

Laboratory environments and rodents' behavioural needs: a review

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Summary

Laboratory housing conditions have significant physiological and psychological effects on rodents, raising both scientific and humane concerns. Published studies of rats, mice and other rodents were reviewed to document behavioural and psychological problems attributable to predominant laboratory housing conditions. Studies indicate that rats and mice value opportunities to take cover, build nests, explore, gain social contact, and exercise some control over their social milieu, and that the inability to satisfy these needs is physically and psychologically detrimental, leading to impaired brain development and behavioural anomalies (e.g. stereotypies). To the extent that space is a means to gain access to such resources, spatial confinement likely exacerbates these deficits. Adding environmental 'enrichments' to small cages reduces but does not eliminate these problems, and I argue that substantial changes in housing and husbandry conditions would be needed to further reduce them.

Keywords Rats; mice; laboratory; housing; stereotypies; enrichment

Rodent housing conditions in laboratories represent an important potential welfare problem. Most animals used in research and testing spend their lives in small cages. Increasingly, some enrichment is provided within these cages, but there remains a significant proportion where enrichment is not provided despite consensus for needed reforms within the scientific community (Wolfe 2005). It is important to not only ask whether enrichment is provided, but also if the approach of within-cage enrichment has shortcomings, because any suffering caused by inappropriate housing will typically be of greater duration than that caused by the experiments themselves (Sherwin 2002).

The number of animals affected is large, and probably increasing. Norway rats (*Rattus norvegicus*) and house mice (*Mus*

musculus) comprise some 90% of all vertebrate animals used in laboratory research. While rodent use in Europe reportedly dropped from 10 to eight million between 1991 and 2002 (CEC 2005), global numbers now appear to be increasing due to a new emphasis on transgenic mice (O'Shea 2000, Fishbein 2001). For instance, one recent estimate puts the number of mice consumed by US laboratories at close to 100 million (Carbone 2004).

Minimum laboratory husbandry standards for rats and mice are prescribed by the European Community (CEC 1986) and in the UK by the British Home Office (1986), whose guidelines state that 'rats and mice should be group-housed unless a particular experiment requires otherwise' (para 3.28), and that (for animals in general) 'bedding and nesting materials should be provided, unless it is clearly inappropriate' (para 3.60). A Council of Europe review of housing standards

[nearly completed at time of writing] states that rodent 'enclosures and their enrichment should allow the animals to manifest normal behaviours' (CEC 2005, p. 20), and strongly recommends nesting materials and nest-boxes, and the further addition of some form of enrichment, such as tubes, boxes and climbing racks.

In the US there are no federal regulations for laboratory husbandry of rats and mice, owing to these animals' exclusion from the Animal Welfare Act (USDA 1995). However, guidelines developed by the non-governmental organization Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) and issued by the National Research Council (NRC 1996) include recommendations for the care and use of rodents, and constitute an important resource for the exchange of information on the care and use of animals in laboratories (Howard *et al.* 2004). Currently, these guidelines recommend specifically for rodents only solid-bottom caging with bedding, though enrichments for all laboratory-housed animals are encouraged.

Strictly speaking, the above guidelines are recommendations and not requirements, and this may be to allow room for exceptions: 'it is not appropriate for a code of practice to set mandatory requirements for housing which must be followed in all circumstances' (British Home Office 1986, para 1.13).

Nevertheless, what little data are available indicate that efforts are being made to meet these recommendations. We are aware of only two current surveys of rodent enrichment. A survey of US National Institutes of Health facilities ($n = 22$) reported that some 90% of rats and mice housed in these facilities receive nesting materials, slightly more than 50% are provided with a structural enrichment (usually a cardboard or plastic shelter), and about 40% and 20% of rats and mice, respectively, receive manipulanda (e.g. chew toys) (Hutchinson *et al.* 2005). The average reported percentage of singly-housed animals was 11% for mice, and 12% for rats (*ibid*). A recent survey assessing welfare of mice in 46 UK animal units (Leach MC,

personal communication, October 2005) found that substrate (e.g. sawdust) was provided by 87% of the units and nesting material (e.g. shredded paper) was provided by 80% of units, with all units surveyed providing either one or the other.

Enrichment items were provided for mice by 63% of units, of these all provided shelters and gnawing material, 21% of units provided other enrichment items such as egg boxes, metal rings on the cage top, wheels and hammocks, and 32% of units provided additional food scattered or placed onto cage substrate (e.g. grain). In addition, 21% of the mice were found to be housed singly in 78% of the units surveyed, of these the majority were male mice (37%). A complete set of results from this survey of UK animal units will be published in early 2006 (Leach & Main 2006).

This paper reviews published empirical evidence – including studies of the animals' preferences – to examine the degree to which laboratory housing conditions may or may not meet the behavioural and psychological needs of rodents in laboratories. Preferences may not denote underlying needs; however, where preferences are expressed for commodities integral to an animal's biology – such as places to hide or nest, and space to forage, disperse, or seek mates – denying those commodities can reasonably be assumed to be deleterious.

Methods

We used an online database (PubMed) to identify studies published in English since 1966 addressing the effects of standard laboratory housing conditions on the behavioural, mental or physical status of small rodents, especially mice and rats. The following root key terms were used: animal, behaviour, caging, deprivation, distress, environmental enrichment, housing, laboratory, mouse, pathology, psychology, rat, single housing, social isolation, standard housing, stress and stereotypy. Other papers were found by scanning the cited literature sections of retrieved papers.

We consulted the most recent caging standards and guidelines issued by relevant

governing bodies and associations for the US (NRC 1996), the UK (British Home Office 1986) and the European Community (CEC 2003). For actual housing conditions being used in laboratories, we extracted relevant data from the methods sections of published papers. Our use of the word 'standard' as applied to housing (e.g. standard housing or standard cage) denotes a commercially produced rodent cage without enrichments [except where indicated].

Rats

A. Social behaviour

It has long been observed that social isolation is deleterious for rats, and that so-called 'isolation stress' alters physiological and behavioural characteristics (Hatch *et al.* 1963). Studies using adrenal weights to estimate stress levels conclude that isolated rats are more stressed than group-housed rats (Brain & Benton 1979). Rats housed alone ($n=8$) were deemed more stressed than rats housed four per cage ($n=8$), as judged by significantly higher heart rates and arterial blood pressures recorded in the solitary rats (Sharp *et al.* 2002).

