

THE Enrichment RECORD

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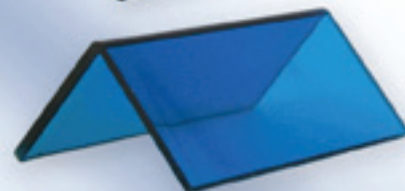


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WE'D LOVE TO HEAR FROM YOU!

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.

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Join the Discussion!

To facilitate informed discussion about environmental enrichment, we have joined the LinkedIn Group called **Laboratory Animal Sciences**. This group allows members of the laboratory animal science community and our readers to interact over a web-based platform to compare ideas and methods. To participate, you will need to create a LinkedIn account and then join the Laboratory Animal Sciences Group.

It's easy! It's free! It's a safe and secure place where you can say what's on your mind.

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New Resource



National Institutes of Health
Office of
Extramural Research



Office of Laboratory Animal Welfare (OLAW) has a new online resource for information on nonhuman primate enrichment and social housing. This resource is provided to assist institutions in enhancing the care and well-being of nonhuman primates. You can find new FAQs, a special online seminar, the OLAW report visits to Chimpanzee facilities, a bibliographic guide developed by USDA, NAL, AWIC and more.

Nonhuman Primate Enrichment and Social Housing Resources
http://grants.nih.gov/grants/olaw/primate_enrichment-social_housing.htm

Contact: hamptonl@OD.NIH.GOV

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In Other Words



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 enrichment on the science

Building the case for integrating enrichment into research design

In a recent conversation with a western-trained lab animal veterinarian working in China, I heard a familiar complaint. Local staff are resisting his introduction of basic enrichment for various species of lab animals because they think he adds to the workload and increases costs. Part of the army of professionals invading mainland China, this lab animal vet wants to introduce best practices and encourage fast-growing animal research and testing programs to consider enrichment fundamental to good animal welfare, good science, and good business.

Of course, we have all encountered similar resistance and the same old arguments wherever in the world we work. There are still many labs where management and PIs consider enrichment problematic and a compromising variable.

One of the ways we can promote enrichment on the merits is to provide scientific evidence that supports environmental enrichment and demonstrates when it advances the science and when it does not. In other words: **let's show them the data!**

Jayne Mackta

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



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Rhoda Weiner, Editor at
rmbw19@verizon.net

SHARING CAN ADVANCE ANIMAL WELFARE

The Enrichment Record wants to create a searchable database containing policies, protocol questions and SOPs for environmental enrichment. Our success depends on your willingness to share this kind of information.

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Environmental Enrichment Fights Cancer and Improves Research Results—



*What Now
for the Biomedical Researcher?*



Article by |

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American Veterinary Medical Association (AVMA) Animal Welfare Division

The article, "Environmental and Genetic Activation of a Brain-Adipocyte BDNF/Leptin Axis Causes Cancer Remission and Inhibition,"* was first published in *Cell* on July 8, and quickly echoed by online editions of major journals including *The Scientist*, *Scientific American*, *Nature* and *ScienceDirect*. Matthew During, Lei Cao, and their team at The Ohio State University had found that environmental enrichment (EE) diminishes the susceptibility of mice to cancer. Compared to the control mice in a standard cage, the EE mice enjoyed a larger living space, group size, nesting materials and apparatus like wheels, toys and tunnels. When the mice were injected with tumor cells the control animals developed malignant tumors within 15 days. By contrast, the mice living in the EE for 3 weeks prior to cancer inoculation showed significant delay in tumor development and 15 percent of the EE mice had not developed tumors after three weeks. In EE mice that did develop malignancies, the tumors were 43 percent smaller than the tumors of the control mice. Longer exposure to an enriched environment (6 weeks) produced even more dramatic effects.

We have long known that enrichment is beneficial for rodents, promoting neurological development supporting the immune system, but the real implications of these differences have never been made so clear. Not only is reduced susceptibility to cancer a very obvious and significant difference, the study that established this effect was unusually thorough and painstaking. The Ohio team fully investigated this effect over the course of 5 years, studying over 1500 mice. They were able to reproduce similar results in two different cancers (melanoma and colon cancer). They studied variations in the environment to see, for example, whether a single factor (e.g. mouse wheels for greater physical activity) has the same protective effect of the complex EE environment (it doesn't). They also studied hormonal changes, looking at gene expression data from serum samples, and did overexpression and hormonal knock down experiments to isolate potential mechanism of protection. The investigators reported slight upregulation of numerous genes associated with stress, but showed significant upregulation and downregulation of key genes, helping to identify the biochemical mechanisms of protection.

During and Cao's study is a sobering demonstration that enrichment is not just a humane option for the animals; it is fundamental to scientific validity. It shows that enriched conditions are vital to model the protective mechanisms of normal biological functioning. This research opens exciting avenues for future investigation, but it also raises very challenging questions. If most of our existing disease models are based on animals with compromised base health and vitality, just how does this affect the validity of these models in understanding the progression of disease in normal 'free range' humans? And if we need to shift our research baselines by adopting enriched housing, just how disruptive will this be to long-running research projects? How many of our existing painstaking disease models will, or should, survive the transition? If a cancer researcher with 20 years of experience (and accumulated animal data) wants or needs to make a shift to enriched environments for research animals, will it take each of them five years and 1500 mice to change to the new paradigm?

