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 (Wolfle 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

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(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 facilites 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 tows) (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 cm^2; 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 m^2 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 m^2 floor area per