

The impact of environmental enrichment on the outcome variability and scientific validity of laboratory animal studies

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Summary

It has been widely accepted for some time that species-appropriate environmental enrichment is important for the welfare of research animals, but its impact on research data initially received little attention. This has now changed, as the use of enrichment as one element of routine husbandry has expanded. In addition to its use in the care of larger research animals, such as nonhuman primates, it is now being used to improve the environments of small research animals, such as rodents, which are used in significantly greater numbers and in a wide variety of studies. Concern has been expressed that enrichment negatively affects both experimental validity and reproducibility. However, when a concise definition of enrichment is used, with a sound understanding of the biology and behaviour of the animal as well as the research constraints, it becomes clear that the welfare of research animals can be enhanced through environmental enrichment without compromising their purpose. Indeed, it is shown that the converse is true: the provision of suitable enrichment enhances the well-being of the animal, thereby refining the animal model and improving the research data. Thus, the argument is made that both the validity and reproducibility of the research are enhanced when proper consideration is given to the research animal's living environment and the animal's opportunities to express species-typical behaviours.

Keywords

Animal welfare – Environmental enrichment – Experimental validity – Reproducibility – Three Rs.

Defining enrichment

The multiple effects that a captive environment can have on the health and behaviour, indeed the overall well-being, of an animal have been recognised for decades. The observance of abnormal behaviour in captive animals dates back to as early as 1928, when severe 'self-mutilation' was observed in a rhesus monkey that had undergone a change in social environment (1). But studies by Professor Harry Harlow (2, 3) beginning in the 1960s definitively confirmed that different constellations of behavioural pathology could be induced by manipulating the degree of social isolation experienced by laboratory monkeys during critical periods of development (4). Other studies in the 1960s, carried

out by Rosenzweig and colleagues (5, 6), demonstrated how complex environments altered neurochemistry and brain weight in rats. It became evident that the barren cage environment was changing the animals in ways that were detrimental to the animal's health and welfare as well as the research data. This realisation led to the reverse trend of providing environmental enrichment to laboratory animals of all species, with a goal of either addressing existing manifestations of abnormal behaviour or preventing its occurrence.

As Coleman *et al.* (7) have noted, the term 'enrichment' has been used variously to refer to specific items placed in the cage with the animals, or, more broadly, to describe a

process to improve animal welfare. Mench (8) notes that environmental enrichment is 'often defined in terms of its purposes rather than simply as a process or a phenomenon'. For example, enrichment has been described as a means to increase the amount of time an animal spends in species-typical activities (e.g. foraging, nest building, etc.), with a concomitant reduction in time spent expressing abnormal behaviour such as stereotypic locomotion and self-injurious behaviour (9). Also, Shepherdson (10) defined environmental enrichment as 'an animal husbandry principle that seeks to enhance the quality of captive animal care by identifying and providing the environmental stimuli necessary for optimal psychological and physiological well-being'. It is critical to take into consideration that inappropriate enrichment can induce fear or stress in an animal, and thus it is most accurate to speak in terms of providing beneficial enrichments, which improve an animal's welfare (11).

There is general agreement that, to be relevant and meaningful, the environmental enrichment programme should be tailored to the species of animal of concern, which requires a sound understanding of the behavioural repertoire of the animal. For some animals (e.g. nonhuman primates), the enrichment programme should also take into account the age of the animals, as young animals are often more playful than adults and could benefit from more active enrichment opportunities. In some species there are also sex and individual differences that should prompt a customised enrichment approach to enhancing the animals' welfare. In addition, the safety of the animals and personnel must be a primary consideration in the selection of enrichment methodologies and the implementation strategy (12). The laboratory environment places several physical and operational constraints on the methods and extent of enrichment offered to research animals, but these limitations have also stimulated creativity and innovation in the development of enrichment options.

Types of enrichment

A more encompassing approach toward enhancing research animal welfare is to consider enrichment as one element of a broader behavioural management programme which comprises the structural environment, the social environment, and animal activity (both physical and cognitive) (13). Keeling *et al.* (14) included sensory and nutritional enrichment as additional categories for improving animal welfare. Behavioural management programmes have been described as encompassing positive reinforcement training, facility and cage design, and positive interactions with staff (15, 16). However, they should also influence husbandry, veterinary and experimental procedures and practices from the time the animal arrives at the institution

through to the end of its life, in accordance with the Three Rs (replace, refine, reduce).

