Spring/Summer 2014
Volume 7, Issue 2

WEILL CORNELL MEDICAL COLLEGE EDITION

InFocus

Center of Comparative Medicine & Pathology
Research Animal Resource Center
Laboratory of Comparative Pathology

SEX MATTERS: NIH to Implement New Policy

The National Institutes of Health (NIH) made a significant announcement concerning gender balance and the use of animals for preclinical studies. New policies, expected to be rolled out in October, will require that both male and female animals and cells be included in preclinical research conducted with NIH support. Later this spring NIH will release guidelines to assist investigators in adhering to the policy.

It has long been recognized that the differences between the sexes needed to be addressed in human clinical studies and a similar policy for human studies was implemented in 1994, see the NIH Revitalization Act (1993) http://grants.nih.gov/grants/guide/notice-files/not94-100.html.

This poses the question as to why it has taken this long to acknowledge that gender bias needed to be addressed in studies using animal models. The answer is not particularly complex. The female mouse's estrous cycle, with its associated hormonal changes, makes it more difficult to analyze data and potentially requires determination of estrous stage during the experiment. Additionally, males are less expensive. For example, a C57BL/6 male mouse is, on average, ~$2.00 less than a similarly aged female mouse; the difference is more significant for other strains. The female athymic nude mouse used extensively in cancer research costs ~ $10.00 more than its male counterpart.

For more information refer to "NIH to balance sex in cell and animal studies", Nature, May 15, 2014, http://www.nature.com/polopoly_fs/1.151

From the Mouse Genetics Core

✓*NSG mice are now available! For more details, please see the weblink (http://onemsk/ski/MouseGenCF/Pages/NSGAnnoucement.aspx)*

✓*The MGCF has begun offering Gene Editing in mice as a service. To obtain detailed information, please go to their weblink (http://onemsk/ski/MouseGenCF/Pages/*

Are YOU inadvertently influencing your research?

A recent study published in *Nature Methods* suggests that **researcher gender** may affect research outcomes. Robert Sorge and a group of international collaborators suggest that olfactory cues associated with male researchers increase stress biomarkers and can have analgesic effects[1].

The group began to investigate this phenomenon after anecdotal reports that pain behaviors were altered by an investigator's presence during behavioral testing. By injectig a potent inflammatory agent (zymosan A) and measuring pain using the recently developed mouse grimace scale[2], they found that mice exhibited significantly lower pain scores when observed by male observers when compared to female observers. Interestingly, male mice showed the greatest difference.

Because the mouse has a well developed olfactory system, the group suspected that the smell of male researchers was causing the effect. To investigate this hypothesis,

Cont. on pg. 2

Environmental Enrichment for the Laboratory Mouse – Part 1

Enrichment is provided to an animal's environment to enhance its psychological and physical well-being[1-3]. The Guide for the Care and Use of Laboratory Animals states: "all animals should be housed under conditions that provide sufficient space as well as supplementary structures and resources required to meet physical, physiologic, and behavioral needs[3]." Enrichment programs use multiple techniques to address the innate behavioral and physiological needs of animals used in biomedical research. The goals of an enrichment program includes: providing animals a sense of security and control over their environment, encouraging desirable behaviors, increasing the diversity of species-typical behaviors, decreasing abnormal behaviors, increasing the utility of the environment, increasing their ability to cope with challenges, and ensuring compliance with regulatory requirements[1-3].

Enrichment for laboratory mice is generally chosen to promote many of the

Cont. on pg. 3

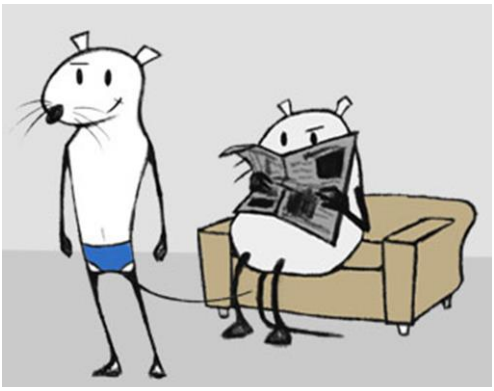
Repeated failures of preclinical animal models: What can be done?

