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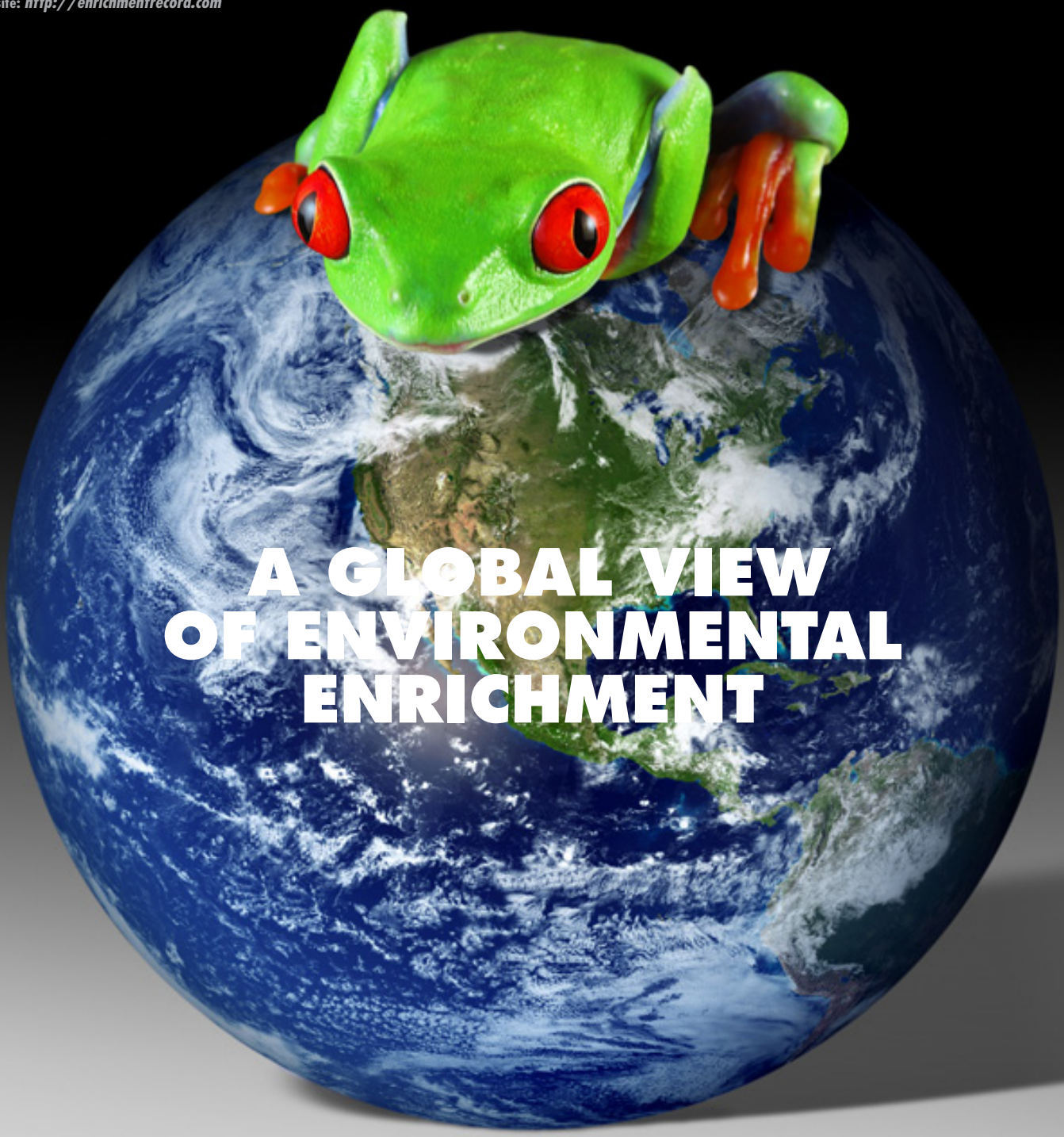

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In Other Words

Penny Hawkins

Research Animals Department
RSPCA, UK

GUEST EDITOR

I've been an avid reader and collector of ***The Enrichment Record*** from the first issue, and I was delighted when Jayne asked me to be the first guest editor. When it comes to promoting enrichment, I get very frustrated by the lack of awareness within the general scientific community of the growing body of literature on enrichment evaluation—and also by the way in which information about housing and care is often regarded as irrelevant, with nowhere near enough exchange of ideas or mention in publications.

But attitudes and awareness are both changing for the better, and ***The Enrichment Record*** does a fantastic job of helping to get the message out there in a way that is accessible to all. I want to use this issue to take the opportunity of having a look at some current issues and 'seizing the day' with respect to promoting enrichment.

This was prompted by the revision of two major documents that influence the way in which millions of laboratory animals are housed and cared for globally; the new European Union (EU) *Directive* (2010/63/EU) and the US Institute for Laboratory Animal Resources (ILAR) *Guide*, which were published in 2010 and 2011 respectively. Both of these include increased emphasis on providing enrichment that is species-appropriate and reflects current knowledge, as explained in the articles by Axel Kornerup Hansen and Dorte Bratbo Sørensen, of the University of Copenhagen, and by the Global Enrichment Committee at Abbott Laboratories, Illinois.

The revisions of the *Directive* and *Guide* should prompt facilities to review their provision of enrichment for all species, and reflect on what they currently provide and how this might be improved upon, and I believe that many will do just that. However, there has been a disappointing backlash from some organisations, which have objected

on the grounds that providing enrichment costs money and they are not convinced of the welfare benefits. My overall impression, on the basis of my involvement in the revision of the *Directive* and the high level of liaison that my organisation has with working scientists and animal technologists and care staff, is that most people do take the view that enrichment benefits animals and that this is worth investing in.

However, many assumptions are made about the impact of enrichment on both welfare and science, and it is of course essential to evaluate these, as discussed in the article by Gilly Griffin of the Canadian Council on Animal Care. Whether providing enrichment can actually help to reduce suffering experienced by animals undergoing procedures is also worth some serious consideration, and is a highly topical question in relation to the focus on the animal's lifetime experience in the new EU Directive. Christina Winnicker and Brianna Gaskill of Charles River have examined this issue for us.

Another topical subject included in this edition is enrichment for genetically altered (GA) mice, which is of major importance in relation to laboratory animal welfare because of the sheer number of animals involved—which is still increasing globally year-on-year[1]. Anne Fawcett of the University of Sydney, New South Wales, explains how enrichment can be tailored to different GA mouse lines with individual needs. Our other animal-specific article is on enrichment for cephalopods, whose use will be regulated EU-wide for the first time when the new Directive is implemented on 1 January 2013. Jennifer Mather, of the University of Lethbridge, explains how to give cephalopods a good quality of life.

Finally—and to come full circle to the beginning of this editorial—it's high time that information about enrichment was fully recognised as an essential component of the materials and methods section of papers, posters and talks. My RSPCA colleague Nikki Osborne gives us a snapshot of the status quo and outlines what improvements are needed to achieve better information sharing for all.

The articles in this edition of ***The Enrichment Record*** have come from people working in a wide range of different countries and contexts. All support the principle that carefully considered and appropriate enrichment promotes better welfare and therefore better science—and their articles illustrate that this is sufficiently important to be translated into legislation and regulatory guidelines. Yet adequate enrichment is still not universally applied. There are likely to be a number of reasons, and thus no one way to tackle this problem—a combination of legislation, advocacy and communication is likely to be required. Perhaps this would be another issue for ***The Enrichment Record*** to tackle!

I've really enjoyed my temporary editorship and I would like to thank all of the authors for agreeing so readily to write for this edition and for making it such a useful and thought-provoking read. We hope you enjoy it!

Penny Hawkins
Research Animals Department, RSPCA, UK

Penny Hawkins, BSc., Ph.D., **The Enrichment Record***'s first guest editor is Deputy Head of the Research Animals Department in the Science Group of The Royal Society for the Prevention of Cruelty to Animals (RSPCA)—the UK's leading animal welfare nonprofit organization. She works to promote refinements to improve animal housing and care—especially rodents and birds—and to assess the welfare of laboratory animals. Other key areas include refining procedures to reduce suffering, animal use in fundamental (basic biology) research, and the ethics of animal experimentation. She is a member of the Animal Procedures Committee (APC), the body that advises the secretary of state on the implementation of the UK Animals (Scientific Procedures) Act 1986. Penny has also been involved in the revision of the European guidelines for laboratory animal husbandry, and the development of the new regulations on animal use for EU Directive 2010/63/EU.*

[1]Zebrafish use is also on the rise—see the Spring 2012 edition of ***The Enrichment Record*** for an article on enrichment for zebrafish by Christian Lawrence.

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- Species specific enrichment
- Enrichment Observations
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- Human—animal relationship
- Choice and control
- Enrichment schedule development
- Implementation and Observation
- Practical exercises
- Group work

For more information and registration, please visit: *http://www.animalconcepts.eu/AnimalConcepts/Events/Entries/2012/9/3_Entry_1.html*

Enrichment & Behavior Forum
September 14-15, 2012
McKimmon Center for Extension & Continuing Education Building at North Carolina State University
1101 Gorman Street
Raleigh, NC 27606

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Highlights include:
- Improvements to the *Guide*— Better Behavioral Management
- Ethologically Relevant Enrichment for rodents, rabbits, and guinea pigs
- Classical Conditioning
- Nesting and Thermoregulation
- Humane handling to reduce fear responses in dogs
- A Better Way Than "The Scruff and Stretch"
- Corral: Natural Horsemanship—Conditioning
- Treating abnormal behavior in primates
- Physiologic and Behavioral Needs of the Laboratory Rabbit
- Behavioral Conditioning for Laboratory Animals
- Enrichment as Animal Welfare at the Conservators' Center

This meeting is approved for 13hrs of continuing education through the North Carolina Veterinary Medical Board.

For registration and information, please visit:
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2 Day: *https://www.123signup.com/register?id=svprt*

Meals are included with registration.
Questions: please contact
Sarah Wall at *sjwall@ncsu.edu*

Harmonisation of the Care and Use of Agricultural Animals in Research
September 26-28, 2012
Gardermoen Airport, Oslo, Norway

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- Update on changes in European legislation and international guidelines
- Positive welfare indicators
- Husbandry and environmental enrichment
- Humane endpoints
- Update on the latest research in comparative medicine
- Groupwork to produce a consensus statement on the way ahead, including research needs

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Sunday, November 4, 2012
(Satellite to National AALAS)
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- Developing an enrichment program
- Negative outcomes of Enrichment
- Social housing
- Behavioral conditioning
- Human-animal interaction and socialization
- Enrichment in a GLP environment
- Determining economic costs and benefits of enrichment strategies
- Regulatory considerations in enrichment programs

http://www.virtualvivarium.com/about-us/upcoming-events/environmental_Enrichment_Symposium.asp

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To facilitate informed discussion about environmental enrichment, we have joined the Linkedin Group called Laboratory Animal Sciences.

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EVENT _____

DATE_____

TIME_____

LOCATION_____

Meeting Announcement

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DATE OF EVENT _____

TIME OF EVENT_____

EVENT LOCATION_____

TYPE OF EVENT

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BRIEF DESCRIPTION OF THE EVENT_____

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Marcel Perret-Gentil, D.V.M., M.S.
University Veterinarian
Director, Laboratory Animal Resources Center
The University of Texas at San Antonio

Abbott Global Enrichment Committee: **Natalie Bratcher**, **Donna Clemons**, and **Leticia Medina**
Abbott Laboratories, Abbott Park, IL, USA

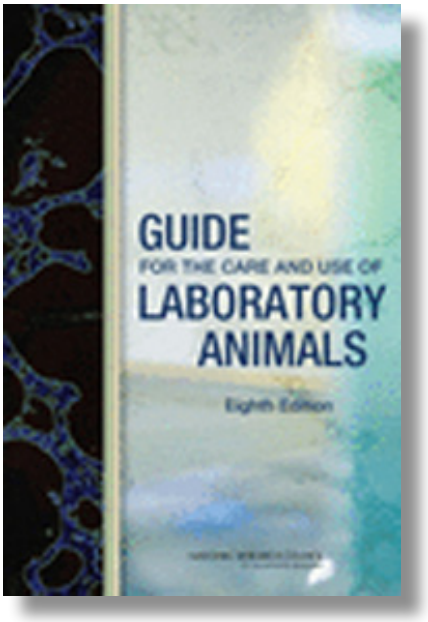
History of Enrichment in the ILAR *Guide for the Care and Use of Laboratory Animals*

Introduction

Throughout the nearly 50 year history of the Institute for Laboratory Animal Research (ILAR) *Guide*, little attention or content was allocated to the subject of enrichment until the current (8th) edition (NRC 2011). Although animal well-being, social environment, psychological well-being and behavioral adaptation have been mentioned in previous editions, guidance on catering for these was not provided. For instance, the 4th edition (1972) refers to physical comfort and well-being, and also to the potential for "psychological discomfort" due to confinement and/or lack of exercise, but enrichment was not specifically discussed in the *Guide* until the 7th edition (1996), where 4 index entries were devoted to the subject. In the 8th edition, however, there are 22 index entries and dozens of references on environmental enrichment, as well as many more in related areas such as socialization and behavior management.

The 8th edition

The goal of enrichment is, as plainly stated in the new *Guide*, to enhance animal well-being. Consensus holds that this is best accomplished through providing housing, social interaction, and/or opportunities for physical or cognitive activity that are stimulating, enable expression of instinctive species-specific behaviors, and do not inadvertently cause physical harm or undue levels of stress.



The *Guide* further instructs that the many species and strains of animals used in research will have varying needs which must be carefully and individually assessed while making judgments about the quality and applicability of enrichment options. Determining the needs of animals is acknowledged as an area where research is needed, and where current understandings may require future adjustment. One example offered in the *Guide* is the use of marbles for mouse enrichment; however, marble burying is an assay used in mice to measure anxiety via defensive burying. While a marble may appear to be enriching, based on the animal's interaction with the object, it may instead be detrimental to well-being.

The impact of enrichment devices and activities on research outcomes is acknowledged as an area in need of additional investigation. Enrichment, like other environmental factors, does appear in some cases to alter an animal's response to experimental treatments. A valid case is also made that lack of enrichment creates conditions of elevated stress reactivity and an abnormal response to experimental manipulation (see Griffin (2012) for additional discussion of these issues).

Effects of housing on well-being

The impact of housing on animal well-being has received increased attention in the 8th edition of the *Guide*. The potential impact of failing to meet animals' needs through appropriate housing and enrichment on both animal well-being and scientific validity is acknowledged with reference to abnormal brain development, physiologic dysfunction, and behavioral disorders. Special emphasis is placed on the importance of sufficient space and structural complexity allowing for escape from aggression, increased space requirements for breeding animals, and space quality with regard to usability (e.g. complex environments may increase activity and thus space requirements). New evidence is referenced to support the need for providing animals with

sufficient resources for thermoregulation, which can impact animal well-being and thus research outcomes. There is also a section dedicated to bedding and nesting materials with discussion and references supporting substrate preference and nest building behavior in rodents. While no specific bedding or nesting material is identified as being the ideal for all species, information and relevant references are provided about various bedding and nesting types and potential impact in various strains.

Social housing as enrichment

The 8th edition of the *Guide* places more emphasis on space allocations and group size, although it states that making absolute recommendations can be challenging because studies have evaluated a range of different factors relating to group size and housing conditions, and their differing impacts on behavior and experimental outcomes. Similar factors are considered for nonhuman primates and the *Guide* acknowledges that species-specific factors must be taken into consideration when determining group composition.

