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The influences of standard laboratory cages on rodents and the validity of research data

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Abstract

Standard cages for laboratory animals are often small, minimalist and barren. Such cages can compromise animal welfare, indicating that there are welfare-based reasons for improving their designs. However, a second issue, that is, whether animals from standard laboratory housing and husbandry conditions provide valid research data, also indicates that cage designs and husbandry methods need to be improved. This paper reviews various influences of standard laboratory cage design and husbandry. These include their effects on the repeatability of studies, models of neuro-degenerative disease, sensory development, physiology, and behaviour, the effects of standard social housing and standard handling, and the effects of maternal experience on the responses of offspring. These studies show that the development and responses of animals from standard laboratory housing and husbandry conditions are often unrepresentative and idiosyncratic, indicating that data are likely to have reduced external validity. An underlying question is whether animals from standard, barren laboratory cages are 'abnormal' and therefore might not provide valid baseline data. In terms of animal welfare, these studies indicate that standard laboratory housing may sometimes be associated both with reduced welfare and with reduced benefits gained from research. It is suggested that in a similar manner to the use of production measures when assessing cages for production animals, laboratory cages could be assessed in terms of their suitability to provide valid research data.

Keywords: *animal welfare, behaviour, housing, laboratory cages, research validity, rodents*

Introduction

Standard cages for laboratory, farm and companion animals are often small, minimalist and barren. Many have argued that such cages can compromise the welfare of animals (eg Kessler & Turner 1999; Mason *et al* 2001; Olsson & Dahlborn 2002; Sherwin 2002; Olsson *et al* 2003), indicating that there are welfare-based reasons for improving their design. However, an issue that is less frequently considered is that animals are housed in cages for a purpose: for companion animals the purpose is human entertainment or companionship, for farm animals the purpose is food or fibre, and for laboratory animals the purpose is scientific research. If a cage design is to be acceptable overall, it should not only avoid compromising welfare, but also achieve its intended purpose. For example, an assessment of the acceptability of cage systems for laying hens almost invariably includes measures of egg production. But, this type of purpose-oriented assessment is rarely, if ever, performed for laboratory cages. The question should be asked whether standard laboratory cages and husbandry yield valid research data. Aspects of this issue have been addressed previously (Poole 1997; Würbel 2001, 2002; Sherwin 2002). The present paper expands on these arguments using recent publications, and gives examples in which authors make uncompromising statements regarding the unsuitability of standard laboratory cages and husbandry for particular types of research. This indicates that there are science-based reasons for improving the design of laboratory cages, in addition to the welfare-based reasons. Emphasis is given to studies on laboratory rodents, since these are the most frequently and widely used animals in research.

What are valid data?

If research data are to be valid, they must meet several conditions, three of which are relevant to the present discussion. Condition 1: the data should represent the responses of animals that are physiologically and behaviourally 'normal', unless the aim of the study is to investigate abnormality. Condition 2: the investigation should be repeatable. It is a fundamental principle of scientific research that a study can be replicated in another laboratory and the findings are consistent. If an investigation is not repeatable, the data are idiosyncratic, external validity is limited, and the probability values ascribed to the significance of differences will have reduced meaning. Condition 3: data should not be susceptible to unidentifiable extraneous variables. These are likely to be peculiar to a laboratory, and therefore would again result in idiosyncratic data and reduced validity.

This paper reviews how standard, barren laboratory cages and standard husbandry methods can influence the biology of animals such that one or more of these three conditions are violated. It is usually assumed that animals from standard housing and husbandry conditions are 'normal'.

However, if they are not, two issues are raised. First, if the responses of the animals in tests outside the home-cage are unrepresentative because of, for example, retarded sensory development, the data will lack external validity. Second, if data from animals housed in standard conditions are used as a control for comparison with a treatment group, the detrimental effects of standard housing could mean that these animals should be considered as a treatment group, rather than as a control.

