Volume 18, JANUARY 2014

THE Enrichment RECORD

Publisher: Global Research Education and Training, LLC • Email: mackta@enrichment.com • Website: http://enrichmentrecord.com

Enrichment:

A HISTORICAL PERSPECTIVE ON SOCIAL HOUSING

FACTS AND DEMONSTRATIONS: EXPLORING THE EFFECTS OF ENRICHMENT ON DATA QUALITY

AGGRESSION IN LABORATORY MICE: POTENTIAL INFLUENCES AND HOW TO MANAGE IT

THE Enrichment RECORD

Please direct all inquiries to
Rhoda Weiner, Editor: *rmbw19@verizon.net*

WE'D LOVE TO HEAR FROM YOU!
We welcome your comments, observations and contributions to *The Enrichment Record*. Contributors include lab animal veterinarians, principal investigators, animal care staff, animal behaviorists, animal technologists and members of the bioscience community who promote the 4 Rs: reduction, replacement, refinement and respect.

Share your story ideas with Rhoda Weiner, Editor at *rmbw19@verizon.net*
Guidelines for authors can be accessed at *http://enrichmentrecord.com/contribute/*

Please give credit where credit is due.
Outstanding animal care is truly a team effort, and we ask you to credit colleagues, published reports, articles, and other reference materials that have contributed to your enrichment article. Great ideas don't happen in a vacuum, and we encourage you to list all sources of inspiration.

The Enrichment Record ***is not a peer-reviewed journal. However, the Editorial Board of this E-Zine is composed of dedicated volunteers who have extensive experience and expertise in the care of laboratory animals. Members of the Board are involved with all aspects of this publication.***

The Enrichment Record is published in October, January, April and July. If you are interested in advertising in *The Enrichment Record*, please visit:
http://enrichmentrecord.com/advertise/

Publisher:
GR8 (Global Research Education & Training, LLC)
http://enrichmentrecord.com
Jayne Mackta, *President & CEO*

IN THIS ISSUE | WINTER 2014

The Enrichment Record
is a quarterly E-Zine/Forum for:

- Discussing environmental enrichment in the optimal care of laboratory animals
- Documenting best practices and approaches for addressing challenges of implementation & assessment at every level
- Sharing data on the impact of environmental enrichmenton the science
- Building the case for integrating enrichment into research design

If you are interested in advertising in *The Enrichment Record,* please visit: *http://enrichmentrecord.com/advertise/* or contact Jayne Mackta, Publisher: *mackta@enrichmentrecord.com*

ADVERTISING DEADLINES
January Issue—*December 1*
April Issue—*March 1*
July Issue—*June 1*
October Issue—*September 1*

ADVERTISING RATES
Single Issue
1/4 page $350
1/2 page $500
Full page $1,000

4 Issues
1/4 page $1,120
1/2 page $1,600
Full page $3,200

The Enrichment Record
is an 8.5" x 11" format.
Full color; 300 dpi pdfs accepted.

Visit out website—
browse past issues:
http://enrichmentrecord.com

In Other Words

Welcome to 2014… the year of sharing the caring.

Time sure seems to fly, especially when you are having fun. Can you believe that we are approaching our fifth year of publication? In preparation for the 20th issue of *The Enrichment Record* in July, we are promoting **Enrichment Program Party Packs** to ensure celebrations break out in labs around the globe throughout the year.

Here's the deal: Contribute $50 to *The Enrichment Record* and receive a box of goodies that will excite all the animals in the lab: human and nonhuman alike. Besides discount coupons for enrichment products, these Party Packs feature recipes for delicious treats as well as colorful stickers and magnets that declare "Caring Happens Here." Whether you want to overcome post-holiday depression, SAD, or plain old winter blahs, spread good cheer, help support *The Enrichment Record*, and even stretch your shrinking budget by ordering your **Enrichment Program Party Pack today**. To order, CLICK HERE.

In addition to making yourselves a party, make yourselves a part of the global discussion. Take a few minutes, and take a survey that explores the effects of enrichment on data quality. This survey was set up by Global Research Education and Training (GR8tt) and the RSPCA Research Animals Department, with the aim of discovering how those involved in animal research view the potential effects of providing environmental enrichment for animals on data quality. It applies to all species and types of facility worldwide. The survey is completely confidential and should take no more than a few minutes—although the first three questions are mandatory, you can then go on to answer as many or as few as you like. Filling out this survey will help us to understand people's views and practices, and to identify any training or resource needs that we can address with the aim of helping to improve both science and animal welfare. To participate, **CLICK HERE.** ***http://goo.gl/osnClp***

So please, spread the word and let the parties begin.

Jayne Mackta

Jayne Mackta, Publisher
President & CEO, Global Research Education & Training, LLC (GR8)

LabDiet®

Your work is worth it.®

Satisfy their *needs*, enrich their *senses*.

Macro-Pack

Provides ideal method of delivering superior and constant nutrition along with animal enrichment.

200 X 75-gram packs per box • Four products available

Monkey Jumble®

A fusion of seeds, nuts, fruits and vegetables for enhanced palatability. The assortment of flavors and textures will keep primates interested over long periods of time.

Monkey Morsels

A tasty mixture of small fruits, seeds, and vegetables that are especially suited to encourage the natural foraging instincts of primates.

Health Products

Specialized products to enrich performance and well-being. Products include PriMade® Hydrating Electrolyte Replenisher, Vitamin Supplement Tablets, and PriLieve® Series medicated tablets for maintenance of health and prevention of disease.

LabTreat® Primate Enrichment Products

Primate enrichment products, perfect for reward, reinforcement, and psychological well-being. Products include Foraging Bits, Primate Crunch®, High Fat Primate Treats, Monkey Delight® Tablets, LabTreat® Enrichment Tablets, LabTreat® OmniTreat® Enrichment Tablets & Pellets, and LabTreat® Primate Enrichment Pellets.

For more information visit ***www.labdiet.com*** or email ***info@labdiet.com***

OUR READERS TELL US

The Enrichment Record is an excellent forum in which caretakers and others can share information about novel ways to enrich the lives of laboratory animals. I particularly like the fact that it provides an opportunity for technicians to publish, as their stories provide valuable insights that would not otherwise be available to those that care for captive animals.

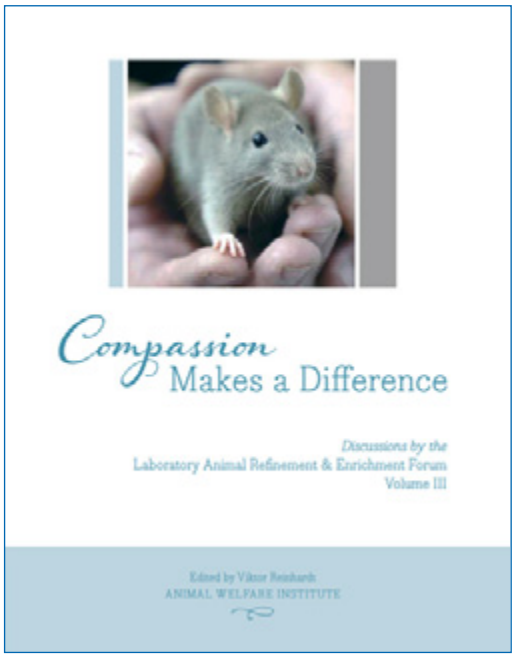
Kristine Coleman,
Oregon Health & Science University
http://www.ohsu.edu/xd/

Resources

AWI is pleased to announce publication of *Compassion Makes a Difference*, the third volume of discussions from the Laboratory Animal Refinement & Enrichment Forum (LAREF), edited by longtime AWI laboratory animal advisor, Viktor Reinhardt. The online forum facilitates the exchange of ideas and the sharing of personal knowledge and experience by animal care personnel who seek to improve the conditions under which animals in research are housed and handled.

In compiling *Compassion Makes a Difference*, Viktor selected the most salient questions and responses posted by forum participants during 2010–2012. Separate sections of the book cover issues relevant to cats and dogs, pigs, sheep and goats, rodents, rabbits, primates, and (new with this volume) cold-blooded animals. The book includes a wealth of practical advice for animal care personnel, as well as touching personal anecdotes concerning the caregivers' attempts to provide enrichment to the animals and shield them from needless stress and suffering.

https://awionline.org/store/catalog/animal-welfare-publications/animals-laboratories/compassion-makes-difference-book



Idea Exchange

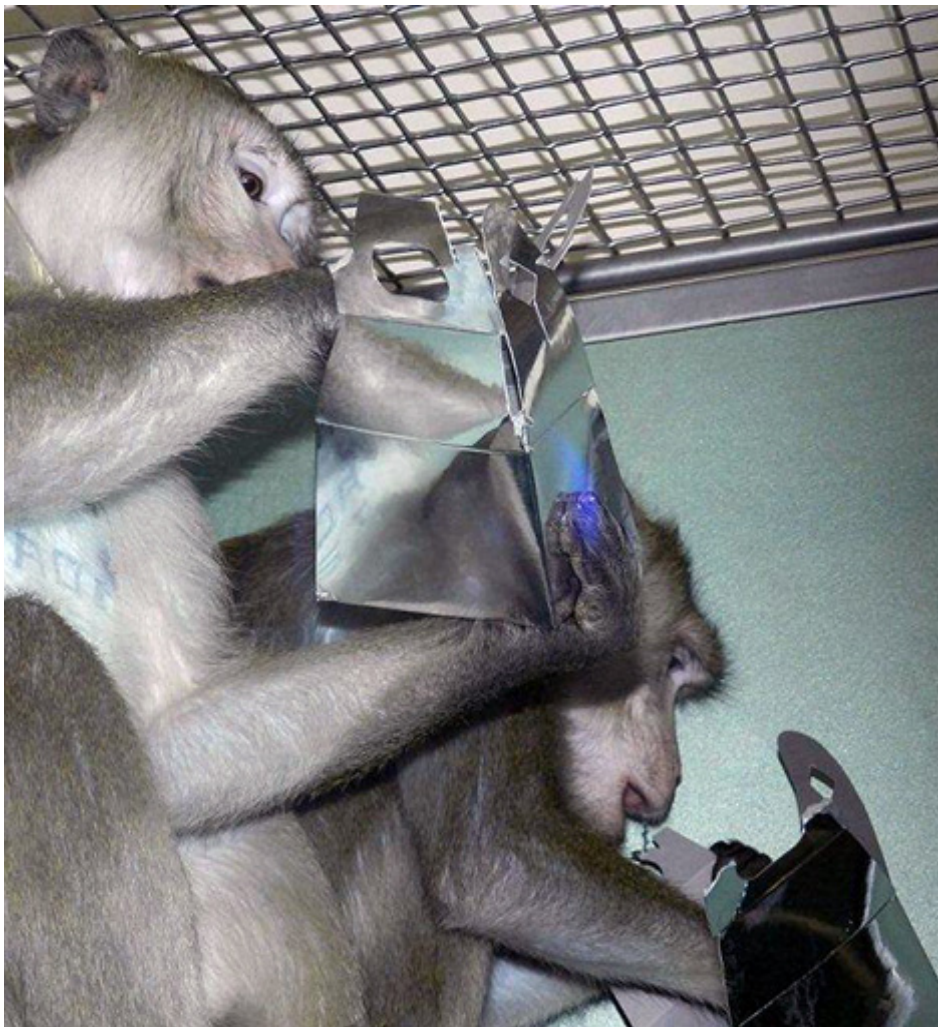
Please provide a photo of your favorite foraging device, manipulanda, puzzle feeder, etc., along with a brief narrative. List the species that it is intended for, describe how the device is used, and include a short statement on the durability, cost, pros and cons. How much time is invested in preparation, and how does that compare with the time invested by the animals? If you have performed any behavioral evaluations and a cost analysis, include that as well.