Both the benefits and potential detriment of social housing under some circumstances are more clearly defined in the 8th edition of the *Guide*, with 'an understanding of species-typical natural social behavior' recognized as key to successful social housing. There is also more detail on factors influencing social compatibility and/or incompatibility across species. The *Guide* provides the example of the potential for social stress in some strains of mice, if dominant animals protect shelters placed inside cages. This is an area where special attention needs to be given to the husbandry and enrichment program as a whole, to ensure that enrichment does not increase behaviors with negative consequences (e.g. fighting) in socially housed animals.

Single housing is referenced in regard to both housing and management, with special attention placed on provision of veterinary care, space, and enrichment for animals if it is necessary to singly house them for veterinary or justified scientific reasons. Single housing of social species is recognized as an exception and exposure to compatible conspecifics, or positive interaction with animal care staff, is recommended.

Exercise

The new *Guide* doesn't differ significantly from the previous edition with regard to discussions about exercise, but it is useful to review what it recommends. In our view, most laboratory animal programs in the US do an adequate job of providing exercise opportunities for dogs, especially when required by law, but exercise for other species is often lacking. Providing opportunities for increased animal activity or exercise is an important aspect of enriching animal housing environments and enhancing animal welfare. The *Guide* highlights that laboratory animals often live in restricted environments and suggests that animals' activity profiles and natural behaviors should be considered when evaluating suitable housing or assessing behavior. Lack of exercise can lead to muscle weakening, obesity and has also been implicated as a cause of stereotypic behaviors. Today, many new cage designs promote exercise of laboratory animals through pair or group housing. Current innovations include primate housing that interconnects for upward, sideways and downward mobility, dog pens, and hanging metal rings that can facilitate rodent exercise within home cages.

Human interaction

Human interaction is mentioned in the last two versions of the *Guide* as providing benefit to dogs, cats, rabbits and many other animals. Development of a socialization program for dogs, cats, rabbits, swine or other species is one way to promote positive human interaction. The benefits of such a program include enhanced socialization of the animals,

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increased exercise, easier animal handling, opportunities for positive reinforcement training and greater employee morale. For facilities that have adoption programs, increased socialization eases the transition from research to the 'forever' home environment.

Psychological well-being of the nonhuman primate

While not addressed in a separate section or chapter of the *Guide*, special requirements for the psychological well-being of these highly intelligent and complex animals are scattered throughout the book. In multiple areas, the benefits of training animals to be cooperative (e.g. with dosing), special housing requirements (e.g. vertical space, perches), behavioral enrichment through use of foraging, puzzle feeders, manipulation of objects, novelty, and social structure are addressed. The need to provide animals with choice, social interaction, avoidance of social conflict, variety, and mental stimulation is emphasized.

Oversight of enrichment

Responsibility for oversight of an environmental enrichment program is addressed for the first time in the 8th edition of the *Guide*. It clarifies that enrichment programs should be reviewed by three different groups of stakeholders including the IACUC, researchers and veterinarians. This approach to oversight recognizes environmental enrichment as an important aspect of a quality animal care and use program. The attending veterinarian and IACUC are charged to assure that the enrichment program appropriately promotes animal well-being. Likewise, researchers must assure that the enrichment provided is consistent with the goals of their research. The *Guide* emphasizes that enrichment can act as an independent variable affecting animal phenotype and possibly experimental outcome, and therefore must be appropriately controlled. This team approach ensures that enrichment is part of a well-designed strategy and is carefully managed.

Although record keeping of enrichment is not specifically addressed in the new *Guide*, it does imply that documentation should exist to facilitate review by the various stakeholders. Some current approaches include developing an Ethics Committee guideline or overview about the enrichment program and how it is managed, or committing the program to specific Standard Operating Procedures (SOPs) for GLP study environments. The *Guide* also clarifies that the program should be updated as needed to reflect current knowledge. Although not specifically stated in the *Guide*, a good practice may be to include a biannual update in the semiannual program review.

Conclusion

With the recent update to the *Guide,* there was some controversy about several of the changes, such as the cost of adopting and implementing new cage sizes for breeding rodents. There was valid concern about the lack of available scientific evidence to prove that the larger cage sizes would provide any real benefit to the rodents. The biomedical and animal welfare advocacy communities must proceed with extreme caution in proposing new guidelines to ensure that they are based on scientific evidence whenever possible. This should further stimulate the scientific community to lead the efforts in providing the scientific data to support acceptable care and use standards for the various laboratory animal species rather than recommendations based on hypothetical grounds alone. Notably, over the past several decades, there have been a growing number of scientific publications about refining the way we care for and work with laboratory animals, providing clear evidence that animal welfare has been increasingly recognized as essential to good science and good management. It naturally follows that given this change, environmental and behavioral enrichment are more strongly emphasized throughout the 8th edition of the *Guide*. As we learn more about the various laboratory animal species, we believe that the way we care for laboratory animals will continue to evolve towards adoption of more naturalistic environments that promote species-appropriate behaviors, and with a better understanding of variability from environmental factors and behavioral stressors. Development of enrichment strategies based on sound scientific data will ensure that enrichment does not become a deterrent to good science and actually promotes better science and animal welfare.

References

Griffin, G. (2012). The Three Es—Evaluating Enrichment is Essential. *Enrichment Record* July 2012.

National Research Council (2011). *Guide for the Care and Use of Laboratory Animals* (8th edn). National Academies Press, Washington, DC.

Article | **Dorte Brabo Sørensen** and **Axel Kornerup Hansen**

Section of Biomedicine, Department of Veterinary Disease Biology,
Faculty of Health & Medical Sciences, University of Copenhagen, Denmark

Drivers for Enrichment in Directive 2010/63/EU

Introduction

The revised European Union (EU) Directive regulating the care and use of animals for scientific purposes was adopted in 2010 and must be transposed into the national law of each EU Member State on 1 January 2013 (European Commission 2010). Like the previous Directive from 1986, the new Directive 2010/63/EU sets out minimum standards for housing and care, but a new development in the legislation is the incorporation of many enrichment initiatives and actions as 'must's and 'shall's instead of the previous 'could's or 'should's.

From an animal welfare point of view this seems—at first glance—to be quite an improvement, as enrichment is recognised as a mandatory need, not a choice. However, even though the legislation has been tightened with the aim of ensuring a more animal welfare focused treatment of our experimental animals, how effectively this is implemented will depend largely on the people responsible for the animals at the user, supplier and breeding establishments. It is our joint responsibility to read the new Directive with an open mind and a will to apply the spirit, as well as the letter, of the law. Undertaking animal experimentation calls for a strict and clear logic, but empathy with the animals and a sincere will to optimize their welfare are also essential.

This paper will focus on the intentions of the Directive and possible interpretations, offering suggestions for areas to focus on when applying its requirements for enrichment. Even though there is much emphasis on animal welfare and the Three Rs throughout the Directive, we will focus on Article 33, on 'care and accommodation', and on Annex III which sets out requirements for animal housing and husbandry. In these sections, concepts like the environment of the animals, their behavioural needs and enrichment are explicitly mentioned.

Care, accommodation and needs of the animal

The wording of Article 33 is very similar to the previous Directive, requiring Member States to *'ensure that…all animals are provided with accommodation, an environment, food, water and care which are appropriate to their health and well-being' and 'any restrictions on the extent to which an animal can satisfy its physiological and ethological needs are kept to a minimum'*. It is important to note that the requirements for appropriate accommodation are general for all species within the scope of the Directive 2010/63, i.e. all vertebrates and cephalopods, which are the species covered. The legislation ascribes equal rights to all, so those species such as dogs, cats and primates that have been considered 'higher animals' are no more important than rodents when providing enrichment. Otherwise, this paragraph is unspecific with respect to what enrichment might comprise.

More detail on housing and enrichment is provided in Annex III and—as with Article 33—the requirements in its general introduction concern all animal species within the scope of the legislation. Annex III differs significantly from the previous Directive in two important ways with respect to enrichment. First, it goes into more detail about what enrichment aims to achieve; second, it uses the term 'shall' rather than 'should'. For example, Annex III Section A requires that *'All facilities shall be constructed so as to provide an environment which takes into account the physiological and ethological needs of the species kept in them'*. The phrase 'ethological

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needs' should encompass the need to perform certain behaviours, even if the functional consequences of a behaviour are already met (for example, hens perform nest-building behaviour sequences during the pre-laying period, even if a previously-built nest is still available). Failure to meet ethological needs may not result in death or decreased reproduction; nevertheless, the animal may suffer as there is a strong internal motivation to perform these behaviours (Hughes & Duncan 1988).

The Annex also states that '*All animals shall be provided with space of sufficient complexity to allow expression of a wide range of normal behaviour*'. This sounds good, but the requirement is open for free interpretation as to the level at which the cages, pens or tanks of the animals can be enriched. How wide is 'wide'? Should we provide caging high enough for rats to rear upright? Should we provide burrowing opportunities for gerbils? Should grazing be an integral part of the daily lives of all ruminant lab animals? And should foraging for food be reinforced by providing suitable substrates with hidden food, e.g. for rooting behaviour in pigs and scratching and pecking behaviour in chickens?

Further guidance on questions such as these is forthcoming from the requirements that: '*Establishments shall have appropriate enrichment techniques in place, to extend the range of activities available to the animals and increase their coping activities including physical exercise, foraging, manipulative and cognitive activities, as appropriate to the species' and animals 'shall be given a degree of control and choice over their environment to reduce stress-induced behaviour. Environmental enrichment in animal enclosures shall be adapted to the species and individual needs of the animals concerned*'. In light of these statements, it seems reasonable to conclude that the kinds of behavioural needs listed above should be catered to when possible. Although there will unfortunately be circumstances when this is not possible[1], some natural behaviours can be facilitated fairly easily. For example, providing foraging activities for animals such as rodents is straightforward, since adding seeds and grains to the clean litter when changing rodents' cages stimulates foraging behaviour, but this is often still not done. Foraging opportunities for rodents can be developed further by using modified 'diet boards' filled with preferred and palatable food that the animals must actively gnaw to obtain, which fulfils natural behaviours without excessive food intake (Kasanen et al. 2009a, 2009b).

Clicker training a pig

In reality, the emphasis on control and choice encapsulates two kinds of enrichment: environmental enrichment and cognitive enrichment (Mantueffel et al. 2009a, Puppe et al. 2007). The term 'environmental enrichment' is traditionally used with reference to features or stimuli in the environment that add complexity, thus allowing or promoting natural behaviour such as foraging and nest building. However, 'cognitive enrichment' relates to environmental features or stimuli that can stimulate perceptive and cognitive processes for operant learning of discriminatory cues, which leads to a better active control of the environment and a positive anticipatory mental state (Mantueffel et al. 2009a). In this way, animals can increase their

cognitive activities and experience control and choice, all of which is likely to lead to better welfare.

It could be argued that simply providing food *ad libitum* also induces control and choice, as the animals are able to decide when to eat. However, if control and choice are to be welfare-enhancers and reduce stress, we need to provide different stimuli to choose from, different situations to choose between, and situations that can be controlled by the animals. Being in control actually means that the animal possesses knowledge of the consequences of his actions, and the lack of such knowledge has been demonstrated to lead to a high level of distress (Mantueffel et al. 2009b).

One way to provide control, choice and knowledge of consequences is to introduce sessions with positive reinforcement training, where animals—by interacting with the animal trainer—can obtain rewards (e.g. food) by performing behaviours asked for by the trainer. In a positive reinforcement training set-up, the animal chooses to either participate or withdraw from the training; moreover, the training *per se* is cognitively stimulating as the animal needs to learn and remember which actions on which cues will lead to rewards (Sørensen 2010). When done correctly, positive reinforcement training also induces a state of anticipation, which has been demonstrated to counteract the effects of social stress in rats and enhance playfulness and reduce aggression in pigs (van der Harst et al. 2005, Dudink et al. 2006).

Finally, Annex III requires that '*enrichment strategies in establishments shall be regularly reviewed and updated*'. It does not say who would actually do this, but Directive Article 27 states that the Animal Welfare Body[2] should '*establish and review internal operational processes as regards monitoring, reporting and follow-up in relation to the welfare of animals housed or used in the establishment*'. Reviewing and updating enrichment strategies must clearly be seen to fall within this task. What is meant by 'regularly' is also not defined, but at least this term provides a tool for the authorities to ensure that enrichment in the animal facilities is based upon current knowledge and is included in project evaluation.

Conclusion

The revised Directive emphasizes the provision of a stimulating and welfare-promoting environment for our laboratory animals, making environmental and cognitive enrichment mandatory. By increasing the emphasis on and explicit reference to enrichment in the new Directive, the European Commission has shown us the right direction. But the actions in practice, the degree of enrichment and the specific items needed for each species are very vague and wide open for interpretation. Basically it is up to us—the researchers, the designated veterinarians, the Animal Welfare Body and the animal technologists and care staff—to keep on working to continuously increase the level and quality of enrichment.

[1]It may also be undesirable; for example high levels of aggression in males of some species may be 'natural' but can also cause serious health and welfare problems in laboratory housing.

[2]A body required under the Directive to carry out certain tasks relating to the application of the Three Rs, animal welfare and facility management at a local level, complementing the central implementation of the national legislation by the competent authority.

References

European Commission (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Union* L276: 33-79.

Hughes, B.O. and Duncan, I.J.H. (1988). The notion of ethological 'need', models of motivation and animal welfare. *Animal Behaviour* 36: 1696-1707.

Kasanen, I.H.E., Inhilä, K.J., Nevalainen, J.I., Väisänen, S.B., Mertanen, A.M.O., Mering, S.M. and Nevalainen, T.O. (2009a). A novel dietary restriction method for group-housed rats: weight gain and clinical chemistry characterization. *Laboratory Animals* 43: 138-148.

Kasanen, I.H.E., Inhilä, K.J., Vainio, O.M., Kiviniemi, V.V., Hau, J., Scheinin, M., Mering, S.M. and Nevalainen, T.O. (2009b). The diet board: welfare impacts of a novel method of dietary restriction in laboratory rats. *Laboratory Animals* 43: 215-223.

Manteuffel, G., Langbein, J. and Puppe, B (2009a). From operant learning to cognitive enrichment in farm animal housing: bases and applicability. *Animal Welfare* 18: 87-95.

Manteuffel, G., Langbein, J., and Puppe, B. (2009b). Increasing farm animal welfare by positively motivated instrumental behaviour. *Applied Animal Behaviour Science* 118: 191-198.

Puppe, B., Ernst, K., Schön, P.C., and Manteuffel, G. (2007). Cognitive enrichment affects behavioural reactivity in domestic pigs. *Applied Animal Behaviour Science* 105: 75-86.

Sørensen, D.B. (2010). Never wrestle with a pig...*Laboratory Animals* 44: 159-161.

Dudink, S., Simonse, H., Marks, I., de Jonge, F.H., and Spruijt, B.M. (2006). Announcing the arrival of enrichment increases play behaviour and reduces weaning-stress-induced behaviours of piglets directly after weaning. *Applied Animal Behaviour Science* 101: 86-101.

van der Harst, J.E., Baars, A.M., and Spruijt, B.M. (2005). Announced rewards counteract the impairment of anticipatory behaviour in socially stressed rats. *Behavioural Brain Research* 161: 183-189.

