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Evaluating Environmental Enrichment is Essential

Introduction

The term environmental enrichment is used widely to refer to 'an increase in the complexity or naturalness of an enclosure, with the goal of improving animal welfare' (Patterson-Kane 2003, based on Chamove 1992 and Newberry 1995). The focus has been largely on the welfare of the animals, with less reference to potential impacts on research data. However, the concept of environmental enrichment actually originated as a research tool for understanding the effects of experience on the brain (Benefiel et al. 2005). The term was coined by Krech et al (1960), who reared a group of rats in a complex environment with daily training in a water maze, while their siblings were raised in isolation, in barren cages. They found biochemical changes in the brains of rats reared in the enriched environment, plus daily training, compared with the rats raised in barren environments. Since then, many studies have revealed changes in physiological and behavioural measures between animals housed in complex environments and those housed in barren cages. This apparent introduction of variation has led to concerns that addressing animal welfare issues through enrichment may have negative impacts on the progress of scientific research. For the purposes of this paper we have focused on rodents, as they represent the majority of animals used in research, and because less emphasis has been placed on improving their environments, in comparison to larger animals (e.g. non-human primates, dogs and cats).

Impact of enrichment on animals

It is generally accepted that animals maintained in barren environments are not behaviourally normal, often exhibiting stereotypies thought to be indicators of boredom or distress. The fundamental aim of improving an animal's environment should be to permit the performance of species-typical behaviours that give the animal some control over the environment, thus promoting physiological (and hence behavioural) homeostasis (Garner 2005). This should be the minimum standard for any animals used for scientific purposes, and increasingly is being required in legislation/regulations/guidelines (European Commission 2010, NRC 2011, Sørensen & Hansen (2012), Abbott Global Enrichment Committee (2012); see also *http://www.ccac.ca/en_/standards*). However, focus in the animal welfare literature is shifting from papers aimed towards simply providing an environment that meets an animal's needs to papers describing opportunities to give animals additional positive experiences. These positive experiences should outweigh the negative ones (even if animals are to be used in experiments where they might experience some harmful procedures), thus giving animals a 'life worth living' (Weary 2012). This growing literature on the benefits of providing an enhanced environment includes many examples published in *The Enrichment Record*.

In general, when environmental modifications have been selected with consideration of the behavioural and physiological characteristics of the animal, enrichment provides welfare benefits. However, it is still necessary to be aware of the presumption that any measure that increases the complexity of an animal's environment will enhance welfare. Weed & Raber (2005) describes some instances where the approaches used can actually be detrimental to an animal's well-being. For example, van Loo et al (2002) found that supplementing rodent cages with a shelter increased aggression, as well as the incidence of physiological indicators of stress in male mice, although providing only nesting material did not. These and other examples underline the need to critically evaluate the impact of any proposed enrichment in terms of its observed, rather than presumed, effect on animal well-being.

Since providing enrichment will affect both animal behaviour and physiology, it is relevant to consider the 'normality' of animals

continued on page 30

 continued from page 29

kept in barren cages. This leads to the question: are rodents raised in standard, barren cages sufficiently 'normal' to be valid models in research—or are they 'abnormal' because of their physiological and behavioural responses to an inappropriate environment? If the latter, then any research being carried out on those animals might be flawed, as any physiological or behavioural measures would be negatively influenced by their daily environmental conditions as well as the experimental treatment (Sherwin 2007).

Impact of enrichment on scientific outcomes

In general, research studies are designed to minimize variability within groups as far as is possible, so that effects of treatments are more readily observed, i.e. the 'signal-to-noise ratio' is large. This also means that the numbers of animals can be minimized. While reducing the numbers of animals involved in invasive procedures is an important goal, consideration also needs to be given to the amount of suffering likely to be experienced by each individual animal. In their seminal 1959 work '*Principles of Humane Experimental Technique*', Russell and Burch were keen to underline the importance of reducing the amount of suffering for each animal, even if that meant using more animals—as the individual animal's experience was the most important factor to be considered (Russell & Burch 1992). It could be argued that if we can provide a life worth living for animals used in science, then reduction of animal use would not be so much of a concern.