Rats show strong motivation for the company of others. Female Hooded Norway rats ($n=6$) lever-pressed an average 73 times for access to a standard cage containing three familiar rats, which was significantly higher than their demand for either a cage provisioned with novel objects and fixed furniture (average 42 lever presses) or a larger cage (average 40 lever presses) (Patterson-Kane *et al.* 2002). Gärtner (1968a,b) found that formerly group-housed rats actively sought the company of other rats rather than eat and sleep alone. Both male and female rats housed singly spent significantly more time performing escape-related behaviour than did rats housed in groups and this pattern persisted throughout the eight-week period of single housing (Hurst *et al.* 1999).

The presence of another rat appears to be reassuring in novel, potentially stressful situations. Solitary-housed rats in standard cages took nearly twice as long to enter a novel arena as did group-housed rats ($n=64$)

kept in standard cages (Zimmermann *et al.* 2001). Male Wistar rats ($n=12$) froze and defaecated significantly less when placed in an open field environment with another (familiar) rat than when placed alone (Hughes 1969). Anti-predator vigilance – itself a possible source of stress when there is nowhere to hide – may partially account for these differences.

There is evidence that thwarting attempts to escape aggressive cagemates is stressful for rats. When unrelated rats ($n=64$) were housed in single-sex groups of eight in an open room (147 × 210 cm) equipped with two propylene cage bases (one inverted as a platform), low status individuals, especially females, spent more time moving around and stretching up their room walls (Hurst *et al.* 1996). These females had very high corticosterone levels, which the authors attributed to the frustration of attempts to leave their enclosures. The authors do not mention having provided any appropriate enrichment, which might have ameliorated these stress-like patterns. In pair-housed male Long-Evans rats ($n=28$), the lighter animal used a PVC conduit (15 cm long × 7.5 cm diameter) more than the heavier animal in 13 of 14 pairs, during both day and night, suggesting that lighter animals might use the conduit as a way to avoid heavier, presumably dominant cagemates (Galef & Sorge 2000). Females ($n=28$) showed no such pattern.

Social housing affords rats opportunities to play. There is a steady growth in scientific interest in animals' subjective and emotional states (e.g. De Waal 1996, Panksepp 1998, Bekoff 2000), including those of rats. Particularly when young, rats are motivated to engage in social play (Knutson *et al.* 1998a), and there is evidence that the activity is pleasurable. When rats play with each other, their brains secrete large amounts of dopamine into the bloodstream, and they make 50 kHz vocalizations, which have been linked to positive affect in social and sexual contexts (Knutson *et al.* 1998a,b, Burgdorf & Panksepp 2001). Rates of 50 kHz vocalizations were significantly higher when

rats were placed in a chamber they had learned to associate with play than in a habituated control chamber (Knutson *et al.* 1998a,b). A series of experiments found that rats solicited tickles and strokes from trusted human companions; the experimenters (Panksepp & Burgdorf 2003) suggest that the 50 kHz calls made during these encounters are the evolutionary antecedents of primate laughter (Panksepp 2005).

Rats also soon learn to anticipate play. Rats placed alone in a Plexiglas chamber following a week of play sessions with a fellow rat became very active, vocalizing and pacing back and forth with apparent excitement, as if anticipating play (Siviy 1998). Pharmacological dopamine blockade in these habituated rats halted all anticipatory activity (*ibid.*).

While social housing of rats is highly desirable and strongly recommended in guidelines and regulations, it is important to recognize that not all social housing situations represent good welfare (e.g. Hurst *et al.* 1996). Though domesticated rats tend to coexist relatively peacefully (e.g. Schuster *et al.* 1993, Hurst *et al.* 1999), preference should be given for housing animals with prior familiarity or relatedness, and consideration given to the influences of density, sex and available resources to meet behavioural needs.

Using a conditioned place preference (CPP) study design, van den Berg *et al.* (1999) found that both juvenile ($n = 18$) and adult ($n = 18$) male Wistar rats showed a significant preference for a box containing a free moving rat compared with either an empty box or a box with a visible rat confined behind a Plexiglas barrier. Juvenile rats ($n = 6$) also became significantly more active when anticipating 30 min of social play with another free moving rat compared with a confined juvenile rat (van den Berg *et al.* 1999). The authors note that the animals' behaviour was in response to the motivational properties of rewards, such as social play and adult social contact (e.g. grooming and crawling over/under), and not aggressive or otherwise negative interactions.

B. Environmental complexity

Rats are sensitive to variations in environmental complexity. Impoverished living environments can lead to impaired brain development (e.g. Bennett *et al.* 1969, Renner & Rosenzweig 1987). Just four days of exposure to environmental complexity (paired or group-housing in cages with wires, shelves, stairs and other playthings) can produce significant changes in wet weight of cerebral cortical samples taken from laboratory-housed rats (Ferchmin *et al.* 1970). Thickness of the occipital cortex increased in female rats given obstacles to food access (Diamond 1988).

Even rats raised in some enriched cages do not show the cortex development of rats housed in a semi-natural environment. Groups of 12 rats living in larger (75 × 75 × 45 cm) cages provisioned with stimulus objects that were changed daily had significantly smaller regions of the cerebral cortex than did a group of 12 rats living for 30 days in a semi-natural environment (9 × 9 × 1 m outdoor enclosure with 30 cm of earth, weeds, stones, branches and pieces of wood, variable food provision and wire mesh lid) (Rosenzweig *et al.* 1978).

The neuroanatomical effects of spacious, more naturalistic living conditions predict a range of associated physiological and behavioural improvements, including cognition and memory (e.g. Paylor *et al.* 1992, Woodcock & Richardson 2000), visual-spatial learning (e.g. Faverjon *et al.* 2002), recovery from brain injury (e.g. Passineau *et al.* 2001), and resistance to stress-induced pathology (e.g. Rockman *et al.* 1986). Environmental stimulation also ameliorates or eliminates prenatal environmental deficits (e.g. Hannigan & Berman 2000) and the cognitive effects of aging (e.g. Kobayashi *et al.* 2002), and delays the onset of behavioural stereotypies (e.g. Callard *et al.* 2000).