Effects on repeatability of research

It is a fundamental principle of valid research that if studies are repeated, their findings should be consistent. This is not always verified. In 1999, Crabbe *et al* (see also Wahlsten 2001; Crabbe & Wahlsten 2003; Wahlsten *et al* 2003) assessed the repeatability of a battery of six phenotypic characteristics (open-field behaviour, elevated plus maze test, water maze test, alcohol preference, locomotion after cocaine injection, and body weight). The battery was conducted on eight transgenic mouse genotypes and on wild-type mice in three laboratories where the apparatus, test protocols and many environmental variables were rigorously equated. Unexpectedly, there were strain differences between laboratories for all of the phenotypic characteristics other than the water maze test and alcohol preference. Furthermore, the pattern of strain differences varied substantially among the laboratories for several characteristics, that is, there were significant interactions between genotype and laboratory. For example, mice tested in the elevated plus maze test in the Edmonton laboratory were less anxious than those tested in the Portland laboratory. Results such as this are perhaps particularly concerning because the elevated plus maze is a widely used test in the development of human psychoactive drugs, for example, anxiolytics (anxiety-reducing drugs). Crabbe *et al* (1999) were unable to give a convincing explanation to account for all of the variation they observed, but concluded that "experiments characterising mutants may yield results that are idiosyncratic to a particular laboratory." Whilst this study did not distinguish between the factors that might have caused the lack of repeatability, it indicated that housing animals in standard, barren housing with standardised husbandry does not necessarily result in an animal model that gives highly repeatable results. Animals from standard, barren cages often exhibit heightened fearfulness, emotionality or reactivity (see 'Effects on behaviour' section), thereby predisposing animals to respond more vigorously to changes in the environment (eg on a behavioural test). Possibly, this increased reactivity along with individual differences in responsiveness increase variability in data, thereby reducing the repeatability of some studies.

Effects on models of neuro-degenerative disease

Standard, barren cages do not always produce the quality of data we might expect in some disease models, for example, those characterised by motor dysfunction. This issue was addressed by Hockly *et al* (2002) (see also van Dellen *et al* 2000; Martinez-Cue *et al* 2002), who reared transgenic and wild-type mice of a Huntington's disease model either in standard housing, minimally enriched housing (standard cage containing a cardboard tube and food on the floor), or highly enriched housing (a larger cage containing nesting material, a running wheel, ladder, wicker ball and a variety of other objects). In the transgenic mice, the disease progressed rapidly, but the decline in performance on a rotating rod test and the loss of peristriatal cerebral volume in the brain were considerably quicker in mice housed in standard cages than in minimally or highly enriched cages. In the wild-type mice, high enrichment considerably enhanced performance on the rotating rod test, although these animals showed no improvement of grip strength compared to less enriched controls, indicating that the enhanced rotating rod performance was not due to increased muscular strength. The authors suggested that enrichment altered gene expression in the normal mouse brain, which modulated the course of the disease. Hockly *et al* (2002) concluded from their study that "environmentally enriched mice may actually mimic human disease more accurately" and that "Housing mice in nonenriched [standard] conditions, especially in single housing units, can cause a marked worsening in disease phenotype or neurological disorders. Mice housed in such deprived conditions are unlikely to prove a good model of human disease, and the effects of 'enrichment' represent partial reversal of the deleterious effect of relative environmental impoverishment."

Effects on sensory development

It is usually assumed that the sensory development of animals in standard, barren laboratory cages is normal, and therefore that any responses dependent on these senses will also be normal. However, Prusky *et al* (2000) indicated that this might not always be true — at least for vision in mice. Mice were raised from birth either in enriched cages (large, clear cages containing stimulating objects and salient visual cues on the walls) or in standard cages (smaller opaque, white cages with no stimulating objects), and their spatial learning and visual acuity were measured as adults. Rearing environment had no significant effect on spatial learning; however, the visual acuity of the mice from the enriched environment was 18% greater than that of mice from the standard, barren conditions. The authors discussed these effects in terms of the plasticity of brain development, and suggested that "rearing animals in small opaque or clear cages does not provide animals with sufficient exposure to the high spatial frequency information necessary for the visual system to develop maximal acuity. Consequently, caution should be exercised when comparing the visually-mediated behavior of animals reared under different conditions." There are many studies in which normal vision is critical (eg elevated maze tests [Cook *et al* 2001]), but for these tests researchers almost exclusively use animals reared under standard, barren conditions. The study by Prusky *et al* (2000) indicates that many of these animals might have inferior vision. This study also raises the ethical question of whether animals raised under standard laboratory conditions are actually reared in sensory deprivation.