This landmark study suggests that the adoption of enriched housing, as a new standard, will be needed to develop fully valid disease models for conditions that afflict otherwise normal humans. Enrichment advocates need to understand that establishing a new baseline for these studies and adjusting the associated models will be an arduous task, but a necessary one. Part of our task as enrichment advocates must be to acknowledge this obstacle and to encourage people to overcome it. We need to give thought to supporting data and expertise sharing and the development of statistical models and analytical tools for transitioning to enriched baselines, and even funding for the research that will be needed to make the change from standard environment to EE-based studies. And it is unlikely that change will end there as even enriched housing may not be enough to study the subtle and shifting health challenges of the future. Shifting baselines may be a fact of life for research for some time to come.

Make no mistake, moving to enriched housing will be difficult for researchers with years or decades of data that will be thrown into doubt and confusion by making this

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change. But it is becoming even more clear that this change is needed, for reasons of animal welfare and scientific validity. For their part, researchers need to fully report housing conditions as an integral part of their research model. And although fully acknowledging housing conditions as a part of research models will be a difficult step, it will be a significant step closer to truly understanding the terrible diseases biomedical research is trying to conquer.

To learn more:

*** Environmental and Genetic Activation of a Brain-Adipocyte BDNF/Leptin Axis Causes Cancer Remission and Inhibition**

Lei Cao,^{1,2} Xianglan Liu,¹ En-Ju D. Lin,¹ Chuansong Wang,¹ Eugene Y. Choi,¹ Veronique Riban,¹ Benjamin Lin,² and Matthew J. During^{1, 2, 3}
Cell—9 July 2010 (Vol. 142, Issue 1, pp. 52-64)

SUMMARY

Cancer is influenced by its microenvironment, yet broader, environmental effects also play a role but remain poorly defined. We report here that mice living in an enriched housing environment show reduced tumor growth and increased remission. We found this effect in melanoma and colon cancer models, and that it was not caused by physical activity alone. Serum from animals held in an enriched environment (EE) inhibited cancer proliferation in vitro and was markedly lower in

leptin. Hypothalamic brain-derived neurotrophic factor (BDNF) was selectively upregulated by EE, and its genetic overexpression reduced tumor burden, whereas BDNF knockdown blocked the effect of EE. Mechanistically, we show that hypothalamic BDNF downregulated leptin production in adipocytes via sympathoneural β -adrenergic signaling. These results suggest that genetic or environmental activation of this BDNF/leptin axis may have therapeutic significance for cancer.

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● **Environmental Enrichment does not Disrupt Standardisation of Animal Experiments.** Hanno Würbel. *ALTEX 24, Special Issue 2007 [Full Text]*

● **Environmental standardization: cure or cause of poor reproducibility in animal experiments?** Richter, S. H., J. P. Garner, et al. *Nat Meth*—2009 (Volume 6, Issue 4: pp. 257-261.)

WHAT'S GOING ON WITH THE NEW DRAFT VERSION OF *THE GUIDE*?

By Karen Froberg-Fejko, V.M.D., President, Bio-Serv

According to Dr. Pat Brown, Director of OLAW, it was recently announced in NIH Guide Notice, NOT-OD-10-102, until the 8th Edition of the Guide is published in its final form, the 1996 Edition will remain the official Guide for the purposes of implementation of the PHS Policy. OLAW will issue guidance on implementation of the 8th Edition of the Guide after it is published. According to Dr. Kathryn Bayne, Global Director, AAALAC International, there have been several meetings by members of the Council on Accreditation to review and discuss the new Guide. Once the new Guide is published in final format, AAALAC will release a complete package of information for accredited institutions that includes specific details on the changes in the guidelines that Council wishes to clarify, copies of new Position Statements and new FAQs, and complete details on AAALAC expectations for implementing the updated Guide (including timeframes for adoption, as AAALAC plans to allow for reasonable transition periods for all institutions subject to the new standards).



Canine Socialization Through the Use of “Playrooms” or Exercise Rooms



Comprehensive enrichment programs for dogs, while not required by regulations, are becoming more common in research facilities. Many institutions are providing chewable enrichment devices and pair or group housing for their canine colonies. Human interaction is a species-appropriate enrichment for dogs, and should be considered in the development of canine enrichment programs. In addition to the importance of human and canine socialization for the animals' well-being, socialization and positive interactions with people can make the animals better research models. Playrooms or exercise rooms provide an excellent means of socialization and exercise for research dog colonies.

Challenges

Playrooms can be costly; they require dedicated space, time and personnel—potentially limited resources. Finding such resources to dedicate to enrichment and canine socialization can be difficult in an environment that is challenged by limits on time, space and personnel. In situations such as this, it is important to reinforce the benefits and positives that can be gained from implementing a socialization program. Despite the challenges, it is possible.

Getting Started

Get organized. Before starting, create a plan for the socialization room. Summarize the amount of staff that would be required and develop a plan for the time and resource investment. If dedicating space for a socialization room is not possible, another option may be the use of interchangeable rooms, or even corridors. It is important to keep in mind that these areas must be cleaned after use, and that time and personnel should be included in the plan. Flexibility may be the key to the success of your socialization program.

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Creativity may also help in starting your program. If manpower is a confounding factor, perhaps assistance can be sought from other departments. Often times, administrative staff or others may be interested in spending some time playing with the dogs.