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Using Enrichment to Improve Welfare and Reduce Suffering

European Directive 2010/63/EU requires that accommodation and care are refined so as to minimize suffering, and that the lifetime experience of the animal is taken into account when assessing the actual severity of procedures. So, with respect to these requirements, how does one go about assessing 'lifetime suffering' and can we use enrichment to reduce it? First, let's consider suffering. Suffering has been defined as 'a negative emotional state derived from adverse physical, physiological and psychological circumstances' (Morton & Hau 2002). The term 'suffering' is typically associated with pain, and implies a longer-lived, chronic, or relentless suppression of physiologic or behavioral stability or fulfillment. Procedures which induce pain, such as surgery, are addressed with medications: anesthesia and analgesia. Depending on the procedure performed, this pain may be short lived, but it is doubtful that anyone would argue that these acute negative experiences are less important to alleviate than chronic ones. It is perhaps best to assume that any negative alteration in animal welfare may constitute some degree of suffering. Therefore, we plan to address any negative alteration in animal welfare in this article. Alterations in animal welfare are generally assessed based on three concepts: biological functioning or physiological alterations;

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natural living or allowing animals to perform natural behaviors; and subjective experiences or positive or negative affective states (Fraser et al 1997, 2000).

Biological functioning:
Any alteration from normal biologic function can be considered an indicator of a change in welfare (Duncan & Fraser 1997). That is, any animal that has disease, or does not reproduce or grow well, potentially has poor welfare. Certainly, a state of poor health, if left unresolved, could be considered to cause suffering. The state of health of research animals is not only a moral concern but can also affect the validity of the research model. Environmental enrichment can certainly have an effect on an animal's overall health. For example, an environment enriched with a variety of structural and manipulatable materials has been shown to decrease tumor development in mouse models of melanoma and colon cancer (Cao et al 2010). At first glance, this may appear discouraging for the implementation of enrichment in tumor development models. However, further investigation has shown that the cause of the decrease is due to upregulation in brain derived neurotropic factor, (BNDF), potentially opening the door to development of treatment strategies involving this pathway (Cao et al 2010). Other studies have shown increased immune function and survival after immune challenge in socially and physically enriched environments (Schapiro et al 2000, Schapiro 2002, Benaroya-Milshtein et al 2007). This evidence suggests that environments deprived of social interaction or enrichments may decrease immune responses, interfering with vaccine testing, immunotherapy work, and other immune-mediated disease models.

Environmental enrichment has also been linked to improved wound healing (Vitalo et al 2012) and improved recovery from spinal cord injury (Berrocal et al 2007). Reduction in the amount of time needed to heal from these tissue damage models is likely to reduce the cumulative suffering the animals may experience. In addition to

cancer, immunity, and wound healing, enriched environments have shown effects on heart rate (HR) and mean arterial blood pressure (MAP). Socially housed rats had resting HR and MAP values consistently lower than their individually housed counterparts, as well as significantly lower HR and MAP increases in response to acute husbandry and experimental procedures (Sharp et al 2002).

Alleviating behavioral frustration:

Our anthropocentric view of the world means that we find it easiest to identify pain or tissue damage as suffering. What about the less obvious scenarios? Is it possible for animals to suffer if prevented from achieving a goal they are motivated to accomplish? While we acknowledge that the definition of suffering can be subjective, and perhaps the use of the word 'suffering' may not be appropriate in this instance, we can still explore the potential use of enrichment to alleviate behavioral frustration. The level of suffering experienced from behavioral frustration may well be less than that experienced from a painful procedure. However, frustration is more likely to endure for longer than the time it takes a wound to heal. Behavioral frustration is also not as easily measured as changes in physiology, but with behavioral paradigms and new methods of measuring motivation, the animal's drive to complete these goals can be quantified and compared. Enrichment is an obvious tool that can be used to alleviate some types of behavioral frustration. Some might argue that animals not living in a natural environment, even if that environment comes with risk and struggle, are suffering. Whether you consider the everyday life of an animal in the laboratory to constitute suffering or not, certainly life in the lab comes with inherent stressors that we have a moral obligation to attempt to alleviate through refinements in techniques, care, and husbandry.

Laboratories often do not allow for control over stressors present in artificial environments. Behavior, particularly in highly adaptable species like mammals, allows animals to control parts of their environment to improve survival in the wild. Mice, for instance, respond to cold with thermotaxis (locomotion away from stressful temperatures), and with huddling and nest building (Gordon 1993, Latham & Mason 2004). However, in the research environment, relocation is unlikely, huddling may be limited or insufficient depending on the number of mice in the cage, and nest building may be ineffective if proper nesting materials are not provided (Gordon 1993, Hess et al 2008, Gaskill et al 2012). Recommended laboratory temperatures are below the mouse's lower critical temperature of 30 ^{o}C (Gordon 1993) and mice of different ages, strains, and sexes prefer different temperatures at different times of day (Ogilvie & Stinson 1966, Eedy & Ogilvie 1970, Gordon et al 1998, Gaskill et al 2009, 2011, 2012). When provided structurally appropriate nesting material, mice will build nests of varying quality depending on their thermal needs (Gordon 1993, Gaskill et al 2011, 2012). In addition, nests provide cover and protection from predators. The absence of a retreat space has been shown to be stressful in various species (Morgan & Tromberg 2007). Thus, the drive to build and maintain a nest is a basic and constant behavioral need for mice, driven by metabolic and behavioral motivations. The provision of materials that allow mice to build a structurally sound nest can potentially alleviate that behavioral frustration, plus allow the animals to behaviorally thermoregulate, reducing cold stress. Providing mice with control over these stressors has been associated with better reproductive performance and improved feed conversion, as well as likely resulting in improved welfare (Gaskill et al 2011).

Chronic and uncontrollable stressors in an animal's environment (whether physical or psychological) can lead to undesirable behaviors such as stereotypies (Mason & Latham 2004, Frasier 2008). The occurrence of stereotypic behavior is often associated with poor welfare and these animals have neurostructural and functional differences from their non-stereotyping counterparts (Garner & Mason 2002). Stereotypic digging in gerbils has been hypothesized to be due to the drive to create a burrow. However, when the right combination of enrichments is provided, such as a tunnel with an end chamber, stereotypic digging is significantly reduced (Wiedenmayer 1997). Thus, in addition to this enrichment

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potentially alleviating behavioral frustration and preventing an abnormal behavior, it may result in neurologically and behaviorally normal, or more stable, animal models. While this may seem most important for neurodevelopmental, neuroanatomy and behavioral models, work like that of Cao et al (2010), showing the effects of BDNF on tumor models, may suggest more wide-reaching effects. Thus alleviation of behavioral frustration, or behavioral suffering, may improve the cumulative welfare of the animals as well as the validity of the science they are used for.

Subjective experiences:

Subjective experiences are even more difficult to measure and assess than behavioral frustration. Therefore, the measurement of affective state, anthropomorphically referred to as feelings or emotions, is a developing area of research. Could social housing or environmental enrichment contribute to the alleviation of either acute or chronic pain (both of which cause negative affective states)? In recent years, a growing body of literature on this subject has supported the 'pain decreasing' effect of enriched environments (Gabriel et al 2010a and b, Pham et al 2010). The ability to decrease the need for or amount of analgesic medication (Pham et al 2010), or to provide analgesic effect through attentional shift (Gental & Tilston 1999) would be a refinement that decreases animal pain in research paradigms where analgesics might interfere with research parameters without interfering with the science—and the approach could be used to augment the effects of analgesics where these are compatible with the protocol. This type of enrichment intervention could also be used for the alleviation of pain in inflammatory induced models where anti-inflammatories may interfere with the mechanism of model creation.

The belief that most animals can experience fear is widely accepted and the alleviation of this negative affective state is built into the 5 freedoms: the freedom from hunger and thirst; the freedom from discomfort; the freedom from pain, injury or disease; the freedom to express normal behavior, and the freedom from fear and distress (Brambell Committee 1965). We believe animals can experience fear, but assessing fear and other less widely accepted affective states, let alone determining if enrichment can improve them, is difficult. However, determining affective states, whether positive or negative, is quickly becoming a hot topic in animal welfare science. One study found that mice in enriched environments self-administer lower doses of anxiolytic drugs than mice in standard laboratory cages (Sherwin & Olsson 2004). This simple measure suggests that animals in unenriched environments are more anxious, and therefore self-administer a drug to reduce that negative state. Another clever behavioral paradigm, that essentially asks animals if a glass is half empty or half full, found that animals in enriched environments are more likely to view a neutral stimulus as positive (Burman et al 2008, Mendl et al 2009). The unenriched animals react to the same stimulus with a negative response, in essence viewing the situation as half empty. Additional evidence that animals can experience positive affective states comes from vocalizations in rats (Burgdorf et al 2008, 2011,Webber et al 2012); for example, 50 khz vocalizations are generally elicited during play between conspecifics, mating, or even when being tickled by humans (Burgdorf & Panksepp 2001, Burgdorf et al 2008, Cloutier & Newberry 2008). If we can measure positive affective states, we can determine the overall psychological well-being of captive animals and move toward actively improving their quality of life.

Conclusions

Enrichments appear to improve welfare through improved biological functioning, the opportunity to achieve behavioral goals, and may also improve overall affective state. We're past the point where it's just an assumption that an enriched environment is better than an unenriched one, and we doubt that anyone would argue that enrichment, when ethologically sound, is anything but good for an animal's welfare. Irrespective of where we draw the line between frustration and suffering, improving biological function, alleviating behavioral frustration, and an improved affective state are all desirable goals.

Enrichment is an environmental alteration: depending on what is being studied and what enrichment is employed, there is a definite possibility of affecting results. We would argue, however, that this shouldn't be justification for the elimination of the provision of enrichment. The question is: will it affect the results for the better? More stable physiology, normal

neurologic development, displays of normal behavior, and an improved affective state should be a win-win for science and animal welfare.

References

Benaroya-Milshtein, N. et al. (2007). Environmental enrichment augments the efficacy of idiotype vaccination for B-cell lymphoma. J. *Immunother*. 30: 517-522.

Berrocal, Y. et al. (2007). Social and environmental enrichment improves sensory and motor recovery after severe contusive spinal cord injury in the rat. J. *Neurotrauma* 24: 1761-1772, doi:10.1089/neu.2007.0327

Brambell Committee. (1965). *Report of the Technical Committee to Enquire into the Welfare of Animals Kept Under Intensive Livestock Husbandry Systems*. London, Her Majesty's Stationery Office.

Burgdorf, J. et al. (2008). Ultrasonic vocalizations of rats (Rattus norvegicus) during mating, play, and aggression: behavioral concomitants, relationship to reward, and self-administration of playback. J. *Comp. Psychol.* 122: 357-367, doi:10.1037/a0012889

Burgdorf, J. and Panksepp, J. (2001). Tickling induces reward in adolescent rats. *Physiol. Behav.* 72: 167-173, doi:10.1016/s0031-9384(00)00411-x

Burgdorf, J., Panksepp, J. and Moskal, J. R. (2011). Frequency-modulated 50 kHz ultrasonic vocalizations: a tool for uncovering the molecular substrates of positive affect. *Neurosci. Biobehav. Rev.* 35: 1831-1836, doi:10.1016/j.neubiorev.2010.11.011

Burman, O. H. P., Parker, R., Paul, E. S. and Mendl, M. (2008). A spatial judgement task to determine background emotional state in laboratory rats, Rattus norvegicus. *Animal Behavior* 76: 801-809.

Cao, L. et al. (2010). Environmental and Genetic Activation of a Brain-Adipocyte BDNF/Leptin Axis Causes Cancer Remission and Inhibition. Cell 142: 52-64, doi:10.1016/j.cell.2010.05.029

Cloutier, S. and Newberry, R. C. (2008). Use of a conditioning technique to reduce stress associated with repeated intra-peritoneal injections in laboratory rats. *Appl. Anim. Behav. Sci.* 112: 158-173.

Duncan, I.J.H. and Fraser, D. (1997). Understanding animal welfare. Chapter 2 in *Animal Welfare* (eds M. C. Appleby and B. O. Hughes). Oxford, CAB International.

Eedy, J. W. & Ogilvie, D. M. The effect of age on the thermal preference of white mice Mus-musculus and gerbils Meriones-unguiculatus. Can. J. Zool. 48, 1303-1306 (1970).

Fraser, D. (2008). *Understanding Animal Welfare: The Science in its Cultural Context*. Oxford, Wiley-Blackwell.

Fraser, D., Jasper, J. and Weary, D. M. (2000). Environmental enrichment to improve animal welfare: goals, methods, and measures of success. In Progress in the Reduction, Refinement and Replacement of Animal Experimentation Vol. 31B *Developments in Animal and Veterinary Sciences* (eds M. Balls, A. M. VanZeller and M. E. Halder), pp 1283-1293.

Fraser, D., Weary, D. M., Pajor, E. A. and Milligan, B. N. (1997). A scientific conception of animal welfare that reflects ethical concerns. *Animal Welfare* 6: 187-205.

Gabriel, A. F. et al. (2010a). Enriched environment and the recovery from inflammatory pain: Social versus physical aspects and their interaction. *Behavioural Brain Research* 208: 90-95.

Gabriel, A. F., Marcus, M. A. E., Honig, W. M. M. and Joosten, E. A. J. (2010b). Preoperative housing in an enriched environment significantly reduces the duration of post-operative pain in a rat model of knee inflammation. *Neurosci. Lett.* 469: 219-223, doi:10.1016/j.neulet.2009.11.078

Garner, J. P. and Mason, G. J. (2002). Evidence for a relationship between cage stereotypies and behavioural disinhibition in laboratory rodents. *Behavioural Brain Research* 136: 83-92.

Gaskill, B. N. et al. (2012). Heat or insulation: behavioral titration of mouse preference for warmth or access to a nest. *PLoS ONE* 7: e32799.

Gaskill, B. N., Lucas, J. R., Pajor, E. A. and Garner, J. P. (2011a). Working with what you've got: Changes in thermal preference and behavior in mice with or without nesting material. *J. Therm. Biol.* 36: 1193-1199, doi:10.1016/j.jtherbio.2011.02.004

Gaskill, B. N., Garner, J. P. and Pritchett-Corning, K. R. (2011b). Energy reallocation to breeding performance through improved behavioral thermoregulation. J. *Am. Assoc. Lab. Anim. Sci.* 50: 771

Gaskill, B. N., Rohr, S. A., Pajor, E. A., Lucas, J. R. and Garner, J. P. (2009). Some like it hot: mouse temperature preferences in laboratory housing. *Appl. Anim. Behav. Sci.* 116: 279-285.

Gentle, M. J. and Tilston, V. L. (1999). Reduction in peripheral inflammation by changes in attention. *Physiol. Behav.* 66: 289-292, doi:10.1016/s0031-9384(98)00297-2

Gordon, C. J. (1993). *Temperature Regulation in Laboratory Rodents.* Cambridge, Cambridge University Press.

Gordon, C. J., Becker, P. and Ali, J. S. Behavioral thermoregulatory responses of single-and group-housed mice. *Physiol. Behav.* 65: 255-262.

Hess, S. E. et al. (2008). Home improvement: C57BL/6J mice given more naturalistic nesting materials make better nests. J. *Am. Assoc. Lab. Anim. Sci.* 47: 25-31.

Latham, N. and Mason, G. (2004). From house mouse to mouse house: The behavioural biology of free-living Mus musculus and its implications in the laboratory. A*ppl. Anim. Behav. Sci.* 86: 261-289.

