Refining laboratory rodent husbandry - recent advances

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Introduction

The three Rs of animal research - Replacement, Reduction, Refinement - first put forward by Russell and Birch (1959) have provided the foundation on which many of the advances in laboratory animal welfare have been built. The desired end-state is the complete replacement of animals in research, however until scientific advances make this feasible, efforts should be taken to refine husbandry procedures in order to minimise pain and distress for the animals involved. This paper will discuss a number of recent studies aimed at addressing some of the welfare concerns associated with laboratory rodents in terms of environmental enrichment, reduction of intermale aggression, and humane euthanasia.

Cage preferences in laboratory rats - the effects of environmental enrichment

Laboratory rodents spend the majority of their time in their home cage (Jennings, Batchelor, Brain, Dick, Elliott, Francis, Hubrecht, Hurst, Morton, Peters, Raymond, Sales, Sherwin, and West, 1998), and so improving and enriching this environment provides a major way of improving the overall welfare of these animals. Patterson-Kane, Harper, and Hunt (2000) conducted a study aimed at investigating the preferences of laboratory rats for the following enrichment options:

1. Increased cage space
2. A large social group
3. Presence of novel objects
4. Presence of fixed wooden columns
5. A semi-enriched cage containing a combination of options 1 to 4

By using boxes that could be accessed via a T-maze and a continuous access box, the preference of rats for each of these enrichment options was tested against an empty cage. Each of the twelve subjects was observed 60 times in each of the five environments, and for each testing procedure (T-maze / continuous access box). The results of the experiment showed that of all the enrichment options tested, only the semi-enriched cage was significantly preferred as measured by both testing procedures. This result is important in that it shows firstly that there are some cage conditions for which the rat shows a preference, and secondly that it is perhaps the interaction of different environmental enrichment devices, rather than the devices themselves, that is responsible for the positive effect of environmental enrichment. The lack of a statistically significant preference for increased cage size alone reinforces the views of Bantin and Saunders (1989) in that an increase in the complexity of space is more important than an increase in space alone.

In the second part of their study, Patterson-Kane et al. focused specifically on the enrichment provided by novel objects. These objects were not arbitrarily chosen as in the first part of their study, but rather they were chosen to provide a substrate for the performance of specific behaviours of the rat, namely resting, chewing, perching, and tunnelling. The testing procedure used was the same as that for the first part of the study. Results showed that the rats did not consistently prefer environments that allowed for the expression of chewing and tunnelling behaviours. Whether this is because these behaviours are not as highly valued as others, or because the objects chosen did not provide suitable substrates for the performance of these behaviours would require further study. The rats did show a statistically significant preference for environments containing enrichment objects that enabled them to display nesting and perching behaviours, and as such the use of these objects could positively improve the welfare of laboratory rodents. These results agree with studies conducted by Van der Weerd, van Loo, van Zutphen, Koolhaas, and Baumans (1996) and Manser, Broom, Overend, and Morris (1997).

Intermale Aggression - The impact of cage cleaning regime on intermale aggression

Laboratory mice are often housed in single sex groups of three to ten animals (Van Loo, Kruitwagen, Van Zutphen, Koolhass, and Baumans, 2000). Group housing is generally preferred to individual housing, as the latter is often associated with behavioural and physiological abnormalities commonly referred to as 'isolation stress' (Brain, 1975). Group housing brings with it its own set of problems however, prime among them being the incidence of aggression, particularly in male groups. It is known that aggression between male mice is increased following disturbances associated with cage cleaning (Jennings et al., 1998). The reason for the increased aggression is believed to be the disruption of olfactory signals in the cage that enable the mice to maintain a stable social environment (Van Loo et al., 2000). Van Loo et al. (2000) studied the effect of various cage cleaning regimes (outlined in table 1) on the incidence and duration of aggressive behaviour.

Category	Cleaning Method
Clean	Clean cage with clean sawdust and nesting material
Sawdust	Clean cage with clean sawdust and nesting material plus a 5-10 g of dirty sawdust containing urine and faeces transferred from dirty cage
Nest	Clean cage with clean sawdust, but nesting material transferred from dirty cage

Twelve groups, each containing three male mice, were subjected to each cleaning regime three times. The behaviour of the groups was recorded on videotape, and from this the latency until first aggressive encounter, and the frequency and duration of encounters were recorded. Statistically significant increases in latencies to first aggressive encounter were reported between the nest cages and both the clean and sawdust cages. Additionally, there were statistically significant decreases in the frequency and duration of aggressive encounters between the animals in the nest cages compared to those in the clean and sawdust cages. Interestingly, there was a slight increase in frequency and duration of aggressive encounters between animals in the sawdust cages as compared to the clean and nest cages, indicating the need for thorough cleaning. The most important finding of this study in regards to laboratory animal welfare is that the transfer of nesting material to clean cages can reduce intermale aggression following cage cleaning.

Euthanasia - Animal welfare aspects of euthanasia of rats with carbon dioxide

Euthanasia literally translated means 'gentle death'. Regardless of the method used, the goal of euthanasia should be a rapid and painless death, with minimal fear and psychological stress (Close, Banister, Baumans, Bernoth, Bromage, Bunyan, Erhardt, Flecknell, Gregory, Hackbarth, Morton, and Warwick, 1996). In laboratory rodents, carbon dioxide is used both as a short duration anaesthetic (Kohler, Meier, Busato, Neiger-Aeschbacher, and Schatzmann, 1999) and also for euthanasia (Close et al., 1996). Hackbarth, Küppers, and Bohnet (2000) studied the welfare aspects of euthanasia of rats with carbon dioxide in their home cage, by testing the hypothesis that a sedated/anaesthetised animal would show less reaction to euthanasia with CO_2 than a conscious animal. The reaction was measured in terms of behavioural responses, blood glucose concentrations, and changes in hormone concentration (ACTH and corticosterone). The study involved the euthanasia of four groups of twelve animals. The first group was sedated with acepromazine prior to euthanasia, the second group was anaesthetised with pentobarbital prior to euthanasia, and finally the two control groups was neither sedated nor anaesthetised. The animals were euthanased in their home cages by the induction of CO_2 at a rate of 6 L/min. Four animals from each group were decapitated 30, 75, and 120 seconds after the beginning of the induction of CO_2 to measure

hormone and glucose concentrations. The results of the study showed that there were no statistically significant differences in the concentration of the stress-induced hormones between the sedated/anaesthetised animals and the conscious animals. Additionally, none of the animals in the study showed any behavioural changes indicating distress or fear, agreeing with the results of Smith and Harrap (1997). An interesting, but not unexpected result revealed by the study was that those animals that were handled and injected prior to euthanasia (the anaesthetised group with pentobarbital and one of the control groups with saline) showed statistically higher corticosterone concentrations than the unhandled animals. Based on these results it can be concluded that euthanasia of rats in their home cage (thereby eliminating the stress of handling) can be recommended as humane.

Conclusions

Implementation of the recommendations of studies such as those discussed above will not only result in improvements in the welfare of these animals, but may also enhance the quality of scientific research conducted with them, since distress in animals can cause physiological changes which can introduce variability and even erroneous results. Refinement of laboratory rodent husbandry should therefore be considered essential for scientists and institutions aiming to produce high quality and ethically sound research.

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