Please send your ideas to: Genevieve Andrews-Kelly at genandr@aol.com

Thank you!

Primates Presents

Tanya Callan, BS, LAT
Veterinary Technician II, Huntingdon Life Sciences



In the world of enrichment, ideas can be limitless. Sometimes, you just have to think outside the box…or in this case, "inside the box"! Who doesn't love a present with the colorful exterior and a surprise interior? This is how the idea of Primate Presents was created. Primates are very intelligent as well as curious. Providing them with an enrichment device such as a colorful or reflective cardboard box filled with foraging material and food treats supplies structural, sensory, and nutritional enrichment. Primates generally inspect these presents at first, or use the reflective boxes as mirrors and then begin opening the boxes. Some primates tend to tear apart the boxes with either their hands or teeth, while others gently open the box as we expect humans would do. Some primates empty the contents by demolishing the box or flipping the box upside down, while others gently forage for treats with the box still intact. The duration of interest solely depends on the animal.

continued on page 6

 continued from page 5

Although this is a single use enrichment device, the cost is still relatively low. Through a wholesale paper supplier (*www.papermart.com*), we purchase a case of 100 gable boxes which cost $25-$36 depending on the color and design. That's only 25¢-36¢ per box! For foraging materials, we shred our unwanted paper (free of staples, tape, laminated paper, etc.) in our facility; cost is FREE! As for treats, we use anything from trail mix and peanuts to fruit snacks and marshmallows that we have in our inventory. For example, a case of 96 individual packages of fruit snacks costs 28¢ or 29¢ per package. Each Primate Present would get the contents of an individual package.

This makes the total cost of a Primate Present only 54¢-65¢ each. The gable boxes arrive flat and need to be popped into box shape, which takes mere seconds, and then need to be filled. The total time it takes to prepare 40 Primate Presents is about 6 minutes. Besides the pros of low cost, minimal man hours and high animal interest, this present isn't just for Cynomolgus monkeys, but for other species, like the Gottingen mini pig, as well. The only con to this enrichment device would have to be the cleanup. Small pieces of cardboard can be washed down the drains, but if the primate keeps the box intact, then technicians will need to manually remove the box. After watching the excitement and natural foraging behaviors displayed by the primates and rooting behavior of the mini pigs, the pros outweigh the cons!

Lauren Foster, Social Media Specialist, Charles River Laboratories

Join The Global 3Rs Conversation

Charles River is committed to laboratory animal welfare as well as the 3Rs (Replacement, Reduction and Refinement), to help limit the number of animals used in research. Recently, these topics have been of particular interest throughout the industry. Related discussions grow increasingly prevalent around us and an apparent need to host such discussions online has risen to our attention. As a worldwide leader in the humane care of laboratory animals, Charles River introduced the "Animal Welfare and the 3Rs" LinkedIn group, with the support of Dr. Marilyn Brown, Corporate Vice President of Global Animal Welfare, members of her team including Christina Winnicker, Director of Enrichment and Behavioral Medicine, and Judy Murray, Senior Manager of Animal Care & Welfare Training, as well as a number of other animal welfare experts.

In addition to their intrinsic value, laboratory animals play a critical role in the discovery of life-saving drugs and procedures. Charles River encourages the commitment to animal welfare to extend beyond mere compliance with applicable local laws and regulations. We work hand-in-hand with the scientific community to understand how living conditions, handling procedures, and stress play an important role in the quality and efficiency of research. As a continuation of our efforts in this field, we're proud to introduce our new LinkedIn group and encourage community members to join in on our discussions.

LinkedIn is a professional social media network with over a million specialty groups where active discussion forums focused around topics relating to every area of interest imaginable take place every day. LinkedIn users can join relevant groups and participate in discussions by posting meaningful content or reacting to posts provided by others.

Joining and participating in LinkedIn groups can be an extremely valuable and rewarding experience, when executed properly. Not only do they provide the opportunity to communicate with people of similar interests or professional backgrounds, but the discussion forums allow members to gather valuable information from other experts, pose questions and receive answers, and expand individual networks by making new connections.

Charles River's new "Animal Welfare and the 3Rs" group was created to facilitate the active exchange of best practices, knowledge and opinions between professionals committed to laboratory animal welfare and the 3Rs. Because of the sensitive nature of the subject matter, we are only granting membership to individuals within the laboratory animal/research community. The objective is to build and expand this community online by providing a safe place to post articles, blog posts, research findings, opinions, questions and any other relevant information worth sharing with our colleagues.

We'd like to invite you to join our "Animal Welfare and the 3Rs" group and to use it as a resource for valuable information and a place to interact with other members of the community. *Click here to join*. We look forward to engaging with you on LinkedIn!

Celebrating Enrichment in the Workplace for the Holidays

Your Publisher was honored to serve as a judge at the first Annual Tox Ops departmental holiday decoration competition at Huntingdon Life Sciences this December. Not at all influenced by the profusion of baked goods and cheerful gift bags, we three wise judges were literally blown away by the amount of effort that went into "enriching" the various work areas that transformed tech rooms and labs into festive explosions of color and light. It was a truly amazing display that was part 5th Avenue store window and part Tim Burton nightmare. Everything we saw was wildly creative, reflecting each department's individual flavor and flair.

Article

Kathryn Bayne, MS, PhD, DVM, DACLAM, DACAW, CAAB

Global Director, AAALAC International

A Historical Perspective on Social Housing

An examination of the evolution of social housing of laboratory animals not only reveals the foundation of the philosophies, management practices and expectations for social housing and how these have changed over time, but also leads to a better perspective to gauge beneficial future directions. Thus, pausing to reflect on changes over time shows the progress made, which Nisbet (1980) described as that "inexorable change over time from lower to higher states of knowledge." As we evaluate our progress in providing social housing to the variety of laboratory animals we study, it is clear that our knowledge base to accomplish this has increased over time, resulting in greater success in our efforts to socially house our research animals and in improving their welfare.

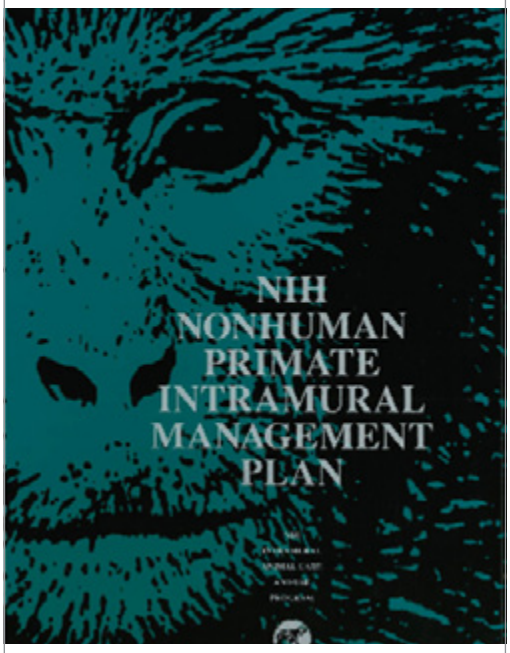
Our industry's current widespread bias toward housing research animals socially whenever possible illustrates a journey in maturing our understanding of the benefits to the individual animal and the research data they produce. The changes in housing conditions of nonhuman primates used in research serve as a good illustration of this journey in management practices. When rhesus monkeys were imported into the U.S. from India, they were placed in groups in holding rooms during the quarantine period. But, the animals were not necessarily familiar with each other, and so many experienced social stress which was compounded by the stress of shipping and the novelty of the environment. One report documented 95 percent of the animals having intestinal parasites and other diseases under these conditions. The adverse effects on the health and well-being of the animals maintained in this type of housing resulted in significant changes in management procedures for imported primates. The accepted methodology was modified such that animals were housed in individual cages to ensure adequate nutrition and to treat (or prevent the spread of) disease conditions. However, the impact on the animals' behavior and welfare was not recognized. In 1978, the challenges associated with housing imported primates in quarantine were made mostly moot when the Indian government banned exportation of nonhuman primates. This action prompted the expansion of domestic breeding colonies, which meant shorter transportation times and a well-defined health history of the primates. These animals typically had a social rearing history, but quarantine procedures still entailed single housing, primarily to facilitate management procedures.

Just seven years later, the Food Security Act of 1985 was passed which directed the Secretary of Agriculture to establish regulations to provide a physical environment adequate to promote the psychological well-being of nonhuman primates. The Act stated that "Social interaction is an integral part of the psychological well-being of nonhuman primates, and we believe it is appropriate to address such social grouping in the context of an overall approach to promoting the psychological well-being of nonhuman primates." In the same vein, the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (IRAC 1985), also published by federal agencies, stated in Principle VII, "The living conditions of animals should

be appropriate for their species and contribute to their health and comfort." The implication that animal housing should not only support animal well-being, but actually to contribute or enhance it, was novel for the time.

Contemporaneous to this pivotal declaration from the federal government was the publication of the 6th edition of the *Guide for the Care and Use of Laboratory Animals* (*Guide*, NRC 1985). The tone taken toward social housing in this report was much more conservative. The *Guide* limited the scope of interactions to animals that are able to communicate, leaving one to wonder what species this excluded. The *Guide* stated, "The effects of social environment on caged animals vary with the species and experience of the animals. They are often more difficult to define than the effects of physical environment." And, "there is little objective evidence for defining adequate care in relation to social environment…. The data are limited and contradictory… and lack sufficient guidance to establish absolute recommendation…."

In light of this philosophically contradictory environment, the Director of the National Institutes of Health (NIH), Dr. James Wyngaarden, instructed the Deputy Director for Intramural Research, Dr. J.E. (Ed) Rall, to develop an NIH Nonhuman Primate Management Plan to address the issue of promoting the psychological well-being of non-human primates used in research.



To that end, in 1987, a survey was conducted of the intramural research community and a set of recommendations was developed based on those survey results. Specifically, the Plan recommended that social housing be considered "an appropriate means of providing enrichment…." Others were also moving forward with plans to socially house their research primates. But, the risks associated with social housing were not fully understood or anticipated. For example, Line et al. (1990) reported ten of thirteen monkeys sustaining injuries during fights in the first eight days following group formation; one female died of general trauma.

As our knowledge about appropriate methods to provide social housing of nonhuman primates increased, so too did the philosophical tone in the *Guide* become biased toward social housing. The seventh edition of the *Guide* (NRC 1996) stated "It is desirable that social animals be housed in groups…. When it is appropriate and compatible with the protocol, social animals should be housed in physical contact with conspecifics."

Social housing stimulates the expression of species-typical behaviors.

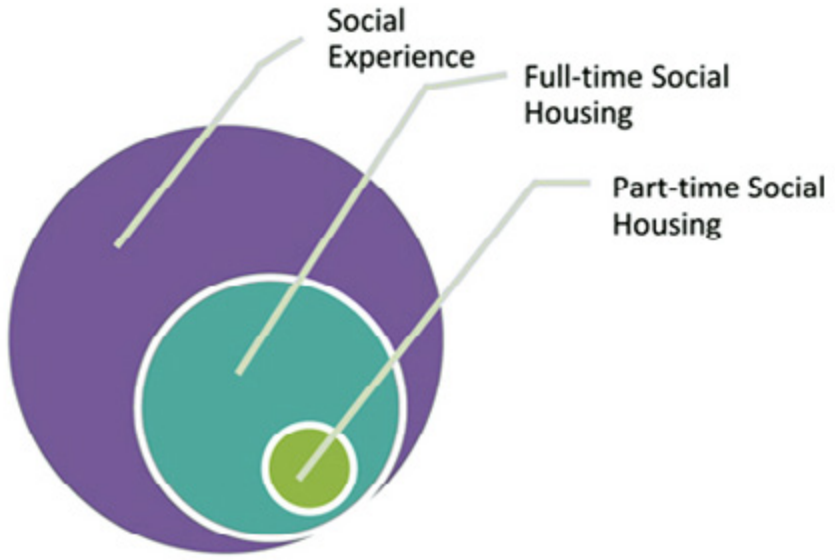
continued on page 10

continued from page 9

This edition of the *Guide* further stated, "Appropriate social interactions among members of the same species (conspecifics) are essential to normal development and well-being…." It was noted that social housing might buffer the effects of a stressful situation, reduce behavioral abnormality, increase opportunities for exercise, and expand species-typical behavior and cognitive stimulation. The *Guide*'s position on social housing matured even further in the eighth edition (NRC 2011). In this latest edition, the *Guide* states that "Single housing should be the exception and justified based on experimental requirements or veterinary-related concerns about animal well-being" and that "The need for single housing should be reviewed on a regular basis by the IACUC and veterinarian."