Publications on rodent welfare present the introduction of 'environmental enrichment' as a good thing for animal welfare, but typically, the potential effects on experimental outcomes are not considered. Keeping environmental conditions standardized has been thought to assist in minimizing variation, so over the years researchers have used standard, barren cages. However, in 1999, Crabbe et al tested this presumption in a multi-centre study looking at various measures of anxiety. Despite best attempts to standardize housing and husbandry conditions, they found significant differences in the measures between laboratories.

Currently, there is a growing literature that reports marked differences in animal models depending on the housing environment. For example, rats with experimentally induced traumatic brain injury living in an enriched environment took less time to find the platform in a Morris Water Maze test than rats with a similar brain injury maintained in individual housing (Hamm et al 1996, Passineau et al 2001). At the time of post-mortem, two weeks later, the brain injury in rats from enriched cages was found to be approximately half the size of that in the individually housed rats (Passineau et al 2001). In another example, transgenic R6/1 and R6/2 mice used to model Huntington's disease (HD)—a genetic disorder that results in motor dysfunction, dementia and death—exhibited less deterioration in motor skills and had a slower loss of cerebral volume when housed in an enriched environment. Similarly, mice living in an enriched environment exhibited a reduction in tumor growth and an increased remission in their cancers (three different models were studied; Cao et al 2010). These examples clearly show that outcomes in animal-based research are affected by the animals' housing environment, whether barren or complex.

Enrichment—How should impacts on scientific outcomes be interpreted?

How should the impacts of enrichment on scientific outcomes, such as the examples above, be interpreted? From the perspective of science, are they positive or negative, or is it not possible to generalize? Since many research studies are based on previously published work, the introduction of a complex environment that changes the parameters of an animal model may mean that the experimental data might not be readily comparable to previous findings. For example, in testing the effects of a drug aimed at improving outcomes from traumatic brain injury, different results might be anticipated depending on whether the animals were maintained in individual housing or group housed in an enriched environment. Similarly, to study potential treatments for HD, one would need to question which environment (impoverished or enriched) should be used. While it has been argued by some authors that a lack of stimulation for animals housed in laboratories may lead to increased variability within a group of animals (Garner 2005), others have proposed that increasing the

complexity of the environment results in an increase in variability (Weed & Raber 2005). Although these findings appear paradoxical, Würbel (2000) has suggested that 'standardization increases the risk of obtaining results that are idiosyncratic to a particular situation'. Therefore, in order to ensure the generalizability of results, it may be scientifically justified to include systematic variation of environmental conditions as part of the experimental design (Cao et al 2010).

Moreover, this new information brings into question the validity of some disease models. In the example of the cancer models above, there has been the suggestion that adoption of enriched housing, as a new standard, is needed to develop fully valid disease models. The rationale is that human patients have a stimulating environment, including socialization and occupational therapy, so translatability may be improved by providing the equivalents for research animals. This will require the establishment of a 'new' baseline for some studies and subsequent adjustment of associated models, a potentially time-consuming, but scientifically necessary task (Olsson & Dahlborn 2002). This approach could also result in experimental animals living with a milder form of the disease for an extended period of time, until the experimental endpoint is reached, which creates further animal welfare and indeed ethical considerations as to what is in the animals' best interests.

In addition, some recent studies have shown that enrichment strategies can also be used to establish earlier experimental endpoints for animal models. For example, a study of an HD mouse model determined that when the HD mice were housed in enriched cages, a decreased use of climbing resources (beam, rope and ladder) reliably preceded the development of clinical signs of disease (Litton et al 2008). These authors concluded that behavioural changes could be used as an early indicator of disease onset. Similarly, another study that used cages enriched with nest-building material determined that deficits in the performance of nest-building can be used as a measure of neurological dysfunction in a chemically-induced mouse model of Parkinson's disease (Sager et al 2010).

How to evaluate the overall impact of enrichment

The above discussion underlines the need to evaluate any enrichment strategies prior to introduction into a research paradigm. This is important to ensure that the enrichment strategy will be beneficial for the animals themselves, as well as understanding the impact of the proposed enrichment on research data.