Mason, G. J. and Latham, N. R. (2004). Can't stop, won't stop: is stereotypy a reliable animal welfare indicator? *Animal Welfare* 13: 57-69.

Mendl, M., Burman, O. H. P., Parker, R. M. A. and Paul, E. S. (2009). Cognitive bias as an indicator of animal emotion and welfare: Emerging evidence and underlying mechanisms. *Applied Animal Behaviour Science* 118: 161-181.

Morgan, K. N. and Tromborg, C. T. (2007). Sources of stress in captivity. *Appl. Anim. Behav. Sci.* 102: 262-302, doi:10.1016/j.applanim.2006.05.032

Morton, D.B. and Hau, J. (2002). Welfare assessment and humane endpoints. In: *Handbook of Laboratory Animal Science: Essential Principles and Practices, Vol 1* (2nd edn) (eds J. Hau and G.L. Hoosier). Seattle, WA: CRC Press, pp 457-486.

Ogilvie, D. M. and Stinson, R. H. (1966). Effect of age on temperature selection by laboratory mice (*Mus musculus*). *Can. J. Zool*. 44: 511-517.

Pham, T. M. et al. (2010). Housing environment influences the need for pain relief during post-operative recovery in mice. *Physiol. Behav.* 99: 663-668, doi:10.1016/j.physbeh.2010.01.038

Schapiro, S. J. (2002). Effects of social manipulations and environmental enrichment on behavior and cell-mediated immune responses in rhesus macaques. *Pharmacology Biochemistry and Behavior* 73: 271-278, doi:10.1016/s0091-3057(02)00779-7

Schapiro, S. J., Nehete, P. N., Perlman, J. E. and Sastry, K. J. (2000). A comparison of cell-mediated immune responses in rhesus macaques housed singly, in pairs, or in groups. *Appl. Anim. Behav. Sci.* 68: 67-84, doi:10.1016/s0168-1591(00)00090-3

Sharp, J. L., Zammit, T. G., Azar, T. A. and Lawson, D. M. (2002). Stress-like responses to common procedures in male rats housed alone or with other rats. Contemp. Topics Lab. *Anim. Sci.* 41: 8-14.

Sherwin, C. M. and Olsson, I. A. S. (2004). Housing conditions affect self-administration of anxiolytic by laboratory mice. *Animal Welfare* 13: 33-38.

Vitalo, A. G. et al. (2012). Environmental enrichment with nesting material accelerates wound healing in isolation-reared rats. *Behavioural Brain Research* 226: 606-612.

Webber, E. S. et al. (2012). Selective breeding for 50 kHz ultrasonic vocalization emission produces alterations in the ontogeny and regulation of rough-and-tumble play. *Behavioural Brain Research* 229: 138-144, doi:10.1016/j.bbr.2012.01.012

Wiedenmayer, C. (1997). Causation of the ontogenetic development of stereotypic digging in gerbils. *Anim. Behav*. 53: 461-470, doi:10.1006/anbe.1996.0296

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Tailoring Enrichment to GA Mice

Introduction

Philosopher and ethicist Professor Bernard Rollin recently wrote that animal researchers should "do the utmost to minimise animal suffering arising in the course of animal research, and maximise the animal's ability to live a life where the interests and needs flowing from its biological and psychological nature are respected" (Rollin 2012). But in the case of genetically altered (GA) mice, biological and psychological nature—or phenotype—may be highly variable. In such cases, flexibility, creativity and information-sharing are required to tailor environmental enrichment (EE) such that the basic needs of these animals are satisfied while ensuring the minimal number of animals is used.

Genetic alteration may adversely affect phenotype

An increasing number of GA mice—including transgenic mice, knock-out and knock-in mice, chimeras and clones—are used in laboratories worldwide. Ethical and welfare issues arise not only due to the use and husbandry of these mice, but also in the creation and breeding of particular strains (Government 2007, Brown & Murray 2006, Fawcett 2012). For example, more animals may be required to create and maintain each GA line, prompting a high rate of wastage (Buehr et al. 2003). On the other hand, by targeting particular aspects of a medical condition using

GA mice, researchers may require fewer animals in a study (Brown & Murray 2006) or be able to reduce severity.

It is important to note that being genetically altered does not necessarily adversely impact the welfare of an individual animal in itself, but the impact of genetic alteration on the animal's phenotype may cause welfare problems due to the expression of modified or deleted genes, the position of a modified gene on the genome, interactions between gene products, disruption of normal physiological processes or a poor fit between the new strain and its environment (Wells et al. 2006). These could be manifested as a physical disability or disorder, behavioural problems such as anxiety, altered social behaviours or a combination of all of these.

For example, in one study which looked at 87 GA mouse strains, 36 per cent experienced discomfort (Thon et al. 2002). For 21 per cent, this was classified as minor (mice with increased aggression, lymphoma or a weakened immune response), while 15 per cent experienced severe discomfort (cystic fibrosis, diabetes, seizures, malformation of the skull or rectal prolapse). In addition to this, 30 per cent of strains had increased mortality, disease incidence and susceptibility to disease.

Genetic alteration may also lead to changes in emotionality, anxiety and predisposition to psychological stress (Cook et al. 2002), all of which may adversely affect welfare. For example, genetic alteration may lead to an aggressive phenotype (Miczek et al. 2001), which is stressful to victims and may necessitate individual housing of mice. Some strains are more likely to develop stereotypies than others (Mason 2006), and may be more likely to do so in a particular environment. To ensure that appropriate steps are taken to minimise suffering, it is vital that investigators understand the impact of genetic alteration on mice and explore all

possible ways to reduce this, including tailored husbandry refinements. This is particularly challenging when little is known about a new GA strain. Where possible, investigators should familiarise themselves with all available data on strain-specific and transgene-mediated health conditions so they can be addressed in a timely manner.

Tailoring EE to GA phenotype

Importantly, phenotype affects husbandry requirements—often dramatically so. It has been hypothesised that mouse behaviour is simpler and less flexible than rat behaviour (Whishaw et al. 2001). The upshot of this difference between mice and rats is that alterations in genotype and phenotype may alter behaviour radically between mouse strains. So while it is accepted that enrichment is provided to encourage and maintain normal, species-specific behaviour, this is complicated by the fact that 'normal' can be very different for different GA lines. Furthermore, it may be necessary to decide for each line what may be 'good' normal (e.g. increased activity levels) and what is 'bad' or harmful normal behaviour (e.g. excessive anxiety or aggression). The challenge for researchers is to devise husbandry and EE strategies that facilitate positive or neutral behaviour and discourage negative behaviour. Simply blocking undesirable behaviour (for example, individual housing for aggressive strains) is not ideal as it does not address the underlying welfare problem and may lead to frustration. It is better to try to predict the behaviour of a specific GA line and plan EE to address any problems.

There are several approaches to help achieve this:

- A literature search should be done on the line in question. For example, if publications mention that a line is especially anxious, it would be prudent to ensure that adequate nesting material and in-cage shelters are provided, and that the husbandry regime and site of cages ensures minimal disturbance[1].
- Collaboration and information sharing between researchers, animal technologists and carers, veterinarians and the ethics or animal care and use committee are essential to ensure that all information about phenotype and appropriate EE is recorded and shared.
- Specific husbandry advice about individual GA strains should be sought from the supplier, institution of origin and databases such as the Mouse Genome Informatics Database (*www.informatics.jax.org*) and Eumorphia (*www.eumorphia.org)*.
- Behaviourally, the majority of GA mice are similar to the background strain. Therefore, when seeking EE, a good starting point would be EE that works well for the background strain (Pascalle van Loo, pers. comm.).
- Investigators should evaluate the effectiveness of EE modifications and be prepared to revise protocols as necessary (see Griffin 2012).

[1]However, in the case of anxiety-inducing genetic alterations, managing the animals may require a 'less is more' approach that avoids exacerbating the neophobic tendencies that mice sometimes exhibit naturally. Providing the basics of shelter to allow the animals to retreat, appropriate materials for nest building and security and food items in the litter to encourage foraging, together with a familiar scent (retaining a small proportion of litter from the soiled cage in the new box) is perhaps all that is needed for their well-being (Peter Johnson, pers. comm.).

Using EE to alleviate adverse phenotypic effects

The majority of modifications made for GA mice (and indeed non-GA strains) are alterations in housing and husbandry rather than EE per se, for example easier access to feed if mobility is reduced; the use of gel feeds as complete nutritional replacements for muscular dystrophy models which cannot climb or stand well and have weak jaw muscles; increased litter changes for diabetic models to deal with their increased urination; and use of cellulose litter for BALB/c models without eyelashes to prevent dust irritation (Jenny Kingham, pers. comm.). This approach has implications for the science as well as welfare, as phenotypic expression may be radically altered by aspects of

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husbandry and housing (Wells et al. 2006).
Some examples:

- Pathology of blood vessels in fibulin-$4^{+/-}$ mice was reduced when mice were provided with larger cages containing a shelf, ladder, exercise wheel and plastic tube, suggesting that approprate EE may counteract negative effects of genotype (Cudilo et al. 2007).
- Vascular dysfunction in the brains of TgCRND8 mice (carrying human $APP_{Swedish+Indiana}$) was counteracted by EE consisting of a wooden scaffolding, plastic inset and additional nesting material, as well as an additional cage accessed by a tunnel (Herring et al. 2008). The additional cage contained gnawing wood and a sisal rope. Additionally, objects including tunnels, balls, soft materials and 'locomotive substrates' including wooden ramps, ladders, plastic stairs and running wheels were provided. Further studies have shown that transgenic Alzheimer disease model mice with access to EE showed a reduced rate of cognitive decline and anxiety-related behaviour (Herring et al. 2011).
- Survival in transgenic Huntington's disease model R6/2 mice was improved in those exposed daily to a playground box containing wheels and other toys (Wood et al. 2010). Importantly, this study found a marked difference in response to EE according to sex and genotype. On this basis, the authors recommend tailoring EE to individual animals.

- Anecdotal reports suggest that transgenic 'waltzer' mice provided with circular guards that they could leap in and out of exhibited reduced anxiety (Hawkins 2002). EE may not alleviate all adverse effects due to the phenotype, in which case researchers will need to consider other strategies, such as modifications in general husbandry and the use of alternate animal models to improve the welfare of mice used in experiments.

Interactions with enrichment items can help to monitor well-being

Phenotyping can provide some information that is relevant to welfare status, but it is not the same as welfare assessment. For this reason, non-invasive, structured welfare assessments should also be carried out to ensure that the needs of GA mice are met. For a list of standard welfare indicators for GA mice, see Wells et al (2006).

Personnel responsible for animal care and husbandry should be trained to observe and assess the effects of EE and to identify adverse or abnormal behaviours (ILAR 2011). This should include monitoring whether GA lines are using enrichment as expected. For example, failure to construct proper nests, avoidance of climbing structures or not using chewing blocks as normal could indicate a welfare problem, in which case a welfare assessment should be undertaken.

The impact of housing, husbandry and EE on mouse welfare and experimental variability should also be assessed for each GA strain used and reviewed on a regular basis to ensure they are consistent with both facilitating animal well-being and the goals of the animal use (ILAR 2011). Consistent husbandry is vital to ensure that the line is not mis-characterised, since environment can have more of an impact on phenotype than genetic alteration.

Conclusion

The welfare of GA mice will continue to improve if information

about husbandry and EE modifications is shared. At present, very little strain-specific information on husbandry or EE is provided in passports or by breeders. Successful EE modifications, along with refinements related to housing, feeding and husbandry, should be included on GA passports to ensure that research is not duplicated or unnecessarily replicated and that the animals get what they need (Osborne et al. 2010). Details of background strains or stock and backcross/intercross generation contained on GA passports may be useful for anyone providing EE for GA mice.

There is also a role for common sense and experience in providing and tailoring enrichment to these animals. In the words of Pascalle van Loo, "tailoring [of EE] is needed when mice exhibit a specific phenotype. Whether this phenotype is the result of GA or inbreeding/mutation is besides the point."

Refinement is also an ongoing process. As Rollin writes, "even if we lack full knowledge of animals' needs and natures...we certainly know enough to come much closer to satisfying those needs than we currently do" (Rollin, 2012). Above all, EE should be based on the best available evidence, in conjunction with structured welfare assessments, to ensure a better standard of welfare for GA mice.

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References and further reading

Brown, M. J. and Murray, K. A. (2006). Phenotyping of genetically engineered mice: Humane, ethical, environmental, and husbandry issues. *ILAR Journal* 47: 118-123.

Buehr, M., Hjorth, J.P., Hansen, A.K. and Sandøe, P. (2003). Genetically modified laboratory animals—what welfare problems do they face? *Journal of Applied Animal Welfare Science* 6: 319-338.

Cook, M. N., Bolivar, V. J., Mcfadyen, M. P. and Flaherty, L. (2002). Behavioral differences among 129 substrains: Implications for knockout and transgenic mice. *Behavioral Neuroscience* 116: 600-611.

Cudilo, E., Al Naemi, H., Marmorstein, L. and Baldwin, A. L. (2007). Knockout Mice: Is It Just Genetics? Effect of Enriched Housing on Fibulin-4(+/-) Mice. *PLoS ONE* 2: e229. doi:10.1371/journal.pone.0000229

Fawcett, A. (2012). Guideline 22: Guidelines for the Housing of Mice in Scientific Institutions [Online]. Department of Primary Industries. Download at: *http://www.animalethics.org.au/__data/assets/pdf_file/0004/249898/Guideline-22-mouse-housing.pdf* [Accessed May 5 2012].

Government, A. (2007). *Animal Welfare Committee Guidelines for the Generation, Breeding, Care and Use of Genetically Modified and Cloned Animals for Scientific Purposes.* In: COUNCIL, N. H. A. M. R. (ed.) Government. Canberra.

Hawkins, P. (2002). Recognising and Assessing Pain, Suffering and Distress in Laboratory Animals: A Survey of Current Practice in the UK with Recommendations. Download at: *http://www.rspca.org.uk/ImageLocator/LocateAsset?asset=document&assetId=1232715011820&mode=prd* [Accessed June 7, 2012].

Herring, A., Lewejohann, L., Panzer, A.-L., Donath, A., Kröll, O., Sachser, N., Paulus, W. and Keyvani, K. (2011). Preventive and therapeutic types of environmental enrichment counteract beta amyloid pathology by different molecular mechanisms. *Neurobiology of Disease* 42: 530-538.

Herring, A., Yasin, H., Ambree, O., Sachser, N., Paulus, W. and Keyvani, K. (2008). Environmental enrichment counteracts Alzheimer's neurovascular dysfunction in TgCRND8 mice. *Brain Pathology* 18: 32-39.

ILAR (2011). *Guide for the Care and Use of Laboratory Animals*, 8th edn. Washington, D.C., National Academies Press.

Mason, G. (2006). Strain differences in cage stereotypies of laboratory mice. In: Mason, G. and Rushen, J. (eds.) *Stereotypic Animal Behaviour: Fundamentals and Applications to Welfare*, 2nd edn Cambridge, MA.: CABI.

Miczek, K. A., Maxson, S. C., Fish, E. W. and Faccidomo, S. (2001). Aggressive behavioral phenotypes in mice. *Behavioural Brain Research* 125: 167-181.

Osborne, N., Bussell, J., Carver, R., Chadwisk, C., Enser, B., Haskings, M. A., Hubreacht, R., Latcham, J., Maconochie, M., Mallon, A.M., Norrington, R. & Wilson, S. (2010). GA Passports: The Key to Consistent Animal Care. Southwater, RSPCA, download at *http://www.rspca.org.uk/sciencegroup/researchanimals/reportsandresources/biotechnology* [Accessed June 11 2012].