Social enrichment

The importance of providing social species of laboratory animals with a suitable social environment cannot be overstated. Recognising that some research projects preclude the possibility of social housing (e.g. infectious disease studies), as noted in several key standards documents (e.g. 17, 18), social housing of research animals should be considered the default method. That being said, for some species the formation of pairs or groups of animals is not without risk due to the potential for aggressive encounters, and thus social housing procedures should be well-established and conducted by knowledgeable personnel. The evidence is clear across the multitude of species used in research that single housing can have a negative effect on social species and that social housing, managed properly, has numerous positive effects on the animals. Primary among these is the reduction or elimination of abnormal behaviour and the opportunity to express species-typical social behaviours.

Structural enrichment

The most common method of providing enrichment is to modify the home cage environment in a manner that expands the range of behaviours that can be expressed by the animal. Lutz and Novak (19) have referred to this as an 'analogous' approach to enriching the environment, where the behavioural outcome is the goal of the enrichment technique. Examples of such enrichments, in the home cage (or pen, kennel, etc.), are as follows: shelters that provide suitable hiding, nesting or sleeping areas for primates, rodents, rabbits and several other species; perches provided to primates; resting boards or beds provided to dogs, cats and ferrets; and nesting material provided to mice. Toys are also regularly provided to some laboratory animals (e.g. primates, dogs, cats, rabbits, ferrets, swine) with the goal of stimulating play or exploratory behaviours. A balance should be sought between providing adequate complexity within the enclosure and ensuring that the items are relevant to the animal, will not harm the animal and do not preclude the care staff from conducting their daily husbandry duties (e.g. daily observations of the animals, cage cleaning, etc.).

Physical and cognitive activity

Depending on the age and health of the animal, opportunities for physical activity may be especially appropriate for some species of research animals. For example, with proper stimulation, dogs and cats will engage in play activities with human caregivers or compatible conspecifics. Nonhuman primates and cats both benefit from the opportunity to

explore and use three-dimensional space created through shelves, swings, ramps, and other climbing surfaces. For many species of laboratory animals, toys will induce physical activity. Stimulation of cognitive activity is also considered a means of providing enrichment. Food enrichment may be offered in ways that require the animal to solve a puzzle or manipulate a toy to retrieve it, search for the food in bedding, or move around in its environment to access food-dispensing sources (e.g. 20, 21, 22). Physical and cognitive activities are an intrinsic benefit of social housing.

Cage space

Consensus has not been reached on the amount of cage space that should be provided to all species of laboratory animals. A review of the literature (23) pertaining to cage space for mice includes salient points that apply to many species. Key among these is reliance on a performance-based approach to allocating cage space that provides sufficient area for exercise and normal social behaviour as well as the inclusion of enrichment items (24). Also, cage space allocation should take into consideration the strain, number and age of the animals in the cage, and their reproductive status, familiarity with each other, and the research work being done with them (25).

Enrichment and within-experiment variation

Concern has been expressed that environmental enrichment may increase within-experiment variation. This concern is based on the hypothesis that a more complex environment produces a greater diversity of phenotypes among the animals of a study population. On the one hand, a more complex environment might create more opportunities for individuals to have more diverse experiences, for example, by providing different niches within an environment so that the animals within a cage are exposed to various environmental conditions. On the other hand, an inadequate environment might increase individual differences, as indicated by the occurrence of abnormal behaviours such as stereotypies, resulting in variable and individual coping responses. Whether phenotypic diversity is a function of environmental complexity and whether this relationship is positive or negative are empirical questions that have never been systematically addressed. However, several studies have examined the effects of various enrichment protocols on within-experiment variation in physiological and behavioural measures (26, 27, 28). None of them identified evidence that enrichment would affect within-experiment variation consistently one way or another.

Present evidence therefore suggests that the welfare of animals can be improved by the provision of suitable environmental enrichment without increasing within-experiment variation, provided the enrichment is appropriate for the animals and does not itself constitute a stressor, in which case variation in experimental results may indeed be increased.

