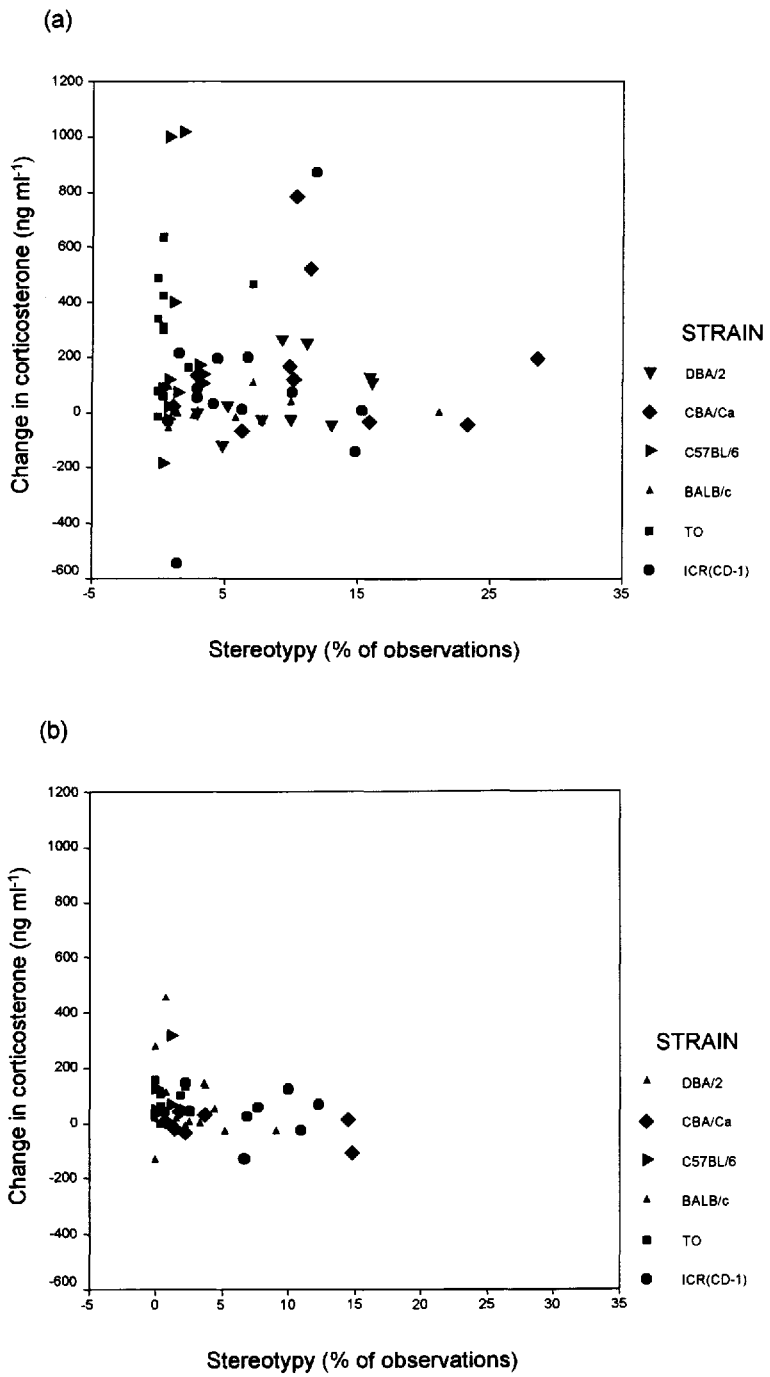

Figure 4 **Correlation between the change in corticosterone titre over the study (ng ml^{-1}) and (bar-related) stereotypic behaviour in mice: (a) mice in enriched cages (r_{68} = 0.05, ns); (b) mice in standard cages (r_{49} = -0.32, P < 0.025).**