Rollin, B. E. (2012). "Enrichment" as a moral presupposition of animal research. *Enrichment Record* 10 (January): 13-15.

Thon, R., Lassen, J., Hansen, A.K., Jegstrup, I.M. and Ritskes-Hoitinga, M. (2002). Welfare evaluation of genetically modified mice—an inventory study of reports to the Danish Animal Experiments Inspectorate. *Scandinavian Journal of Laboratory Animal Science* 29: 45-53.

Wells, D., Playle, L., Enser, W., Flecknell, P., Gardiner, M., Holland, J., Howard, B., Hubrecht, R., Humphreys, K., Jackson, I., Lane, N., Maconochie, M., Mason, G., Morton, D., Raymond, R., Robinson. V., Smith, J. and Watt, N. (2006). *Assessing the Welfare of Genetically Altered Mice: Full Report of GA Mouse Welfare Assessment Working Group*. Download at *http://www.nc3rs.org.uk/GAmice* [Accessed June 12 2012].

Whishaw, I. Q., Metz, G. A. S., Kolb, B. and Pellis, S. M. (2001). Accelerated nervous system development contributes to behavioral efficiency in the laboratory mouse: A behavioral review and theoretical proposal. *Developmental Psychobiology* 39: 151-170.

Wood, N. I., Carta, V., Milde, S., Skillings, E. A., Mcallister, C. J., Ang, Y. L. M., Duguid, A., Wijesuriya, N., Afzal, S. M., Fernandes, J. X., Leong, T. W. and Morton, J. (2010). Responses to Environmental Enrichment Differ with Sex and Genotype in a Transgenic Mouse Model of Huntington's Disease. *PLoS One* 5(2):e9077

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Enrichment and Cephalopods

Enrichment of the lives of cephalopod molluscs is a new idea born on the advance of knowledge about their intelligence and cognition and the formulation of regulations for their care in the European Union. This paper will discuss different reasons why we should care about cephalopods in captivity. It will then outline enrichment possibilities for the common cephalopods in captivity—the octopuses, cuttlefish and squid. While there are some suggestions and 'lab lore', there is little formal research to prove that enrichment makes the lives of cephalopods better, and the paper will end with a plea for more research.

Introduction

Before we discuss enrichment for any animals, we should think about our philosophical views towards them. The 'Contractarian' viewpoint suggests that what we do doesn't matter because animals are just 'things' (Mather 2011). Still, how we behave towards animals affects how we are perceived, so we might care for them appropriately because it 'looks good'. The rules now say, for instance, that in the European Union we must consider the welfare of cephalopods. A second philosophy is the 'Utilitarian' one, which suggests that all our actions have both harms and benefits, and that we should act to maximize the benefits and minimize the harms, although in practice we tend to look at our benefits as primary. Keepers

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of zoos and aquariums believe that captivity for animals benefits the humans who would otherwise never see them, and then indirectly benefits the animals in our awareness of the need to preserve them. A third viewpoint is the 'Rights' one, which suggests that animals have an inherent right to a full and rich life, and since we put them in captivity, it's our obligation to make sure that while they are there, we should make their lives as full as possible. There are some hitches in this philosophy, as the 'right' of a cat to reproduce may lead to a litter of unwanted, abandoned kittens.

All of us have some mixture of these philosophical attitudes, but it gets complicated when we talk about invertebrates (Mather & Anderson 2007). Although invertebrates constitute about 98% of the animals on the planet, the word 'animal' is translated by most people to 'vertebrate' (or even 'mammal'). Many people simply don't see invertebrates (Ponder 1992). We only think of enrichment as enhancing the lives (Sheperdson 1998) of complex, intelligent animals, and certainly enrichment for a clam or a coral animal is difficult to imagine. But cephalopods are different; they have all these characteristics that we assume 'dumb' invertebrates don't have. Besides, we know so very little about invertebrates, including cephalopods, that even if we want to enrich their lives, we may not know how (Davis, Roberts & Ayre 1999).

In this context, why provide enrichment for these invertebrates? The first reason is that regulations (see the Canadian Council of Animal Care and EU Directive) and organizations such as the American Zoo & Aquarium Association are beginning to demand enrichment for cephalopods (Peters, Rehling & Anderson 2005). That's very contractarian; we have to do it because it's written in the rules. Second, we may use enrichment because there's a set of practical outcomes, quite utilitarian. Cuttlefish given an enriched early environment grow larger and have better memory performance as adults (Dickel, Boal & Budelmann 2000)—that's what researchers want. Similarly, mudflat octopuses given habitat enrichment are more active and colourful, good for the aquarium audience (Beigel & Boal 2006). Third, bored animals tend to be destructive, escape (Wood & Anderson 2004) or perform stereotyped behaviours (Anderson & Wood 2001), which is not useful for the commercial enterprise, audience or even the animals themselves. And lastly, we can expect that enriched cephalopods and other animals will have an increased 'quality of life', a measure almost impossible to quantify in animals we know as little about as cephalopod molluscs. This is clearly paying attention to the animal's rights to a full life. One measure that can be quantified is whether a captive animal can be released into its natural habitat and live as it normally would—a common conservation outcome for zoos and aquariums with threatened animals. The release of a female giant Pacific octopus from the Seattle Aquarium stands out (Anderson 2000). As cephalopods are semelparous and reproduce at the end of their lifespan, they can be released into the wild to find mates and lay or fertilize eggs. This was apparently successful, as she was tracked underneath the aquarium pier for 40 days, during which food remains and potential mates were spotted.

Why should cephalopods, of all the invertebrates, be given enrichment? They are intelligent animals, with a brain/body ratio higher than that of most fishes and reptiles, even some birds. They are excellent at learning (Wells 1978). They have distinct personalities (Mather & Anderson 1993), solve complex problems (Anderson & Mather 2007) and even play (Mather & Anderson 1999) in situations where they may be bored (Wemesfelder 1993). They may even have a simple form of consciousness (Mather 2008). But enrichment must be tailored to the physical and social needs of the particular species. In practice, three groups of coleoid cephalopods—the Octopus family, the Sepioid

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cuttlefish and the Loliginid squid—are commonly found in captivity. General guides to the care of cephalopods in captivity are Boyle (1991) and an update review by Moltschaniwskyj et al. (2007), which cover many details but say little about enrichment.

Octopuses are common in captivity, and their propensity to remain in a confined space makes them amenable to this situation (Wells 1978). Yet shelter is important for them. An appropriate-sized pot or set of bricks will do, but they prefer a shelter that fits their volume fairly closely and has a small entrance (Mather 1994). Octopuses may block the entrance by items such as shells and small rocks, a clear example of tool use. Provision of 'building' materials allows octopuses to arrange shelter to their own preferences and rearrange them and enrich the physical space. They are able to tolerate frozen food from a variety of prey species, and enrichment might include giving them novel foods such as hard-boiled eggs or chicken. Yet live food is preferable for an enriched life of an octopus. If prey animals such as clams or snails can be introduced into the tank when the octopus is not near or is asleep, they will likely bury or hide, so the octopus will have to use normal hunting behavior to find them (Wood & Wood 1999). In addition, preparation of whole animal food can be a long procedure (Anderson & Mather 2007). Hours and much manipulation are spent getting access to a clam or disarticulating a crab and consuming the food from the body and appendages. Aquarists sometimes construct elaborate devices with a food reward inside, and the octopus commonly takes only seconds to gain access.

Octopus in the Seattle Aquarium being fed lots of clams in a study. © Seattle Aquarium

Yet an animal will retain such a device after the food is consumed, exploring it with its arms and manipulating pieces (Wood & Wood 1999). Octopuses are great explorers, and any novel item enriches their lives.

Conversely, social enrichment is not appropriate for octopuses. It is essential to research species-specific cephalopod social behaviour, as most cephalopods are cannibalistic, and confinement together in close quarters may lead to stress, escape responses or even death. This is true of octopuses from the tiny paralarval stage to adulthood. The necessity to seek partners for mating (sexes are separate and permanent), occurs only at the end of their lifespan. The opportunity to mate is part of enrichment, and when the female lays her thousands of eggs, they will be fertile. She will brood them nevertheless, and allowing her to carry out this stage of her lifespan is also enrichment. But mating encounters have to be monitored to prevent attack deaths.

Cuttlefish are similar to octopuses in seeking a benthic habitat, but different in other ways. They are masters of bottom-matching camouflage but also show skin displays in great complexity matched to different circumstances (Hanlon & Messenger 1988). Yet they prefer to dig into sand-mud bottoms in the daytime and remain unseen to predators. Given an inadequate amount of sand, they will repeatedly dig (Mather 1986),

so enrichment should include enough sand for appropriate cover. And young cuttlefish given sand and/or a varied background will learn to dig or camouflage themselves appropriately and more quickly (Poirer, Chichery & Dickel 2004; 2005). A varied background generally enriches the cuttlefish, leading to better growth and faster learning capacity (Dickel et al. 2000). They can be fed thawed frozen food, but feeding them with live food can be more appropriate for enrichment. Darmaillacq et al's (in press) papers show that young cuttlefish have the ability to learn a great deal about appropriate prey species, so provision of live food (fish and crabs) is an obvious enrichment. Of course, this raises both ethical and legal issues, since fish are often protected by law and, while crustaceans are not generally protected by animal welfare legislation, procedures on them were almost regulated under the new EU Directive. It could be argued that providing dead food actually simply shifts the blame, since dead crustaceans or fish were caught and killed sometime, somewhere, and probably without much consideration (see Braithwaite 2010). As an additional benefit to the cuttlefish, they have to learn to evade the defenses of prey such as pinching crab claws. Many prey species will dig into sand or maneuver away from an approaching cuttlefish, so much energy will be expended in learning how to catch the prey (Boal, Wittenberg & Hanlon 2000). It's not clear to what extent cuttlefish manipulate their physical environment—studies simply haven't been done.

Cuttlefish are also solitary, and, particularly in the juvenile stage, are cannibalistic, so social housing is not appropriate. As adults, males eagerly seek out females and advertise their sex to others with a striped Zebra skin pattern. Since the reflective surface of an aquarium wall can be sufficient to elicit this display, low-level enrichment might consist of giving them a reflective surface such as a mirror. But the enrichment of giving them mating opportunities is easy to provide. Pairs mate quickly and repeatedly, then males guard the female from other males and conduct display contests (see Hanlon & Messenger 1996, a good general guide to cephalopod behavior). Females lay small eggs singly on any available surface, and enrichment here will consist of giving them a variety of structures on which to do so. As they have no parental care and both sexes die soon after mating, enrichment does not include the opportunity for parental care.

Loliginid squid are animals of the open waters, and enrichment for them may involve environmental restriction rather than enhancement. As they are not near the bottom, squid need no special substrate to enrich their lives. Instead, their fast jet escape response at disturbance means that their habitat must be simplified. Disturbance by visitors or animals in neighboring tanks may trigger an escape response, and the resultant impact might damage their skin, causing wounds that become infected and result in death. Squid need to be kept in large volumes of water, and the aquarium sides should be blackened or a circular 'raceway' provided so that they never bang into the edges of the tank. Provision of live food is again a useful form of enrichment, and dead unconsumed food may sink to the bottom of the tank, decay and cause bacterial growth. No one has provided items for squid to manipulate; they are fast learners and have the eight grasping cephalopod arms as well as elastic tentacles to shoot out for food capture, but have not been proved to explore or play with them. Most squid swim in schools with conspecifics, but no one has studied whether depriving them of this opportunity is detrimental. However, it makes sense to give social squid the benefit of the doubt and group house them unless there is sound scientific or veterinary justification not to.

Opportunities for enrichment during early life stages of most cephalopods are rare because the young are often very tiny paralarvae that float in the plankton. Efforts to raise these tiny animals are seldom successful (this is especially true of *Idiosepius*), and much effort is devoted to just keeping them alive. A few octopus species, such as *O. maya* and *O. bimaculoides*, have 'large' eggs, where large is around 17 mm long. Since cephalopods lay from a hundred up to tens of thousands of eggs at a time, we can assume that survival to adulthood is unlikely. Cuttlefish have large young, which may be 2 cm long at hatching. Newly hatched cuttlefish are benthic like the adults and behave similarly (Darmaillacq et al, in prep). They are an excellent model for studying the influence of habitat and experiential characteristics on the developing animal, including enrichment effects. For instance, newly hatched

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cuttlefish have a very narrow range of preferred prey for the first several weeks of life—mysid crustaceans. Yet when they are exposed to appropriate sized crab prey even while still in the egg, the hatchlings instead prefer them (Darmaillacq et al. in prep). Thus enrichment simply by exposure will widen their prey choices. There are both short and long term effects of environmental enrichment in young cuttlefish, and their learning capacity in matching to their environment is probably a model for that of all the cephalopods.

That we know little of the biology of most cephalopod species is a huge barrier to keeping them in captivity, let alone to enriching their lives there. One paper on enrichment in an octopus species and several on one cuttlefish do not provide us with much information. Yet the manipulations are fairly simple: provide an experimental group with a specific or several general physical and /or biological stimuli, give a control group a poorly enriched environment, wait a few weeks and measure the results (Dickel et al. 2000; Bielgel & Boal 2006; Poirer et al. 2004; 2005). A small sample size is not necessarily a barrier to enrichment studies, as Wutchik (pers. comm.) is presently using a single-subject pre-post study to look at enrichment for a giant Pacific octopus. It is critical that we move beyond anecdotes (Wood & Wood 1999; Anderson & Wood 2001; Peters et al. 2005) and conduct the studies to prove what keepers of cephalopods already know, that the animals' lives are enhanced by the provision of environmental enrichment.

References

Anderson, R. C. (2000). The release of a captive-reared giant Pacific octopus. *Drum and Croaker* 31: 7-10.

Anderson, R. C. and Mather, J. A. (2007). The packaging problem: Bivalve mollusk prey selection and prey entry techniques of Enteroctopus dofleini. *Journal of Comparative Psychology* 121: 300-305.

Anderson, R. C. and Wood, J. B. (2001). Enrichment for giant Pacific octopuses: Happy as a clam? *Journal of Applied Animal Welfare Science*, 4, 157-168.

Biegel, M. & Boal, J. G. (2006). The effect of habitat enrichment on the mudflat octopus. *The Shape of Enrichment* 15: 3-6.

Boal, J. G., Wittenberg, K. M. and Hanlon, R. T. (2000). Observational learning does not explain improvement in predation tactics by cuttlefish (Mollusca, Cephalopoda). *Behavioral Processes* 52: 141-153.

Boyle, P. R. (1991). *The UFAW Handbook on the Care and Management of Cephalopods in the Laboratory*. London, UK, Ennisfield, Universities Federation for Animal Welfare.

Braithwaite, V. (2010). *Do Fish Feel Pain?* Oxford, UK, Oxford University Press.

Darmaillacq, A-S., Joze-Alvez, C., Belanger, C. and Dickel, L. Cuttlefish preschool: how to learn in the perihatching period. In: A-S Darmaillacq, L. Dickel and J. A. Mather, (Eds), *Cephalopod cognition*, in preparation.

Davis, A. R., Roberts, D. and Ayre, D. J. (1999). Conservation of sessile marine invertebrates: You do not know what you have got until it's gone. In: Ponder, W. and Lummey, D. (eds). The other 99%: *The conservation and biodiversity of Invertebrates*. Mosman, NSW, Royal Society of New South Wales.