Rats raised in more complex environments appear to show less fear of novelty than do rats in standard or more impoverished environments. When raised in a large (200 × 100 × 180 cm) split-level cage with bedding, cover, tubes, wood and

burrowing opportunities rats ($n = 24$) entered a novel arena significantly earlier than did rats ($n = 72$) raised in standard commercial (Makrolon, $33 \times 55 \times 19$ cm) cages with or without bedding, nest-boxes and tubes, and were significantly more active (exploring) during their first time in the arena (Zimmermann *et al.* 2001). Male Long-Evans rats ($n = 68$) reared in perceptually impoverished cages (complete darkness with constant white noise) explored a novel open field environment less than did rats ($n = 72$) reared in a perceptually more stimulating environment containing mazes, ramps, sand boxes, beach balls, mirrors, toys and flashing coloured lights (Gardner *et al.* 1975). Female Hooded Norway rats ($n = 35$) kept in a larger cage (two adjoined $20 \times 23 \times 45$ cm cages) with two large nest-boxes, branches, cardboard box, running wheel, several plastic containers, straw and tissue paper initially explored and habituated to a novel environment significantly sooner than did rats pair-housed in standard ($20 \times 23 \times 45$ cm) or solitary ($23 \times 23 \times 26$ cm) cages (Patterson-Kane *et al.* 1999). The non-enriched rats showed no decline in fear responses in successive trials, suggesting a failure to habituate and depressed learning and memory.

Rats anticipate access to more complex housing. Male Wistar rats ($n = 24$) engaged in significantly more arousal behaviours and explored and moved about significantly more in anticipation of being put in a more stimulating cage (higher, with extensions, a shelter, a tunnel with passages and holes with inserted wood pieces, and a bin filled with old bedding) or with a sexually-receptive female than did 24 males anticipating a forced swim or being moved into a standard commercial cage (Techniplast Makrolon IV: floor area 1875 cm²; height 18 cm) (van der Harst *et al.* 1999).

Nesting is widely acknowledged to be an important behaviour for female and male rats (Patterson-Kane *et al.* 2001). Twelve inbred Hooded Norway rats (6 males, 6 females) housed in standard cages in groups of four preferred a cage with nesting material to a cage without (Patterson-Kane *et al.* 2001). Five female Wistar rats housed in a

group always showed a significant preference for a cage containing a nest-box regardless of nest-box design (Patterson-Kane 2002a). Male Sprague Dawley rats (group size of 3) preferred to spend time in nest-boxes than in other parts of their cage, and they favoured opaque or semi-opaque designs (Manser *et al.* 1998). Preference studies have also shown rats to prefer solid flooring to grid flooring regardless of previous experience (Manser *et al.* 1995), and that they will work as hard to reach a solid floor to rest on as they will to explore a novel environment (Manser *et al.* 1996).

Because rats are highly inquisitive, any new element introduced to their cage is a source of interest. This may help explain why, on average, the rats ($n = 20$) spent four times longer in a 'high' complexity environment (highest density and diversity of chains hanging from cage roof) than in a medium or low complexity environment, engaging in significantly higher rates of ambulation and resting activity (Denny 1975). Male Wistar rats kept in a standard cage with ($n = 10$) and without ($n = 10$) a propylene cage insert strongly preferred the altered cage to the empty one in two-way choice tests lasting 8 h for each subject (Townsend 1997). The enriched rats spent more time exploring and resting in the altered cage than did their standard-housed counterparts (*ibid.*).

C. Mobility

Depending on the animal's weight, UK and US housing requirements and recommendations for rats provide between 0.010 and 0.080 m² floor area per animal, and minimum cage height of 18 cm (Table 1).

Current standards largely reflect current practice. Commercially available caging systems, in which probably most laboratory-housed rodents are kept, adhere fairly closely to regulatory (minimum) standards and guidelines. Floor area for five laboratory studies on rats published between 1996 and 2002 (randomly selected from papers cited elsewhere in this review, which include enriched housing conditions) provided between 0.022 and 0.105 m² floor area per

Table 1 Living space for rats: comparison of laboratory housing standards, laboratory practice, and home ranges reported from wild populations

Source	Floor area (m ²)	Cage height (cm)
Standards and recommendations*		
National Research Council 1996 (USA)	0.011-0.045	18
Home Office Code of Practice (UK)	0.010-0.080	18-20
Laboratory practice		
Chmiel and Noonan (1996)	41 × 25 cm=0.103	19
Galef and Sorge (2000)	33 × 30 cm=0.099	17
Hurst <i>et al.</i> (1996)	38 × 23 cm=0.087	15
Patterson-Kane (2002a)	48 × 38 cm=0.182	20
Zimmerman <i>et al.</i> (2001)	55 × 33 cm=0.182	19
Wild populations		
Jackson (1982)	3000-8000	NA
Stroud (1982)	2400	NA

*Per animal

animal, and cage height between 15 and 20 cm (Table 1).

Rats' natural history might predict that they will value space. In the wild, average home ranges for *R. norvegicus* have been measured from < 10 to 8000 m² (Jackson 1982). But there are few studies addressing the perceived value of space to rats. I was unable to find any studies specifically addressing this question in Norway rats, save the following preference studies.

Female and male rats ($n = 10$) tested in a T-maze preferred larger cages to smaller ones both in isolation and with other cagemates (Patterson-Kane 2002b). Individually-housed rats ($n = 8$) showed no statistically significant preference for either side of a pair of cages joined via a PVC pipe, one cage being slightly higher (23 cm) than the other (16.8 cm high) (Galef & Durlach 1993). That this study found no preference may be due to the fairly negligible difference between the two compartments, or that the rats might have perceived their PVC mediated enclosure not as a choice between two cages of different heights, but as a single living space with two compartments.

Mice

D. Social behaviour

Notwithstanding the need for social distancing between certain individuals, mice

are a highly social species and almost invariably seek the company of conspecifics (Jennings *et al.* 1998, Sherwin 2002). Behavioural symptoms of 'isolation stress' (also termed 'isolation syndrome') in mice include aggression, stereotypies, convulsions, nervousness and handling difficulty (see van Loo *et al.* 2001). Physiological symptoms include lower immunocompetence, higher tumour incidence, gastric ulcerations, hypersensitivity to toxins and increased pathology (e.g. 'scaly tail') (*ibid.*).