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Organ weights

Overall organ weights (taking body weight into account as a covariate) differed significantly between strains (multivariate $F_{40, 753} = 23.56$, $P < 0.001$) due to strain variation in the weights of all organs with the exception of the adrenals (Table 4). There was also a significant effect of housing treatment on organ weights, illustrated in Table 4, (multivariate $F_{8, 172} = 3.69$, $P < 0.001$). This effect was due to the seminal vesicles being heavier in mice housed in enriched cages ($F_{1, 179} = 4.82$, $P < 0.05$), except in C57BL/6 mice; to adrenal weights being noticeably heavier in standard cages in all strains except ICR (CD-1) and CBA/Ca ($F_{1, 179} = 6.06$, $P < 0.025$); and to heart weights being heavier ($F_{1, 179} = 7.89$, $P < 0.01$) in mice that were housed in standard cages largely due to an increase in ICR (CD-1) mice. There was an interaction between strain and cage type (multivariate $F_{40, 713} = 1.74$, $P < 0.005$) due to the different effects enrichment had on strains with regards to heart weight ($F_{5, 179} = 2.69$, $P < 0.05$) and the near significant effects on spleen weight ($F_{5, 179} = 2.27$, $P = 0.05$).

Table 4 **Organ weights expressed as mean (± SEM) g^{10-2}, split by strain and cage type (enriched vs standard).**

Organ	ICR (CD-1)	TO	BALB/c	C57BL/6	CBA/Ca	DBA/2
Adrenals						
Enriched	0.54 ± 0.03	0.50 ± 0.07	0.46 ± 0.03	0.41 ± 0.03	0.60 ± 0.06	0.49 ± 0.03
Standard	0.52 ± 0.04	0.61 ± 0.05	0.64 ± 0.23	0.59 ± 0.03	0.54 ± 0.05	0.54 ± 0.03
Heart						
Enriched	16.47 ± 0.42	17.56 ± 0.47	13.95 ± 0.37	14.06 ± 0.49	11.51 ± 0.26	14.34 ± 0.21
Standard	18.20 ± 0.53	17.98 ± 0.41	13.35 ± 0.23	13.96 ± 0.36	11.74 ± 0.17	14.92 ± 0.40
Kidneys						
Enriched	60.77 ± 1.64	69.48 ± 2.06	50.00 ± 1.26	35.73 ± 0.85	47.53 ± 0.74	43.87 ± 0.80
Standard	64.98 ± 2.17	67.24 ± 1.31	48.09 ± 1.09	34.82 ± 0.63	47.54 ± 1.13	44.61 ± 1.12
Preputial						
Enriched	19.40 ± 1.44	14.62 ± 0.91	12.60 ± 0.83	11.06 ± 0.93	7.33 ± 0.38	9.95 ± 0.38
Standard	18.70 ± 1.29	15.13 ± 1.88	10.37 ± 0.74	10.98 ± 0.62	7.49 ± 0.35	10.29 ± 0.43
Seminal vesicles						
Enriched	26.41 ± 0.84	31.65 ± 1.86	20.41 ± 0.63	21.23 ± 1.04	18.47 ± 1.1	23.93 ± 0.76
Standard	25.38 ± 1.04	28.78 ± 1.46	20.30 ± 0.97	21.81 ± 0.78	16.64 ± 0.76	20.72 ± 0.92
Spleen						
Enriched	16.97 ± 2.70	12.96 ± 0.81	11.89 ± 0.52	9.12 ± 0.39	5.93 ± 0.24	8.94 ± 0.85
Standard	11.90 ± 0.56	15.13 ± 1.88	11.94 ± 0.53	8.15 ± 0.40	7.70 ± 2.21	9.12 ± 0.44
Testes						
Enriched	28.79 ± 1.47	21.88 ± 0.66	23.97 ± 0.65	23.58 ± 0.31	16.05 ± 0.02	23.21 ± 0.27
Standard	26.85 ± 0.77	22.07 ± 0.58	23.83 ± 0.66	22.92 ± 0.59	15.00 ± 0.49	23.02 ± 0.51
Thymus						
Enriched	4.03 ± 0.30	3.47 ± 0.23	3.04 ± 0.16	5.21 ± 0.25	3.22 ± 0.16	2.80 ± 0.10
Standard	3.48 ± 0.22	3.71 ± 0.22	3.17 ± 0.18	5.36 ± 0.24	3.02 ± 0.13	3.04 ± 0.16