Dickel, L., Boal, J. G. and Budelmann, B. U. (2000). The effect of early experience on learning and memory in cuttlefish. *Developmental Psychobiology* 36: 101-110.

Hanlon, R. T. and Messenger, J. B. (1988). Adaptive coloration in young cuttlefish (Sepia officinalis L.): The morphology and development of body patterns and their relation to behaviour. *Philosophical Transactions of the Royal Society of London B* 320: 437-487.

Hanlon, R. T. and Messenger, J. B. (1996). *Cephalopod Behaviour*. Cambridge, UK: Cambridge University Press.

Mather, J. A. (1986). Sand-digging in Sepia officinalis: assessment of a cephalopod mollusc's "fixed" behavior pattern. *Journal of Comparative Psychology* 100: 315-320.

Mather, J. A. (1994). "Home" choice and modification by juvenile Octopus vulgaris (Mollusca: Cephalopoda): Specialized intelligence and tool use? *Journal of Zoology*, London 233: 359-368.

Mather, J. A. (2008). Cephalopod consciousness: Behavioral evidence. *Consciousness and Cognition* 17: 37-48.

Mather, J. A. (2011). Philosophical background of attitudes towards and treatment of invertebrates. *ILAR Journal* 52: 205-212.

Mather, J. and Anderson, R. C. (1993). "Personalities" of octopuses (Octopus rubescens). *Journal of Comparative Psychology* 107: 336-340.

Mather, J. A. and Anderson, R. C. (1999). Exploration, play and habituation in Octopus dofleini. *Journal of Comparative Psychology* 113: 333-338.

Mather, J. A. & Anderson, R. C. (2007). Ethics and invertebrates: A cephalopod perspective. Diseases of Aquatic Organisms 75: 119-129. Moltschaniwskyj, N, A., Hall, K., Lipinski, M. R., Marian, J. E. A. R., Nishiguschi, M., Sakai, M. and Warnke, K. (2007). Ethical and welfare considerations when using cephalopods as experimental animals. *Review of Fish Biology and Fisheries* 17: 455-476.

Peters, A. Rehling, M. and Anderson, R. C. (2005). Mission possible: Enriched cephalopods. *AZA Communique* September 2005: 21-23.

Poirer, R., Chichery, R & Dickel, L. (2004). Effects of rearing conditions on sand digging efficiency in juvenile cuttlefish. *Behavioural Processes*, 67, 273-279.

Poirier, R., Chichery, R. & Dickel, L. (2005). Early experience and postembryonic maturation of body patterns in cuttlefish (Sepia officinalis). *Journal of Comparative Psychology*, 119, 230-237.

Ponder, W. F. (1992). Bias and biodiversity. *Australian Zoologist*, 28, 47-51.

Sheperdson, D. J. (1998). Tracing the path of environmental enrichment in zoos. In D. L. Sheperdson, J. D. Melles & M. Hutchins (Eds), *Second nature: Environmental enrichment for captive animals* (pp 15-29). Washington, D. C.: Smithsonian Institute Press.

Wells, M. J. (1978). *Octopus: Physiology and behaviour of an advanced invertebrate*. London, UK: Chapman & Hall.

Wemelsfelder, F. (1993). Concept of animal boredom and its relationship to stereotyped behavior. In Mason, G. & Rushen, A., Eds., *Stereotypical animal behavior: Fundamentals and application to animal welfare* (pp 65-95). Wallingford, UK: CABI.

Wood, J. B. & Anderson, R. C. (2004). Interspecies evaluation of octopus escape behavior. *Journal of Applied Animal Welfare Science*, 7, 95-106.

Wood, J. B. & Wood, D. A. (1999). Enrichment for an advanced invertebrate. *The Shape of Enrichment*, 8, 1-5.

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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

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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

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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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References

Abbott Global Enrichment Committee. (2012). History of enrichment in the ILAR *Guide for the Care and Use of Laboratory Animals. The Enrichment Record*, July, 2012, vol. 12, 10-13.

Baumans, V., Van Loo, P.L.P., & Pham, T.M. (2011). Standardisation of environmental enrichment for laboratory mice and rats: Utilisation, practicality and variation in experimental results. *Scandinavian Journal of Laboratory Animal Science*, 37(2), 101-114.

Benefiel, A. C., Dong, W. K., & Greenough, W. T. (2005). Mandatory " enriched" housing of laboratory animals: The need for evidence-based evaluation. *Institute for Laboratory Animal Research Journal*, 46(2), 95-105.

Cao, L., Liu, X., Lin, E-J. D., Wang, C., Choi, E. Y., Riban, V., . . . During, M. J. (2010). Environmental and genetic activation of a brain-adipocyte bdnf/leptin axis causes cancer remission and inhibition. *Cell* 142(1), 52-64.

Chamove, A. S. (1992). Environmental enrichment: a review. *Animal Technology*, 40:155-177.

Crabbe, J. C., Wahlsten, D., & Dudek, B. C. (1999). Genetics of mouse behavior: Interactions with laboratory environment. *Science*, 284(5420), 1670-1672.

European Commission (2010). Directive 2010/63/EU of the European Parliament and of the council of 22 September 2010 and of the council on the protection of animals used for scientific purposes." *http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:%200033:0079:EN:PDF*

Garner, J. P. (2005). Stereotypies and other abnormal repetitive behaviors: Potential impact on validity, reliability, and replicability of scientific outcomes. *Institute for Laboratory Animal Research*, 46(2), 106-117.

Hamm, R.J., Temple, M.D., O'Dell, D.M., Pike, B.R. and Lyeth B.G. (1996). Exposure to Environmental Complexity Promotes Recovery of Cognitive Function after Traumatic Brain Injury. *Journal of Neurotrauma* 13(1):41-47.

Institute for Laboratory Animal Research & National Research Council. (2011). Guidance for the description of animal research in scientific publications (pp. 1-31). Washington DC: The National Academies Press.

Korevaar, D. A., Hooft, L., ter Riet, G. (2011). Systematic reviews and meta-analyses of animal studies. *Laboratory Animals*, 45(4): 225–230.

Krech, D., Rosenzweig, M. R., & Bennett, E. L. (1960). Effects of environmental complexity and training on brain chemistry. *Journal of Comparative & Physiological Psychology*, 53, 509-519.

Litton, K., A. Acevedo, W. Browne, J. Edgar, M. Mendl, D. Owen, C. Sherwin, H. Würbel and C. Nicol. (2008). Towards humane end points: Behavioural changes precede clinical signs of disease in a huntington's disease model. *Proceedings of the Royal Society-Biological Sciences*, 275, 1895-1874.

National Research Council (2011). *Guide for the Care and Use of Laboratory Animal*. Eighth Edition. Washington, DC: The National Academies Press.

Newberry, R. C. 1995. Environmental enrichment: increasing the biological relevance of captive environments. *Applied Animal Behaviour Science* 44:229-243.

Olsson, I. A., & Dahlborn, K. (2002). Improving housing conditions for laboratory mice: A review of "environmental enrichment". *Laboratory Animals*, 36(3), 243-270.

Osborne, N. (2012). Reporting enrichment in research papers. *Enrichment Record*, July, 2012, vol. 12, 34-35.

Passineau, M. J., Green, E. J., & Dietrich, W.D. (2001). Therapeutic effects of environmental enrichment on cognitive function and tissue integrity following severe traumatic brain injury in rats. *Experimental Neurology*, 168, 373-384.

Patterson-Kane, E. G. (2003). Shelter enrichment for rats. *Contemporary Topics in Laboratory Animal Science*, 42(2), 46-48.

Patterson-Kane, E. G. (2004). Enrichment of laboratory caging for rats: A review. *Animal Welfare*, 13 (Supplement), S209-S214.

Russell, W. M. S., & Burch, R. L. (1992). The principles of humane experimental techniques (originally published in 1959).*http://altweb.jhsph.edu/publications/humane_exp/het-toc.htm*.

Sager, T.N., Kirchhoff, J. , Mørk, A., Van Beek, J., Thirstrup, K., Didriksen, M., & Lauridsen, J.B. (2010). Nest building performance following mptp toxicity in mice. *Behavioral Brain Research*, 208(2), 444-449.

Sherwin, C. M. (2007). Validating refinements to laboratory housing: Asking the animals. Commissioned article on NC3Rs web-page. *http://www.nc3rs.org.uk/news.asp?id=759* (pp. 1-13).

Sørensen, D.B. and Hansen, A.K. (2012). Drivers for enrichment in Directive 2010/63/EU. *Enrichment Record*, July, 2012, vol. 12, 13-15.

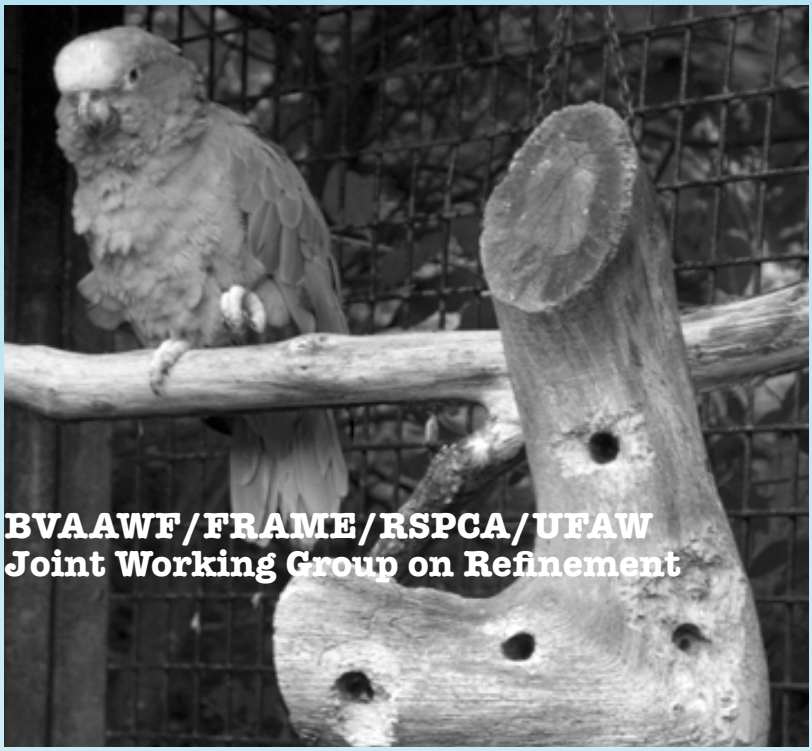
Van Loo, P. L.P., Kruitwagen, C. L. J. J., Koohaas, J. M., Van de Weerd, H. A., Van Zutphen, L. F. M., & Baumans, V. (2002). Influence of cage enrichment on aggressive behavior and physiological parameters in male mice. *Applied Animal Behaviour Science*, 76, 65-81.

Weary, D. M. (2012) A Good Life for Laboratory Animals—How Far Must Refinement Go? World Congress on Alternatives and Animal Use in the Life Sciences (8th). Montreal, Canada, August 21-25, 2011, *Alternatives to Animal Testing and Experimentation Proceedings* 1:11-13.

Weed, J. L., & Raber, J. M. (2005). Balancing animal research with animal well-being: Establishment of goals and harmonization of approaches. *Institute for Laboratory Animal Research Journal*, 46(2), 118-128.

Würbel, H. (2000). Behaviour and the standardization fallacy. *Nature Genetics*, 26(3), 263.

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

Nikki Osborne, Research Animals Department, RSPCA, Southwater, West Sussex, UK

Reporting Enrichment in Research Papers

The provision of environmental enrichment, whilst good for animal welfare, can sometimes be a contentious issue with regard to scientific data. Some argue that the inclusion of enrichment increases the number of experimental variables and therefore has a negative impact on the science, whilst others argue that only those results achieved in an appropriately enriched environment, when the animal is displaying a range of natural behaviours, can be said to present the 'true' nature of any effect. Whichever of these opinions is closest to your own, in my view, a good way to inform the debate is to ensure that all published research includes details of any environmental enrichment that the experimental animals have experienced.

But is this happening? Following an in-depth, but unlikely to be comprehensive PubMed search using 53 different 'animal model' and 'environmental enrichment' search term variations, I have in my hand a list of 126 references from 64 different journals. Not a lot, when you consider that I didn't include any time frame limits, and less still when reading of the abstracts reduces this number to just 87 references, in 46 journals, that actually provide details and data on environmental enrichments for a range of species; primarily mice and rats. The vast majority of these papers report the effect of environmental enrichments (not including social housing/enrichment) on specific phenotypic effects or behaviours, with a few including details of environmental enrichments, when discussing current best practice in housing and care. I am, of course, aware that this quick search will not have picked up any papers that include details of enrichments within the materials and methods sections, as these are not indexed, but it does illustrate how difficult it can be to find the limited amount of research published in this field. On a brighter note, *The Enrichment Record* does a fantastic job of filling what is clearly a very big hole in this regard, but we really need to get the majority of authors including enrichment details within all published research in order to accumulate a sufficient body of data with which to analyse and address the many questions surrounding the use, mis-use, pros and cons of environmental enrichment. So how can this be achieved?

The RSPCA's approach has been to see whether journals have publication policies on the use of animals in the research that they publish, and if so, whether they included any reference to the 3Rs, enrichment, or other animal welfare related issues. Most of the headline results from our surveys have already been published, but to summarise... the publication policies of a total of 868 different English language peer-reviewed journals publishing primary research involving the use of animals were surveyed from January 2007-2010. This figure is equivalent to 40% of the total number of relevant journals (3,000+) in 2010 (Osborne et al. 2010). Of these 868 journals, over 57% had no meaningful policies relating to the use of animals in the research they published, meaning that we confirmed that they didn't have a policy or that it only included the word 'animal' at some point. Most relevant to *The Enrichment Record* readers is the fact that only 18 out of 868 journal policies gave any mention to the 3Rs, either with or without using the term specifically. Of these, 8 policies referred to all 3Rs, 5 mentioned 2 of the 3Rs (either replacement & reduction, or reduction & refinement) and 5 policies mentioned 1 of the 3Rs. None of the policies included environmental enrichment and only 2 policies mentioned disseminating best practice by publishing improvements. Armed with this data, we have gone on to produce, with input from some journal editors and publishing societies, some simple '*Publication Policy Principles*' and a '*Good Practice Model: Instructions to Authors*' (Osborne et al. 2010). These are aimed at providing journal editors with a short list of points relating to the publication of research involving animals that they can consider when next updating their editorial/publication policies. Or, for those currently without a policy, we provide an instruction to authors that can be copied,

pasted and edited to cater to an individual journal's needs.

We believe that journal editors are well placed to influence the content of the papers submitted to them for publication, because authors will ensure that the paper they submit is prepared in a journal's preferred style and format. So, why not also stipulate required content? An argument against this approach is that the author of the paper, not journal editor, is best placed to know what details are pertinent to include within the description of the study. Where this argument falls flat is revealed by a number of studies that have analysed what information is included within published research papers (Smith et al. 1997, Gomez & Conlee 2007, Kilkenny et al. 2009). Fifteen years ago, Smith et al. (1997) looked at the information authors included within the methods sections of 149 biomedical science papers taken from eight journals. This may not be a statistically significant sample, but their conclusion was that '*Our study points to the need for journals to establish more rigorous guidelines and editorial procedures, in order to ensure adequate reporting*.' These comments were reiterated by Kilkenny et al. (2009) who analysed the quality of reporting in 271 publications and again concluded that '*we believe there is a need to develop reporting standards specifically for research using animals, with the aim of enhancing the transparency of reporting and encouraging both researchers, and those journals responsible for publishing this research, to adopt and adhere to them*.'