Despite concerns about aggression, mice have been shown to prefer dominant company to no company at all (van Loo 2001, van Loo *et al.* 2001). Adult male mice ($n = 60$) showed a significant preference for dwelling in a standard cage inhabited by another male mouse compared to dwelling in an equivalent but uninhabited cage or a barren central cage. Young mice (7-8 weeks old) showed no such preference, but did so as they became older (van Loo *et al.* 2004). Another cohort of subordinate male mice showed a significant preference for a cage inhabited by an unfamiliar male over a similar cage with no occupant (van Loo *et al.* 2001).

Mice also show preference for familiar over unfamiliar mice. Subordinate male mice ($n = 12$) showed a significant preference for their dominant cagemate, and vice versa, compared with an unfamiliar male (van Loo *et al.* 2001). The authors caution that the

mice used in this study had been successfully group-housed for a relatively long time before testing, and that no extreme injuries were observed during that time.

E. Environmental complexity

Increased environmental complexity for caged mice has been shown to enhance brain cell genesis (e.g. Ehninger & Kempermann 2003), slow disease progression (Hockly *et al.* 2002), increase neuronal metabolic activity (e.g. Turner *et al.* 2002), and improve behavioural expression (e.g. Marashi *et al.* 2003), as well as reduce the prevalence of behavioural stereotypies (e.g. Powell *et al.* 2000) and ameliorate learning and memory impairments (e.g. Need *et al.* 2003). Male mice ($n = 128$) housed in a standard-sized cage with bedding, nest-boxes and nesting materials, tubes, and opportunities to climb and gnaw ate more food, gained weight faster, and were heavier than cohorts housed in unenriched conditions (Van de Weerd *et al.* 2002). Mice ($n = 72$) provided with nesting material ate less but weighed more than cohorts without nesting material, a result which the authors attributed in part to better thermoregulation (Van de Weerd *et al.* 1997a).

Several studies indicate the mouse's liking for spatial complexity provided by platforms and vertical partitions, which provide opportunities for climbing, chewing and manipulation (Jennings *et al.* 1998). Male BALB/c mice ($n = 10$) to whose cages was added a polypropylene insert with two raised platforms and an in-built shelter explored significantly more and bar-gnawed significantly less than did mice ($n = 10$) pair-housed in standard polypropylene cages (Leach *et al.* 2000).

Play behaviour is a reliable indicator of good psychological welfare in mammals (Broom & Johnson 1993). There is good evidence that house mice, particularly juveniles, engage in both locomotor and object play (Wolff 1981, Walker & Byers 1991). There is also evidence that greater environmental complexity and space encourage play in laboratory-housed mice. Male CS mice ($n = 12$) housed in groups of four in spacious glass terraria

($100 \times 40 \times 34.5$ cm) containing ladders, platforms, a climbing tree and ropes, exhibited significantly higher rates of locomotor play behaviour (performing horizontal leaps and vertical hops) than did mice ($n = 12$) housed in standard cages ($37.5 \times 22 \times 5$ cm) with or without a plastic insert and wooden scaffolding (Marashi *et al.* 2003).

There is pharmacological evidence that mice housed in standard cages are more anxious or stressed than mice in more complex cages. Standard-housed female C57BL/6J mice ($n = 30$) drank significantly more water that contained an anxiolytic (midazolam, 0.08 mg/mL) than did mice ($n = 18$) housed in cages fitted with a PVC nest-box, a running wheel, two cardboard tubes and two sheets of absorbent paper for nesting material (Sherwin & Olsson 2004).

Even so-called 'enriched' cages may still represent an unnatural degree of monotony. While stereotyped wire-gnawing was significantly higher in adult male ICR mice ($n = 16$) housed in barren cages, the behaviour was nevertheless abundantly present in animals ($n = 16$) whose cages had been furnished with a toilet roll tube (Würbel *et al.* 1998a). At 34 days old, mice in both cohorts spent equivalent time wire gnawing, and by 80 days, gnawing time for the enriched group had nearly doubled (to 400 s per 4320 s observation period). Although the standard-housed mice in Sherwin and Olsson's (2004) study drank the most water containing an anxiolytic, mice in the other two treatment cages ('enriched' and unpredictable) still consumed more treated than untreated water, suggesting that all of the cage conditions induced anxiety and/or that they made the animals more prone to addictive behaviour.

By the same token, enrichments in some situations may not always improve welfare. For example, in a study of 66 male DBA/2J mice, inter-male aggression and plasma corticosterone levels increased when standard cages (3 mice per cage) were modified with a floor platform creating several corners and burrow-like passages beneath (Haemisch *et al.* 1994). The study authors believe that the animals' social

structure shifted from hierarchical to territorial in the modified condition, which in turn suggests that the mice found the sub-platform space both defensible and worth defending compared with a simple open box with nowhere to hide. Marashi *et al.* (2003) reported higher concentrations of corticosterone in enriched-housed ($n = 12$) than standard-housed ($n = 12$) male CS mice, but still conclude that 'an environmental enrichment is beneficial for male mice as long as the spatial conditions are generous enough to allow coping with the increased aggression brought about by the enrichment' (p. 281).

Preference studies show that mice in laboratories favour a variety of environmental features still commonly absent in laboratory housing conditions. A review of 40 studies published between 1987 and 2000 concluded that mice prefer more complex cages, and will work for nesting material, shelter, raised platforms, a running wheel and larger cages (Olsson & Dahlborn 2002). While merely adding structure to a standard cage had limited effects on behaviour, providing a considerably larger and more complex cage had significant effects, including increased activity or reduced signs of anxiety in open field trials, exploration tests and elevated plus maze trials, or a reduced latency to emerge in emergence tests (Olsson & Dahlborn 2002).

Preferences for hiding shelters and nesting materials have been repeatedly demonstrated in mice, of which 22 strains are known to build non-breeding nests (Sherwin 1997). Mice are strongly motivated to build such nests not only for breeding, but also for temperature and light regulation (Jennings *et al.* 1998).