As enrichment was shown to increase testosterone titres in aggressive strains, we examined whether there were any effects of enrichment, status or strain on organ weights, especially with regard to organs that may play a part in territorial responses. While there was no impact of enrichment on organ weights ($F_{8, 72} = 0.87$, ns), or any effect of status ($F_{1, 79} = 0.87$, ns), there were significant differences between the aggressive strains in the weight of the preputial glands ($F_{2, 79} = 8.26$, $P < 0.005$), seminal vesicles ($F_{2, 79} = 5.98$, $P < 0.005$), testes ($F_{2, 79} = 27.87$, $P < 0.001$) and kidneys ($F_{2, 79} = 5.35$, $P < 0.01$).

Discussion

It is interesting that sleeping accounted for the largest proportion of the time budget in each strain. It is not known if time spent sleeping in the dark period (when mice should be most active) reflected activity over the full 24h. However, such apparent inactivity could be of welfare concern if caused by a lack of stimulation within cages (but see relationships between activity and organ pathology in rats [Hurst *et al* 1996]).

Strains differed in their behaviour and physiology in a complex manner. Strain variation in aggression may be particularly relevant when assessing welfare because it is potentially injurious and because of the pivotal role it plays in the social organization of male mice (Hurst 1993). The frequency of its expression within captive environments may indicate variation in the ability of males from different strains to show adaptive plasticity in their naturally selected territorial response in an artificial situation. In the standard and enriched cages used in the current study, both outbred strains (ICR [CD-1] and TO) exhibited aggression, as did the inbred BALB/c (which were also aggressive in 'putative' tests [Jones & Brain 1987]), but the other inbred strains, C57BL/6, CBA/Ca and DBA/2, were rarely aggressive. In contrast to other studies that measured aggression between familiar males which had been housed in larger cages with more complex enrichment (McGregor & Ayling 1990; Barnard *et al* 1996b), mice in our enriched cages were no more aggressive towards one another than mice housed in standard cages. Sandnabba (1997) showed that high levels of aggression in mice were associated with an urge to enlarge the home range or territorial area and gain exclusive dominance. In this study, testosterone (which has been linked with competitive ability, see Zielinski & Vandenburgh [1993]) increased significantly more over the study in mice in enriched cages, due to elevation of its levels in aggressive strains. The seminal vesicles, which secrete fluid into the vas deferens, were also heavier in enriched-housed mice, suggesting anticipated reproductive opportunity in a more competitive environment.

Although aggression and testosterone both play a role in the naturally selected territorial responses of mice, they appear to be influenced independently by cage size and complexity in captivity. In relatively small cages housing single-sex groups, such as were used in this study, mice may adopt a behavioural strategy of relative tolerance towards their cage mates after aggressive attempts to exclude potential competitors from the area fail and familiarity increases. However, adding resources to these cages stimulates hormonal aspects of the territorial response. The increased corticosterone levels in enriched-housed mice may indicate stress related to increased territorial motivation (although standard-housed mice had heavier adrenals). Increasing cage size allows more spatial separation between mice, which may encourage the aggressive territorial response (although space remains too limited for territory establishment). Together with the addition of resources likely to be coveted (enrichment), larger cages may stimulate increased aggression in male mice.

Why might strain differences in aggression and social behaviour arise? Bisazza (1981) found that outbred Swiss mice formed territories within enclosures but inbred C57BL/6 and BALB/c mice did not. As in this study, Bisazza found C57BL/6 mice to be relatively pacific and BALB/c to be aggressive. This suggests an effect of inbreeding on the ability to establish and maintain territories, possibly because genetic similarity affects communication mechanisms. Mice primarily communicate using urinary odour cues that convey individuality and status information, enabling territories to be established and maintained (reviewed by Brown [1985]). As individuals within an inbred strain are isogenic, mice may be unable to recognize odour cues from other individuals as different to self, affecting their

ability to establish and maintain territories. Inbreeding wild mice reduces their competitive ability (Eklund 1996). From an inclusive fitness perspective, animals should respond more favourably towards animals that are of greater genetic similarity to themselves than towards those that are less genetically similar (but see Barnard *et al* [1991]); similarity in odour cues may provide a mechanism by which this occurs. The aggressive response of BALB/c mice (the only inbred strain to show levels of aggression and change in testosterone titres comparable to outbred strains), may, therefore, be even greater towards genetically dissimilar mice or mice whose signals could be recognized as different to self.