Of note, AAALAC International, which uses the *Guide* in its assessments of animal care and use programs, has taken an even stronger stance, stating in one of its very few Position Statements that "Social housing will be considered by AAALAC International as the default method of housing unless otherwise justified based on social incompatibility resulting from inappropriate behavior, veterinary concerns regarding animal well-being, or scientific necessity approved by the IACUC (or comparable oversight body)." AAALAC offers this further guidance: "When necessary, single housing of social animals should be limited to the minimum period necessary and, where possible, visual, auditory, olfactory and, depending on the species, protected tactile contact with compatible conspecifics should be provided. In the absence of other animals, additional enrichment should be offered, such as safe and positive interaction with the animal care staff, as appropriate to the species of concern; periodic release into larger enclosures; supplemental enrichment items; and/or the addition of a companion animal in the room or housing area." AAALAC has also issued a "Frequently Asked Question" related to this subject, which notes that the total social experience of an animal is larger than that offered solely through full-time social housing. AAALAC notes that the entire social experience of the animal can include interactions with other animals in the room, personnel, etc. AAALAC states that there is a spectrum of social experiences that can be made available to an animal, which, when properly managed, can significantly enhance the welfare of the animal.

The Institutional Animal Care and Use Committee (or comparable oversight body) and veterinarian should periodically review the strategies for providing social housing or other social experience to the animals at the institution to ensure conformance with the *Guide*.



The relative social experience of a laboratory animal.

AAALAC expands on this approach in a podcast available on its website *http://www.aaalac.org/education/index.cfm*

The ever-broadening scope of social housing of research animals is increasingly encompassing the diversity of species used in research, including dogs, cats, rabbits, a variety of rodent species, agricultural animals, aquatic animals, and others.

Socialization of dogs promotes normal behaviors such as play.

For some of these species, such as cats and dogs, a concomitant effort is underway to reduce the "institutional" appearance of the housing environments by designing the enclosures to be colorful, include sight-lines and be highly enriched. Baumans and Van Loo (2012) have described a similar evolution for the housing of rodents used in research. They describe the changes in cages for socially housed rats over a 50 year period—reflecting back on how cages used circa 1960 had a low cage height and were mostly wire-bottom, to the use of solid-bottom bedded cages in common use circa 1990, to the highly enriched cage environment currently in use.

These examples illustrate the point that social housing is increasingly being considered in the context of facility design, cage design, cage space, and cage/pen complexities (e.g., enrichment strategies). In this way, to some degree, the past is prologue. Our animal housing systems are returning to an approach that is predominantly based on the natural social behavior of the animal, but unlike the methods of social housing used decades ago, our methods are based on an increased understanding of the species' behavior, more attention is paid to ensuring animal health is not compromised, and the welfare of the animal and quality of the research animal model are overarching factors in animal housing decisions.

References

Baumans V and Van Loo PLP. 2012. How to improve housing conditions of laboratory animals: the possibilities of environmental refinement. *Vet J.* 2013 Jan;195(1):24-32.

Interagency Research Advisory Committee (IRAC). 1985. U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. Federal Register, May 20, 1985, Vol. 50, No. 97, Office of Science and Technology Policy.

Line SW, Morgan KN, Roberts JA, and Markowitz H. 1990. Preliminary Comments on Resocialization of Aged Rhesus Macaques. *Lab Prim News* 29(1):8-12.

National Research Council. (1985). *Guide for the Care and Use of Laboratory Animals.* Washington DC: National Academies Press.

National Research Council. (1996). *Guide for the Care and Use of Laboratory Animals*. Washington DC: National Academies Press.

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

Nisbet R. 1980. *History of the idea of Progress.* Basic Books, New York.

Penny Hawkins, BSc, PhD

Research Animals Department, RSPCA, UK

Facts and Demonstrations: Exploring the Effects of Enrichment on Data Quality

There is widespread support within the scientific community for the concept that better welfare equals better science. It is also broadly accepted that environmental enrichment improves welfare. I have seen much evidence of support for both of these concepts in the literature, at meetings and when I visit animal facilities and see enrichment increasingly becoming the norm. However, this degree of acceptance is still not universal. For example, there are inconsistencies with levels of enrichment that are provided, even within the same facility at times. I still encounter researchers who do not regard enrichment as the default and have to be persuaded to provide it. There are also still, unfortunately, plenty of examples of materials and methods sections in papers that simply say something along the lines of "animals were housed in standard cages with soft wood litter and lab chow *ad libitum*", with no explanation as to why the animals were not provided with anything else.

There are three categories of frequently encountered reasons for resistance to enrichment. First, some individuals and organisations are opposed on economic grounds, as evidenced by some of the responses* to the increased emphasis on enrichment in the most recent US *Guide for the Care and Use of Laboratory Animals* (NRC 2011). Second, some have a perception that the welfare benefits to animals, with respect to particular refinements or enrichment as a whole, are not yet proven. This is often used to try to help justify the first point. For example, the American Psychological Association's (APA 2011) comment to the National Institutes of Health on the new *Guide* stated that "solid bottom cages particularly for rats and mice are based on weak to nonexistent evidence"—yet a classic paper in the animal behaviour literature found that rats housed on grid floors will lift 83% of their body weight to gain access to a solid floor (Manser et al. 1995).

Neither of these objections is sustainable. However, the third reason for resistance to enrichment relates to concerns that experimental variability will increase, or a confound will be introduced, affecting data quality. This does merit further examination. There are three areas of concern:

1. the validity of the science within an individual project, if another variable is introduced;
2. whether or not the data can be compared with those obtained from studies conducted without enrichment; and
3. an ethical issue—whether greater variability will necessitate an increase in animal numbers to ensure that results are significant, trading off refinement and reduction against one another.

Assumptions are often made about all three of these points, hence the title of this paper: "Facts and Demonstrations". This quote from John Ruskin, the nineteenth century artist, social critic and philanthropist, sums up the kind of approach that should be taken when confronted with pre-judgements about enrichment and its effects on the science: **"the work of science is to substitute facts for appearances, and demonstrations for impressions".** This can be applied to all three of the concerns listed next.

* See *http://grants.nih.gov/grants/olaw/2011guidecomments/web_listing.htm*

Issue 1:
The effect on scientific validity within the project

Many international regulations and guidelines do encourage or even mandate enrichment. For example, the US *Guide* presents an enriched environment as the default position. The European Union (EU), Directive 2010/63/EU, which regulates animal use throughout all 28 Member States, requires that restrictions on the animals' abilities to satisfy their physiological and behavioural needs be kept to a minimum (EC 2010). However, both US and EU standards acknowledge that there may be scientific justification for modifying or withholding enrichment. The problem lies in the nature and level of the justification that is accepted by regulators, and/or by ethical or animal care and use committees. This can range from requiring evidence from the literature, or pilot studies, to back up assertions that data quality will be affected, to simply accepting a researcher's assumptions at face value.

So, what is genuine scientific justification for restricting or withholding enrichment? It is essential to ask this question—including asking yourself if you are a scientist who houses animals without enrichment as a matter of tradition. Much can be achieved if assumptions and the status quo are creatively challenged (Fig.1).

Photo: Novo Nordisk

Figure 1: This picture shows a dog used in pharmacokinetic studies who is regularly able to exercise outside in a secure area, with a group of other dogs, even between procedures.

There has always been a steady stream of studies and reviews in the literature that have looked at the effects of refinement, including enrichment, on data variability and standardisation (e.g. Eskola et al. 1999, Baumans et al. 2003, Würbel et al. 2005, Mikkelsen et al. 2010, Toth et al. 2011). It has been found that enrichment has effects on variability or data quality that are significant, or that it has effects that are not significant, or that there are no detectable effects at all. This demonstrates that it is not possible to make sweeping assumptions about the effects of enrichment on data quality. What is really important is making sure that findings of such studies are interpreted, and acted upon, appropriately.

That is, even if enrichment does have a significant effect on data, restricting or withholding it may not be the right thing to do.

This is an important point not only for animal welfare reasons, but also because of the current climate of reflection and debate about the translatability and validity of animal "models". Systematic reviews of preclinical studies have indicated that there are significant issues that need to be addressed with respect to translatability, and there is increasing recognition that "standard" housing and care might actually be causing problems. For example, a recent review paper on optimising translation of

continued on page 14

continued from page 13

preclinical studies for central nervous system disorders cited the use of sedentary, unstimulated animals as a limitation of current approaches, suggesting that therapies showing promise in standard-housed animals should be retested under conditions of greater environmental complexity, in order to improve construct validity (Burrows & Hannan 2013). From an ethical point of view, it seems to me it would be better to dispense with the "standard housed" stage altogether.

To further illustrate the translatability issue, here are three recent papers that have set a good example with respect to interpreting data from animals provided with enrichment*. If the authors of these three studies had decided to withhold enrichment in the belief that the effects on data quality were negative, one could argue that the models would have been less valid, so that important, relevant results would not have been obtained.

Example 1: fibulin-4+/- mice

Fibulin-4 is a protein that is expressed by vascular smooth muscle cells, and is essential for maintaining arterial integrity. Homozygous fibulin-4+/- knockout mice die before birth from arterial haemorrhage, but heterozygous animals appear outwardly normal—although gaps are present between smooth muscle cells in the aorta and the endothelium also shows signs of damage. Figure 2a depicts gaps (shown by green arrows) and fragments coming away from the endothelium (shown by red arrows). In a controlled study, it was found that housing the heterozygous fibulin-4+/- mice in large cages, with a tunnel and wheel, significantly reduced the gaps and maintained the integrity of the endothelium, as in Figure 2b (Cudilo et al. 2007).

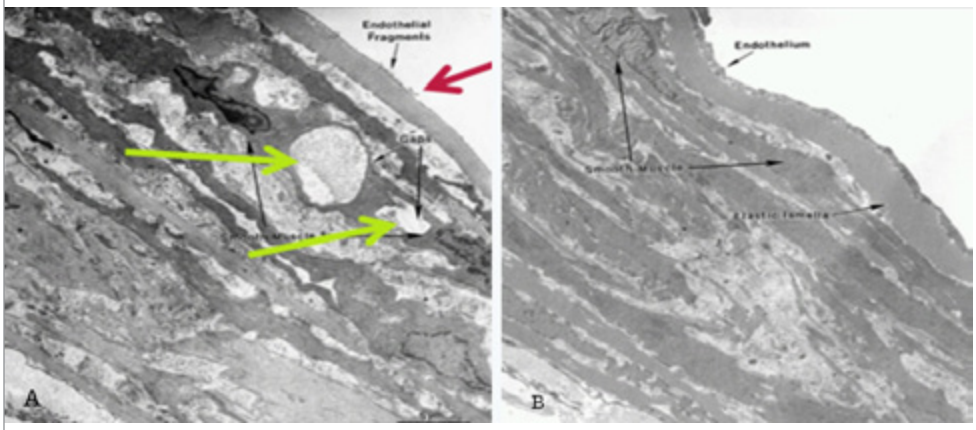


Figure 2: Electron micrographs of sections of aorta from fibulin-4+/- mice housed in (a) standard and (b) enriched cages

The authors' interpretation of these results was that findings assumed to be due simply to genetic differences may have been wrongly interpreted. This is because environmental factors, such as housing conditions, play a more important role in determining this phenotype than was previously thought. Potential mechanisms for this are discussed; for example, it could be that the better housing environment creates conditions in which a surrogate protein could inhibit gap formation. Alternatively, environmental factors could change the methylation patterns of the genes, altering their transcriptional capabilities. Importantly, the authors consider the implications of their findings for human patients with conditions such as the connective tissue disorder Marfan syndrome.