So what reporting standards are there and do they include a reference to environmental enrichment? In 1985, the GV-SOLAS Working Committee for the Biological Characterization of Laboratory Animals published '*Guidelines for specification of animals and husbandry methods when reporting the results of animal experiments*' (GV-SOLAS 1985). Perhaps, unsurprisingly, these do not include reference to environmental enrichment; however, the guidelines published by Festing & van Zutphen in 1997 definitely do (Festing & van Zutphen 1997). More recently, several groups have revisited reporting standards publishing '*the ARRIVE guidelines*' for bioscience research reporting (Kilkenny et al. 2010) and the '*gold standard publication checklist*' for animal studies (Hooijmans et al. 2010). Similarly, ILAR (2011) published '*Guidelines for the description of animal research in scientific publications*', with all of these specifying the need to report on the presence and type of environmental enrichment. Likewise, the provision of environmental enrichment has been included as part and parcel of contemporary good practice within the guidance associated with the new revised European Directive on the use of animals in scientific procedures.

I am therefore hopeful that this will prove to be an important turning point, when the provision and reporting of environmental enrichment for laboratory animals hits the scientific mainstream. If authors don't report it because journals ask them to, then maybe they will do so to demonstrate that their research conforms to the good practice standards set out in both the Directive and ILAR *Guide*—and that they recognise better welfare and better science go hand in hand.

References

Osborne, N.J., Phillips, B.J. and Westwood, K. (2010). Journal editorial polices as a driver for change—animal welfare and the Three Rs. *Proceedings of the Eleventh FELASA Symposium and the 40th Scand-LAS Symposium*, 14-17 June 2010, Helsinki, Finland. FELASA, *http://www.felasa.eu*

Festing, M.F.W. and van Zutphen, L.F.M. (1997). Guidelines for reviewing manuscripts on studies involving live animals. In L.F.M. van Zutphen and M. Balls (Eds), *Animal Alternatives, Welfare and Ethics* (pp. 405-410). Amsterdam: Elsevier.

Gomez, L.M. and Conlee, K.M. (2007). An analysis of reporting pain and distress recognition and alleviation in scientific journal publications. *AATEX 14*, Special Issue: 171-177.

GV-SOLAS Working Committee for the Biological Characterisation of Laboratory Animals (1985). Guidelines for specification of animals and husbandry methods when reporting the results of animal experiments. *Laboratory Animals* 19: 106-108.

Hooijmans, C.R., Leenaars, M. and Ritskes-Hoitinga, M. (2010). A gold standard publication checklist to improve the quality of animal studies, to fully integrate the Three Rs, and to make systematic reviews more feasible. *ATLA* 38: 167-182.

ILAR (Institute for Laboratory Animal Resources). (2011). *Guidance for the Description of Animal Research in Scientific Publications.* National Research Council Institute for Laboratory Animal Research. Washington, DC: The National Academies Press.

Kilkenny, C., Parsons, N., Kadyszewski, E., Festing, M.F.W., Cuthill, I.C. et al. (2009). Survey of the quality of experimental design, statistical analysis and reporting of research using animals. *PLoS ONE* 4(11): e7824.

Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M. and Altman, D.G. (2010). Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biology* 8(6): e1000412.

Smith, J., Birke, L. and Sadler, D. (1997). Reporting animal use in scientific papers. *Laboratory Animals* 31: 312-317.

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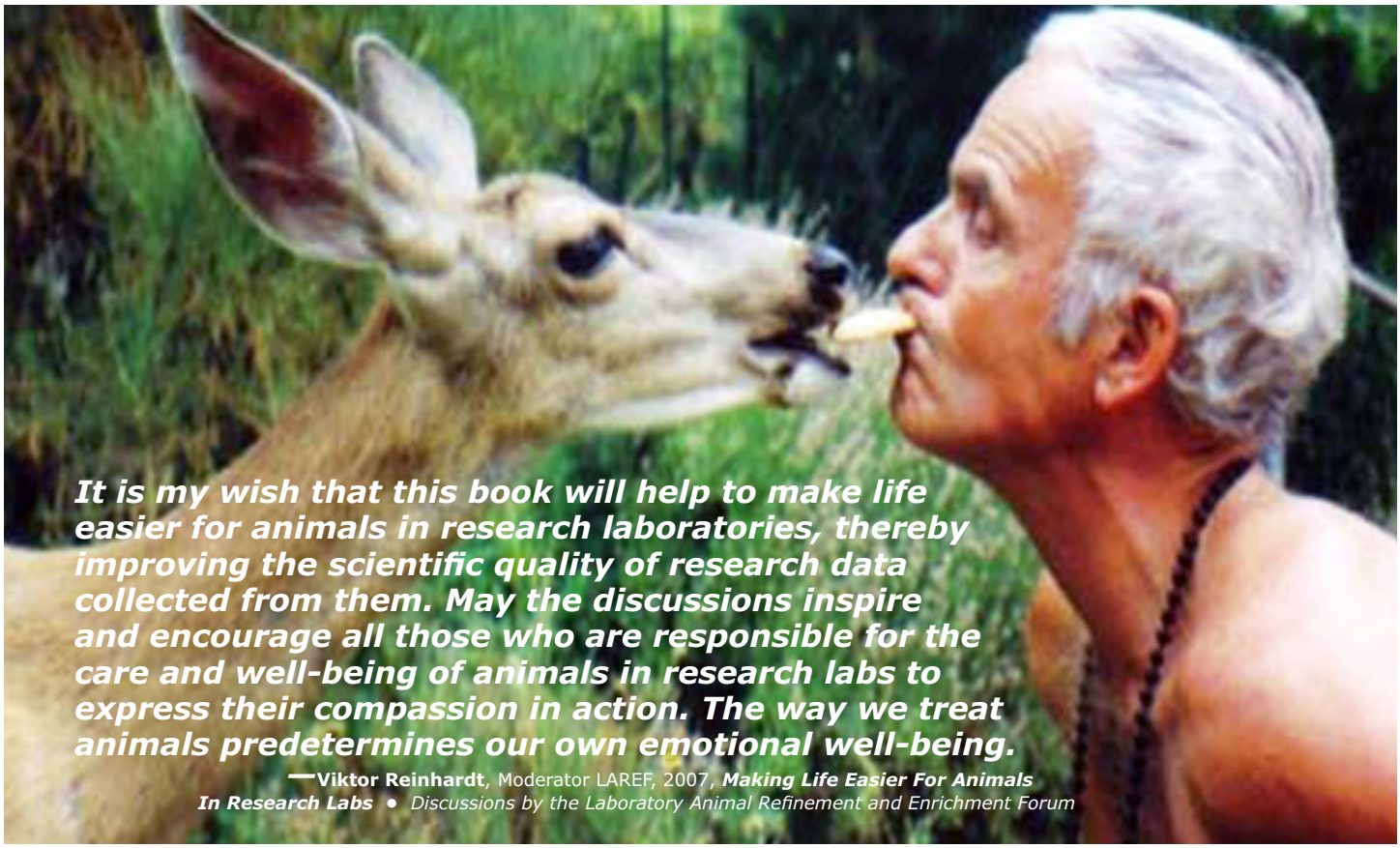

Internationally acclaimed author, teacher and skier, Dr. Viktor Reinhardt grew up in the German Alps. "I was always awestruck by animals," he says. "As a kid, I collected vegetable and fruit scraps from local restaurants and took them to deer-feeding stations where I observed and photographed the wildlife."

Dr. Reinhardt left high school before graduating. "I never liked school," he explains. "I questioned my teachers and thought a lot of the stuff was useless. Even biology was too dry." He apprenticed himself to a local carpenter, enjoyed the work, and was relieved to be finished with school. However, a year later he realized that being a carpenter wouldn't get him where he wanted to be—working with animals. So… he returned to school to get the degree necessary to pursue his vision.

He recalls seeking advice from famed ethologist Konrad Lorenz, whose work he greatly admired. When asked how to become an ethologist, Lorenz said, "you don't become an ethologist…you have to *be* an ethologist." Then he suggested studying veterinary medicine, because it would allow earning a living as a practitioner or a researcher.

Good advice! Reinhardt received his D.V.M. degree from the University of Munich in Germany and his Ph.D. in Ethology from the Max Planck Institute of Physiology of Behaviour, Seewiesen, Germany.

Prior to his current volunteer position, Dr. Reinhardt served as Laboratory Animal Advisor, Animal Welfare Institute, Washington, DC; Attending Veterinarian and Ethologist, Wisconsin Regional Primate Research Center, Madison, Wisconsin; Research Fellow, Department of Veterinary Anatomy, University of Saskatchewan, Canada; Scientific Assistant, Department of Anatomy, Physiology and Hygiene, University of Bonn, Germany; Lecturer, Department of Animal Physiology, University of

Nairobi, Kenya; and Scientific Assistant, Department of Animal Physiology, University of Munich-Weihenstephan, Germany. He joined the Animal Welfare Institute (AWI) in 1994.

In 1997, Dr. Reinhardt created the 8th edition of AWI's *Comfortable Quarters for Laboratory Animals*, a book providing guidance on the humane housing and handling of individual animal species in research facilities. The book was well received; in 2002, he prepared the 9th edition, still in use today.

In 1998, AWI published Reinhardt's *Environmental Enrichment for Caged Rhesus Macaques*, a photographic documentation and literature review. The 3rd edition, now called *Environmental Enrichment and Refinement for Nonhuman Primates Kept in Research Laboratories*, was published in 2008. All of Reinhardt's publications (well over 200 at last count!) are designed to increase awareness among scientists, animal care personnel and the public of the importance of improving the living and handling conditions of animals in research laboratories.

For Dr. Reinhardt, observing undisturbed animals is fascinating. He is "humbled that animals of very different species and people share the same emotional and behavioral roots." "For me," he says, "it has always been a privilege to be with animals, to gain their trust and to gradually get some insight into their emotions. Observing animals is often like looking into a mirror; you learn much about yourself."

AWI Policy on Research and Testing with Animals states:

Research must not be conducted on animals unless, at minimum, the methodology fulfills the three "Rs" of Russell and Burch, including the following:

Animals are maintained in an optimum, species-appropriate environment.

Animals are under the care of professionally trained, compassionate personnel.

Animals' pain, physical discomfort, maladaptive behaviors, fear and anxiety are prevented or, at least minimized by considerate and scientifically sound experimental design and appropriate use of anesthetic, analgesic or tranquilizing drugs.

Dr. Reinhardt attributes his focus on environmental enrichment to an experience in Wisconsin. While applying for a job, he saw a shocking sight—hundreds and hundreds of monkeys alone in single barren cages. For ethical and scientific reasons, he decided to provide companions for the animals. He was warned that it could be dangerous, but his idea was to allow the animals to establish a relationship in a safe way… so that they could live together and not fight. By 1991, 92% of the colony's 749 macaques lived in compatible pairs.

"Housing monkeys in compatible pairs or groups is a necessity…not a luxury," he says. "Since 1991, federal regulations require that facilities have an enhancement plan which must include provisions to address the social needs of nonhuman primates."

Thoughts on the future of environmental enrichment

"Good environmental enrichment allows confined animals to express species-appropriate behaviors. In addition, many animals in laboratories can be trained to work *with* rather than *against* researchers and personnel. When animals are cooperative during procedures, there is little or no stress, and stress is a significant variable that affects research data. In addition, working with a cooperative animal is much safer for the handler."

Dr. Reinhardt's two free databases:

Annotated Database on Environmental Enrichment and Refinement of Husbandry for Nonhuman Primates

Annotated Database on Environmental Enrichment and Refinement of Husbandry for Rodents, Rabbits, Cats, Dogs, Ferrets, Farm Animals, Horses Birds, Fishes, Amphibians and Reptiles
http://www.awionline.org/content/refinement-databases

And…his wife, Annie Reinhardt and his daughter, Catherine Reinhardt-Zaccair are highly valued associates. As a family, they have made many important contributions to the field of animal behavior!

There's an old saying that "You can't dance at two weddings at once." You also can't attend all the meetings and conferences taking place that offer the latest information in the field of laboratory animal science. **Meeting Up** will provide summaries of panels, workshops and symposia covering topics relevant to Environmental Enrichment. If you want more information about any of the presentations described or want to contact the presenters, let us know and we will be happy to connect you: info@theenrichmentrecord.com

PRIM&R Conference

Workshop Moderators:
Christina Winnicker, *D.V.M., Director, Enrichment & Behavioral Medicine, Charles River Laboratories* & **Jennifer Camacho**, *LVT, RLATG Enrichment Manager, Massachusetts General Hospital*

The IACUC's Role in Reviewing and Promoting Enrichment Strategies

(*Animal Well-Being and the Three Rs Track*)
Tuesday, March 20, 2012

This workshop highlighted the basic information on animal behavior, regulatory requirements and ethologically relevant laboratory enrichment practices, while allowing attendants to have a focused dialogue. The workshop consisted of approximately 25 individuals, predominately with IACUC roles at their respective institutions. The attendants were asked, "Who feels that their IACUC has a good understanding of the behavioral needs of the species they are working with?" Approximately 75% of the audience did not feel that their IACUCs were adequate in this area. The resulting conversation identified the ability of the IACUC to serve as an influential member of authority, promoting animal welfare through environmental enrichment.

Dialogue Summary:
The role and responsibility of the IACUC, including what is required, minimal, optimal, and beneficial.

IACUC is responsible for providing assurance of animal well-being through validating the effectiveness of processes currently managing and assessing the enrichment program or evaluating a programmatic initiative designed to monitor the effectiveness of processes to promote well-being.

What are considered the best practices for nonhuman primates, i.e. complex and novel environments or social housing and rodents, i.e. social housing and species-typical behavior?

General recommendations for NHPs and Rodents include:

- Social housing for NHPs in compatible pairs, combined with cognitive enrichment stimuli through a variety of novel instruments and manipulanda
- Social housing and nesting material for rodents

It is important to recognize that the laboratory environment is designed for function and human comfort, not necessarily what is normal or naturalistic to the animal. Considerations should be made to simulate laboratory appropriate naturalistic behaviors in a captive environment—recognizing that space is a limiting factor that will hinder naturalistic care and species-specific group formations. Some examples included;

- Providing a visual block for NHPs (who need or want it) to get away
- Pair or group housing rabbits with limited space will yield success with females, but is very difficult with adult males
- Zebrafish utilize enrichment as a block from antagonistic/aggressive behavior

Performance criteria and how to use assessment data
IACUCs are responsible to ask the questions

- Are we providing "best practices" that support animal well-being?

- Are those processes effective?
- How do we know?
- What's next?

The use of exemptions
IACUCs are responsible for requiring valid justification for exemptions to processes (enrichment or otherwise) that promote animal well-being. In addition, IACUCs are also responsible for reviewing such exemptions on no less than an annual basis.

Scientific justification, such as neurological, endocrine and physical variables that will alter the science, must be critically evaluated to determine which state (before or after enrichment/social housing) better translates to the human population.

Controlled variables do not translate to the human population.

Reporting requirements
Reporting is required to OLAW within 10 days for any protocol suspension and for any deviations to the *Guide*, or regulatory non-compliance that is "serious or continuous". OLAW & USDA representation recognize the subjective nature of the reporting language and anticipate releasing guidelines to further specify occurrences that require and benefit reporting actions.

