The effect of daily disturbance on the breeding performance of mice

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Summary

The United Kingdom Home Office Code of Practice for the housing and care of breeding animals requires that, *'the general well-being of all animals must be checked at least once daily'*. However, excessive daily disturbance of rodent breeding colonies could be counter-productive to animal welfare if it increases pre-weaning mortality. An experiment involving 100 breeding cages of BALB/c mice compared daily inspection of the mouse cages, but without disturbing the mice within the nest, with daily inspection in which every individual was studied even if this involved removing the cage lid and disturbing the nest.

No statistically significant differences were found between the two groups in breeding performance or pre-weaning mortality, though the disturbed group produced marginally fewer offspring and had slightly higher mortality. Average weaning weight did not differ between the groups, but sexual dimorphism at weaning was significantly increased in the disturbed group. It is concluded that there are unlikely to be any welfare benefits in an inspection regimen that involves disturbance of breeding mice, provided the cage is inspected daily.

Keywords Mice; BALB/c; disturbance; welfare; environment

Normal practice in all animal facilities is to check animals daily, enabling the technicians to evaluate the condition and well-being of the animals in their care. The inspections highlight mechanical problems such as malfunction of automatic watering systems, leaking water bottles, physical problems, e.g. poor condition, injuries and signs of aggression. If there are any specific problems identified, appropriate remedial action can be taken immediately by the animal technician to rectify the situation.

The Code of Practice for 'The Housing and Care of Animals in Designated Breeding and Supplying Establishments' (Home Office 1995) states in section 3.3 that *'the general well-being of all animals must be checked at least once daily. Special care must be taken to ensure adequate monitoring of animals housed above head height and in the lower tiers of cage racks'*. This has been taken literally by some welfare groups who have suggested that each animal in each cage, whether single or group-housed, should be observed each day. Whilst larger animals such as rabbits, dogs, primates can usually be seen clearly within their cage or pen, rodents, especially in breeding groups with young pups and nesting material, can be more difficult to observe individually without physical disturbance, which may be counter-productive to the animal's welfare (see Figs 1 and 2). In rodent breeding colonies of known microbiological status the Code of Practice has been interpreted as requiring a visual check of the cage and the visible animals with as little physical disturbance as possible. Routine collection of data on production,

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Accepted 9 October 2001

Fig 1 Enriched mouse cage, even with lid removed, mice are not visible without disturbing the nesting material

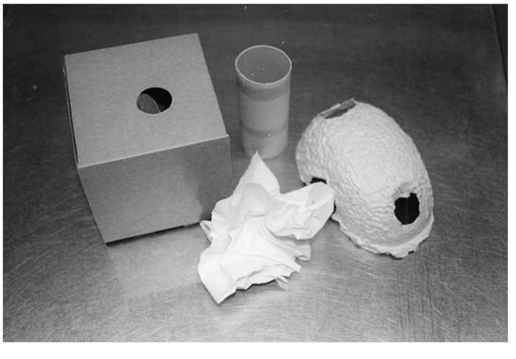

Fig 2 Mouse enrichment materials

mortality, litter size, body weight and other factors is also used to monitor the well-being of breeding colonies, and experience suggests that this regime of minimal disturbance is not detrimental to the welfare of the animals.

Over the last 10 years improvements in cage design and the increasing use of environmental enrichment devices in the form of nest boxes, nesting material, tunnels etc, have been widely accepted as desirable (Chamove 1989, Peters & Festing 1990, Hobbs *et al.* 1997, Jennings *et al.* 1998, Van de Weerd *et al.* 1998, Baumans 1999, Dean 1999, Eskola & Kaliste-Korhonen 1999). However the insertion of these materials into the cage, allowing the animal choice and the ability to manipulate its microenvironment has meant that it has become increasingly difficult to visually check individual animals without needing to disturb them. Similarly, the increasing use of closed caging systems, such as flexible film isolators and individually ventilated cages (IVCs), with the inclusion of environmental enrichment has made the individual checking of every animal on a daily basis without disturbing the animal even more difficult. The use of more absorbent bedding in these systems allows the animals to be left for longer intervals between cleaning; with lower pup mortality in those breeding groups cleaned every 14–21 days compared to those cleaned every week (Reeb-Whitaker *et al.* 2001).

Previous unpublished work at Harlan UK has shown that strains with poor breeding performance, such as A/JOlaHsd and SWR/OlaHsd, react poorly to physical disturbance, resulting in lower productivity and increased pre-weaning mortality. Following a regime of minimum disturbance, cleaning at 14-day intervals and not cleaning cages with newborn litters the productivity increased from 0.38 and 0.50 to 0.88 and 0.92 young weaned per female per week in these two strains, respectively.

With these problems in mind we designed an experiment to study the effects of disturbance on breeding mice. 'Disturbance' in this case involved daily inspection of all animals within a cage, compared with the normal practice of only disturbing the cages when checking for litters, changing bedding and nesting materials and weaning young.

Materials and methods

A total of 100 cages of inbred BALB/cOlaHsd mice, a popular and prolific strain, were mated in trios (two females and one male) and divided at random into two equal groups, designated 'Controls' and 'Disturbed'. All cages were cleaned weekly, at which time births were recorded and offspring were weaned and weighed. The normal husbandry checks were carried out daily on all cages to confirm the general well-being of all animals, this is also a specific requirement of the Animals (Scientific Procedures) Act 1986. Cages in the *Control Group* were checked daily but without disturbing the animals, thus if the animals were in the nest and unseen but the cage appeared normal they were judged not to need further disturbance to check on their welfare. Cages in the *Disturbed Group* were checked daily and every animal was visually inspected; this meant removing each cage lid and, where necessary, the nesting material to allow the animals to be observed. Suckling females were not necessarily lifted from their litters, providing the technician was satisfied that the litter was in good condition.