Enrichment and between-experiment variation

The hypothesis that enrichment might increase within-experiment variation is also used to argue that enrichment might compromise the reproducibility of experimental results. However, reproducibility is not determined by variation within experiments but by variation between experiments, and a recent multi-laboratory study showed that even extensive enrichment had no adverse effect on between-experiment variation, demonstrating that a more complex environment does not compromise reproducibility (27, 29). Others are concerned that more complex housing conditions would inevitably lead to greater differences in the environmental conditions between laboratories, because different laboratories would choose different enrichment items, use different products, arrange them differently within cages and differ in how often they replaced them. However, it is unlikely that these differences represent a significant problem, given the variation in environmental conditions that exists anyway among different laboratories. There are many environmental factors that simply cannot be standardised across laboratories. It is therefore unavoidable that different laboratories have different local environmental conditions, and enrichment is just one more factor that may vary between laboratories. Given that environmental variation among laboratories (and even between experiments within the same laboratory) is a matter of fact, results will only be reproducible if they can be generalised to at least the range of conditions manifest in different laboratories.

Assessment of the value of enrichment

Potential concerns with enrichment

The provision of environmental enrichment may result in unintended consequences for both animals and research results (30). Some of the concern regarding negative impacts of enrichment may simply be based on a problem with semantics. Specifically, any addition to the cage environment seems to be automatically labelled as

enrichment, whether the actual definition of enrichment is achieved or not. However, there have been reports of animals being physically harmed by enrichment (31, 32, 33), though such incidents are infrequent. Toth *et al.* (34) have issued a caution regarding the unintended impacts on rodent research of the provision of enrichment, but the literature is also replete with examples of the harmful effects of singly housed animals living in barren environments. Thus, enrichment should be provided in a manner that considers the health and welfare of the animal as well as the research in which the animal is being used.

Improving welfare and science through enrichment

For enrichment to be considered a meaningful addition to standard animal care practices, a harm–benefit analysis should be undertaken. This analysis should be inclusive of effects on the animal and on the research. Clearly, some types of research will only be slightly affected by the often subtle impact on the animal, while others will be exquisitely sensitive to small changes in the animal's physiology. In many instances, the inclusion of enrichment in an animal's cage has led to intriguing discoveries regarding the effect of environmental complexity on a particular animal model of human disease, leading to new theories of pathogenesis as well as potential treatment adjuncts.

Several fundamental questions are germane to the implementation of enrichment. The most basic of these is whether the animal 'uses' the enrichment. Such use can be described as moving the object around, contacting or manipulating it, entering it, acquiring food or other resources from it, or changing its configuration. Next, it should be determined how the animal is using the enrichment. For example, if the animal is defending the enrichment to the point of increasing its aggressive behaviour toward other animals, an alternative form of enrichment, or possibly other approaches to improving the animals' welfare, should be considered. Finally, and importantly, there is the need for a sound understanding of the potential ramifications of the enrichment technique on the animal's biology and whether this may have consequences for the intended research use of the animal. The scientific literature is rich with studies that have evaluated this topic (e.g. 35, 36, 37, 38, 39, 40, 41, 42), although much remains to be done.

Enrichment offered with the best of intentions can have a negative impact on the animal. The animal's response appears to depend on the type of enrichment and on the species, strain, sex and age of the animals. It also depends on whether housing is individual or social, and whether the enrichment is provided in the home cage or the animal is moved to a separate enrichment space. The responses can include stress, fear and anxiety, aggression, injury due to the enrichment itself, and potentially undesirable consequences of the introduction of contaminants into the animal's environment. Yet, the evidence is clear that a sterile cage environment results in animals that have physiological, neurological, immunological and behavioural abnormalities, which are certainly of welfare concern, but also raise doubts regarding the validity of results obtained from such research subjects. It is apparent that not providing an adequate housing environment has both welfare and scientific implications, and so does providing inappropriate enrichment. Accordingly, a measured approach to optimising cage environments should be taken. In addition to establishing a team of professionals at the institution to assess the specific circumstances and determine the proper strategy for animal housing, consideration should be given to implementing enrichment in an incremental manner, if possible, so that the impact on the animals and the research can be reviewed and any necessary adjustments in the programme made accordingly.