Creating Quantifiable and Objective Indices of Animal Well Being
(*Animal Well-Being and the Three Rs Track*)
Wednesday, March 21, 2012

The participants in this workshop were interested in learning what others were doing to measure animal welfare, assessing whether their current methods are working and finding tools to assess animal welfare.

The point that measuring pain and distress is different than measuring animal well-being was discussed. The importance of understanding normal behavior in order to detect when behavior changes are abnormal was also discussed.

Physiologic measures were discussed: hormones, heart rate, body condition scoring, reproductive parameters, cortisone levels. The cons were that some measures were too cumbersome and unrefined to detect subtle changes; they didn't give immediate "cage side" information. Additionally, there is cost and a longer time commitment associated with using them. Respiratory rate and heart rate changes may occur too late in the disease process: for welfare and humane endpoints, an earlier detection would be better for the animals.

Behavioral metrics worked well for technicians familiar with the normal behavioral repertoire of their animals, and astute ones with enough time to do proper observations could pick up on small or subtle changes in behavior. Qualitative assessments, such as "personality" (or temperament) in non-human primates, was discussed as a potential indicator of welfare. The question of how to utilize this information, considering that individuals would be very different from each other, was brought up, but the point of this type of assessment is to look for changes in an individual over time, rather than compare individuals to each other. Qualitative assessments could be used to alleviate a particular behavior type, such as fear from human interaction, through a human interaction acclimation program. The decrease in fear displays or fear behaviors can be used to assess the effectiveness of that program or the progress of the animal.

Measuring activity when observers are absent, particularly during night hours when rodents would be most active, was also discussed. Night video and activity wheels were suggested as ways to quantify these activity periods, and the idea of using a 'sentinel' cage, or providing a single cage with the monitoring equipment that would act as a proxy for the other cages, was suggested as a way to make monitoring cost effective. Utilization of enrichment items, such as nest building, was also suggested as a way to measure animal activity or condition, though this has not yet been validated.

The question of how to train astute technicians was considered. Several excellent suggestions were put forward:

- Creating a culture of openness in reporting to allow technicians to know that it's both important and OK for them to speak up when they think something is wrong
- Fostering a culture of high performance standards
- Allowing technicians enough time in their clinical condition checks to focus on observations
- Creating and using a record keeping system, such as cage card observations or

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monitoring forms that would allow multiple observers to follow a case through time
- Having researchers describe their protocols in layman's terms and explain what observations were expected
- Developing a picture book of clinical signs, and cross training staff to teach clicker-training (operant conditioning) to improve behavioral observation skills

MSMR Laboratory Animal Enrichment Symposium

Alan B. Dittrich, Ph.D.
President, Massachusetts Society for Medical Research, Inc.

The 4th annual Massachusetts Society for Medical Research Enrichment Symposium, "All Creatures Big and Small" was held in Boston as an all-day event on March 23, 2012. The symposium boasted over 220 attendees and 14 vendor exhibitors!

Paul McKellips, Executive Vice President for the Foundation for Biomedical Research, kicked off the one-day event with a dynamic talk on the importance and necessity of animal research and the increased approval rating it is gaining among the public. He also spoke candidly about the criticisms surrounding animal research from various groups, showed video and noted the danger that research facilities and personnel have encountered by being in this profession.

Christian Lawrence, Aquatic Resources Program Manager from Children's Hospital, presented "Using Data on Natural History and Behavior to Build Better Management Strategies for Laboratory Zebra Fish". He stressed the importance of understanding natural behaviors and environments and applying them to the lab setting and presented examples and data showing improvements in behavior and breeding when incorporating changes representing their natural habitat.

Kimberly Wasko, from Drexel University College of Medicine, transformed rabbit enrichment to a whole new level. She showed video and pictures of rabbit exercise pens, playgroups, and super-enriched housing.

Brianna Gaskill, Postdoctoral Research Scientist from Charles River Laboratories, spoke in two separate sessions on mice and rat enrichment.

Natalie Bratcher, 3Rs Scientist and Alternatives Coordinator from Abbot Laboratories gave an inspiring talk on Abbott's canine program. It focuses heavily on staff engagement and animal welfare.

The speakers rounded out with Jamie Wells, Animal Husbandry Supervisor from Tufts University, speaking on pig enrichment ("Are Your Pigs Happy?") and Evelyn Skoumbourdis, Veterinary Technician who traveled to the Symposium from Thomas Jefferson University, offering ideas for seemingly "Unenrichable" non-human primates.

The event was another huge success and, as always, we would like to give a special thanks to our vendors for their continued support and fantastic raffle prizes.

The 2012 Enrichment Extravaganza

Dhaval K. Vyas
Biologist/Environmental Enrichment Program Centers for Disease Control and Prevention (CDC), National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Scientific Resources (DSR)

The 2012 Enrichment Extravaganza arrived in Atlanta, Georgia at the campus of Emory University on April 24. This all-day event brought together the laboratory animal community in an environment where ideas were shared for improving the welfare of laboratory animals. The Enrichment Extravaganza was possible because of support from Huntingdon Life Sciences; Covance; Primate Products, Inc.; Shepherd Specialty Papers; Allentown, Inc.; Animal Specialties & Provisions; Purina Lab Diet; Bio-Serv and The Andersons Bedding Products. The Enrichment Extravaganza began with a morning plenary session, followed with a poster session and ended with several workshops.

Dr. Mollie Bloomsmith, from the Yerkes National Primate Research Center, began the day's activities with opening remarks. Dr. Bloomsmith is recognized for her contribution to the behavioral management of several species of primates at Yerkes. She and her team have published several studies examining the effects of environmental enrichment for laboratory primates. Dr. Bloomsmith set the tone for the meeting by asserting that attention towards enrichment in laboratory animal care is at its

highest. With this affirmation in place, the meeting commenced as three speakers presented topics addressing enrichment for a diversity species.

Dr. Kris Coleman flew across the country from the Oregon Regional Primate Research Center to present her work incorporating temperament into the behavioral management of non-human primates. Even though it is well established that primates have unique personalities and peculiarities, management plans often overlook temperament as a variable.
The temperament is defined by how an individual adapts to changes in its environment and can be measured by how it reacts to novel stimuli.
Dr. Coleman's studies used a 6-point scale that categorized temperament on levels of rhesus macaques from shy to bold. Once individuals were assigned a temperament score, experiments were conducted to determine how temperament affected three aspects of behavioral management: social housing, positive reinforcement training and abnormal behavior management.
Rhesus macaques with bold temperament levels had the most successful socialization histories and the more similar the temperament of two macaques, the greater the likelihood of compatibility. Positive reinforcement training is an effective tool as it allows for shaping of numerous behaviors; however, temperament can affect the success of training strategies. Dr. Coleman confirmed that the shyest monkeys were the least trainable using positive reinforcement training during a target training task; therefore, alternative training methods were required. The presence of abnormal behaviors often has an origin during the early stages of development. Infant rhesus macaques that scored on the shy end of the temperament scale developed abnormal behaviors more so than the bolder infants. Based on Dr. Coleman's results, management strategies that ignore temperament are incomplete. In addition to age and sex, differences in personality need to be incorporated in order for a proper administration of animal care. The incorporation of individual differences into decisions made by veterinarians and colony managers creates a more comprehensive approach to the care of primate colonies.

There is abundance in the breadth of literature on the care of laboratory primates and rodents. For zebrafish, the bank of information is less fruitful. The biomedical world has benefited for decades from the use of zebrafish as a model for numerous studies; yet, the care of this species has lagged behind the science. Christian Lawrence was aware of this discrepancy and presented a solution for providing proper care to zebrafish. As a fish ecologist, Christian Lawrence is familiar with the biotic and abiotic requirements for fish in their natural habitats. A zebrafish in Children's Hospital Boston, which has a population of approximately 500,000 zebrafish, finds itself in unfamiliar conditions.
In order to derive the most appropriate care for zebrafish, Mr. Lawrence stressed the importance of examining three components: natural history, normal behavior and measures of well-being. To combat an environment consisting of barren fish tanks, the information from a review of zebrafish natural history provides caretakers with appropriate options for enhancing their housing and diet. Instead of making haphazard decisions on how to augment an empty fish tank, knowledge of zebrafish ecology enables personnel to choose specific variables to manipulate. An understanding of zebrafish behavior is critical to identifying impaired individuals. The life of a zebrafish is quite a drama where offspring are on the menu, social hierarchies are mediated by aggression and hormones control behaviors. In addition to behavior, two other measures of well-being include the rate of growth and physiology. When compromised, zebrafish depress their growth, reproduction and immune function. Once caretakers get a grasp on all three aspects of zebrafish, abnormal individuals are easily identified and managers can begin to determine the source of the problem(s).
Mr. Lawrence showed videos of normal zebrafish compared to abnormal individuals in fish tanks. The fish that were normal swam slowly throughout the fish tank and were not bunched; however, the abnormal fish swam in rapid spurts on the bottom of the tank and were in tight clusters. It was apparent that without knowledge of zebrafish natural history and behavior, it would be impossible to determine which group was normal versus abnormal. The care of zebrafish at Children's Hospital Boston has benefited from Mr. Lawrence's work and the fish tanks are density controlled and

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supplemented with live diets and plastic plants. Utilizing information from field biology and natural history is vital for the management of any species. In labs where non-traditional species are common, a meticulous review of the species' natural history and behavior is critical to providing proper care.

Enriching the lives of laboratory animals is often an expensive endeavor. As president of Bio-Serv, Dr. Karen Froberg-Fejko is familiar with the business side of enrichment. For many institutions, money is the limiting factor that affects the diversity and composition of enrichment programs. Dr. Froberg-Fejko reminded everyone that if the animals' behavioral needs are kept in mind, enrichment can be affordable and effective. Using results from numerous studies, Dr. Froberg-Fejko showed how the proper adjustments to the husbandry of rodents can result in cost savings and better science. Mice and most other rodents have a lower critical temperature (LCT) around 26° Celsius; temperatures below the LCT can result in cold stress. Unfortunately, many institutions house their mouse colonies at temperatures lower than the mouse's LCT. To compensate for cold temperatures, mice build meticulous nests out of various materials. The addition of shredded paper and tissue nesting pads to the cages of mice yielded results that benefited the facility, the studies and the mice. When given nesting material, mice tended to eat less since they expended less energy to stay warm; decreased food consumption resulted in lower expenditure for food. Warmer mice showed a reduction in stress caused by being exposed to temperatures below the LCT. For studies that rely on proper immune function, the validity of the data collected from mice with nesting material was stronger as a result of reducing cold stress. Images from a thermal camera vividly captured the heat profile from mice inside of a nest. The glowing orange and red forms were unmistakable signs of warm mice. A simple mix of shredded paper and tissue nesting paper was enough to create considerable benefits to facility mangers, technicians and researchers. Dr. Froberg-Fejko emphasized that adjustment to the macro- and microenvironments for our animals must be centered on an understanding of their normal behaviors. If behavioral normalcy is kept in mind when selecting enrichment ideas, the care and use of every species will result in rewards for animals and people.

The Enrichment Extravaganza switched gears from the morning plenary sessions to a poster session. Facilities from across the country were represented in posters that covered a diverse selection of topics. A clever method of treating lesions on the hand of a bonnet macaque was displayed by Paula Austin and Casey Coke-Murphy of Vanderbilt University Medical Center Division of Animal Care. Using a shaping plan and positive reinforcement, the researchers trained the patient to dip its hand in a solution containing Dexamethasone® and a food reward. While "fishing" for the treats, the monkey coated its hand in the medicine and the lesions receded. Kaile Bennett, Megan Nowland, Valerie Hill and Gerry Hish showed how the University of Michigan Medical School utilized an "enrichment drive" to acquire donated milk jugs for swine and paper towel rolls for mice. The two winners of the poster contest were from Rutgers University and Yerkes National Primate Research Center. Leslie Sheppard Bird, David Reimer and Elizabeth Dodemaide from Rutgers University's Laboratory Animal Services presented a method of delivering hay to rabbits by placing it inside of balloon whisks hung in the cage. The rabbits spent time manipulating the whisks to retrieve hay and the whisks kept the hay from contacting animal waste in the cage. This cost-effective delivery option would benefit anyone looking for an engaging enrichment option for rabbits. Forage boards are recognized as being one of the most effective enrichment ideas for non-human primates. Katie Chace, Jaine Perlman and Buddy "James" Jordan identified a few flaws in the traditional turf used for most forage boards and presented an alternative option. Turf made from high density polyethylene material was found to be stronger and more practical than the softer turf. The new turf retained the same benefits as the traditional turf and it appeared to be a suitable replacement for institutions with destructive primates. These and other posters that were presented at the 2012 Enrichment Extravaganza may be found on the poster

repository (*www.vetbiotech.com/posters2.php*), which is a joint venture by *The Enrichment Record* and the Veterinary Bioscience Institute.

After an enjoyable lunch with various savory desert options, the crowd dispersed into the afternoon workshops. Workshops were divided into three groups, with each group having three simultaneous presentations. Once the first workshop group was over, the second workshop with its set of three presentations began. The topics discussed were diverse and included sessions on enrichment for ferrets, positive reinforcement training for swine and socialization of dogs. The workshops created an opportunity for the audience to engage the speakers and each other by sharing their own experiences. Questions and discussions were encouraged, which produced useful insights into how personnel in various facilities carried out enrichment. The workshops were an appropriate ending to a day dedicated to providing a greater perspective on the direction of enrichment. The speakers and presenters were successful in demonstrating how vital enrichment is for the proper care and use of animals. Attendees were exposed to enrichment ideas for a diversity of species and new methods for solving common problems. The 2012 Enrichment Extravaganza continued its tradition of creating a forum where the enrichment community can assemble and benefit from one another.

Letter From

Lisa M. Kelly, RLATG, *Training Coordinator*
Office of Animal Care and Use
Boyd Graduate Studies Research Center, University of Georgia

The 2012 Emory Enrichment Extravaganza was great! I was so happy to represent LAWTE at this terrific event. The positive energy and the depth of caring that this group has for laboratory animals is truly inspiring. From primates to fish, there was so much good information. It is clear that there is a revolution in our industry of conscientious people who want to show the world that we take our obligation toward animal welfare very seriously.

Here are just a few of the key points that I took back to my home institution…

Did any of you know that a wire balloon whisk could be a great enrichment device??? Just fill it with hay and rabbits have a great source of nutrition and entertainment, without compromising their ability to be corprophagic. Milk jugs with a couple of small holes and little feed make great enrichment devices for pigs. Hours of fun without all of the dietary issues common with sweet treats. Pretty cool…especially at my budget conscious university.

Also, enrichment for fish!!! Now that is an area that I hadn't really considered. And the best part was that Christian Lawrence spelled out how to sell the idea to your investigators using an argument that makes dollars and "sense" for them.

It was clear that the enrichment arena is expanding. We are getting more creative and more focused on innovative ideas that combine better living for our research animals with better data for our scientists. The world of research is changing. LAWTE and similarly minded individuals are showing that no one cares more about these animals or is working harder to improve their world.

Thanks for LAWTE's support! I would encourage anyone who is able to attend an enrichment event. You will come away with some great ideas and a sense of pride for all of the good people in our industry.

Enrichment RECORD

The Enrichment Record is a quarterly E-Zine created by the Laboratory Animal Research Community as an online forum for:

- Discussing environmenta enrichment in the optimal care of laboratory animals
- Documenting best practices
- Sharing data on the impact of environmental enrichment on the science
- Building the case for integrating enrichment into research design