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Effects on physiology

Several studies have shown that characteristics of standard, barren cages can influence physiology, indicating that the responses of animals from such cages may not always be 'normal'. Steyermark and Mueller (2002) examined the influence of cage size on the metabolism of two species of mice. For the larger species (*Peryomyscus californicus*), the daily energy expenditure and food intake of animals housed in large cages were greater than those of animals housed in smaller, standard cages. In contrast, in a smaller species (*Peromyscus eremicus*), energy expenditure and food intake were unaffected by cage size. The authors commented that "if body size and cage size interact to affect behaviour and physiology, physiologic values reported as 'normal' or 'absolute' may instead be relative measures." Rather disconcertingly, they also noted that cage size was not reported in 43% of a selection of peer-reviewed papers! Kuhnen (1999) housed golden hamsters (*Mesocricetus auratus*) in standard cages of four different sizes and in enriched cages of three different sizes. Both increased cage size and the provision of enrichments decreased baseline rectal temperature with effects of similar magnitude. They also increased the fever response to the injection of lipopolysaccharide, although in this case, the effects of increasing cage size were greater than were those of providing enrichment. The authors argued that housing animals in small cages induced chronic stress, which influenced thermoregulation, and stated that "The findings demonstrate that the results of some physiological experiments are significantly influenced by the pre-experimental housing conditions." Because laboratory animals are generally housed in small cages, many studies might be prone to this type of influence.

Krohn *et al* (2003) used telemetry to measure the heart rate, blood pressure and body temperature of rats housed in cages with grid floors, plastic floors or with bedding. Their study revealed significant differences in systolic and diastolic blood pressure, heart rate and body temperature, which persisted for the two weeks of observations. It was argued that these differences indicated that grid floors and plastic floors (either can be standard in some studies) were more stressful for the animals than bedding.

Effects on behaviour

Standard laboratory cages and husbandry can influence both home-cage behaviour and the responses of animals in external behavioural tests.

Home-cage behaviour

Sherwin and Olsson (2004) reported that mice housed in standard, barren home-cages self-administered greater quantities of anxiolytic than mice housed in enriched cages, indicating that underlying anxiety might be greater in animals housed in standard conditions. Van Loo *et al* (2002) reported that the absence of nesting material (standard in many laboratories) increased aggression amongst mice, and both cage shape and cage size can influence the mating behaviour of male rats (Saito *et al* 1996). Steyermark and Mueller (2002) reported that cage size had species-specific effects on feeding behaviour and on the metabolism of mice, and Poon *et al* (1997) found that when kept in smaller (standard) cages, bouts of spontaneous nocturnal locomotion in mice were less intense and distinct and that the first bout of the period disappeared. Standard cages either are white or transparent; however, Sherwin and Glenn (in press) reported that home-cage colour influenced food intake and the body weight of mice.

Behavioural tests

Standard cage conditions can influence a range of responses outside the home-cage. In tests of emotionality (eg elevated plus maze, open-field, shuttle box), rodents from standard, barren cages often behave in a manner indicative of being more anxious or fearful than animals from enriched or larger cages (Syme & Hughes 1972; Chamove 1989; Prior & Sachser 1995; Roy *et al* 2001; Chapillon *et al* 2002; Kohl *et al* 2002; Larsson *et al* 2002; Schrijver *et al* 2002). This could explain contradictory findings in studies in which emotionality might influence animals' responses, and where the effects of their previous housing have not been adequately considered.