Alternatively, the pacific nature of some inbred strains may be due to other non-discriminatory shared strain characteristics (see Barnard *et al* 1991). The genetic and physiological basis of social behaviour and communication in different strains of laboratory mice is not clearly understood and requires further study. It is worth noting that some of the inbred strains which were relatively pacific in this study have been described as aggressive by other researchers (eg DBA/2 mice were described as aggressive by Jones & Brain [1987]; Haemisch & Gärtner [1994]; and Haemisch *et al* 1994). However, while aggression was relatively constant towards familiar cage mates, it increased towards intruders whose odour cues may have become recognizable as different to self (Haemisch & Gärtner 1994), or otherwise altered by housing conditions such as isolation (Jones & Brain 1987). Festing (1979) discussed how substrain differences arise between laboratories due to genetic differences resulting from mutation, residual heterozygosity and genetic contamination. Behavioural traits are not of interest to most researchers, and thus may be subject to incidental accumulation of variation between breeding establishments, whereas particular efforts may be made to standardize traits such as spontaneous formation of particular tumours. Thus, researchers and animal care staff should familiarize themselves with the particular behavioural characteristics of their substrain in order to assess its welfare. This may also be important for standardization between biomedical studies, as significant between-strain differences in behavioural parameters, such as sleep, could have a variety of effects on physiological responses.

In this study, enrichment generally appears to have stimulated interest towards the cage environment, with mice showing a greater tendency explore the internal cage environment. However, mice in enriched cages performed more stereotypies involving bars set in the cage lid, behaviour which is thought to develop from frustrated attempts to explore the external environment and/or leave the cage (Würbel *et al* 1996). This is surprising, as non-enriched environments are usually considered to be 'poorer', and are assumed to induce such behavioural abnormalities (Haemisch *et al* 1994). Our cage enrichment may have increased the motivation of mice to perform another natural behaviour pattern – exploration (possibly associated with motivation to increase the home range, discussed by Sandnabba [1997]) – which was frustrated by the confinement imposed by the cage, resulting in stereotypic behaviour. There was no relationship between (bar-related) stereotypy and corticosterone levels in the enriched cages, but, in the standard cages, mice performing more stereotypic behaviour had lower corticosterone titres. Thus, there may be environmental differences in the apparent function of bar-related stereotypies in mice. In standard cages this behaviour pattern could be interpreted as a coping response by which animals in behavioural 'limbo' (McFarland 1989) deal with a deficient environment. The increase in motivation to explore, apparently stimulated by enrichment, may persist – so 'coping' does not occur. The periodic opening of the cage lid for maintenance procedures may reinforce such motivation. Environmental influences on the motivational basis of stereotypies could relate to the enhancing effect that enrichment has on learning ability (Wainwright *et al* 1994).

While there were no differences between strains in their physiological responses to enrichment, strain differences in physiology may be relevant to welfare. Inbred strains generally exhibited lower IgG responses over the study than outbred ones, particularly in standard cages. It is important to consider this in conjunction with caging effects on aspects of the male territorial response. Barnard *et al* (1996a) found that when the immunocompetence of mice was challenged, aggression and testosterone levels were reduced to a greater extent in mice with low IgG levels. In non-challenged groups, as in this study, there was no relationship between IgG levels and aggression. In evolutionary terms, it may 'pay' mice whose immunocompetence is challenged to maintain testosterone levels and aggression when resources encourage competitive behaviour, since despite the cost to immunocompetence, acquiring these resources may increase reproductive opportunity. Indeed, in a study where reproductive opportunities were indicated by the addition of female odours (Barnard *et al* 1997), immunocompromised mice maintained their aggression levels despite the potentially costly effects to survivorship. In laboratories, few individuals receive real reproductive opportunities, but as this likelihood is unlikely to be perceived by mice this naturally selected behaviour pattern is likely to persist in laboratory mice.