All members of a sample of 39 female TO mice built nests using paper towel and/or cellulose fibre bedding when these were provided (Sherwin 1997). When sawdust was present (for 11 days), male TO mice ($n = 6$) preferred to use it to build nests to sleep in than to sleep in any of three available tubes. When sawdust was then removed (12 days), five of the six mice took to sleeping in the tubes, then reverted to sawdust when it was

once again made available (Sherwin 1996a). Sherwin (1996b) found that male TO mice ($n = 6$) defaecated non-randomly in their cages and preferred a substrate with sawdust to a bare plastic floor for this purpose; he concluded that conventional cage designs do not provide mice with a sufficient environment to allow selective hygienic behaviours. Mice also prefer solid to grid floors, and given a choice will use the non-favoured surface (mesh or grid) to defaecate and urinate (Jennings *et al.* 1998). Mice (both sexes, $n = 48$) of two strains (C57BL/6JlcoU and BALB/cAnCrRyCpbRivU) showed clear preferences for certain types of nesting materials over others, but always preferred nesting material to no material (Van de Weerd *et al.* 1997b).

Mice (both sexes, $n = 47$) of two strains (C57BL/6JlcoU and BALB/cAnCrRyCpbRivU) spent significantly more time (69%) in a cage with nesting material (two pieces of Kleenex tissues) than in a cage with an empty perforated metal nest-box (25%) (Van de Weerd *et al.* 1998). In a second experiment, 24 female mice (housed in groups of 6) spent more than 67% of their time in a cage with nesting material, despite the floor being gridded (Van de Weerd *et al.* 1998). When mice (BALB/cAnNCrIBR, $n = 60$) were given a choice between an inhabited standard cage and a similar uninhabited cage with nesting material, all age groups showed a strong preference for the latter option (van Loo *et al.* 2004).

In standard cages, mice often make their nest in the shadow of the food hopper and/or drinking bottle (Baumans *et al.* 1987, Sherwin 1996b), apparently making the best of a poor situation. Given a choice of four commercial cages, female Zo:WK mice ($n = 17$) showed a significant overall preference (77%) for the one cage (Cambridge) which, unlike the others, was opaque and included a shelter (Baumans *et al.* 1987).

F. Mobility

The small size of typical laboratory cages precludes practically any opportunities to exercise or explore. Depending on the

Table 2 Living space for mice: comparison of laboratory housing standards, laboratory practice, and home ranges reported from wild populations

Source	Floor area (m ²)	Cage height (cm)
Standards and recommendations*		
National Research Council 1996 (USA)	0.004-0.010	13
Home Office Code of Practice (UK)	0.006-0.020	12
European Community Directive 86/609†	0.006-0.010	NA
Laboratory practice		
Nevison <i>et al.</i> (1999)	45 × 13 cm=0.059	14
Leach <i>et al.</i> (2000)	45 × 13 cm=0.059	11.4
Sherwin and Nicol (1997)	27 × 10 cm=0.027	12
van de Weerd <i>et al.</i> (1997)	25 × 15 cm=0.038	18
Würbel <i>et al.</i> (1998a)	22 × 16 cm=0.035	14
Wild populations		
Lidicker (1966)	139	NA
Quadagno (1968)	365	NA
Chambers <i>et al.</i> (2000)	2-80000	NA

*Per animal
†Jennings *et al.* (1998)

animal's weight, UK, US and EU housing requirements and recommendations for mice provide between 0.004 and 0.020 m² floor area per animal, and minimum cage height of 12 cm (Table 2).

As with rats, these standards mostly reflect commercially available caging systems, and current practice. Floor area reported in five randomly selected laboratory studies on mice published between 1997 and 2002, including 'enriched' housing conditions, provided between 0.005 and 0.075 m² per animal, and cage height between 12 and 18 cm (Table 2).

Mice appear highly motivated to enter additional space when it is provided, and this seems most likely attributable to a desire to patrol and explore (Sherwin & Nicol 1996, Sherwin 1996c). T/O mice (*n* = 18) worked for access to a range of 'resources' (food, other mouse, shelter and space) including ones they did not find valuable (because they did not remain there once they got there) (Sherwin & Nicol 1996). Mice (*n* = 18) were willing to pay a cost to access resources (food, extra space and shelter), then spent only brief periods with them (Sherwin 1996c). Female mice (*n* = 17) placed individually in a barren central cage adjoining four different cages with resources first explored the central cage for usually 5-15 min, before visiting the test cages,

which they did in quick succession (Baumans *et al.* 1987).

Economic demand and preference studies also indicate that mice value space. Trained female CB57 mice (*n* = 24) housed in groups of three in standard laboratory cages continued to work by pressing a lever over a six-day period to gain access to additional space, even though it lacked food, water and other mice (Sherwin 2004). Male mice (*n* = 7) presented with cages of various sizes showed a statistically significant preference for more floor space by making more visits to larger cages, spending more time in them, and performing more lever switch actions to gain access to them (Sherwin & Nicol 1997).

Behavioural stereotypies

G.1. Stereotypies in *Mus musculus*

The link between abnormal or impoverished housing conditions and the development of behavioural stereotypies is well established. Stereotypic behaviours are repetitive, unvarying and apparently functionless behaviour patterns commonly seen in animals kept in close confinement. They are believed to reflect animal suffering (Mason 1991a) and are common in some rodents caged for research, including mice, chinchillas, black rats, deer mice, field voles,

bank voles and gerbils (Garner & Mason 2002). Stereotypies are highly variable in origin and expression among different species, strains and individuals (Garner & Mason 2002, Mason & Latham 2004). They can be extremely prevalent in certain species or strains; about 98% of laboratory-caged male ICR mice, for instance, are prone to stereotypic behaviours (Garner & Mason 2002), and stereotypies are estimated to afflict some 50% of all laboratory-housed mice (Mason & Latham 2004).