The effects of standard, barren cages on behaviour can occur due to influences on neural development and plasticity. Enriched cages can enhance neurogenesis (van Praag *et al* 2000; Fernandez-Teruel *et al* 2002; Kohl *et al* 2002), or, stated conversely, standard barren cages can limit neurogenesis. The implications of this effect could be profound given that the links between neural development, behaviour and physiology are still not fully understood. Certainly, there are many reports that housing in enriched cages compared to standard, barren cages can improve recovery from brain trauma (eg Passineau *et al* 2001; Puurunen & Sivenius 2002; Wagner *et al* 2002). Hockly *et al* (2002) reviewed evidence that the use of running wheels increases neurogenesis and improves learning, long-term potentiation and synaptic plasticity. Garner and Mason (2002) reported that stereotypic bar-mouthing in caged voles (*Clethrionomys glareolus*) was correlated with inappropriate responding (ie increased perseveration) in studies investigating cognition and activity, and suggested that a single underlying deficit consistent with disinhibition of responses was implicated. They stated that "stereotypic animals may experience novel forms of psychological distress, and that stereotypy might well represent a potential confound in many behavioural experiments." Since standard, barren laboratory cages and standard husbandry methods can be related to a greater incidence of stereotypies (Würbel *et al* 1998; Powell *et al* 1999, 2000; Callard *et al* 2000; Würbel 2001), many studies might be confounded in this way.

The effects of standard, barren cages on behaviour are not limited to vertebrates nor necessarily related to hugely complex environmental changes. Carducci and Jakob (2000) housed jumping spiders (*Phidippus audax*) in small or large cages that either were or were not enriched. The enrichment was simply a 15 cm dowel rod stretching from one top corner of the cage to the diagonally opposite bottom corner. Carducci and Jakob tested the spiders in a detour test and an open-field test, and assessed their responses to a video of prey. Spiders from the small cages progressed less distance in the detour test and responded less vigorously to the video-taped prey. Spiders from the non-enriched small cages were less active in the open-field test and responded to video-taped prey from shorter distances. The authors concluded that "This study should serve to caution researchers working on arthropods. The fact that we found effects of both cage size and environmental complexity on the behaviours we examined suggests that negative effects of laboratory rearing may be mitigated by careful design of housing conditions."

Effects of the standard social environment

Standard laboratory husbandry often entails housing gregarious animals in isolation or solitary animals in groups. But, housing animals without accounting for their normal social behaviour can result in responses that almost certainly reduce the validity of the data gained from the animals.

Korz and Gattermann (1999) studied the effects of social housing on mercury toxicity in the golden hamster. Mercuric chloride reduced body weight gain in both isolated and group-housed hamsters. During the post-application period, however, singly housed hamsters recovered, whereas the group-housed hamsters continued to show reduced body weight gains. The authors argued that this was due to high levels of social stress in the grouped hamsters, which elevated their susceptibility to intoxication. They commented that "This study highlights the need to carefully consider the housing conditions which can influence the results of teratological experiments." Those with experience of animal behaviour might suggest that this was a predictable result considering that hamsters are generally solitary animals (but see Arnold & Estep 1990); however, even a cursory examination of non-behavioural research reveals that the social behaviour of animals is often overlooked or disregarded with respect to housing, thereby exposing the study to the risk of reduced validity.

Perez *et al* (1997) conducted a study on the effects of individual housing on a range of biochemical parameters in female rats. Rats were either group-housed for days 1–21 (Group A), or group-housed for days 1–7, singly housed for days 8–14 and then group-housed again for days 15–21 (Group B). For the Group B rats, food intake increased and plasma triglyceride decreased during the second week when they were isolated compared to when they were group housed, and plasma glucose levels declined when they were returned to group housing in the third week. The levels of plasma triglyceride in isolated Group B rats were lower than any measurement for the Group A rats, although there was no difference in body weight (also see Morgan 1973). The authors suggested that the variations in biochemical parameters related to isolation housing could have been due to modified feeding patterns, adaptive changes in behaviour such as increased anxiety, or increased physical activity. Furthermore, they concluded that "individual housing of female rats provokes variations in certain biochemical parameters, and if this is not taken into account in performing different scientific studies, it could give rise to unreliable or even dubious results."