Many laboratory mice do undergo some form of immune challenge in the form of toxicological testing. As discussed earlier, enrichment can raise levels of aggression and testosterone. However, Barnard *et al* (1996b) showed that testosterone, but not aggression, was downregulated (ie levels were reduced) in parasitized mice in enriched cages, perhaps to reduce its impact on immunocompetence when animals are already immunocompromised. While not reflected in a decrease in their total IgG, mice in enriched cages showed reduced resistance to *Babesia microti*. Enriching the cages of aggressive, high testosterone-producing mice (in which immunocompetence may be reduced, particularly where both aggression and hormone levels may be further increased by the provision of female odour or reproductive experience), may have particular welfare implications.

In this study, where the immunocompetence of mice was not challenged, the higher IgG response of enriched-housed mice may indicate that their immune systems were stimulated, although differences in titres in differentially housed mice may also be the result of immunodepression in standard cages. Any apparent housing effects on immunological parameters are likely to be of interest to biomedical researchers.

Animal welfare implications

The drive to improve animal welfare, which is the aim of most environmental enrichment programmes, is laudable and should be encouraged – but with caution. Enrichment, as used in this study, could be interpreted as improving the welfare of male mice, as it apparently encouraged aspects of a naturally selected response (territoriality) and increased interest in the environment within and external to the cage. However, these responses were encouraged within a highly artificial environment, and may not be appropriate. The increase in bar-related stereotypy within enriched cages may have resulted from increased interest in the external environment that was frustrated by confinement. The effects of artificial selection on the behavioural and physiological responses of each strain should also be considered. In particular circumstances, such responses may be detrimental to individuals.

Enrichment is also likely to have an impact on the lives of female mice. However, as females have different life history strategies and, therefore, motivational priorities, care must be taken in extrapolating these results to females. As advocated by Barnard and Hurst (1996), we must use our knowledge of the naturally selected responses of animals and how

circumstances impinge on their functional design to predict the potential effects of manipulating their captive environment on their welfare.

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References

Barnard C J, Behnke J M, Gage A R, Brown H and Smithurst PR 1997. Immunity costs and behavioural modulation in male laboratory mice (*Mus musculus*) exposed to the odours of females. *Physiology & Behavior 62:* 857-866.

Barnard C J, Behnke J M and Sewell 1993 Social behaviour, stress and susceptibility to infection in house mice (*Mus musculus*) – effects of duration of groups and aggressive behaviour prior to infection on susceptibility to *Babesia microti. Parasitology 103:* 183-192

Barnard C J, Behnke J M and Sewell J 1994a Social behaviour and susceptibility to infection in house mice (*Mus musculus*) – effects of group size, aggressive behaviour and status related hormonal responses prior to infection on resistance to *Babesia microti. Parasitology 108:* 487-496

Barnard C J, Behnke, J M and Sewell J 1994b Social behaviour, stress and susceptibility to infection in house mice (*Mus musculus*); effects of duration of grouping and aggressive behaviour prior to infection on susceptibility to *Babesia microti. Parasitology 107:* 183-192

Barnard C J, Behnke J M and Sewell J 1996a Social status and resistance to disease in house mice (*Mus musculus*) – status related modulation of hormonal responses in relation to immunity costs in different social and physical environments. *Ethology 102:* 63-84

Barnard C J, Behnke J M and Sewell J 1996b Environmental enrichment, immunocompetence and resistance to *Babesia microti* in male laboratory mice. *Physiology & Behavior 60:* 1223-1231

Barnard C J and Hurst J L 1996 Welfare by design: the natural selection of welfare criteria. *Animal Welfare 5:* 405-434

Barnard C J, Hurst J L and Aldhous P 1991 Of mice and kin: the functional significance of kin bias in social behaviour. *Biological Reviews 66:* 379-430