Several behavioural stereotypies have been described in laboratory-caged *M. musculus*, including bar gnawing, bar circling and bar jumping (Nevison *et al.* 1999). Wire-gnawing in male ICR mice ($n = 64$) was observed to be extremely fast, repetitive and invariant within individuals (Würbel & Stauffacher 1996). Each individual performed it in one or two preferred spots, but the frequency of stereotypies varied considerably across individuals. Stereotypies may result in self-injury (Ödberg 1986), and may disrupt maternal behaviour resulting in impaired growth and increased offspring mortality (see Garner & Mason 2002).

Stereotypical wire-gnawing in ICR mice develops from single bites at the cage lid during exploratory climbing (Würbel *et al.* 1996), while stereotypical jumping originates from exploratory rearing at the cage wall (*ibid.*). These findings suggest that mice experience at least some degree of suffering due to thwarting of exploration imposed by confinement.

There is evidence that wire-gnawing in mice derives, in part, from attempts to escape confinement. Juvenile, sub-adult and adult male mice ($n = 32$) directed more stereotyped gnawing towards cage bars that were occasionally removed for husbandry purposes than towards fixed bars (independent of their location on the cage top or side) (Nevison *et al.* 1999). Mice also chewed significantly more at bars through which airborne odour cues could pass from the outside (i.e. not covered with Perspex), further suggesting that chewing reflects a desire to pass through the barrier (Nevison *et al.* 1999). The authors surmise that even though bar chewing in their study never

resulted directly in escape, these mice may still have associated their bar chewing with occasional 'success' given that bars are removed for husbandry (*ibid.*).

It has been suggested that cage gnawing and jumping stereotypies may aid in coping with the stress of confinement (Cooper & Nicol 1991). Several studies have determined, however, that behavioural stereotypies fail to reduce stress indicators in mice. Corticosterone levels did not decline when male mice ($n = 32$) chewed their cage bars (Nevison *et al.* 1999). Outbred male ICR mice ($n = 20$) prevented from gnawing for 10 days had chronic stress levels (measured as serum corticosterone, tyrosinehydroxylase activity and phenylethanolamine *n*-methyltransferase activity) equivalent to those of controls ($n = 20$) who were able to continue gnawing (Würbel & Stauffacher 1996). This study therefore found no evidence that stereotypic wire-gnawing reduces chronic stress.

If wire-gnawing relieved stress, we might also expect a 'rebound' effect following frustration of stereotyped behaviours. Würbel *et al.* (1998b) blocked stereotypic wire-gnawing in 35 outbred male ICR mice by replacing their cage lids with closely spaced bars. When this blocking was later removed, mice did not rebound by gnawing more than before, nor was duration of blocking a factor in post-inhibitory behaviour. These findings further suggest that wire-gnawing is not a coping behaviour in ICR mice (Würbel *et al.* 1998b).

Wire-gnawing in recently weaned mice may reflect, in part, attempts to escape and return to the mother (Würbel & Stauffacher 1997). If so, then lower weaning age and weight might correlate with higher stereotypy rates due to increased motivation to nurse. When 32 outbred male ICR mice were weaned at the standard 20 days post-partum, mice lighter than the median weight at weaning had significantly higher wire-gnawing stereotypy levels than did heavier mice (Würbel & Stauffacher 1997). A related study found that both premature weaning (17 days instead of 20) and low weaning weight resulted in significantly higher adult wire-gnawing stereotypies

(measured at 80 days) (Würbel & Stauffacher 1998).

G.2. Stereotypies in other rodents

R. norvegicus is generally not known to exhibit stereotypies in laboratory conditions, unless they are drug-induced. In this section I outline the occurrence of stereotypies in rodent species not discussed above, with particular reference to housing conditions. Stereotypies described for bank voles (*Clethrionomys glareolus*) include repetitive bar-mouthing (Garner & Mason 2002), jumping, looping (somersaulting from the cage top) and weaving (pacing to and fro over the same point, with frequent rears when turning) (Cooper & Nicol 1991). Both form and location of the bar-mouthing stereotypy were idiosyncratic to individuals ($n = 8$), for whom the proportion of active time spent bar-mouthing ranged from 3.5% to 28.1% (Garner & Mason 2002).

Stereotypy in bank voles has been linked to deficits in brain structure and function. Bar-mouthing stereotypy in singly-reared, standard-housed bank voles ($n = 8$) correlated positively with latency to extinction of a maze task; stereotypic animals persisted in responding rapidly to a previously rewarded maze arm. The least stereotypic animal extinguished in 26 maze trials, and the most stereotypic took 244 (Garner & Mason 2002). These findings are consistent with prior damage to the central nervous system (Garner & Mason 2002). The authors suggest that stereotypic animals, like human clinical patients, might feel the frustration of being unable to turn decisions and preferences into actions (*ibid*).

Stereotypies derive from chronic environmental deprivation (Mason 1991a,b). In a study of both lab-reared and wild-caught bank voles and their offspring ($n = 47$), all housed singly in standard cages from the start of the experiment, stereotypies (rearing, weaving, jumping, looping, pacing and rearing, figure of eight) developed in all but the wild-caught adult cohort. At 10 days post-weaning, adult lab-reared voles ($n = 12$) spent significantly more time stereotyping than did pups ($n = 23$), and after 60 days,

three of nine (33%) wild and seven of 14 (50%) lab-reared pups had developed locomotor stereotypies (Cooper & Nicol 1996). Ödberg (1987) found that most (though not all) bank voles performed less stereotypy when raised in or moved from a small cage to a bigger and more complex enclosure.

Powell *et al.* (1999) described three behavioural stereotypies in deer mice (*Peromyscus maniculatus*): repetitive jumping, patterned running and backward somersaulting. Sixteen deer mice housed two or three per standard laboratory mouse cage developed stereotypy at a significantly faster rate than did 15 deer mice housed in larger cages fitted with a running wheel, habit trails, nesting and hiding enclosures, nesting material, and sunflower seeds (Powell *et al.* 1999). More than 50% of the mice in standard cages exhibited stereotypy by week 8 of the study, and 62.5% by week 16. Stereotypies developed in seven of the 15 more complex-housed animals, with patterned running exceeding that of the standard-caged animals (Powell *et al.* 1999).

