A review of recent research exploring the effects of common husbandry methods on the wellbeing of the laboratory mouse (*Mus musculus*): How science is working to improve animal welfare

Reviews recent research on laboratory-mouse husbandry, discussing how scientific discoveries enhance welfare.

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Introduction

Laboratory mice are kept in strictly controlled environmental conditions to minimise possible sources of variability in experiments but it is also important to find a balance with animal welfare and even carer satisfaction (Baumans & Van Loo, 2013). Ongoing research aims to both understand the possible impact of mouse behaviour and physiology on experimental outcomes, and find ways to improve welfare.

Discussion

Gaskill *et al*. (2013) explored the benefits of nesting material on thermoregulation and quantified the amount to sufficiently reduce cold stress for three common strains of mice (C57BL/6, CD-1 and BALB/c). Mice of the same strain (n=24 each strain) were housed in same-sex families of three and given 8g Enviro-Dri nesting material (FiberCore, Cleveland, OH, USA) or none. ng.

The four-week study found higher-quality nests were dome-shaped and provided good insulation, demonstrated by thermal imaging. These mice had reduced weekly food consumption, reflecting lower energy requirements, and BALB/c mice showed a reduced expression of uncoupling protein 1mRNA in brown adipose tissue, indicating reduced non-shivering thermogenesis. Males of all strains built better nests, ate less food, had lower core body temperatures and higher end bodyweights than females. Nesting males also had lower plasma T_4 concentrations than controls, reflecting a lower metabolic rate. The authors postulated that no difference in thermoregulation was detected in females due to their higher thermal comfort range, so the 8g of nesting material provided was insufficient to significantly reduce cold stress. They concluded that although different strains appear to use different behavioural and physiological thermoregulatory strategies, 8g of nesting material was sufficient to alleviate thermal stress in males at 20°C ambient temperature, but more is required by females.

Gaskill *et al*. (2013) noted that core body temperatures increased when cages were being disrupted and during handling, reflecting physiological changes during simple husbandry procedures. Another study (Gouveia & Hurst, 2013) specifically examined the effect of handling methods on mice anxiety and behaviour. They expanded on a previous study (Hurst & West, 2010), which demonstrated reduced anxiety by handling mice using a “home” tunnel left permanently in their home cage, by examining whether familiarity with the tunnel was significant.

Two strains of mice (ICR(CD-1 and C57BL/6) were divided into groups (n=8x2 mice in each group for each strain) based on handling method: 1. Tail handling; 2. Tunnel handling: i) home tunnel, ii) shared tunnel experienced (prior exposure to tunnel) and iii) shared tunnel only (no prior exposure to tunnel). Mice were handled over 9 days, their voluntary interactions with the handler analysed and anxiety levels measured in an elevated plus maze (EPM) on Day 10. Because handling procedures were distinct from one another, blind handling was not possible. However, unconscious handler or observer bias was deemed highly unlikely to have a significant effect based on a previous study (Hurst & West, 2010).

All tunnel-handled mice, regardless of strain, sex or tunnel familiarity, habituated to the handling method, with the home tunnel treatment adapting faster. They displayed increased voluntary interactions with the handler over time and reduced anxiety behaviour in the EPM test. Tail-handled mice failed to habituate to handling, consistently avoiding interaction with the handler and demonstrating greater anxiety behaviour in the EPM test. However, only a single measure of anxiety was made at the end of the treatment period. It may be preferable to assess anxiety levels over time using, for example, faecal corticosterone concentrations. The use of multiple measures helps to give a more complete picture of anxiety.

Kalliokoski *et al*. (2013) utilised many parameters to measure three different forms of stress in mice: acute, oxidative and emotional. They examined whether male BALB/c mice (n=16) could habituate to individual housing in metabolism cages, comprising a small living area on wire mesh with no bedding, shelter or enrichment – all factors that have been shown individually to induce stress or discomfort in mice (Kalliokoski *et al*., 2013; Baumans & Van Loo, 2013).

A range of biochemical, clinical and behavioural measurements were made, but a full description of the rigorous methods employed is beyond the scope of this essay and readers are encouraged to read Kalliokoski *et al*. (2013) to fully appreciate their detailed methodology. Compared to standard-housed mice, metabolism-caged mice had an abnormal physiology with: very high faecal corticosterone metabolite (CORT) concentrations; elevated hypothalamic-pituitary-adrenal axis activity; post-experiment elevated core body temperature (stress-induced hyperthermia); various changes possibly due to thermoregulatory demands, including increased feed intake, higher piloerection and an increased metabolism, reflected in high concentrations of creatinine and oxidised nucleic acid metabolites. Metabolism-caged mice thus had a significantly increased metabolism that would be detrimental to their health and skew experimental results. Accordingly, the authors recommended that if metabolism cages are to be used, minimal time be spent in them.

While each paper focuses on a different aspect of laboratory-mouse husbandry, all attempt to better understand the animal's wellbeing using measurable, specific variables. Kalliokoski *et al*. (2013) had the most rigorous methodology and went a step further, using statistical analyses to assess the efficacy of their measurements as parameters for measuring stress. They concluded that faecal CORT output, fur scorings and stress-induced hyperthermia were good tools for evaluating the wellbeing of mice, encouraging their use in future studies. Feed intake, water intake and bodyweight were not found to be useful stand-alone welfare parameters, and it was recommended that such measurements be interpreted with caution. Accordingly, the studies of Gouveia & Hurst (2013) and Gaskill *et al*. (2013) may benefit from future work using more accurate stress measures.

Conclusion

It is evident that mice are highly reactive to their environment, displaying behavioural and physiological changes that may skew experimental results and compromise their wellbeing. Each paper provides compelling arguments for environmental refinement (provision of adequate nesting material, tunnel handling and reduction in metabolism-cage use) to improve laboratory-mouse welfare based on the results of rigorous scientific method with distinct, measurable parameters. For confined laboratory animals it is important to find balance between animal welfare and experimental validity (Baumans & Van Loo, 2013) and, encouragingly, research in this field is ongoing.

References

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