Documentation of socialization is very important. Document progress as well as failure. Socialization is not without risks, such as potential injuries from fighting, resource guarding, or other behavioral problems. By documenting such issues, the program can be refined to help prevent recurrences. Sometimes we learn more from failures than successes. The success stories, if documented, may play an important role in gaining and maintaining support for the program.

Never underestimate the importance of staff morale! Morale is important in creating a positive work environment, and socializing dogs can have a huge impact on staff morale.

All enrichment projects are potentially difficult to implement at the onset; the challenge is to move past the initial difficulty, provide evidence and documentation to emphasize the benefits of the socialization, and incorporate these goals into the daily schedules.

Zen Pen

By Laura Shulder and Jackie Ogbin,
Veterinary Technicians, Huntingdon Life Sciences

At Huntingdon Life Sciences, we have developed a program to meet the exercise and socialization needs of our canines, as required by the Animal Welfare Act and recommendations from the "Guide for the Care and Use of Laboratory Animals." Our program, entitled "Zen Pen" is a 30-minute weekly play session with at least two technicians. Four to five dogs are chosen from our in-house colony and placed in a gated hallway with a plastic play structure, a tunnel and a variety of toys. Our Veterinary Services department has the overall responsibility of scheduling, setting up, breaking down and cleaning the designated Zen Pen area.

Exercise and socialization are essential for the psychological well-being of canines and allow for visual, auditory, olfactory and tactile contact. This method of enrichment allows our dogs time to socialize within a larger group, while also providing them socialization with our technicians. Overall, Zen Pen promotes the well-being of our dogs and provides the opportunity for our canines to be better models for handling and training.





DOES LACK OF ENRICHMENT INVALIDATE SCIENTIFIC DATA OBTAINED FROM RODENTS?

Introduction

According to the U.S. Department of Agriculture (USDA), rodents comprise approximately 90% of all animals used in research today. In order to ensure the validity of data collected from these animals, it is crucial that they are kept under conditions that do not cause them stress. Stress adversely affects every physiological system, thereby introducing a confounding variable into experimental designs.

One potential source of stress is the animal housing environment. Chronic environmental stress due to lack of enrichment imposes a host of adverse physiological consequences on rodents, including an increase in corticosterone levels and the development of repetitive behaviors (e.g. excessive grooming, digging, rearing, yawning, and fighting/biting). However, research rodents are often housed in small plastic cages that lack items common to their natural environment and limit their opportunities to perform their natural behaviors.

In this article, Dr. Baldwin describes two studies, one on rats (Brauner et al, in press) and one on mice (Cudilo et al, 2007), demonstrating that lack of enrichment can totally change physiological and pathological measures, leading to erroneous conclusions.

HEART RATE VARIABILITY IN RATS

Background

Heart rate variability (HRV) is how the heart rate varies with time resulting from a variety of factors including neural input from the parasympathetic and sympathetic nervous systems. Sympathetic activation tends to produce low frequency (LF: 0.05 -0.15 Hz) oscillations in heart rate, whereas parasympathetic activation produces higher frequency (HF: 0.15-0.40 Hz) oscillations. By comparing the relative 'powers' of the two frequency ranges of oscillations, LF/HF, it is possible to determine an animal's sympathovagal balance. A significant increase in LF/HF is representative of increased stress.

Heart rate variability is commonly used to predict clinical outcomes in trials involving treatment of heart disease in humans. This is relevant to rodents because rats are considered a good model for cardiovascular disease. It is important that potential factors that can confound HRV in rats used



for research are identified in order to avoid imprecise results in drug trials. Enrichment is not the only component of the cage that could impact a rodent's stress. The size of the cage relative to the number of animals in that cage could also significantly impact stress. The goal of this study was to evaluate the effects of cage size and enrichment on LF/HF of rats housed in one of two standard sizes of rodent cages and provided with or without two enrichment items (tube and shelf).

Experiment

Before the experiment, the 10 rats were housed in pairs in large, enriched cages because our previous preliminary studies (Baldwin et al, 2005) showed that rats housed in large, enriched cages demonstrated less aggressive nocturnal behavior than those housed in small, un-enriched cages. The cages were located in a university animal facility with a 12 hour light-dark cycle (lights on at 6 AM and off at 6 PM). One of each pair of rats was pre-implanted with a telemetric transducer (C50 PXT, Data Sciences International, St. Paul, MN) to allow remote measurement of ECG from which LF/HF was derived.

At the start of the experiment, the rats were housed in the small un-enriched cage (SU) and (after the first 3 week assessment) were randomly assigned to each of the other three cage conditions [small enriched (SE), large un-enriched (LU), and large enriched (LE)] until they had experienced each condition once. All of the cages contained a layer of pine shavings as bedding. Large cages provided a floor area of 3.5 cm² per gram weight (350 g rats) or 4.0 cm²/g (500 g rats), and small cages provided the rats with a floor area of 2.5 cm² per gram weight. The enrichment items consisted of a polyvinyl chloride tube and a wire mesh shelf to increase the complexity of the cage while stimulating the rodent's natural species-specific behaviors (nesting behaviors and subordinate rat escape behaviors). During the first week of each cage condition, the rats acclimated to their new surroundings. For the next two weeks ECG data were collected and the rats were videotaped for behavioral analysis twice a day (8 AM and 8 PM) for 10 minutes, three days a week. Rat behaviors involving activity were classified from video recordings by means of an established Rat Ethogram and the percentage of total time (AM and PM) each rat spent performing active behaviors was evaluated.