They suggest that programmes of mild exercise could benefit these patients and even help to inform preventative strategies before aneurysms occur. This is a constructive way to interpret differences in animals housed in an enriched environment—accepting that results are different, considering why this might be and exploring ways of increasing translatability and

• Given that the RSPCA's ultimate goal is total replacement with humane alternatives, citing studies that involve animal use as "good examples" does present something of a dilemma. But it is unethical to use animals in protocols that are flawed, as this is causing avoidable suffering and wasting lives. Also, these studies support the case for providing enrichment on scientific grounds, which will lead to welfare benefits for animals while their use continues, an immediate aim of the RSPCA.

benefit. Mice on future studies should also benefit, if they have better quality environments to live in.

Example 2: tumour growth

The second example concerns the effects of an enriched environment on levels of the hormone leptin, on signalling proteins known as eicosanoids, and on the pro-inflammatory mediator COX-2 (Nachat-Kappes et al. 2012). It was hypothesised that these factors would have varying effects on the development and progression of mammary tumours, depending on whether the mice were housed in a standard or enriched environment. The study found that mammary tumour weight was significantly reduced in the enriched environment. In addition, normal mammary glands of mice in the standard environment had more COX-2 positive cells, suggesting an increased inflammatory state of the mammary gland under those conditions. The enriched mice had a marked decrease in intratumoral COX-2 activity and an increase in the plasma ratio of adiponectin/leptin levels, both of which have previously been associated with resistance to tumours.

The authors note that standard housing affects basic biological processes, such as mammary gland development and pathogenesis. They conclude that their study provides "evidence for the need of strong recommendations for future directives on the use of enriched environment in experimental animal models". They also cite Martin et al. (2010), which makes the case that many laboratory rodents are metabolically morbid; that is, sedentary, obese, glucose intolerant and on a trajectory to premature death. In fact, Martin et al. suggest that the standard, overfed, sedentary control animal might be a better model for overweight and sedentary human subjects, but may be inadequate for normal weight humans, making the point that patients with cancer, vascular disorders and neurodegenerative diseases are often otherwise fit and cognitively stimulated. Returning to the paper evaluating tumour development, the authors also indicate that their research provides evidence supporting the positive impact of physical and social well-being with respect to managing cancer progression in human patients.

What do you think? *The Enrichment Record* and RSPCA have put together a survey to gauge people's views on the effects of enrichment on data quality. We would greatly appreciate your input and will feed back and discuss the results in a future edition. Please take part at http://goo.gl/osnClp

Example 3: amyloid plaques

The last of the three examples relates to $APP^{Swe}/PS1^{L166P}$ mice used in Alzheimer's disease research (Montarolo et al. 2013). In this study, the authors begin by citing reports that humans who undertake enriched social, physical and/or cognitive activities have a reduced risk of developing Alzheimer's disease. One of the aims of this project was to use the effects of enrichment to try to better understand these protective effects of exercise and stimulation. As in the studies mentioned previously, $APP^{Swe}/PS1^{L166P}$ mice in "standard" housing were compared with those in an enriched environment. The authors found that enrichment transiently accelerated the deposition of the amyloid plaques that are diagnostic of Alzheimer's, but there was a protective effect on cognitive deterioration for the enriched mice. The performance deficit in the Morris water maze was significantly reduced for those housed in enriched cages.

continued on page 16

 continued from page 15

The authors note how enrichment can modulate both symptoms and pathological progression in $APP^{Swe}/PS1^{L166P}$ mice. The results from animals with enrichment confirmed the dissociation between amyloid burden and cognitive deterioration that has been observed in human patients. The authors also review the results of other studies using a variety of different enrichment and exercise protocols, from the aspect of using enrichment to help understand the effects of the environment on pathology.

Addressing animals' needs makes for better science

Considering the above examples, it is hardly surprising that animals in "standard" laboratory housing are not physiologically or psychologically normal. Nor is it surprising that this can affect not only welfare but also scientific quality. To illustrate this, Table 1 provides some examples of the conflicts between the needs of mice and requirements of humans in a laboratory setting.

Sources: Latham and Mason (2004), Burn (2008). Castelhano-Carlos & Baumans (2009), Hurst & West (2010; tail capture), Gaskill et al. (2009; temperature)

Table 1: Conflicts between the needs of laboratory mice and humans

Mice are ...	... but
Nocturnal and crepuscular	they are housed in bright light
Highly dependent on smell and scent markings	their markings are completely destroyed whenever the cage is cleaned
Sensitive to ultrasound	there are many sources of ultrasound in the laboratory, and these are not always checked and minimised
Able to feel more secure when touching objects (thigmotaxis)	they are often housed in barren cages
Master diggers	they have no opportunity to burrow
Highly social (sex and strain dependent)	they are often housed in inappropriate groups or singly
Capable of covering long distances	they are housed in small cages
Omnivorous, trying new foods from different feeding sites	they are fed boring, monotonous diets from hoppers
Made extremely anxious when captured by the tail	most people catch them by the base of the tail
Most comfortable at a temperature of 26° to 34°C	many facilities house them at colder temperatures, and/or do not provide sufficient nesting material

The aspects of mouse behaviour outlined in the table demonstrate just how undesirable a "standard" cage can be from a mouse's point of view. For example, repeated scent markings can actually build up into permanent little "pillars" that are an important feature of the environment for mice, yet these patterns of scent markings are destroyed whenever cages are cleaned. Although there is an obvious need to clean cages for health reasons, some other behaviours can be catered for by altering light regimes, using a bat detector to detect troublesome ultrasound, and providing refuges. The "need" to burrow could perhaps be reduced if appropriately designed tunnels and shelters are provided, but the inability to control social interactions and range over large areas is likely to be a significant stressor. The range sizes of wild mice can be anything from 2 m^2 to 10,000 m^2—but a standard cage can be just 0.035 m^2. Sixty of these cages could fit into 2m^2. Eating plain lab chow from a hopper, with no opportunities to forage, is not only boring but also leads to obesity, with serious implications for validity as mentioned earlier.

Most of the information on mouse behaviour in Table 1 is from Latham and Mason (2004) and Burns (2008), which are well worth reading if you want to understand the mouse and rat better. Castelhano-Carlos and Baumans is an excellent review of the effects of the environment and husbandry procedures on rat well-being and should be required reading for everyone involved in the care and use of any species. For example, the authors cite a study that found rat plasma corticosterone levels doubled as a result of metal cages being banged in the animal room, and remained at these levels for 2 to 4 hours. This would clearly affect data quality if scientific procedures were conducted within this period, to say nothing of the impact on welfare.

It is sometimes argued that strains of laboratory mice have been inbred for generations and such "wild-type" behaviours are no longer relevant, but this is effectively countered by the "Ratlife" documentary and website (*www.ratlife.org*). This is a film that follows a group of laboratory rats released into a semi-wild enclosure, and it demonstrates that natural behaviours are still innate after many generations in the laboratory.

Looking at this another way, rather than worrying about the effects of enrichment on data quality, conditions that cause poor animal welfare should be the prime concern. Inadequate housing, husbandry and care, and unrefined procedures, can lead to stress and abnormal behaviours that are certain to be experimental confounds in themselves (see Box 1).

continued on page 18

Box 1: Effects of timing on anxiety and cognition

DBA mice are thought to be an animal model of anxiety and have also been reported to exhibit cognitive dysfunction in spatial memory tests. However, behavioural inhibition, an indicator of anxiety, was significantly reduced when mice were tested using a modified hole board during the dark (or active) phase as opposed to the light phase. The cognitive performance of the mice was also better at times when they would naturally be awake and active (Roedel et al. 2005). The authors make the point that light conditions can be confounding factors, yet this is often not taken into account. How many papers even state the time and lighting conditions when behavioural or cognitive tests like these were run?

 continued from page 17

Although the examples considered so far in this paper relate to positive effects of refining husbandry on data quality, reducing stress during procedures can also benefit the science, especially when aiming to avoid restraint.
As an example, retinoic acid, which is used in developmental studies, is usually administered by oral gavage. This involves stressful capture and restraint as well as the distressing gavage procedure. However, after some trial and error with different baits, it has proved possible to give retinoic acid in a chocolate treat which the mouse voluntarily eats when briefly placed in a cage base (Maconochie et al. 2012). This is not only less stressful for the animal but the bioavailability of the retinoic acid is also improved, and there is a good dose-response relationship.

Enrichment as a welfare indicator

Besides the scientific benefits, there are other advantages associated with providing enrichment. Giving animals enrichment items also gives them an additional means of communicating their state of well-being, or whether they are suffering. In Figure 3 (Arras et al. 2007), the mouse in the top two pictures has undergone a surgical procedure but has had adequate perioperative analgesia. He has built a discrete nest and has defaecated outside it, as shown by the circles (arrows). The mouse in the bottom two pictures has not had adequate pain management and has not made a proper nest; his faeces are also deposited within the nesting material. He would clearly benefit from some analgesia, but it would have been much harder to tell if the nesting material had not been present. This is not just about welfare; there could well be an impact on the science too, for example, if further interventions were begun before animals had fully recovered from surgery.

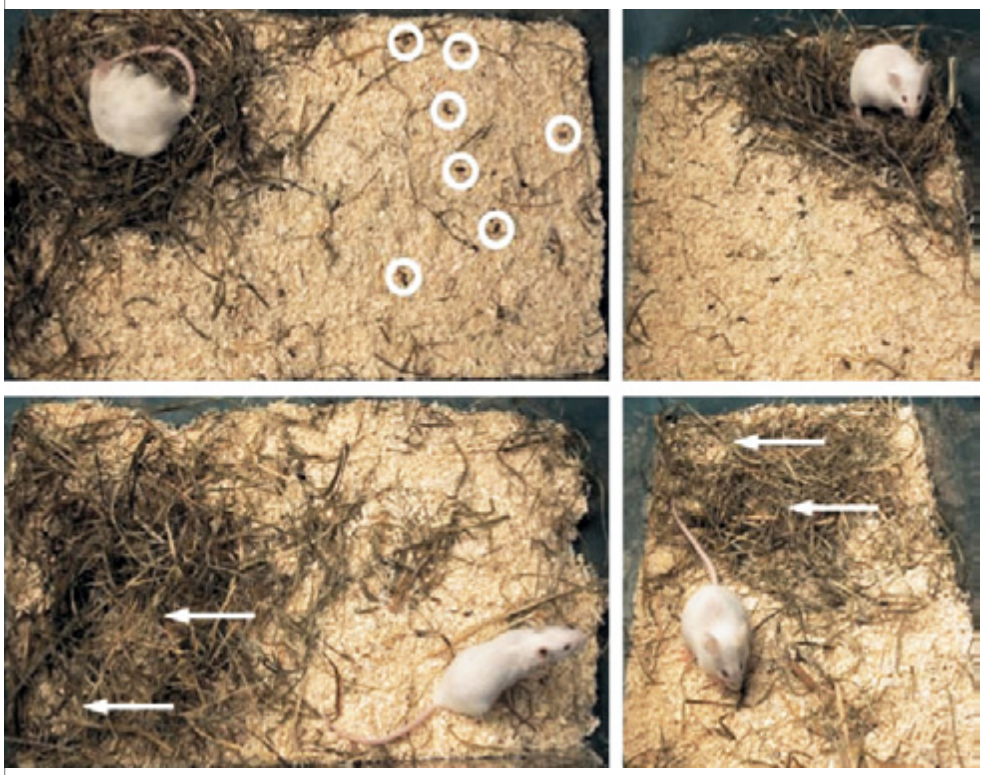


Figure 3: Mouse nest quality as an indicator of postoperative pain

The concept of using enrichment as a welfare indicator is starting to become more widespread. For example, nest scoring is used in Alzheimer's research; in Figure 4, the left hand nest scored 1, and the right hand nest scored 5 out of 5 (Deacon 2012). Burrowing behaviour is a useful and well validated early indicator of pain or sickness behaviour (Jirkof et al. 2010). Although this requires a tube full of burrowing material, which is not often routinely supplied as enrichment, it could be useful in pilot studies as a means of identifying and recognising suffering so that protocols can be refined.