From the perspective of animal welfare, it is important to ensure that any enrichment is scientifically valid for the species (Baumans et al 2011). This requires sound, scientifically-conducted studies, properly designed with appropriate numbers of animals and statistical analysis of results. These can include behavioural observation studies, with analysis of the time budget an animal gives to a particular enrichment device; preference testing, where the animal is asked to choose between different environments (for example a cage containing a shelter or one without); and motivational testing where the extent to which an animal is prepared to work to access the enriched environment or object is measured, as an indication of the importance of the resource to the animal. Although there have been suggestions that relying on animal preferences may not be the ideal indicator of what is of most value to animal well-being (Benefiel et al 2005), in general these approaches provide a good scientific basis for making informed decisions about what is relevant for an animal's welfare.

Some studies employing the synthesis of evidence approach (Korevaar et al 2011) have surveyed the literature for publications that report on the impact of enrichment for particular species. For example, Olsson & Dahlborn (2002) reviewed the effects of cage supplementation and produced five pages of comparison tables, resulting in the conclusion that nesting material was the most important improvement for mice. These syntheses will become increasingly important as we try to determine what actually matters to an animal.

It is similarly important to validate any environmental enrichment as part of a research protocol, as these refinements can potentially impact research outcomes (Patterson-Kane 2004). Unfortunately, there is currently limited published

continued on pag 32

information concerning the actual environmental conditions in which research animals are housed (Institute for Laboratory Animal Research & National Research Council (2011), see also Osborne 2012). This means that it can be difficult to replicate studies, and the impact of any husbandry refinements on the research data is unknown. As discussed above, the growing evidence that research results are quite dependent on the animals' environment requires that the conditions under which the research was carried out are well described. Where inclusion of enrichment is shown to have an impact on research results, the evaluation of the effects of the enrichment should also be carried out in a systematic manner so that the results can be published and used to establish new baselines. In addition, for some animal disease models, systematic reviews of available knowledge may assist in better targeting the enrichments and validations that will improve the model and improve the quality of data generated.

For both types of evaluation—benefit to animals and the effect on science—communication and teamwork is needed between all individuals involved in research studies, i.e. researcher, animal technologist and veterinarian, with advice/oversight by the ethics or animal care and use committee as appropriate.

Concluding statements

Housing laboratory animals in environments aimed at meeting species-specific needs is increasingly required by regulations and guidelines worldwide, setting new standards for laboratory animal welfare. In addition, there is much more interest in providing animals with more complex environments where they have the ability to exert some measure of control. These complex environments also aim to provide animals with positive experiences, thus improving their overall quality of life. However, before these are introduced into an experimental paradigm, it is vital to ensure that the proposed changes have a positive impact on the animals' well-being. As an animal's environment can have a profound impact on their physiological and psychological state, and therefore research results, it is important that housing and husbandry conditions are properly described in the literature. This is even more important when complex environments are provided, as there is the potential for change to current data on animal models of disease. The use of enriched environments offers the possibility of more robust animal models, and the ability to detect early onset of disease, and has the potential to improve both the quality of an animal's life as well as the quality of scientific data.

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Resources

BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement

Whenever animals are used in laboratories, minimizing pain and distress should be as important an objective as achieving the experimental results. This is important for humanitarian reasons, good science, economic reasons and satisfying broad legal principles.

The Joint Working Group on Refinement (JWGR) was convened by the British Veterinary Association Animal Welfare Foundation (BVAAWF), the Fund for the Replacement of Animals in Medical Experiments (FRAME), the Royal Society for the Prevention of Cruelty to Animals (RSPCA) and the Universities Federation for Animal Welfare (UFAW) to facilitate refinement by making up-to-date information on good practice available. The JWGR has a broad range of members, with representatives from science and industry, veterinarians and animal welfare bodies. With the goal of making significant reductions in the overall impact of research on animals, it has produced a series of comprehensive reports setting out good practice for a range of husbandry and care practices and experimental procedures, including husbandry refinements for mice, birds, animals in telemetry procedures, dogs and primates.

For further information, including downloads of some of the eleven reports in the JWGR series, see *http://www.rspca.org.uk/sciencegroup/researchanimals/implementing3rs/refinement*

JWGR reports were also used as a basis for many of the RSPCA's Good Practice Guidelines for laboratory animal housing and care, which aim to provide easy to use 'checklists' for members of ethical and animal care and use committees. These can be downloaded at

http://www.rspca.org.uk/sciencegroup/researchanimals/ethicalreview/housingandcare