Spani *et al* (2003) used implantable transmitters to record several physiological parameters from two groups of mice, housed either individually or in pairs. They reported that even after several weeks of habituation to these conditions, the singly housed mice had higher heart rates and tended to have a reduced body temperature, although there were no differences in activity. Furthermore, the singly housed mice had more frequent but shorter resting bouts, indicating disruption of the normal circadian sleep pattern. The authors concluded that "It is therefore important to control for the effects of the social (as well as non-social) environment, especially in experiments that are sensitive to these effects." Nagy *et al* (2002) showed that, compared to group-housed mice, singly housed animals were smaller, had less soft-lean tissue and bone mineral content, and lower bone mineral density. Schapiro (2002) reviewed the effects of social manipulations on the immune responses of rhesus macaques and concluded that "Given the immunological effects of single caging alone, it seems likely that single caging effects may be a potential confound in many such studies."

In terms of behaviour, Schrijver *et al* (2002) and Morgan (1973) showed that, compared to group-housed rats, singly housed rats were more active under several conditions of environmental novelty, but exhibited longer latencies in an emergence test, indicating greater fearfulness. In addition, rats reared and housed as singletons were less able to learn a task reversal than rats reared and housed in groups (Morgan 1973).

Effects of standard handling

In large and busy laboratories, handling of the animals is usually limited to the minimum required for the experimental procedure (eg injection, drug administration). Therefore, standard handling is relatively brief and infrequent and is prone to being associated with a negative experience by the animal. Several studies have shown that standard handling influences data in a manner that can be interpreted as indicative of reduced validity. Nerem *et al* (1980) investigated the effects of handling on the development of atherosclerosis in rabbits. Control rabbits were reared under standard husbandry conditions whereas a second group was visited on regular occasions throughout the day by the experimenter who handled, stroked, played with, and talked to the rabbits. Both groups were given the same diet to induce atherosclerosis, and the aortas of the rabbits were later removed and examined for indications of the disease. Compared to the control rabbits, the more frequently handled rabbits showed a 60% reduction in the aortic surface area exhibiting lesions, although serum cholesterol levels, heart rate and blood pressure were comparable. The authors wrote "It is possible that the results obtained by different laboratories for essentially the same experiment are contradictory solely because of a difference in socio-psychological environment. If this is true, then it might also explain anomalous results within a single laboratory." These findings also raise the possibility that if, for some reason, animals (groups or individuals) are

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handled differentially by staff, this might influence the data. This could be unknown by the principal investigator, who might then unwittingly ascribe differences in responses as treatment effects rather than as handling effects.

If studies are to be repeatable and to have scientific validity, they should not be susceptible to extraneous variables unless these variables can be identified and replicated. Therefore, a critical factor for improving scientific validity is to exclude extraneous variables, or to identify them and their influences. Chesler *et al* (2002) reported on the results of a computational analysis designed to achieve this. They examined an archival data set of 8034 mice in a common test of nociception, the tail-flick/withdrawal test. In this test, the tail of a mouse is placed in water at 49°C and the latency for the animal to withdraw or vigorously flick its tail is recorded. The authors used Classification and Regression Tree (CART) analysis to identify and rank predictors of the latency to tail withdrawal, and reported eight factors that were significant predictors (Table 1). The influence of several of these predictors was expected; for example, differences in nociception attributable to sex and genotype are widely known (Chesler *et al* 2002). However, other factors were less expected to influence the response. One of these, stocking density, is directly related to standard cage design and husbandry, and was actually a better predictor of tail withdrawal than the sex of the mouse — a previously established predictor of the behaviour. Remarkably, the strongest predictor of nociception was the identity of the experimenter. This was despite all 11 of the experimenters having been trained by one of the authors or by a graduate student trained by them. The authors were uncertain what precisely differentiated experimenters in the present study, but commented that "Differential animal handling, perhaps inducing stress differences, is likely to be responsible. Indeed, different types of restraint greatly affect sensitivity on this assay." Animals from standard, barren cages are sometimes more emotional or reactive than animals from enriched housing (see 'Effects on behaviour' section), which raises the possibility that the differences in reactions to handling by the experimenters and resultant effects on nociception could have been caused by exaggerated responses, due at least partly to standard, barren caging conditions.