Figure 4: Nest quality as an indicator of the progression of Alzheimer's disease in mice

Issue 2:
Comparability of data with previous studies conducted without enrichment

Whether data obtained from animals provided with enrichment is comparable with data from animals in "standard" conditions is often raised as a concern within regulatory toxicology, although the issue may arise in any field of biomedical research. However, it is essential not to make any assumptions about comparability without conducting objective evaluations to test these. In practice, whatever the research area, data may be comparable, or any differences may be systematic so that it is possible to take them into account, for example, by using appropriate statistical analysis. Alternatively, data quality may be improved by providing enrichment because animals are more physiologically and psychologically normal, as discussed in the previous section. For scientific reasons, data quality should generally be the prime consideration and should take precedence over comparability.

With respect to regulatory toxicology in particular, it is important to look critically at regulatory requirements and what they actually say with respect to enrichment. For example, the OECD states that "proper conditions should be established and maintained for the storage, housing, handling and care of biological test systems", which can be interpreted as providing appropriate housing including enrichment (OECD 1998). The UK Good Laboratory Practice guidelines are more explicit and state that care, housing and containment should "prevent stress and other problems which could affect the test system and the quality of data" (Department of Health 1999). The ICH also recognises that data from unstressed animals will be of better quality (ICH 2000). These statements are borne out by the experience of a major UK Contract Research Organisation, which supplies enrichment as the default and says that most clients readily accept this, provided that enrichment is not abruptly provided or changed during a study.

Issue 3:
Potential for enrichment to lead to increases in animal numbers

This is primarily an ethical issue, as it requires a balance to be struck between animal numbers and suffering. While increasing numbers is diametrically opposed to the aspiration to implement Reduction, it should not be assumed that numbers will have to increase if enrichment is provided. A pilot study can evaluate this one way or the other. Sometimes objections are raised to pilot studies on the grounds that they increase the total number of animals used. However, it is often possible to design pilot studies so that the data can be used as part of the data set for the completed study, and no additional animals will ultimately be needed.

continued on page 20

 continued from page 19

Even if pilot studies or other analyses demonstrate that more animals would be required, reduction is not necessarily everything. Refinement can "trump" reduction if it means that each individual will suffer less, so increasing numbers but reducing severity may be the right thing to do.

Whatever the final experimental and husbandry protocols, it is essential to explain what has been done to refine housing and care, in papers, posters and talks. Otherwise, in the absence of any explanation, it appears as though no efforts were made to research, consider and implement refinement. Including information on enrichment (and refinement in general) helps to promote good practice amongst colleagues and to reassure the public that efforts are made to minimise suffering, improve welfare and improve science. The ARRIVE guidelines set out useful guidance on the kind of information to include (Kilkenny et al. 2010), and it is also helpful to explain where it has not been possible to refine for justifiable scientific reasons, or where numbers have increased as a result of refinement.

It is good practice to apply this open approach to the public communication strategy relating to animal use at your facility, in the form of information on refinements and how these are considered, evaluated and applied, which can be presented on websites and in other materials such as annual reports and Corporate Social Responsibility statements. Communicating with the public about refinements such as enrichment, and the scientific and animal welfare benefits, is important because (1) most people are concerned about animal use, and the suffering this can cause (Ipsos MORI 2010), and (2) the public directly or indirectly funds research, so is entitled to know whether its donations and taxes are being spent on good quality, well-designed research projects.

Action points:

Facts...

- Do not assume that refinement will adversely affect data or results without actually evaluating whether this is the case, for example by conducting pilot studies.
- Interpret findings of evaluation or pilot studies with an open mind:
  - if the data from animals provided with enrichment are different, this does not mean that they are less relevant, or flawed;
  - it may be that the data from animals in standard housing were flawed; or
  - it may be possible to accommodate the differences by adjusting the experimental design or data analysis.
- Take part in discussion and dialogue among user groups and within the literature on enrichment and its effects, as this is helping to improve communication and openness about the issue.
- If working in a regulatory arena, look critically at regulatory requirements and ensure they have not been misinterpreted with respect to whether enrichment can be provided.

... and demonstrations

- Write up publications according to the principles in the ARRIVE guidelines—which also need to be taken into account at the project design stage, so as to ensure that appropriate information is recorded.
- Include details of refinements in materials and methods sections, challenging any requirements to remove detail. If it really was not possible to refine for justifiable scientific reasons, briefly explain and demonstrate why.
- Keep up with current thinking and approaches to refinement and its impact on both science and animal welfare.
- Actively make sure that there are adequate information channels at your facility for accessing information about enrichment and its implications for experimental design and analysis. This could be via an individual such as the Named Information Officer in the UK, or through an effective ethical review or animal care and use committee that includes one or more members who have the necessary interest and expertise.
- Please fill in our survey at *http://goo.gl/osnClp*

Conclusion

Enrichment should be embraced as essential for humane science, regarded as a requisite for more valid science, taken for granted as the norm unless there is compelling scientific justification, and its application promoted within the scientific community and to the wider public. Things have certainly changed for the better, but there is still much work to be done.

Penny Hawkins, BSc, PhD, *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 Animals in Science Committee (ASC), the body that advises the secretary of state on the implementation of the UK Animals (Scientific Procedures) Act 1986.*

References

American Psychological Association (APA) (2011) *Comments Submitted to NIH on the Proposed Eighth Edition of the Guide for the Care and Use of Laboratory Animals.* Download at *http://www.apa.org/science/leadership/care/comments-nih.aspx*

Arras M, Rettich A, Cinelli P, Kasermann HP & Burki K (2007) Assessment of post-laparotomy pain in laboratory mice by telemetric recording of heart rate and heart rate variability *BMC Veterinary Research* 2007, 3:16 doi:10.1186/1746-6148-3-16, open access at: *http://www.biomedcentral.com/1746-6148/3/16*

Baumans V, Augustsson H, van de Weerd HA & Kruitwagen CLJJ (2003) Environmental enrichment does not affect the variability of animal experimentation data in the Light/Dark test. 3R-Info-Bulletin 22, download from *http://www.forschung3r.ch/en/publications/bu22_print.html*

Burrows EL & Hannan AJ (2013) Towards environmental construct validity in animal models of CNS disorders: optimizing translation of preclinical studies. *CNS Neurol Disord Drug Targets* 12: 587-592

Burn CC (2008) What is it like to be a rat? Rat sensory perception and its implications for experimental design and rat welfare. *Appl Anim Behav Sci* 112: 1-32

Castelhano-Carlos MJ & Baumans V (2009) The impact of light, noise, cage-cleaning and in-house transport on welfare and stress of laboratory rats. *Laboratory Animals* 43: 311-327

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

Deacon R (2012) Assessing burrowing, nest construction, and hoarding in mice. J. Vis. Exp. (JOVE) 59: e2607, doi:10.3791/2607, open access with video clips at: http://www.jove.com/video/2607/assessing-*burrowing-nest-construction-and-hoarding-in-mice* (last viewed 25 November 2013)

Department of Health (1999) *The Good Laboratory Practice Regulations* 1999. Statutory Instrument 1999 No. 3106. London: The Stationery Office

EC (European Commissi0n) (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

Eskola S, Lauhikari M, Voipio H-M, Laitinen M & Nevalainen T (1999) Environmental enrichment may alter the number of rats needed to achieve statistical significance. Scand J *Anim Sci* 26: 134-144

Gaskill BN, Rohr SA, Pajor EA, Lucas JR & Garner JP (2009) Some like it hot: mouse temperature preferences in laboratory housing. *Applied Animal Behaviour Science* 116: 279-285

Hurst JL & West RS (2010) Taming anxiety in laboratory mice. *Nature Methods* 7: 825–826, doi:10.1038/nmeth.1500

ICH (International Conference on Harmonisation) (2000) S7A: *Safety Pharmacology Studies for Human Pharmaceuticals.* CPMP/ICH/539/00. Geneva: ICH

Ipsos MORI (2010) *Views on Animal Experimentation*. London: Department for Business Innovation and Skills

Jirkof P, Cesarovic N, Rettich A, Nicholls F, Seifert B & Arras (2010) Burrowing behavior as an indicator of post-laparotomy pain in mice *Front Behav Neurosci* 4:165. doi: 10.3389/fnbeh.2010.00165, open access at: *http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2965018/*

Kilkenny C, Browne W, Cuthill IC, Emerson M & Altman DG (2010) Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *PLoS Biol* 8: e1000412. doi:10.1371/journal.pbio.1000412 or download quick checklist at *http://www.nc3rs.org.uk/ARRIVE*

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

Maconochie M, Cadot S & Frenz D (2012) A novel method for retinoic acid administration reveals differential and dose-dependent downregulation of Fgf3 in the developing inner ear and anterior CNS. *Developmental Dynamics* 241: 741-758

Manser CE, Morris TH & Broom DM (1995) An investigation into the effects of solid or grid cage flooring on the welfare of laboratory rats. *Laboratory Animals* 29: 353-363

Martin B, Sunggoan J, Maudsley S & Mattson MP (2010) "Control" laboratory rodents are metabolically morbid: Why it matters. PNAS 107: 6127-6133, open access at *http://www.pnas.org/content/107/14/6127.full*

Mikkelsen LF, Sørensen DB, Krohn T, Lauritzen B, Dragsted N, Hansen AK & Ottesen JL (2010) Clinical pathology and cardiovascular parameters are not influenced by housing rats under increased environmental complexity. *Animal Welfare* 19: 449-460

Montarolo F, Parolisi R, Hoxha E, Boda E & Tempia F (2013) Early enriched environment exposure protects spatial memory and accelerates amyloid plaque formation in APPSwe/PS1L166P Mice. *PLoS ONE* 8(7): e69381. doi:10.1371/journal.pone.0069381

Nachat-Kappes R, Pinel A, Combe K, Lamas B, Farges M-C, Rossary A, Goncalves-Mendes N, Caldefie-Chezet F, Vasson M-P & Basu S (2012) Effects of Enriched Environment on COX-2, Leptin and Eicosanoids in a Mouse Model of Breast Cancer. *PLoS ONE* 7(12): e51525. doi:10.1371/journal.pone.0051525

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

OECD (Organisation for Economic C-operation and Development) (1998) *OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring Number 1: OECD Principles on Good Laboratory Practice* (as revised in 1997). ENV/MC/CHEM(98)17, Paris: OECD

Roedel A, Storch C, Holsboer F & Ohl F(2005) Effects of light or dark phase testing on behavioural and cognitive performance in DBA mice. *Laboratory Animals* 40: 371-381

Toth LA, Kregel K, Leon L & Musch TI (2011) Environmental enrichment of laboratory rodents: the answer depends on the question. *Comparative Medicine* 61: 314-321 (see also *http://enrichmentrecord.com/2012/01/22/viewpoints/*

Würbel H, Lipp H-P, Nitsch RM, Wolfer DP, Morf S & Litvin O (2005) Environmental enrichment does not disrupt standardization. 3R-Info-Bulletin 30, download at *http://www.forschung3r.ch/en/publications/bu30.html*

Brianna Gaskill, PhD

Postdoctoral Research Scientist, Charles River Laboratories

Aggression in Laboratory Mice: Potential Influences and How to Manage It

Mouse aggression is a hot topic and is the first question asked when I give a talk on mouse behavior. "My mice are killing each other, what do I do?" There are two obvious reasons why this behavior is so troubling to anyone who works with laboratory mice. The first is concern for the animals. Excessive aggression leads to wounding, pain, and inevitably suffering. The common solution to alleviating unwanted aggression is through solitary housing, which is likely to cause other sources of stress for mice[1], which are social animals. Second, is concern for the science. Aggression, pain, and social isolation can alter physiological parameters, such as circadian rhythm, glucocorticoid levels, and immune function[2-6], creating variability and issues of scientific validity. The best solution for aggression is prevention instead of reaction. However, before aggression can be prevented, we need to know what the purpose of the behavior is, and what causes and influences it.