Berry R J 1987 House mouse. *Biologist 34:* 177-186

Bisazza A 1981 Social organisation and territorial behaviour in three strains of mice. *Bollettino do Zoologia 48:* 157-16

Brain P F 1988 Hormones and aggression in infra-human vertebrates. In: Brain P F and Benton D (eds) *The Biology of Aggression* pp 181-213. Sijthoff and Noordhoff International: Alphen aan den Rijn, The Netherlands

Brain P F 1989 Social stress in laboratory mouse colonies. In: *Laboratory Animal Welfare Research: Rodents. Proceedings of a Symposium Organised by Universities Federation for Animal Welfare and Held at the Royal Holloway and Bedford New College, University of London, Egham, Surrey, 22nd April 1988* pp 49-61. Universities Federation for Animal Welfare: Potters Bar, UK

Brown R E 1985 The rodents ii: suborder Myomorpha. In: Brown R E and Macdonald D W (eds) *Social Odours in Mammals. Volume 1* pp 345-457. Clarendon Press: Oxford, UK

Chamove A S 1989 Cage design reduces emotionality in mice. *Laboratory Animals 23:* 215-219

Crowcroft P 1955 Territoriality in wild house mice, *Mus musculus. Journal of Mammalogy 36:* 299-301

Crowcroft P 1966 *Mice all Over*. Foulis: London, UK

Crowcroft P and Rowe F P 1963 Social organization and territorial behaviour in the wild house mouse (*Mus musculus* L.). *Proceedings of the Zoological Society of London. 140:* 517-531

Dawkins M S 1988 Behavioural deprivation: a central problem in animal welfare. *Applied Animal Behaviour Science 20:* 209-225

Eklund A 1996 The effects of inbreeding on aggression in wild male house mice (*Mus domesticus*). *Behaviour 133:* 883-901

Estep D Q, Lanier D L and Dewsbury D A 1975 Copulatory behaviour and nest-building behaviour of wild house mice (*Mus musculus*). *Animal Learning and Behaviour 3:* 329-336

Festing M F W 1979 *Inbred Strains in Biomedical Research*. Macmillan Press: London, UK

Haemisch A and Gärtner K 1994 The cage design affects intermale aggression in small groups of male laboratory mice: strain specific consequences on social organization, and endocrine activations in two inbred strains. *Journal of Experimental Animal Science 36:* 101-116

Haemisch A, Voss T, and Gärtner K 1994 Effects of environmental enrichment on aggressive behaviour, dominance hierarchies and endocrine states in male DBA/2J mice. *Physiology & Behavior 56:* 1041-1048

Hennesey M B and Foy T 1987 Nonedible material elicits chewing and reduces the plasma corticosterone response during novelty in mice. *Behavioural Neuroscience 101:* 237-245.

Home Office 1989 *Code of Practice for The Housing and Care of Animals Used in Scientific Procedures*. HMSO: London, UK

Hurst J L 1993 The priming effects of urine substrate markers on interactions between male house mice, *Mus musculus domesticus*, Schwartz and Schwartz. *Animal Behaviour 45:* 55-81

Hurst J L, Barnard C J, Hare R, Wheeldon E B and West C D 1996 Housing and welfare in laboratory rats: status dependent time-budgeting and pathophysiology in single-sex groups maintained in open rooms. *Animal Behaviour 52:* 335-360

Hurst J L, Barnard C J, Nevison C M and West C D 1997 Housing and welfare in laboratory rats: Welfare implications of isolation and social contact among caged males. *Animal Welfare 6:* 329-347

Jones S E and Brain P F 1987 Performances of inbred and outbred laboratory mice in putative tests on aggression. *Behaviour Genetics 17:* 87-96

Lynch C B and Possidente B P Jr 1978 Relationships of maternal nesting to thermoregulatory nesting in house mice (*Mus musculus*) at warm and cold temperatures. *Animal Behaviour 26:* 1136-1143

Mackintosh J H 1989 Aggression in confined populations. In: Brain P F, Mainardi D and Parmigiani S (eds) *House Mouse Aggression* pp 119-134. Harwood Academic Publishers: London, UK