Results

There was no difference in LF/HF between the four cage conditions when considered independent of sleep/wake cycle but LF/HF increased when the rats were awake and active ($p < 0.05$, $F = 32.3$) (Figure 1A). Since the HF component (primarily parasympathetic nervous activity)

FIGURE 1A:
AVERAGED OVERALL CAGE CONDITIONS,
LF/HF INCREASED IN THE PM

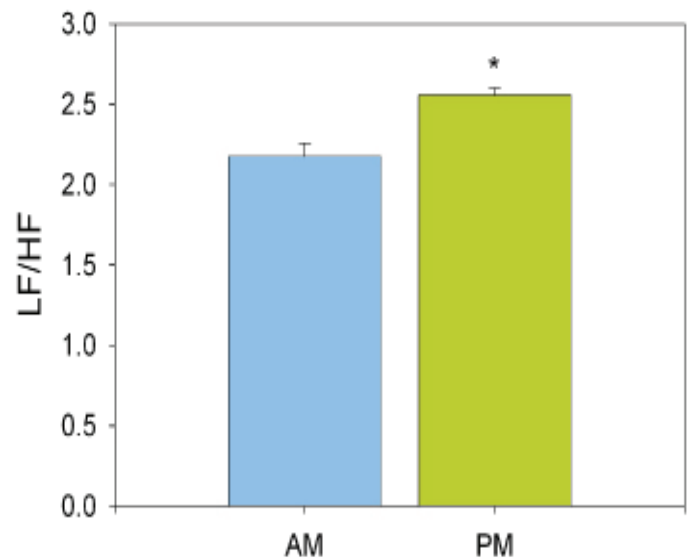
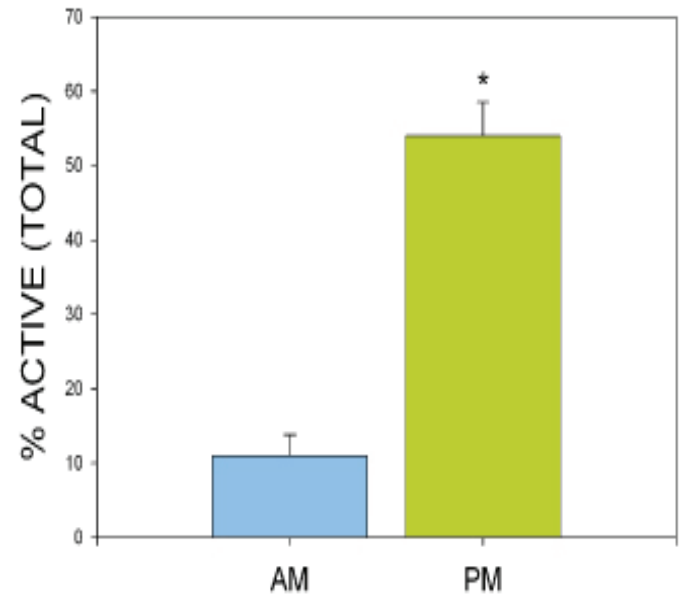


FIGURE 1B:
AVERAGED OVERALL CAGE CONDITIONS,
ACTIVITY INCREASED IN THE PM



was not different, regardless of cage condition or time of day, the increase in LF/HF ratio reflects an increase in sympathetic nervous activity (SNA). The amount of time spent in the active state increased during the evening ($p < 0.05$, $F = 80.47$) (Figure 1B). The increase in LF/HF seen when the rats were awake compared to asleep was driven by the un-enriched cage condition ($p < 0.05$, $F = 5.63$) as no significant change in LF/HF (PM vs. AM)

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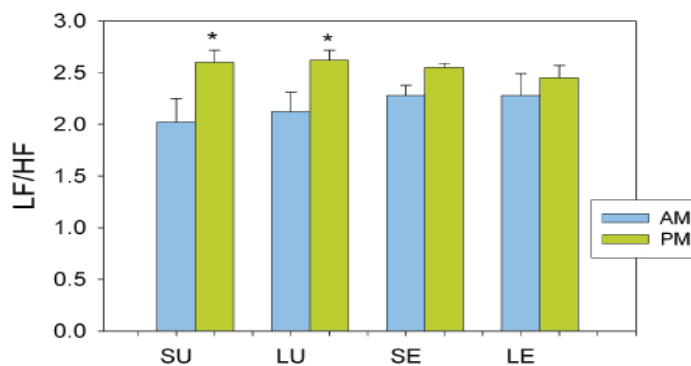


FIGURE 2:
LF/HF, AM VS PM FOR ALL FOUR CAGE CONDITIONS

was observed in the enriched environment (Figure 2). On the other hand, the differences in activity levels observed between AM and PM were seen in both enriched and un-enriched conditions ($p < 0.05$). In summary, the data suggest that enrichment significantly reduces the difference in LF/HF experienced by the rats throughout the sleep/wake cycle in the un-enriched cage condition and that this effect cannot be explained by a reduced variation in activity levels.

The increases in LF/HF and activity seen when the rats were awake occurred for both the small and large cage conditions (Figure 2). Thus an increase in cage size above the recommended minimum, regardless of the presence or absence of enrichment, was not sufficient to reduce the difference in LF/HF experienced by the rats throughout the sleep/wake cycle.

What does this study tell us?