Aggression is a natural behavior related to territories and the resources they contain[7-8]. Resources could include food, a water source, or access to breeding females. The spatial and temporal distributions of these resources strongly influence the development of territoriality even within the same species. If resources are in short supply or spread out, this will increase the amount of space needed to support an individual or group[7]. For instance, territory size can be as large as 80,000 m^2 for mice in wheat fields where resources are patchy or scarce[9] and as small as a few square meters in mice living commensally with people where high quality resources are frequently found[10]. Because of the stability of resources found in close proximity to humans, commensal territories remain relatively stable and are more vigorously defended compared to wild territories where it is more difficult to patrol and defend vast areas[10]. Other aspects of the environment, such as temperature, influence the rigidity of territory boundaries[11]. Dominant mice owning a territory are likely to tolerate an outsider when temperatures are low and both mice benefit thermally from huddling[11]. Similar results have been found in laboratory mice; less aggression occurs in cooler temperatures[12]. Unfortunately, laboratory temperatures already induce cold stress[13-14], which can effect various physiological systems altering scientific results[15-18]. Thus cooling laboratories further is not a solution to reducing or alleviating aggression.

The natural history of the wild house mouse also provides insight into motivations and needs of the domesticated laboratory mouse. Vulnerability to predation has shaped the behavior and life history strategies of mice[19]. They are nocturnal, showing peaks of activity at dawn and dusk[20-22], and avoid brightly lit and open spaces[19,23]. Mice live in complex and variable social systems.

They generally live in loose kin groups called 'demes' which include a male, 1-2 breeding females (which are usually related), subadults, and pups[10-11]. One male owns a territory but other adults, including females, will defend it fiercely[11]. Groups of bachelors can be found when dispersion is restricted or there are no available territories to claim[11]. These animals tend to be subordinate to animals holding territories and relatively more active during the day when territory holders are asleep[11].

As a social animal, mice exhibit many types of behaviors that maintain social structure. Affiliative behaviors, such as grooming others, function to strengthen social bonds. Mediated aggression is an agonistic interaction which solidifies dominance hierarchies but avoids fighting. An example of this is a dominant mouse mounting a subordinate, eliciting a submissive behavior from the subordinate. This type of aggression can account for up to 15% of daily activity[24]. If a subordinate responds to a threat with aggressive behavior instead of submission, the mice engage in escalated aggression. This particular type of aggression can result in bite wounds, castration, or death if a mouse does not display an appropriate subordinate signal to end the fight[24-27]. In the wild, aggressive interactions between territory holders (dominant mice) and intruders (subordinates) consist of a frontal attack by the owner and the intruder fleeing. Chasing ceases once line of sight is broken or the intruder has left the boundaries of the territory[11]. Since exiting a territory or breaking line of sight is difficult to achieve within the confines of a standard laboratory cage, chasing duration may be exacerbated in the laboratory. Interactions between two territory holders are more likely to result in injurious fighting[11], but this is unlikely to happen in the laboratory, where territories are limited to cages. Inter-male dominance hierarchies are found, again when territories are restricted[11]. Van Loo et al.[28] found that group size influenced agonistic interactions and wounding more than housing density. In that particular study, conducted with one inbred strain, groups of 5 or 8 mice had more wounds than groups of 3[28]. Dominance hierarchies in large groups of mice are less stable[29], thus resulting in more status fluctuations and likely escalated aggression. Kinship and familiarity have also been found to reduce aggressive social interactions[30-31]. Aggression toward strangers has been documented as early as 32-36 days of age[32]. Therefore, it may be beneficial to keep siblings together throughout life or combine unrelated groups before this age.

A physical structure or cover is necessary for territory formation[11,33]. Aspects of physical structures in the environment are utilized by wild mice to ambush intruders through holes, choke-points, and elevated platforms[11]. Research on the provision of retreat spaces in laboratories has produced mixed results. Cardboard tubes have been found to reduce wire-gnawing stereotypies[34] but the authors could not directly attribute the reduction to the fact that the mice utilized the tube as a retreat space since the tube was also used as nest building substrate. Nest boxes and hard plastic shelters are very popular, easily cleaned, and make the cage seem "enriched". These structures generally increase male aggressive interactions[24,35] but have also been found to increase longevity[35]. Providing a shelter increased aggression, indicating that mice perceived this item as a coveted resource that should be defended[24]. Howerton et al,[24] found that shelters increased the incidence of escalated, injurious aggression and destabilized the dominance hierarchy within the cage. This study and only a few others[36-37] have utilized home cage behavioral monitoring instead of separating, disturbing, or placing mice in an unfamiliar

continued on page 24

 continued from page 23

setting. When aggression is measured after disturbing or stressing the mice or altering the environment, this may potentially be measuring different types of aggression or motivations, making comparisons to normal husbandry difficult[24].

Nesting material is preferred by mice over nest boxes[38] and mice are willing to work to gain access to it[39-40]. Different materials can be manipulated and combined to create a flexible structure that decreases cold stress and provides both structural and occupational enrichment for mice. Nesting material and its transfer at cage change has been found to reduce aggressive interactions[31,41]. It is important to note that one study did report increased aggression when mice were provided with compressed cotton nesting material[42]. However, the mice in that study did not spend much time in contact with the material and usage was not described. The mechanism behind the mitigation of aggression by nests has not been specifically tested. Van Loo et al[31] postulate that specific pheromones deposited within the nest may be the reason for this behavioral response compared to behaviors seen after the transfer of soiled bedding. This is not surprising since scent and pheromones play such an instrumental role in rodent communication[43-44]. Urinary pheromones are used to determine the "maleness"of rivals, mark territory boundaries, and can elicit aggression[43-44]. Importantly, nest sites are kept clean of urine and feces and perhaps free of these aggression eliciting pheromones [31,45].

Another potential influence on mouse aggression is weaning and the early life experiences leading up to it. In the wild, weaning is a gradual process. Pups begin to eat solid food between 10-17 days of age and nursing is significantly reduced after 21 days[10,46]. Maternal interactions, however, can continue up to 4 weeks after pups are born. Even after pups are weaned, they are likely to remain in the natal nest until sexual maturity, continuing to interact with adult females and the dominant male. Curley et al.[46] found that pups weaned at 28 days were more likely to engage in longer bouts of social interaction than pups weaned at 21 days of age. They also found that pups were increasingly mounted by their dam as they neared weaning[46]. This is likely the adult demonstrating their dominance over the pup, while exposing the pup to social cues. Whether pups need this exposure prior to separation to properly react to certain social cues has not been formally tested. If it is necessary, it's possible that mice without those experiences do not understand how to stop aggression before it escalates to injurious fighting. One study did find that pups weaned early (14 days of age) had more wounds than pups weaned at 21 days[47]. This particular study, unlike normal husbandry, isolated mice for 4 weeks before regrouping them with their wean group. Therefore, these isolation-induced results may not be directly applicable to aggression seen in continuously housed mice.

Aggression is a naturally occurring behavior of mice that can provide benefit to those that display it in the wild[11]. Many factors appear to influence these negative social interactions between mice, making mitigation in the laboratory more complicated. Based on the current literature and the natural history and motivations of mice, a few management strategies may help keep escalated aggression in the laboratory to a minimum[41]. First combine small stable groups at 3-4 weeks of age, so they are familiar with one another prior to puberty. Avoid providing mice, males in particular, with items or enrichments that can be monopolized and guarded by dominant mice, such as rigid shelters. Last, transferring nesting material at cage change may maintain some olfactory cues related to identification without transferring aggression-eliciting pheromones.

References

1 Gonder, J. C. & Laber, K. A renewed look at laboratory rodent housing and management. *Ilar Journal* 48, 29-36 (2007).

2 Pham, T. M. et al. 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 (2010).

3 Spani, D., Arras, M., Konig, B. & Rulicke, T. Higher heart rate of laboratory mice housed individually vs in pairs. *Lab. Anim.* 37, 54-62 (2003).

4 Keeney, A. J. & Hogg, S. Behavioural consequences of repeated social defeat in the mouse: preliminary evaluation of a potential animal model of depression. *Behav. Pharmacol.* 10, 753-764 (1999).

5 Miczek, K. A. Aggressive and social stress responses in genetically modified mice: from horizontal to vertical strategy. *Psychopharmacology* 147, 17-19, doi:10.1007/s002130051132 (1999).

6 Barnard, C. J., Behnke, J. M. & Sewell, J. Environmental enrichment, immunocompetence, and resistance to Babesia microti in male mice. *Physiol. Behav.* 60, 1223-1231 (1996).

7 Macdonald, D. W. The ecology of canrivore social behavior. *Nature* 301, 379-384 (1983).

8 Gray, S. J., Jensen, S. P. & Hurst, J. L. Effects of resource distribution on activity and territory defence in house mice, Mus domesticus. *Anim. Behav.* 63, 531-539 (2002).

9 Chambers, l., Singleton, G. & Krebs, C. Movements and social organisation of wild house mice (Mus domesticus) in the wheatlands of northwestern Australia. *Journal of Mammology* 81, 59-69 (2000).

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

11 Crowcroft, P. *Mice all over.* (G.T. Foulis and CO LTD, 1966).

12 Greenberg, G. The effects of ambient temperature and population density on aggression in two inbred strains of mice, *Mus musculus. Behaviour* 42, 119-130 (1972).

13 Gordon, C. J. Temperature regulation in laboratory rodents. (Cambridge University Press, 1993).

14 Cannon, B. & Nedergaard, J. Nonshivering thermogenesis and its adequate measurement in metabolic studies. J. *Exp. Biol.* 214, 242-253 (2011).

15 Kokolus, K. M. et al. Baseline tumor growth and immune control in laboratory mice are significantly influenced by subthermoneutral housing temperature. *Proceedings of the National Academy of Sciences*, doi:10.1073/pnas.1304291110 (2013).

16 Yamauchi, C., Fujita, S., Obara, T. & Ueda, T. Effects of room temperature on reproduction, body and organ weights, food and water intakes, and hematology in mice. *Exp. Anim.* 32, 1-11 (1983).

17 Gaskill, B. N. et al. Impact of nesting material on mouse body temperature and physiology. Physiol. Behav. 110, 87-95, doi:10.1016/j.physbeh.2012.12.018 (2013).

18 Rudaya, A. Y., Steiner, A. A., Robbins, J. R., Dragic, A. S. & Romanovsky, A. A. Thermoregulatory responses to lipopolysaccharide in the mouse: dependence on the dose and ambient temperature. *American Journal Of Physiology-Regulatory Integrative And Comparative Physiology* 289, R1244-R1252, doi:10.1152/ajpregau.00370.2005 (2005).