Deer mice ($n = 70$) housed singly in standard (29 × 18 × 13 cm) cages exhibited significantly higher levels of stereotypy (repetitive jumping, backward somersaulting and patterned running) than did deer mice ($n = 64$) housed in large (609 × 480 × 100 cm), cages furnished with cloth dividers, bedding, nesting squares, mesh cylinders and PVC pipe fittings (Powell *et al.* 2000).

The opportunity to dig in sand did not prevent development of stereotypic digging behaviour, whereas provision of a burrow without digging substrate did prevent stereotypic digging in Mongolian gerbils (*Meriones unguiculatus*) (Wiedenmayer 1997). Thus, stereotypic digging in gerbils seems to arise not from a need to perform digging, but from goal-directed behaviour to attain a burrow. This finding supports a cognitive element of the motivations that underlie some behavioural stereotypies (Dawkins 1988).

Male roof rats (*Rattus rattus*; $n = 22$), pair-housed since weaning in standard wire-mesh cages (25 × 76 × 20 cm), performed an

average of 3477 backflips during a 24 h period at approximately 30 days of age, more than double the rate of 28 subjects (average 1511 backflips) housed in the same sized cage provisioned with a wooden nest-box (13 × 25 × 20 cm) (Callard *et al.* 2000). This finding suggests that providing a nest-box improves but does not eliminate welfare concerns in this housing situation. In the same study, none of 16 pair-housed rats exhibited a backflipping stereotypy when placed in an otherwise standard wire-mesh cage whose height had been increased from 20 to 91 cm (Callard *et al.* 2000). However, by 60 days of age, all of 10 males kept in this enlarged cage were performing a repetitive circling behaviour near the cage top. When these 10 males were returned to the standard cage, all were backflipping within two days. In these experiments cage design modifications are shown to reduce, but not eliminate the performance of repetitive behaviours widely thought to denote compromised welfare in roof rats. The authors entertain the possibility that increasing cage height made performing backflips more difficult, but add that 'the similar circling behaviour (albeit horizontally-oriented) suggests that the factors motivating repetitive locomotor behaviours had not been suppressed' (*ibid.*, p. 150).

Discussion

Deprived environments

The data reviewed here permit some general conclusions about the psychological response of rats and mice to laboratory conditions. For rats, physiological and behavioural studies indicate that social isolation is detrimental to both males and females, and that the company of others can be enriching and beneficial. Rats also value and benefit from a number of resources commonly absent in laboratory housing. Few studies have addressed the possible importance of space to rats, though limited evidence suggests they value it, too.

Like rats, mice prefer company to isolation, which has been repeatedly shown

to be both physiologically and behaviourally harmful. And while aggressive behaviour is a concern in especially male mice of some strains, studies reviewed here suggest that these problems might be resolved by creative husbandry improvements, rather than by isolating the offending males. In addition to consideration of prior familiarity or relatedness, and the influences of density, sex, age, strain, and available resources to meet behavioral needs, husbandry measures can also be implemented, such as transferring nesting material to the clean cage following cage cleaning, which may significantly reduce agonistic encounters (van Loo *et al.* 2000). Providing more space than that of typical housing would further ameliorate such concerns, because small cages thwart opportunities for antagonists to avoid one another. In practically all studies reporting aggression in captive male mice, animals were housed in small, commercial cages.

Studies also demonstrate the desirability of various other resources to caged mice. In the case of space, a dearth of data leaves open the possibility that mice treat it only as something to explore and patrol. However, given that exploration is a natural response to an absence of needed resources (e.g. escape routes, shelter, nesting material, desirable food, gnawing substrates and mates), it may be that limited space does compromise welfare in conventional laboratory housing situations.

Studies repeatedly show that a shelter and nesting material (as distinguished from a sawdust 'bedding') are virtually indispensable resources for rats and mice. Reproductive female house mice are so highly motivated to nest-build that a ball of cotton wool makes an effective trap bait (Randall 1999).

Yet, while awareness of rodent behavioural needs is improving, the provision of basic resources is still wanting in many laboratory rodent housing systems. Systematic surveys of housing provisions for rodents in laboratories have been few, but recent efforts in the UK and parts of the US suggest that significant numbers of rats and mice are still being housed without nesting

materials and/or shelters in their cages, and that at least one in 10 animals is housed alone.

To the extent that deprivations persist, laboratory conditions are compromised and may cause impairments in neural and behavioural development in rats, and behavioural stereotypies in mice and other rodents. Available evidence suggests that specific causation of stereotypies varies among different species, but that it arises generally from the frustration of natural behaviours that the animals are highly motivated to perform, such as burrowing, foraging, hiding, nesting, escaping, exploring and gnawing (Dawkins 1988, Wiedenmayer 1997, Nevison *et al.* 1999). Stereotypies are virtually unknown in free-living wild animals, which indicates that laboratory conditions are an underlying cause of these abnormal behaviours (Sherwin 2002). It is widely agreed that stereotypy in human psychiatric patients is highly stressful for the sufferer (Russell 2002). Mason (1991a) lists four bases for the belief that animal stereotypies also indicate suffering: (1) the contexts in which they develop, (2) behaviour patterns from which they arise, (3) factors influencing their development and subsequent performance, and (4) the fact that some stereotypies involve self-damage. These characteristics, while not providing incontrovertible evidence that stereotypic behaviour denotes a suffering animal, are nonetheless highly suggestive.

Lack of control

Several authors have suggested that lack of control over their environments may be an important factor in the compromised welfare and abnormal behaviour of animals kept in standard laboratory conditions (Wiepkema & Koolhaas 1993, Dawkins 1998, Olsson & Dahlborn 2002, Van de Weerd *et al.* 2002). In the wild, rats and mice must regularly make choices and decisions, such as finding food or mates, building nests and avoiding predators. It follows that they exercise considerable control over their lives. The laboratory environment provides scant opportunities to make natural decisions or

choices (Mench 1994). Movement is restricted by close confinement, feeding regimens preclude opportunities to forage and manipulate food (activities that make up a significant portion of these species' existence), and social circumstances either preclude contact with others (solitary housing) or prevent animals from effectively ousting or avoiding incompatible cagemates. Lack of shelter further prevents animals from escaping bright lights or perceived threats. Rats have been found to be less fearful when allowed to control their own light, food and water supply (Joffe *et al.* 1973), and it is well established that lack of behavioural control paired with aversive stimuli can produce pathological levels of stress in animals (Selye 1974).