Addition of enrichment, regardless of cage size, significantly reduced the apparent diurnal rhythm in LF/HF. This finding is not surprising because HRV is a very sensitive physiological measure that is affected by emotions. Interestingly, another study showed that when miniature swine were housed together in pairs instead of in isolation, the diurnal rhythm of LF/HF also disappeared (Kuwahara et al, 2004). These results suggest that the apparent diurnal rhythm of LF/HF is an artifact in caged animals, only seen when animals are prevented from performing species-specific behaviors.

ARTERIAL PATHOLOGY IN KNOCKOUT MICE

Background

Fibulin proteins play an important role in maintaining the mechanical properties of artery walls. Fibulin-4 is an extracellular matrix protein expressed by vascular smooth muscle cells and is essential for maintaining arterial integrity. Fibulin-4^{-/-} mice, in which both fibulin-4 genes are knocked out, die just before birth due to arterial hemorrhage, but fibulin-4^{+/-} mice, in which only one gene is knocked out, appear to be outwardly

normal. A colleague of Dr. Baldwin's, Dr. Lihua Marmostein, asked Dr. Baldwin whether she would perform experiments to determine if the fibulin-4^{+/-} mice showed normal arterial structure on a microscopic scale.

Experiments

Dr. Baldwin performed preliminary experiments on fibulin-4^{+/-} mice housed in the usual way (four mice per cage in standard cages (26 cm (length) x 16 cm (width) x 12 cm (height)) containing bedding but no enrichment). Electron microscopy showed localized regions of disorganized extracellular matrix and collagen fibers or 'gaps' between some of the medial smooth muscle cells in the mouse aortas. Similar experiments performed on wild-type mice with both fibulin-4 genes intact showed that the smooth muscle cells of the aorta were closely connected to each other and the media was more compact. The number of gaps per square mm was more than ten times greater for fibulin-4^{+/-} mice (172 ± 43 (SEM)) than for wild-type mice (15 ± 8) ($p < 0.01$, $n=8$).

Dr. Baldwin was rather disturbed by the sterile, unstimulating conditions in which the mice were housed and decided to repeat the experiments on mice housed, two per cage, in larger cages (33 cm (length), 25 cm (width) x 25 cm (height)) that contained a shelf, ladder, exercise wheel and a plastic tube. In the enriched cages where the mice could run, climb and nestle in the tunnel, the number of gaps in the fibulin-4^{+/-} mice (35 ± 12) was reduced almost to wild-type amounts and was significantly lower than for fibulin-4^{+/-} mice in the standard cages ($p < 0.05$, $n=8$).

What does this study tell us?

Dr. Baldwin's team demonstrates for the first time a connection between a genetically determined, vascular disease and environment affecting the degree of manifestation of disease symptoms. The study also sheds light on the fact that scientists should pay careful attention to housing conditions and bear in mind that differences in lifestyle could account for varying results. Thus, research findings assumed to be attributed to genetic differences might be interpreted incorrectly, neglecting the role of environmental factors.

Acknowledgements

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Formal nursing training for animal care is as important as formal nursing training for the care of people. Enrichment for all, in lab and in life, is crucial.—Kimberly Wasko



An award-winning Veterinary Surgical Nurse, lecturer, educator and Surgical Research Specialist, Kimberly Wasko is responsible for all emergency care for animals undergoing surgery at Drexel's College of Medicine, as well as administering anesthetics and analgesics pre-op, intra-op and post-op. In addition, she designs protocols using animal models, develops surgical techniques for administering anesthetics, analgesics and fluid therapy (an under-recognized but important component of general anesthesia for rodents), solves medical problems concerning wound care, and serves as a consultant to collaborators testing materials that will be used in animal surgery.

Kim chose nursing to support the importance of animals in our lives—as pets, police assistants, aides to those with disabilities, therapists, and research participants. "I went into research to make the animals' lives better," she says. "My goal is to provide the best quality of care, to make their existence in research the best it can be before they make the ultimate sacrifice. The benefits to people are the ultimate end result."

"Just because they're small doesn't mean they shouldn't be treated equally."

Hundreds of animals—mice, rats, dogs, swine and rabbits—live in Drexel's Vivarium, where environmental enrichment, combined with a state-of-the-art approach to surgery and anesthesia create animals that are optimally conditioned for research. Toward "making their existence in research the best it can be," Kim and her colleagues implemented a proactive environmental enrichment program that addressed the psychological and physical needs of their animals, reduced stress, improved quality of care and humane animal welfare, and, ultimately, created less variability in research results. As Kim stated in her article *Enrichment Adventures: Taking the Initiative—Creating a Program That Works*, "the most humane habitat possible for all research animals should be provided. With a proper understanding of enrichment, animals can be enriched without affecting results or compromising experimental design. GO ENRICHMENT!"

Kim is extremely appreciative of her colleagues and mentors for their support of her enrichment efforts. "Ari Brooks, M.D., Chief of Surgical Oncology, Drexel University College of Medicine, Francis Kralick, D.O., Neurosurgeon, Director of Complex Spinal Surgery, Hahnemann Hospital, and Arye Rosen, Ph.D., Academy Professor, Electrical Engineer," she says, "are awesome!"