19 Jennings, M. et al. Refining rodent husbandry: The mouse—Report of the Rodent Refinement Working Party. *Lab. Anim.* 32, 233-259 (1998).

20 Gordon, C. J., Becker, P. & Ali, J. S. Behavioral thermoregulatory responses of single- and group-housed mice. *Physiol. Behav.* 65, 255-262 (1998).

21 Rowe, F. in *Biology of the house mouse* (ed R. J. Berry) 575-590 (Academic Press, 1981).

22 Southern, H. N. *House mice*. Vol. 3 (Oxford University Press, 1954).

23 Gray, J. A. *The psychology of fear and stress*. 2nd edn, (Cambridge University Press, 1991).

24 Howerton, C. L., Garner, J. P. & Mench, J. A. Effects of running wheel-igloo enrichment on aggression, hierarchy linearity, and stereotypy in group-housed male CD-1 (ICR) mice. *Appl. Anim. Behav. Sci*. 115, 90-103 (2008).

25 Mouse Ethogram, *www.mousebehavior.org*

26 Grant, E. C. & Mackintosh, J. H. A comparison of the social postures of some common laboratory rodents. *Behaviour 21*, 246-259 (1963).

27 Van Abeelen, J. H. F. Mouse mutants studied by means of ethological methods.1. Ethogram. *Genetica* 34, 79-& (1963).

28 Van Loo, P. L. P., Mol, J. A., Koolhaas, J. M., van Zutphen, B. F. M. & Baumans, V. Modulation of aggression in male mice: influence of group size and cage size. *Physiol. Behav.* 72, 675-683 (2001).

29 Poole, T. B. & Morgan, H. D. R. Differences in aggressive behviour between male mice (*Mus musculus*) in colonies of different sizes. *Animal Behavior* 21, 788-795 (1973).

30 Kareem, A. M. & Barnard, C. J. The importance of kinship and familiarity in social interactions between mice. *Anim. Behav.* 30, 594-601, doi:10.1016/s0003-3472(82)80073-0 (1982).

31 Van Loo, P. L. P., Kruitwagen, C. L. J. J., Van Zutphen, L. F. M., Koolhaas, J. M. & Baumans, V. Modulation of aggression in male mice: Influence of cage cleaning regime and scent marks. *Anim. Welf.* 9, 281-295 (2000).

32 Scott, J. P. Agonistic behavior of mice and rats: A review. *Am. Zool.* 6, 683-701 (1966).

33 Mackintosh, J. Factors affecting the recognition of territory boundaries by mice (*Mus musculus*). *Anim. Behav.* 21, 464-470 (1973).

34 Würbel, H., Chapman, R. & Rutland, C. Effect of feed and environmental enrichment on development of stereotypic wire-gnawing in laboratory mice. *Appl. Anim. Behav. Sci.* 60, 69-81 (1998).

35 Swetter, B. J., Karpiak, C. P. & Cannon, J. T. Separating the effects of shelter from additional cage enhancements for group-housed BALB/cJ mice. *Neurosci. Lett.* 495, 205-209, doi:10.1016/j.neulet.2011.03.067 (2011).

36 Nevison, C. M., Hurst, J. L. & Barnard, C. J. Strain-specific effects of cage enrichment in male laboratory mice (Mus musculus). *Anim. Welf.* 8, 361-379 (1999).

37 Eskola, S. & Kaliste-Korhonen, E. Aspen wood-wool is preferred as a resting place, but does not affect intracage fighting of male BALB/c and C57BL/6J mice. *Lab. Anim*. 33, 108-121, doi:10.1258/002367799780578273 (1999).

38 Van de Weerd, H. A., Van Loo, P. L. P., Van Zutphen, L. F. M., Koolhaas, J. M. & Baumans, V. Strength of preference for nesting material as environmental enrichment for laboratory mice. *Appl. Anim. Behav. Sci*. 55, 369-382 (1998).

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

40 Roper, T. J. Nesting material as a reinforcer for female mice. *Anim. Behav.* 21, 733-740 (1973).

41 Van Loo, P. L. P., Van Zutphen, L. F. M. & Baumans, V. Male management: coping with aggression problems in male laboratory mice *Lab Anim* (Lond) 37, 300-313 (2003).

42 Kaliste, E. K., Mering, S. M. & Huuskonen, H. K. Environmental modification and agonistic behavior in NIH/S male mice: Nesting material enhances fighting but shelters prevent it. *Comp. Med*. 56, 202-208 (2006).

43 Brown, R. E. in *Social odours in mammals* Vol. I eds Richard E. Brown & David W. MacDonald) Ch. 9, 345-457 (Oxford University Press, 1985).

44 Arakawa, H., Cruz, S. & Deak, T. From models to mechanisms: Odorant communication as a key determinant of social behavior in rodents during illness-associated states. *Neurosci. Biobehav*. Rev. 35, 1916-1928, doi:10.1016/j.neubiorev.2011.03.007 (2011).

45 Blom, H. J. M. et al. in *Evaluation of housing conditions for laboratory mice and rats* (ed H. J. M. Blom) 81-99 (Febodruk, 1993).

46 Curley, J. P. et al. The meaning of weaning: Influence of the weaning period on behavioral development in mice. *Dev. Neurosci.* 31, 318-331 (2009).

47 Kikusui, T., Takeuchi, Y. & Mori, Y. Early weaning induces anxiety and aggression in adult mice. *Physiol. Behav.* 81, 37-42, doi:10.1016/j.physbeh.2003.12.016 (2004).

Brianna Gaskill
Postdoctoral Research Scientist, Charles River Laboratories

Don't just put something in a cage because it looks pretty. Do your research!

Author, instructor, mentor, presenter, award winner and Kansas State football fan who continues to bleed purple, Brianna Gaskill is dedicated to enhancing the care of research animals. A Postdoctoral Research Scientist at Charles River since 2011, Brianna focuses on designing and conducting research on internal husbandry, enrichment and best practices to improve efficiency and welfare in laboratory species. Prior to accepting her current position, Brianna served as a graduate research assistant at Purdue University.

A passionate supporter of environmental enrichment, (Yoga is her personal environmental enrichment), Brianna is committed to creating an environment that allows animals to express behaviors they would in the wild, especially those that give them control over stressors. "Environmental enrichment," Brianna says, "gives animals an outlet for their species-specific behavior, allows them to have some control over their environment, achieve goals and alleviate stress. Behavior is a wild animal's survival mechanism!"

Brianna has paid particular attention to alleviating and balancing cold stress for mice (ability to maintain heat) and heat stress for lab animal workers (ability to stay cool!). She primarily works with rodents, focusing on demonstrating how good welfare is good science.

"My proudest moment as a researcher," Brianna says, was knowing that my work demonstrating that nesting material reduces cold stress was instrumental in the provision and harmonization of environmental enrichment for all of Charles River's breeding animals in North America. Positively impacting millions of animals' lives is a dream come true.

"My research created a win-win situation. Animals provided with the right amount of nesting material, at a negligible cost, had increased reproduction. More pups means less breeding mice are needed to make the

reproduction quota."

A member of the American Association for Laboratory Animal Science, the Animal Behavior Society and the International Society for Applied Ethology, Brianna holds a PhD in Animal Behavior and Well-being from Purdue University and a BS in Animal Sciences from Kansas State University.

Brianna realized from a young age that she had a moral responsibility to make animals' lives better. She served as a volunteer at a local zoo in her late teens, was introduced to the role of animal behavior in a college course, and "absolutely fell in love with using behavior to design biologically relevant enrichments and improve animal lives."

Inspired as an undergraduate by Janice Swanson, a professor in animal behavior, Brianna became interested in welfare and environmental enrichment in graduate school, when she realized that understanding how animals function in the wild could enhance their lives in a laboratory. "The true power of enrichment", she says, "is to provide animals with control or the perception of control."

Thoughts on the Future of Environmental Enrichment

I believe we are moving in the right direction; today, environmental enrichment is more readily provided. However, lab animal managers must evaluate the effectiveness of enrichment before they provide it to large numbers of animals.

Showing that EE will be effective is dependent on the lab animal team knowing the species they are working with. Understanding the animals' natural history, interaction with others and social makeup plays a huge part in figuring out how to create enrichments that will be most influential.

While EE is often the last item in the budget, it's crucial to demonstrate that this is a good way to spend our money—the animals use the object and it benefits them. It is important in order to justify the expense in today's tough economic times. Does this enrichment make sense biologically or practically? I suggest trying it out to see if it works in a small subset of animals before large-scale implementation.

These small studies still cost money. I would love to see an increase in grants available for researchers, technicians, or managers who want to investigate a type of enrichment or its impact.

VIRTUAL LAB ANIMAL SCIENCE CONFERENCE
February 5 & 6, 2014
Complimentary
Register at *http://goo.gl/sC7Tbe*

4TH *ENRICHMENT RECORD* WEBINAR
February 19, 2014 at 2:30 PM (EDT)
Pragmatic Ethics for Laboratory Animal Welfare: What does "as far as can reasonably be demanded" mean?
Presenter: Raymond Anthony, PhD
http://enrichmentrecord.com/february-webinar-registration/

6TH ANNUAL MSMR ENRICHMENT SYMPOSIUM
March 6, 2014
Boston Marriott Newton, MA
Registration opens mid-January
For information,
contact Lynne Walsh
at 978-251-1556
http://www.msmr.org/

ASSOCIATION OF ZOOS & AQUARIUMS (AZA) AND INTERNATIONAL MARINE ANIMAL TRAINER'S ASSOCIATION (IMATA) ANNUAL CONFERENCE
September 12-18, 2014
Orlando, FLA
http://www.aza.org/annualconference/

Please send notification of your Upcoming Meetings to Rhoda Weiner at *rmbw19@verizon.net*

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*

The 2013 RSPCA/UFAW* Rodent Welfare Group meeting

Penny Hawkins, Research Animals Department, RSPCA

The RSPCA/UFAW Rodent Welfare Group holds a one-day meeting every autumn so that its members can discuss current welfare research, exchange views on rodent welfare issues and share experiences of the implementation of the 3Rs with respect to rodent use.

The 20th Rodent Meeting was held on 9 October 2013, at the Animal Health and Veterinary Laboratories Agency, Surrey, UK. As the 20th wedding anniversary is the 'China' anniversary we began with a presentation on *Animal Welfare Issues in China* by **Paul Littlefair** of RSPCA International. Paul gave an overview of the factors that have driven improved treatment of animals in general within China, and explained how Chinese researchers increasingly acknowledge that poor laboratory animal welfare undermines the validity of scientific results. Since 2007, the RSPCA's International and Research Animal Departments have worked closely with academic institutions and laboratory animal science associations in China, to promote laboratory animal welfare, particularly ethical review and the 3Rs. This includes sharing materials, providing conference speakers and delivering training.

Huw Golledge of Newcastle University gave a presentation on *Aversion to Rodent Euthanasia Agents*. Carbon dioxide is widely used to kill rodents, but is associated with animal welfare issues. If a rising concentration is used, with a flow rate of around 20 % chamber volume per minute, rats and mice should become anaesthetised before the concentration of CO2 reaches painful levels (NB the American Veterinary Medical Association now recommends a 10 to 30 % fill rate). However, recent behavioural research has shown that they may experience distress because the concentration of CO2 will still reach levels that are aversive to them. Anaesthesia with isoflurane before exposure to CO2 has been suggested, but unfortunately rats (and maybe mice) learn aversion to isoflurane—so if they have been anaesthetised previously, isoflurane may then be as aversive as CO2 (see Wong et al. (2013), *Biol. Lett.* doi:10.1098/rsbl.2012.1000). Unfortunately, the search for a completely 'humane' inhaled agent is ongoing.