Table 1 The rankings of factor-importance from the Classification and Regression Tree (CART) analysis to identify predictors of latency to tail withdrawal in mice in a nociceptive test. The factor scores are relative to the highest-ranked factor, which is given the arbitrary score of 100 (from Chesler *et al* 2002).

Factor	Number of factor levels	Score
Experimenter	11	100.0
Genotype	40	78.0
Season	4	35.8
Cage stocking density	7	20.4
Time of day	3	17.4
Sex	2	14.6
Humidity	4	12.0
Order of testing	7	8.7

Effects of maternal experience

An extraneous factor that is rarely considered in research is the influence of maternal experience on the responses of offspring. In research, rodents are generally purchased from breeding laboratories in which the mother has been reared under standard laboratory husbandry conditions. If these conditions have adverse effects on the mother and these effects can influence the responses of her offspring, affected offspring might be used in research that is subsequently confounded by the mother's experience, all without the knowledge of the experimenter.

Denenberg and Whimbey (1963) showed in a cross-fostering study that the handling of female rats in infancy influenced the behaviour and physiology of their subsequent offspring. Compared to pups reared by non-handled mothers, pups reared by handled mothers weighed more and defecated more in an open-field test. Skolnick *et al* (1980) used a model of stress in which the premature weaning of rat pups greatly increased their susceptibility to restraint-induced gastric erosions. When prematurely separated female rats grew to adulthood with stock males, their normally reared F1 progeny also had increased susceptibility. Cross-fostering studies showed that prenatal rather than postnatal factors transmitted this susceptibility to the F1 progeny. Similarly, the offspring of female rats stressed during gestation are likely to be more reactive to restraint (Ward *et al* 2000) and to have impaired cognitive processes (Chapillon *et al* 2002). This vertical transmission of influence on responses (also see Fleming *et al* 2002) means that even if an investigator attempts to reduce the effects of standard, barren husbandry within an experiment, the resulting data might still have reduced validity because of the influence of maternal experience.

Conclusions and animal welfare implications

Economic pressures and the reductionist approach to science mean that standard laboratory cages are generally small and barren, and that the handling of laboratory animals is minimised. I have shown here that housing or rearing animals in such conditions can cause responses that result in research data having limited external validity. This has profound ethical and welfare implications. It is widely believed that placing animals into standard, barren laboratory cages can cause suffering; however, this is usually considered to be a justifiable imposition because of the benefits gained from the data. But, if the validity of the data is reduced as a result of standard housing and husbandry, then it could be argued that the animals might have experienced suffering for limited gain. What can be done to reduce these influences of standard cages and husbandry conditions? One approach is to incorporate systematic variation into the environment (eg Würbel 2001, 2002). Although it has been argued that increasing variation will increase the variability of the data and that, as a consequence, more animals will be needed for a given experiment, evidence for this is equivocal (eg Gartner 1990; Mering *et al* 2001; Tsai *et al* 2002). Another approach is to provide evidence that the cages and husbandry methods used will not have these adverse effects. When cages are being developed for farm animals, an assessment of their suitability is partly determined by the productivity of the animals in the system. The same approach could be used for laboratory cages, which could be assessed for their suitability for research by examining the external validity of the data gained from the animals housed in them. Cage manufacturers or scientists could be asked to prove the suitability of cages intended for use in research. Potentially, funding organisations or ethical committees could request evidence that the cages to be used in a research programme will result in data with robust external validity. This would ensure that if animals are to be maintained in standard housing conditions that might cause suffering, their suffering would be offset by the benefits gained from the research.

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