Kimberly Wasko has been involved in Veterinary Medicine for more than 20 years. At Drexel University, in addition to her position as a Certified Veterinary Surgical Nurse, she also serves as the Director/Manager of the In Vivo Division of Translational Research within the Department of Surgery and the Director of Animal Laboratory Surgical Education (ALSET). An author and frequent presenter at national and international symposia, Kim is currently completing a Bachelor of Science degree in Biology from Thomas Edison College and pursuing a M.S./Ph.D. degree in Biomedical Health Systems at Drexel University School of Biomedical Engineering, Science and Health Systems. She recently received the Technician Authors Support Program Award (TASP) from the Delaware Valley Branch, American Association Laboratory Animal Science and serves as an IACUC Member at Drexel.

In training for:
Iron Girl Triathlon

Favorite authors:
Christopher Moore, Julia Child, Ferran Adria

Dear friend:
Ripples—adopted from pet rescue



Favorite pastime:
Gardening

Person she'd like to sit next to at a dinner party:
Gil Birmingham

European (EU) Compliant Housing Environment for Nonhuman Primates in a Toxicology Laboratory

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¹In-Life Toxicology Department and ²Veterinary Services Department, Huntingdon Life Sciences, Mettlers Road, East Millstone, NJ, USA

Abstract

As a global company, Huntingdon Life Sciences made the decision to install EU Appendix A (see Table 1) compliant nonhuman primate cages at its Princeton facility. This investment represented a substantial evolution of standards commonly employed within a North American contract research organization.

Table 1: Minimum enclosure dimensions as per European Appendix A Guidelines for accommodation and care of animals*

	Minimum enclosure size (m ²)	Minimum enclosure volume (m ³)	Minimum volume per animal (m ³)	Minimum enclosure height (m)
Animals less than 3 yrs of age**	2.0	3.6	1.0	1.8
Animals from 3 yrs of age***	2.0	3.6	1.8	1.8
Animals held for breeding purposes****			3.5	2.0

* Animals should only be kept singly under exceptional circumstances
 ** An enclosure of minimum dimensions may hold up to three animals
 *** An enclosure of minimum dimensions may hold up to two animals
 **** In breeding colonies no additional space/volume allowance is required for young animals up to 2 years of age housed with their mother

In the design and construction of these cages, careful consideration had to be given to preserving and, where possible, enhancing technical practices established over many years, including routine techniques such as husbandry, restraint, dosing and blood collection. Animal welfare components have been greatly enhanced in the new caging system by providing benefits which include increased 'vertical flight' space, a 360-degree view of the room, flexible pair/group modular units, visual barriers and ground foraging opportunities.

The additional space afforded by EU standards, and the cage design itself, provides greater opportunities for installing cage furniture, without having a negative impact on cage space or study requirements. Design materials were chosen to limit use of stainless steel in areas of high contact with animals, in favor of warmer colored solid laminate sheets. Minimizing use of a barren all-steel environment in the cage design not only decreases noise, but also has a positive impact on technicians working in the animal rooms.

Implementation of EU compliant cages at Huntingdon Life Sciences' North American laboratory provides our customers with seamless standards of animal care and welfare in the United States and Europe.

Introduction

Housing systems for nonhuman primates should provide the animal with appropriate space for species specific behavior, social interaction, the flexibility to provide pair and/or group housing. A decision was made to install cages that meet the EU housing requirements in order to provide our animals with increased space and to provide supplementary enrichment in order to promote the psychological well-being of the animals.

In addition to increasing the cage space, we had to keep in mind the impact this has from a technical and scientific perspective. A larger cage presents challenges to the staff in relation to handling/restraint for blood collection, dose administration, husbandry and other routine tasks. From a scientific perspective we had to consider the impact on study data and whether or not the technicians could adequately identify and document any associated clinical signs observed in toxicology studies.

Materials

- Cages and verandas constructed of stainless steel and solid laminate sheets
- Bedding/foraging material

Discussion

The quality of housing provided to laboratory animals can have a profound impact on their health and welfare. When using animals in research it is important to promote good welfare in addition to attaining scientific results. By utilizing cages that met the EU housing standards we were able to provide our primates with the following:

- Pair and/or group housing to provide companionship
- Additional space to accommodate a range of normal behaviors (swinging, climbing, perching, etc.)
- Visual barriers as refuge from other primates
- Solid floors with bedding to promote foraging
- Verandas to provide a 360-degree view of the room
- Additional cage height to accommodate the primates need for vertical flight response

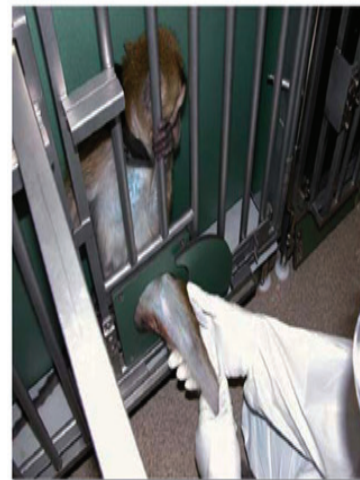
The use of the larger cages does present challenges in a toxicology laboratory. During the design process we wanted to make sure the technicians were able to perform data collection with minimal training and/or re-training. This required that we build cages with our current procedures in mind while still meeting the animal welfare standards we set out to achieve.

The cages were designed so animals could be housed in quads.

A quad consists of 4 cage units; three standard cages with a false back in each cage and one cage with a veranda (no false back). The quad is equipped with vertical and horizontal access, allowing us to move the animals within the unit to provide group housing or separation for data collection.