Claire Richardson of Newcastle University outlined research into *Assessing Welfare in Mouse Disease Models*. The use of animals as disease 'models' presents problems with respect to implementing humane endpoints, because it may be necessary for animals to become sick in order to answer the experimental question. However, it is still possible to set limits on disease progression. Enhanced clinical monitoring, using minimally invasive radiofrequency identification (RFID) technology, is being used to define biomarkers and refine endpoints. RFID chips, which transmit body temperature, are implanted into mice already involved in disease studies, so that data can be obtained without causing any additional suffering apart from implanting the chip. Results so far are promising and research is ongoing to identify relevant biomarkers in different models such as lymphoma and liver fibrosis.

Sarah Allden and **Tania Boden** of UCB presented on *Refining Rheumatoid Arthritis—A 'Joint' Approach*. Different polyarthritis 'models' in mice differ in speed of onset and severity of the arthritis, so there are no generic approaches to welfare assessment or refinement. Welfare

*Royal Society for the Prevention of Cruelty to Animals, Universities Federation for Animal Welfare

scoring sheets must be tailored to each model, which triggered discussions at UCB—involving both scientists and animal technologists —to determine how best to modify animal assessment and monitoring. Effective observation and good communication helped to achieve consensus about welfare indicators and terminology, so significant refinements could be made to both procedures and monitoring systems. Enrichment has also been reviewed and tailored to the different models, all of which has led to reductions in the severity of polyarthritis models at UCB.

Colin Hendrie of the University of Leeds posed the question: *Are Behavioural Scientists Aware of the Natural History of the Animals They Work with and Does it Matter if They're Not?* He outlined the animal welfare and scientific reasons for understanding and catering for laboratory rodent behaviour. Rats are a colonial species and generally do well when socially housed, but male mice are highly territorial and often fight when housed in groups. Social rank differences also increase variance in experiments, as 'dominants' and 'subordinates' differ in behaviour, physiology and immune responses, which should be taken into account in the experimental design. But a recent meta-analysis of 100 research papers using mice and rats found that 99 failed to mention whether animals were socially housed or not—and the one that did, got it wrong. There are some major educational tasks ahead.

A special session in the afternoon focused on *Welfare Assessment of Genetically Altered (GA) Rodent Lines.* **Dominic Wells**, of the Royal Veterinary College, introduced the session by outlining the importance of effective welfare assessment of GA lines; both to enable prompt identification and alleviation of any health or welfare problems, and to provide information for GA 'passports'. However, welfare assessment of GA rodents can raise specific issues, e.g. it may be difficult to predict adverse phenotypes and their welfare impact, and some adverse effects may not become apparent until certain developmental stages. Dominic presented an overview of available guidance on welfare assessment, as an introduction to set the scene for some discussion and practical examples of assessments.

Nikki Osborne, of the RSPCA Research Animals Department, outlined *How and Where to Start with GA Severity Assessment*, by reporting some of the conclusions of a meeting on the topic convened in September by the Wellcome Trust Sanger Institute. This was attended by regulators (from the UK Home Office Inspectorate) and a range of facility staff including researchers, animal technologists an veterinarians. The convenors of the Wellcome Trust meeting are preparing a full report, which will include summaries of current practice for observing, assessing and monitoring animals, as well as explanations of legal requirements for reporting under the recently revised UK legislation.

The Importance of Welfare Assessment in an Ageing Rodent Programme was explained by **Marie Hutchison** of the Mary Lyon Centre at the UK Medical Research Council's Harwell facility. Mice on the ageing programme can live for 18 months, so effective welfare assessment is essential to detect signs of suffering either due to age or an adverse phenotype. Full 'nose-to-tail inspections' are carried out of each animal, in addition to standard phenotyping protocols. Standardised wording is used to ensure consistency and continuity (*http://www.mousewelfareterms.org*). These observations, and the accurate records that accompany them, enable subtle indicators to be picked up. This has led to refined humane endpoints and reduced severity.

In the last talk of the day, **Steve Ryder** of the Home Office Inspectorate gave more detail on how the revised UK law will be implemented with respect to *Actual Severity Assessment and Retrospective Reporting*. A major difference is that the actual severity of all procedures must be assessed and recorded after the end of each regulated procedure. Actual severity is judged from the day-to-day records of each animal's health and welfare, and should reflect the worst experience of each individual. There are some complicating factors issues with GA rodents, including classifying neonatal deaths, poor fertility, late weaning and innate mortality in background strains. The Home Office is currently producing guidance to help with this.

After the presentations, an interactive 'TurningPoint' session was used to find out more about current practice at delegates' establishments and provide some hypothetical examples of adverse effects that could be observed in a breeding colony of GA mice. A full report of the Rodent Welfare Meeting will be submitted to *Animal Technology and Welfare*, the journal of the UK Institute for Animal Technology. For more information see *http://www.rspca.org.uk/sciencegroup/researchanimals/implementing3rs/rodentwelfaregroup* or contact *research.animals@rspca.org.uk*

continued on page 30

 continued from page 29

PRIMATE WELFARE & TRAINING WORKSHOP

Sabrina Brando, Animal Concepts & Marit Vernes, Biomedical Primate Research Centre

Around 30 participants from Europe got together from September 30th to October 2nd, 2013 at the Biomedical Primate Research Centre in Rijswijk, The Netherlands to attend the "Primate Welfare & Training Workshop". Co-hosted by AnimalConcepts and EUPRIM-Net, this conference focused on improving animal welfare through animal training and offered participants possibilities for in-depth learning and discussions on these topics.

Positive reinforcement training has long been used to collect research samples, get animals to participate in daily care and to facilitate group housing and transport to testing areas. All of us involved in animal training know that training is easy and fun for all parties if it goes well. But we also know there are many challenges and obstacles to attain or maintain behaviour over time. Some procedures are more difficult, repetitive or long-lasting than others, and some involve pain, distress and anxiety. Training animals to shift to testing areas together with a companion can give support and increase confidence. Training animals in a social group might be more challenging for both animals and trainers but can have many benefits. Positive interactions with more confident members of the group can have positive results on shy individuals who learn about positive human interactions by watching the others. Spending time with animals to develop positive relationships is important, as is good nutrition and good housing conditions. Primates have been

BPRC

trained to participate in research data collection like repetitive blood sampling, salvia samples, weights, as well as cognitive and behavioural studies.

The programme was filled with lectures on different aspects of training laboratory primates: big social group management in breeding colonies, using positive reinforcement training to achieve voluntary participation, use of reinforcement schedules and the importance of staff communication and documentation.

BPRC gave insight into their training programme by sharing training philosophies and showed live training via webcam connections. Primate behaviour and personality were not to be left out as topics for lectures, as we know these can influence training speed, success and research results. To conclude, there were workshop-sessions for problem solving, writing of training plans, and loads of possibilities for Q&A sessions.

We also discussed the welfare aspects and challenges of using food and water restrictions on individuals in research projects. The idea for a dedicated seminar

BPRC

on food and water use and restriction has been in the pipeline for a while and we are pleased to announce that it is scheduled take place at BPRC on May 23rd and 24th, 2014. More information can be found on *www.animalconcepts.eu*

Conference speakers included Mark Prescott (NC3RS), Annet Louwerse (BPRC), Sabrina Brando (Animal Concepts), Eva-Marie Wergård (KI) and Marit Vernes (BPRC). We would like to thank BPRC and EUPRIM-Net for making this seminar possible and all the speakers and participants for their input and participation; it was a successful seminar!

For more information, please contact Sabrina Brando at *sbrando@animalconcepts.eu*

7TH ANNUAL INNOVATIVE ENRICHMENT SYMPOSIUM

Stefanie Nelsen, Behaviorist
SNBL USA

Massachusetts's General Hospital's Center for Comparative Medicine hosted the 7th Annual Innovative Enrichment Symposium on October 27, 2013, as a pre-conference symposium to the 64th AALAS National Meeting in Baltimore, MD. This symposium aimed to present evidence-based enrichment options to better enhance the lives of laboratory research animals. Seasoned symposium organizer, Jennifer Camacho, designed the day to include a morning enrichment excursion to the Maryland Zoo, and an afternoon of presentations from the following speakers: Melissa Dragon (Pfizer); James Weed, PhD (NIH); Darcy Hannibal, PhD (UC Davis); and Kristine Coleman, PhD (OHSU).

Julie Grove, the Animal Behavior and Training Coordinator at the Maryland Zoo, guided the group through an amazing behind the scenes tour at the zoo. Positive Reinforcement Training (PRT) demonstrations of rhinos, zebras, chimpanzees, and lemurs were provided. Most of the training involved teaching the animals to cooperate with the zookeepers and veterinary staff for various tasks including daily shifting, weights, entering a transport box, and even vet-related procedures such as ultrasounds. Julie also described the various types of enrichment (in addition to the PRT) that they utilize at the zoo, and, as it was "Boo at the Zoo", most of what was observed at this time was Halloween themed enrichment. Following the human enriching trip to the zoo, the group settled in for an afternoon of informative presentations detailed next:

Melissa Dragon discussed Environmental Enrichment in a GLP Facility, and how GLPs can affect the EE Plan. She described challenges faced, as well as ideas and ways to overcome them. She also addressed how important it is to know all the stakeholders in order to get buy-in for the proposed program. Predicting the questions they may have and providing the answers they seek, in a format that "speaks" to them, is invaluable. Effective enrichment in a GLP facility may be more costly on all resource fronts, but it can and should be done.

James Weed, PhD, provided a great wealth of knowledge on Rodent Behavior and Behavioral Management. He began by discussing the history of animal welfare and the 3Rs, and then described how to provide an effective enrichment program for rodents that not only takes into account the natural history of rodents but also provides them with opportunities to express their normal behavioral repertoire. He summed up his presentation with examples of utilizing preference tests to scientifically evaluate the effectiveness of rodent enrichment.

Darcy Hannibal, PhD, described the process of developing an evidence-based enrichment program for a Titi monkey colony. She emphasized the following for EE plan development:

- Know the natural history of the species
- Identify the problems that need to be addressed
- Find enrichment options that address or prevent these problems
- Quantitatively assess the enrichment to determine if it is addressing and/or preventing the problems
- Implement a program that is effective for the species

Kristine Coleman, PhD, discussed Incorporating Positive Reinforcement Training into Behavioral Management Programs for Nonhuman Primates. She explained that training is simply teaching, and is rooted in Operant Conditioning techniques (i.e. PRT, NRT, and Punishment). She further described the evidence-based benefits of PRT, such as reducing the need for sedation, increasing safety, and reducing abnormal behavior. Finally, she explained how nonhuman primate temperament can be used to predict which animals may be easier to train and which animals may need extra training efforts.

Sifaka eating pumpkin

THE Enrichment RECORD

GR8 Enrichment Webinar Series
Series Sponsor: CRL

Wednesday, February 19, 2014
Pragmatic Ethics for Laboratory Animal Welfare: What does "as far as can reasonably be demanded" mean?

Presenter: Raymond Anthony, PhD

Dr. Raymond Anthony is Associate Professor of Philosophy at the University of Alaska Anchorage. He specializes in Ethical Theory, Animal-Environmental-Food Ethics, and the Philosophy of Technology. Recipient of a National Science Foundation grant for climate ethics and an USDA grant to develop teaching aids for animal welfare ethics education, he serves as ethics advisor for the American Veterinary Medical Association's Animal Welfare Committee, Panel on Euthanasia and Panel on Humane Slaughter.

CLICK HERE TO REGISTER

Individuals: $50

Single site group rate: $100

Global Corporate (multiple sites*): $150

*You must provide contact info for each site

For more information or questions, contact: *Jayne at Mackta@gr8tt.com*