Mancini G, Carbonara A O and Heremans J F 1965 Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry 2:* 235-254

Manosevitz M and Montemayer R J 1972 Interaction of environmental enrichment and genotype. *Journal of Comparative and Physiological Psychology 38:* 191-201

Manosevitz M and Pryor J B 1975 Cage size as a factor in environmental enrichment. *Journal of Comparative and Physiological Psychology 89:* 648-654

Manser C E 1992 *The Assessment of Stress in Laboratory Animals*. RSPCA: Horsham, UK

Martin P and Bateson P 1986 *Measuring Behaviour: An Introductory Guide*. Cambridge University Press: Cambridge, UK

McFarland D J 1989 *Problems of Animal Behaviour*. Longman: Harlow, UK

McGregor P K and Ayling S J 1990 Varied cages result in more aggression in male CFLP mice. *Applied Animal Behaviour Science 26:* 227-281

Newberry R C 1995 Environmental enrichment: increasing the biological relevance of captive environments. *Applied Animal Behaviour Science 44:* 229-243

Rodgers R J 1989 Physiological consequences of defeat. In: Brain P F, Mainardi D and Parmigiani S (eds) *House Mouse Aggression* pp 233-252. Harwood Academic Publishers: London, UK

Rushen J and de Passillé A M B 1992 The scientific assessment of animal welfare: a critical review. *Canadian Journal of Animal Science 72:* 721-743

Sandnabba N K 1997 Territorial behaviour and social organization as a function of the level of aggresiveness of male mice. *Ethology 103:* 566-577

Scharmann W 1991 Improved housing of mice, rats and guinea pigs – a contribution to the refinement of animal experiments. *ATLA 19:* 108-114

Sherwin C M 1997 Observations on the prevalence of nest building in non-breeding TO strain mice and their use of two nesting materials. *Laboratory Animals 31:* 125-132

Sherwin C M and Nicol C J 1997 Behavioural demand functions of caged laboratory mice for additional space. *Animal Behaviour 53:* 67-74

Southwick C H and Clark L H 1968 Interstrain differences in aggressive behaviour and exploratory activity of inbred mice. *Communications in Behavioural Biology – A 1:* 49-59

Van Oortmerssen G A 1971 Biological significance, genetics and evolutionary origin of variability in behaviour within and between inbred strains of mice (*Mus musculus*). *Behaviour 36:* 1-92

Van de Weerd H A, Baumans V, Koolhaas J M and Van Zutphen L F M 1994 Strain specific behavioural response to environmental enrichment in the mouse. *Journal of Experimental Animal Science 36:* 117-127.

Van de Weerd H A, Van Loo, P L P, Van Zutphen L F M, Koolhaas J M and Baumans V 1997 Nesting material as environmental enrichment has no adverse effects on behaviour and physiology of laboratory mice. *Physiology & Behavior 62:* 1019-1028

Vom Saal F S 1989 Perinatal testosterone exposure has opposite effects on adult intermale aggression and infanticide in mice. In: Brain P F, Mainardi D, Parmigiani S (eds*) House Mouse Aggression* pp 174-204. Harwood Academic Publishers: London, UK

Wainwright P E, Huang Y S, Bulman-Fleming B, Levesque S and McCutcheon D 1994 The effects of dietary fatty acid composition combined with environmental enrichment on brain and behaviour in mice. *Behavioural Brain Research 60:* 125-136

Wolfensohn S and Lloyd M 1994 *Handbook of Laboratory Animal Management and Welfare.* Oxford University Press; Oxford, UK

Würbel H, Stauffacher M and Van Holst D 1996 Stereotypies in laboratory mice: Quantitative and qualitative description of the ontogeny of 'wire-gnawing' and 'jumping' in Zur:ICR and Zur:ICR NU. *Ethology 102:* 371-385

Zielinski W J and Vandenburgh J G 1993 Testosterone and competitive ability in male house mice, *Mus musculus*: laboratory and field studies. *Animal Behaviour 45:* 873-891