At the front of the three cages with a false back is a bleeding port, that enables us to collect blood samples (see Figure 1). The bleeding port is opened and the size of the opening can be adjusted to accommodate animals of various sizes. The animal is then positioned with its leg through the port where we can access the femoral vein for collection.

Figure 1: Primate positioned for blood collection through the bleeding port

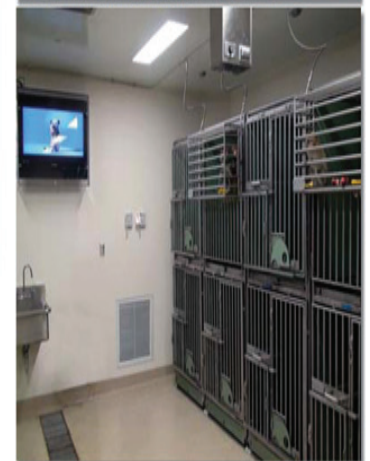
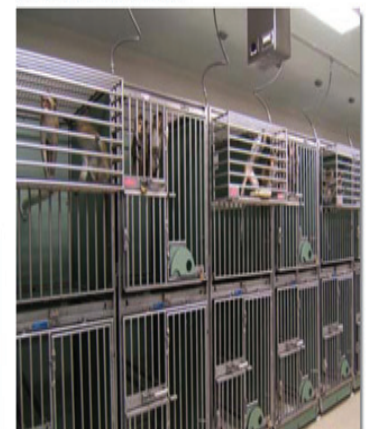


The design of the doors and false back also allow us to restrain the animals in a similar fashion for dose administration, body weights, physical observations and other procedures required by study protocol. These cages can also assist in identifying animals that may have changes in activity level as there is additional room for the animals to move within the cage. This provides us with an opportunity to easily recognize animals with increased or decreased activity levels, in addition to other behavioral signs observed in toxicology studies.

Daily cleaning associated with these cages has presented us with some challenges. The addition of bedding, foraging material and a fixed cage design has resulted in the husbandry process taking longer than expected.

Although cleaning does take longer we feel that the additional space provided to the animals far outweighs the extra time to clean and sanitize the animal room.

Figure 2: Verandas allow for enhanced socialization and a 360-degree view of the room



Overall the use and implementation of the cages was a success. We were able to provide our animals with increased space and the room to perform species specific behavior. In addition to the welfare benefits we were able to successfully perform a variety of toxicology studies using this enhanced cage design.

Effects of International Transit and Relocation on Cortisol Values in Cynomolgus Macaques (*Macaca fascicularis*)

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²The Michael E. Keeling Center for Comparative Medicine and Research, The University of Texas M.D. Anderson Cancer Center, Cool Water Drive, Bastrop TX 78602 USA

Introduction

Cynomolgus macaques (*Macaca fascicularis*) are imported into the USA from China to support research initiatives in many environments (e.g., university, pharmaceutical). As a responsible and progressive company, Huntingdon Life Sciences is continually promoting and implementing improvements in animal welfare and nonhuman primate procurement standards. The present study investigates the effects of a habituation program on cortisol levels in cynomolgus monkeys after an international journey from China to a quarantine facility in the USA, and finally, to their final destination in the USA.

Materials & methods

- Subjects were 20 (10M/10F) cynomolgus macaques that were transferred from a breeding colony in China.
- Subjects were raised in same sex social groups and were then singly housed for pre-export quarantine in China for approximately 45-60 days.
- Upon initial arrival into quarantine in the USA, 10 subjects were housed with a social partner and 10 subjects were singly housed for approximately 35 days.
- Socially housed subjects were maintained in pairs throughout the study and singly housed subjects were subsequently paired after approximately 7 days at the final destination.
- Subjects went through a 4-week habituation program using positive reinforcement techniques when they arrived at the final destination. The program consists of taking rewards from a caregiver's hand, cooperative feeding and desensitization to prepare subjects for study-specific interactions.
- Serum cortisol was collected at multiple time points throughout the journey at approximately the same time point during the day (8-11AM).
 - Location and Blood sample number:
 - China - Sample 1
 - Quarantine in the USA - Samples 2 and 3
 - Final destination in the USA - Samples 4, 5, 6 and 7
- A 2x2 (x7) mixed models ANOVA was used to explore the effects of subject sex, housing condition, and time point.



Results

Figure 1: Mean cortisol of all males and all females (N=20). There was a significant sex and time point interaction ($p < 0.05$). There was a significant sex and time point interaction bordered on significance ($p < 0.058$). There was a main effect of time point ($p < 0.001$).

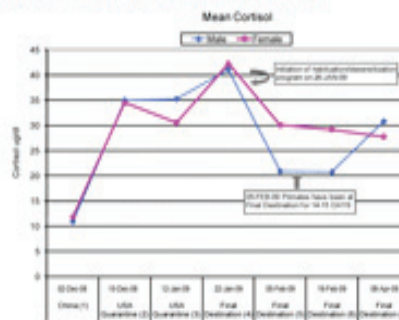


Figure 2: Mean cortisol of paired vs singly housed females (N=10). There was a significant decrease in cortisol between time points 4 and 7 ($p < 0.001$). There was a trend for socially housed females to have lower cortisol levels than singly housed females ($p < 0.058$).