The experiment was carried out in a fully barriered building. The breeding room had 15–20 changes of pre-heated air filtered to 0.3 μ and passed via trunking into the room where a positive pressure was maintained. A cycle of 12 h artificial light and 12 h darkness was maintained with phased dusk and dawn periods of half-light. Relative humidity was 50–60% and temperature was maintained at 20–21°C. Harlan Teklad 2018S autoclaveable rodent diet (Harlan Teklad, Blackthorn, Bicester, UK) was fed *ad libitum*. Water was supplied by automatic drinking valves (Edstrom Industries Inc. Waterford, WI, USA). Softwood shavings, 5 mesh (J Rettenmaier and Söhne GmbH & Co, Rosenberg, Germany) were used as bedding. Shredded paper, paper wool (Datesand Ltd, Manchester, UK) was supplied to each cage weekly as a nesting material. Moulded polypropylene cages with stainless steel lids type M2 (North Kent Plastic Cages Ltd, Erith, Kent, UK) $33 \times 15 \times 13$ cm with an internal floor area of 330 cm^2 were used to house all animals.

Data were collected on the number of mice born, number of litters, number of mice weaned and weaning weight for each cage. Deaths of adult mice were also recorded. All matings were made up on the same day; both groups were housed in the same animal room and looked after by the same animal technicians. The males were removed after 168 days, and any females pregnant at that time were allowed to rear the resulting litter, giving a total breeding period of about 180 days, which is the normal useful productive life of this strain. If a breeding female died very early or very late in the course of the experiment, she was replaced. However, in a few cases both breeding females died due to a malfunction of the automatic watering system, and in these cases breeding data from the cage were discarded as this was judged to be the best way of avoiding potential bias. However, weaning weight data on pups weaned before the malfunction were included, as this could not have biased the weaning weight results.

Data on breeding performance were analysed using a one-way analysis of variance, after checking for homogeneity of variance and normality of the residuals. Individual weaning weights were analysed by a two-way general linear model analysis of variance.

Results

Data from five cages in the Disturbed Group and from three cages in the Control Group were discarded due to death of the breeding females, as noted above. The Disturbed Group produced a total of 477 litters and 2448 pups weaned from 45 cages, whereas the Control Group produced 493 litters and 2622 pups weaned from the 47 cages used in the subsequent analysis.

The breeding results on a per cage basis are given in Table 1. Average productivity was over 0.8 young per female per week weaned, which is slightly above average for this relatively prolific strain. The Disturbed Group produced slightly fewer pups per cage, had a slightly lower litter size at birth, weaned slightly fewer pups and had a slightly higher pre-weaning mortality. However, in no case were the differences statistically significant ($P > 0.05$). Weaning weights are shown in Table 2. The analysis of variance showed that female pups weighed significantly less than males ($F_{1.5060} = 6.8$, $P = 0.009$), there was no significant main effect for the treatment ($F_{1.5060} = 0.3$, $P = 0.56$) but there was a significant ($F_{1.5060} = 4.3$, $P = 0.03$) treatment by sex interaction. The effect of disturbance was to increase sexual dimorphism at weaning. This effect was largely due to a relative increase in weaning weight in males from

Table 1 Breeding results

Group	No. of cages	Born per cage	Litters per cage	Born per litter	Weaned per cage	PEI*	% PW mortality
Disturbed	45	54.4	10.6	5.18	50.9	0.848	6.2
Control	47	55.8	10.5	5.41	53.1	0.885	5.1
SD†		10.7	2.0	0.84	10.6	0.177	6.7
P-value		0.54	0.79	0.18	0.32	0.325	0.41
$F_{1.90}$		0.4	0.1	1.8	1.0	1.0	0.7

*'Production Efficiency Index', young weaned per female per week
†Pooled within-group standard deviation

Table 2 Weaning weights*

Group	Males			Females		
	No. of pups	Mean weight (g)	Standard deviation	No. of pups	Mean weight (g)	Standard deviation
Disturbed	1227	9.70	1.48	1249	9.50	1.44
Control	1220	9.59	1.46	1368	9.57	1.47

*Note that the table contains data on 199 control and 84 disturbed offspring that came from breeding cages not included in the analysis of breeding performance

the Disturbed Group, which may have been the result of the smaller (though not statistically significant) litter size in that group, allowing each pup to get more milk.

Discussion

The purpose of the inspection regime given in the Code of Practice is to maximize the welfare of the animals. It should, therefore be interpreted in such a way as to achieve that objective in the light of all available evidence. In the present study, involving quite large numbers of breeding cages, there was no evidence that daily inspection which involved disturbance to the extent that every individual mouse within a cage had to be inspected every day was more beneficial to the animals than one in which each cage was studied, but the animals were left undisturbed if they chose to remain in their nests. In fact, if anything, the Disturbed Group had a slightly lower productivity and slightly higher mortality than the Control Group, though as the difference was not statistically significant, this could have been due to chance. It should be borne in mind that the BALB/c is a relatively robust inbred strain. Less hardy strains with poorer breeding and rearing performance may well react significantly less well to a disturbed husbandry regime.

Acknowledgments We wish to thank Senga Cameron, Joanne Willoughby, Margaret Rennie and Nichola Clarke for their invaluable help in collecting the data.

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