It is increasingly recognized among those in the zoological parks community – where enrichment issues parallel those in laboratory animal research – that the variables of complexity and variety are key to successful enrichment programmes (Shepherdson 2001, ZooCheck Canada n.d.), and that giving animals optimal opportunity to 'earn' a living by taking control of their lives benefits welfare (Martin 1999). Varied environments allow animals to learn how their own actions affect their environment, which fosters behavioural competence and enhances the animal's ability to cope with the challenges of captivity (Shepherdson 2001).

Naturalistic enrichment

The implementation of enrichment strategies for rats and mice is a welcome change which should be strongly encouraged. However, 'enrichment', as currently practised, is not a complete solution to animal welfare problems (Olsson & Dahlborn 2002). Many environmental enrichment studies report that a substantial proportion of animals nevertheless develop behavioural stereotypies in the 'enriched' condition (e.g. Powell *et al.* 1999, 2000, Würbel *et al.* 1998b, Leach *et al.* 2000, Callard *et al.* 2000). Zimmermann *et al.* (2001) concluded

that simple forms of enrichment do not adjust for a lack of environmental stimulation.

A more humane approach aims to be in tune with the animal's natural history in the wild or feral condition. Naturalistic environments introduce meaningful biological complexity, fulfil animals' ethological needs, and help to foster normal behavioural and brain development (Würbel 2002). Würbel (2002) has proposed that populations of mice be bred and maintained in species-typical societies in naturalistic environments. Such a 'field-study' approach is being used by a Swiss team of investigators working in Russia, where mice lived in large open-roofed squared outdoor pens of about 400 m² with two shelters (2 × 2 × 1 m) filled with hay, and several wooden boxes (Dell'Omo *et al.* 2000). Food (undescribed) and water are supplied *ad libitum* and an electric fence bars entry from terrestrial predators (*ibid*). This housing method promptly detected deficits in transgenic mice that had not been recognized in conventional laboratories (Vyssotski *et al.* 2002).

Scientific rigour

Because impoverished conditions constrain behaviour and retard brain development in rodents, resulting in altered brain function (Würbel 2001, 2002, Turner *et al.* 2002), the potential exists that scientific rigour may be compromised in experiments performed with these animals. For example, animals with behavioural stereotypies have been characterized as poor models of normal physiological and behavioural functioning, for which they often provide highly variable and unreliable backgrounds for genetic or pharmacological manipulations (Garner & Mason 2002). The study of Crabbe *et al.* (1999), which found significant variability in results despite painstaking attempts to standardize protocols across three laboratories, 'clearly revealed the practical impossibility of standardization to guarantee reproducibility of results' (Würbel 2002, p. 4). A retrospective analysis of a large data archive (on thermal nociception in mice)

found that the experimenter performing the test was a more important source of variability than was mouse genotype (Chesler *et al.* 2002). When rats from the same breeding colony within the same room of the same laboratory were tested on the same equipment (elevated plus maze), results varied significantly according to the animals' familiarity with the human handler. Familiar handlers generated more consistent test results than did unfamiliar handlers (Van Driel & Talling 2005).

Other studies tend to corroborate these results. Housing complexity varied inversely with behavioural phenotypic variability in the rats studied by Zimmermann *et al.* (2001). In a study of 128 male mice, housing in larger cages provided with materials for nesting, hiding, climbing and gnawing did not increase variability in any parameters measured, a relationship that appears to have held true for prior studies (Van de Weerd *et al.* 1994, 1997a) whose data were re-evaluated (Van de Weerd *et al.* 2002). A study of female mice ($n = 432$) from two inbred strains (C57BL/6J and DBA/2) and their F₁ hybrids found that environmental enrichment (a Techniplast 'Mouse House' plus twice-weekly addition of a permanent hard and a temporary soft enrichment object) did not increase variability of results across three laboratories, countering concerns that enrichment undermines standardization (Wolfer *et al.* 2004). This is not to say that standard-housed rodents are incapable of generating reproducible results, or that enrichments do not influence scientific outcomes (e.g. Tsai & Hackbarch 1999, Mering *et al.* 2001). A current review (Bayne 2005) of enrichment effects on the physical, neurological and physiological health of rodents indicates that they appear almost always to be salubrious. Thus, such effects may be viewed as desirable to the extent that enriched subjects are healthier and more normal.

Conclusions

There is growing recognition of the inherent problems of depriving rodents the space and

resources to carry out natural behaviours, such as exploring, foraging, running, escaping hiding and hygiene maintenance. A recent survey of animal facilities at the US National Institutes of Health indicates that a slight majority of rats and mice at these facilities are now being provided with nesting and structural (shelter) enrichment (Hutchinson *et al.* 2005). Other indicators that rodent housing conditions are improving include the availability of commercially produced resources for nesting, shelter, gnawing and play (Key 2004), and a sharp rise since the late 1980s in the number of citations using keywords 'environmental enrichment' and 'rodent' (Hutchinson *et al.* 2005). Considering that two decades ago environmental rodent enrichment was scarcely being discussed, these are laudable trends. But practically all laboratory-housed rodents continue to live in small 'shoe-box' cages, many of which afford little or no opportunity to explore, hide, forage or exercise control over their social milieu. Implementing enrichment strategies involves practical and cost-associated challenges to institutions whose rodent systems are already in place. As several papers reviewed here illustrate, even in so-called 'enriched' cages, detrimental effects imposed by laboratory housing systems persist. Nonetheless, both scientific and ethical arguments support an approach more in tune with these species' living environments in the wild.

The evidence reviewed here supports the conclusion that the welfare of laboratory-caged rodents is compromised to the extent that they are confined, isolated, prevented from performing highly motivated behaviours, and allowed to develop stereotypies. Adequate knowledge exists to warrant significant improvements in the housing and enrichment of rodents used in research and testing. Furthermore, if it is argued that even with such improvements the lack of a naturalistic environment causes a considerable amount of animal suffering and discomfort, then this is a significant part of the ethical argument for reducing and ultimately eliminating animal experimentation. To the extent animals are used in

laboratory research, the broad incorporation of such improvements is warranted.

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