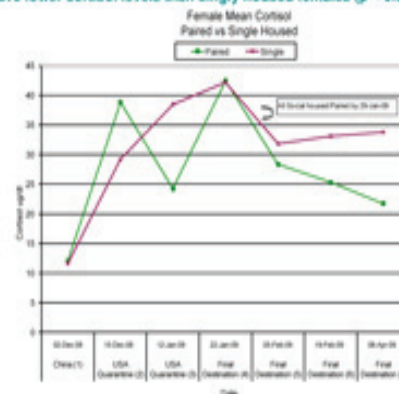
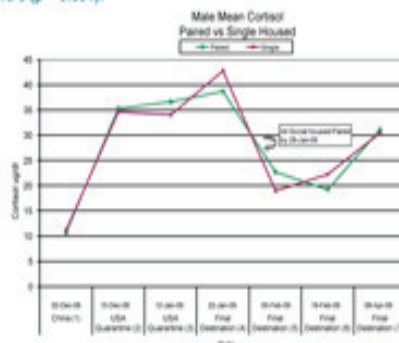


Figure 3: Mean cortisol of paired vs singly housed males (N=10). There was a significant decrease in cortisol between time points 4 and 6 ($p < 0.001$).



Conclusion

- Females that have a social partner, minimally on arrival to quarantine USA and through to final destination, appear to respond less negatively to environmental changes than females with no social partner.
- Pair housing during their international journey appears to have no effect on cortisol levels for males.
- Many research primates withstand a long international journey to reach their final destination. This study provides some insight into potential effects of social housing and habituation programs on physiological responses to transport.
- These data may aid in the establishment of optimal time requirements for transported primates to habituate to their final destination.
- For purposes of refinement and ideal animal welfare conditions, users of nonhuman primates in research should consider optimizing the environments of their animals from their birth to their final destination, implementing habituation programs beginning at the monkeys' place of birth.



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Acknowledgements

Thank-you to all the primate facilities along the way for your support in this project.

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Skin Workshop/ Skin Deep Symposium October 22, 2010 Rutgers University

In acknowledgement of the European ban on testing personal care products on animals, the 3rd Annual Skin

Workshop/Skin Deep Symposium at Rutgers University (Piscataway, NJ) is a full day of presentations and panels on alternatives and product development.

The morning features prominent scientists on "Alternatives to Animal Testing". Speakers include Dr. Bozenak Michniak-Kohn, Director, Laboratory for Drug Delivery & Industrial Membership Program, NJ Center for Biomaterials at Rutgers, Priya Batheja, Ph.D. of Capsugel/Pfizer in Boston, Nava Dayan, Ph.D. of Lipo Chemicals, Inc., and Dr. Richard Mendelsohn, of Rutgers-Newark. Jayne Mackta of GR8 and *The Enrichment Record* will also speak.

The afternoon includes a keynote presentation by Dr. Neal Walker and Dr. Stuart Shanler of Vicept Therapeutics, who will share their experience in reformulating oxymetazoline hydrochloride (Afrin) to become a topical therapy for patients with Rosacea. A panel on tech transfer and intellectual property issues in personal care products will follow.

Complete information and registration are available at www.biosciencecollaborative.com, or call 609-203-6852. Posters are invited.



Mighty Mouse Mini Symposium November 18, 2010 Rutgers University

A full day program to address the interaction of animal models and data. Concepts include data collection, management,

and analysis, deriving maximum value from all data collected, and applying fresh perspectives to experimental design.

Complete information will be available soon at www.biosciencecollaborative.com

Join the email list to be included in outreach and invitations to present, or call 609-203-6852.

Johns Hopkins CAAT to Hold Workshop on Dog Use January 12-13, 2011, Baltimore, MD

The Center for Alternatives to Animal Testing at the Johns Hopkins Bloomberg School of Public Health will hold a workshop on the Critical Evaluation of the Use of Dogs in Biomedical Research and Testing on January 12-13, 2011 in Baltimore, MD. CAAT will bring together experts to closely examine the current use of dogs in both research and testing. Dr. James Serpell, Director of the Center for the Interaction of Animals and Society at the University of Pennsylvania School of Veterinary Medicine and an expert in the area of dog behavior and dog-human interactions, will deliver the keynote address.


Speakers from areas of research where dogs are used will discuss their choice of dogs as experimental animals, the rationale for their use in this research, and address the question of whether there are alternatives to their use. Similarly, there will be representatives from pharmaceutical companies who use dogs in drug discovery and testing to discuss the reasons for their use. In addition, individuals from regulatory agencies that require animal testing will specify when/if dog data are required.

Other speakers will address areas of research and testing on dogs that are done for the benefit of dogs, e.g. dog diseases, veterinary pharmaceuticals and nutritional products. In another session, those who work with dogs in a laboratory setting will focus on the special needs of dogs in a research setting, given their need for human interaction. We will also examine the application of the principles of the 3Rs; how dogs can be replaced whenever possible, dramatically curtailing their use where appropriate, and eliminating any pain or distress they might experience in areas where their use remains essential. More information about the workshop will be available on the Altweb site (www.altweb.jhsph.edu).

Meeting Reporters Needed

We are looking for volunteers to write summaries of meetings, workshops, and conferences addressing any aspect of environmental enrichment for lab animals. Meeting organizers are welcome to assign a recorder. To request "Guidelines for Meeting Up Summaries," send your name, contact and meeting information to info@theenrichmentrecord.com

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