The provision of species-appropriate enrichment to support research animal welfare is an ethical imperative and the production of high-quality data on these animals is critically dependent on the provision of an environment that meets both the demands of the research and the health and well-being of the animal. Good animal welfare through appropriate environmental enrichment ultimately leads to better science.



L'impact de l'enrichissement de l'environnement sur la variabilité des résultats et la validité scientifique des études recourant aux animaux de laboratoire

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Résumé

On sait depuis longtemps que l'enrichissement de l'environnement dans lequel sont maintenus les animaux utilisés à des fins scientifiques, en l'adaptant aux besoins de chaque espèce, est important pour leur bien-être, mais jusqu'à présent on ne s'était guère intéressé à l'impact que cet enrichissement pouvait avoir sur les résultats de la recherche. Cet état des choses a changé, en même temps que l'enrichissement de l'environnement est devenu une pratique plus systématique dans les élevages. En plus d'être appliqué pour les besoins des grandes espèces d'animaux de laboratoire, tels les primates non humains, il est aujourd'hui également utilisé pour améliorer les conditions de vie des petits animaux de laboratoire, par exemple les rongeurs, qui sont utilisés en bien plus grand nombre et dans un très large éventail d'études. Des objections se sont pourtant élevées contre l'enrichissement, au motif que celui-ci aurait un impact négatif sur la validité des études et sur leur reproductibilité. Néanmoins, il est évident que lorsque l'on définit l'enrichissement de manière précise en se basant sur une connaissance sérieuse de la biologie et du comportement des animaux ainsi que sur les contraintes de l'expérimentation, il est tout à fait possible d'améliorer le bien-être des animaux de laboratoire sans compromettre le but visé par les études. En réalité, c'est plutôt l'inverse qui se produit : un enrichissement approprié améliore le bien-être de l'animal, ce qui permet de sélectionner de manière plus judicieuse le modèle animal et d'améliorer ainsi les données obtenues au cours de l'expérimentation. Ainsi, les auteurs soutiennent qu'une prise en compte appropriée du cadre de vie dans lequel sont maintenus les animaux de laboratoire et la possibilité offerte à ces animaux d'exprimer le comportement propre à leur espèce contribuent à améliorer aussi bien la validité que la reproductibilité des expérimentations.

Mots-clés

Bien-être animal – Enrichissement de l'environnement – Règle des « trois R » – Reproductibilité – Validité d'une expérimentation.



Influencia del enriquecimiento del medio en la variabilidad de los resultados y la validez científica de los estudios con animales de laboratorio

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Resumen

Es un hecho sabido desde hace tiempo que un enriquecimiento del medio adaptado a cada especie es importante para el bienestar de los animales de investigación, pero al principio se prestó escasa atención a su influencia en los datos resultantes de las investigaciones. Ahora esto ha cambiado, a medida que se iba extendiendo el uso sistemático del enriquecimiento en el ámbito

de la zootecnia. Además de aplicarse al cuidado de animales de investigación de gran tamaño, como los primates no humanos, ahora se está utilizando para mejorar el medio de los pequeños animales de investigación, como roedores, que en general se utilizan en cantidades sensiblemente mayores en muy diversos estudios. Ello ha generado cierta inquietud por la posible influencia negativa del enriquecimiento en la validez y reproductibilidad de los experimentos. Sin embargo, cuando se utiliza una definición concisa de «enriquecimiento», aunada a una profunda comprensión de la biología y conducta del animal y de las limitaciones inherentes a la investigación, resulta obvio que es posible mejorar el nivel de bienestar de los animales de investigación enriqueciendo su medio sin por ello comprometer los fines últimos del proceso. De hecho, está demostrado lo contrario: un enriquecimiento adecuado aporta mayor bienestar al animal, perfeccionando con ello el modelo animal y mejorando así la calidad de los datos resultantes de las investigaciones. Esto avala la idea de que tanto la validez como la reproductibilidad de las investigaciones mejoran cuando se presta la debida atención al medio en que vive el animal de investigación y a sus posibilidades de expresar las conductas propias de su especie.

Palabras clave

Bienestar animal – Enriquecimiento del medio – Reproductibilidad – Tres erres – Validez experimental.



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