Last year, the *New York Times* published an article entitled "Mice Fall Short as Test Subjects for Some of Humans' Deadly Ills"[1]. This article was based on the findings of Seok et al published in the *Proceedings of the National Academy of Sciences*, which concluded that a higher priority should be given to focus on the more complex human conditions instead of relying solely on mouse models to study human inflammatory diseases[2]. For example, by first characterizing the genomic changes associated with the human disease, animal models would be evaluated and selected by how well they reproduce human disease on a molecular basis, rather than by just phenotype[2]. There are numerous additional published studies reporting on the high percentage of drugs failing human clinical trials when they were shown to be effective in animal models. For example, clinical trials in oncology have the highest failure rate compared with other therapeutic areas with more than 80% of therapeutics failing when tested in people[3]. Indeed, the situation is becoming even more dire with success rates for Phase II trials falling from 28% to 18% in recent years, with insufficient efficacy and unexpected toxicities being cited as the most frequent reasons for failure[4]. Basing clinical trials on flawed preclinical data is not only expensive, but also does disservice to patients whose last hopes may rest on an experimental drug.

An example is the SOD1G93A mouse (a commonly used model for Amyotrophic Lateral Sclerosis [ALS]). Since its advent, at least 50 publications describe therapeutic agents that extend the lifespan of this animal model. However, to this date, only one therapy, Riluzaole (which provides only a two month life extension), has demonstrated significant impact in clinical trials[5]. This begs the question, why is there such a high failure rate when extrapolating results from preclinical animal models?

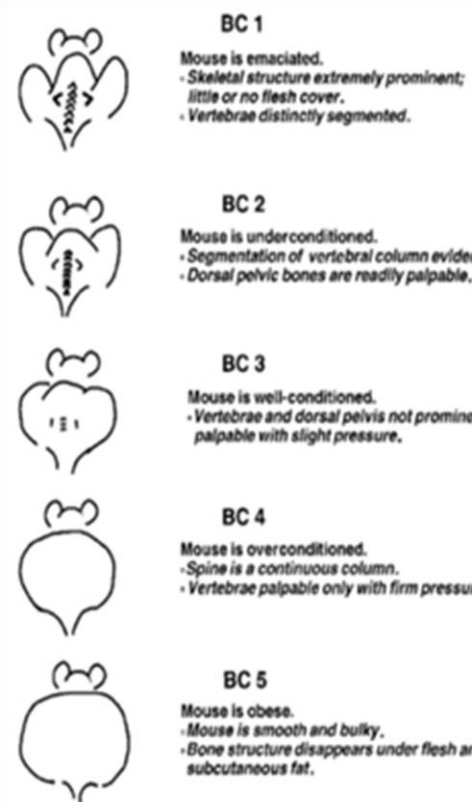
In response to these recognized failures, consortia and committees have been established in various areas of biomedical research to help improve the success of

Cont. on pg. 4



Is your mouse fit or fat…or neither? Body scoring may help provide a more insightful assessment of health status of your mice.

Image credit:http://www.pbs.org/wgbh/nova/body/marathon-mouse.html



Body condition scoring: a rapid and accurate method for assessing health status in mice. Mollie H. Ullman-Cullere and Charmaine J. Foltz, 1999. Laboratory Animal Science 49(3).

Does the mouse nose know? Male investigators may influence how a mouse reacts to pain.

Image credit:http://universitam.com/ academicos/?p=10266

Body Condition Scoring: A highly effective tool

Body weight is often used to provide a quick and easy assessment of general health in rodents. As prey species, rodents attempt to hide signs of illness. Body weight can be a more sensitive indicator of poor health than simple observation, as decreased food and water intake often occur before the animal shows outward signs of illness. Body weight can be used as an experimental variable and can help define humane endpoints: 10-20% weight loss generally requires euthanasia. Advantages of using body weight in this manner include objectivity and reproducibility between personnel. It is also fast, quantitative, and requires only simple inexpensive equipment. However, body weight does not always provide an accurate assessment of the animal's well-being. Animals with large tumors of substantive weight can be extremely ill but, due to their tumor burden, show no decrease in body weight. Similarly, animals with fluid accumulation or organ enlargements due to disease may be quite thin but not lose weight.

Body condition scoring is an alternative, and often a preferred, health assessment tool to body weight. Body condition scores (BCS) are a semi-quantitative measurement that reflects the level of fat stores and muscle mass in an animal. Body condition scoring is also simple, quick, and requires no specialized equipment. Staff can learn to perform scoring accurately with a limited training. Using visual observation and palpation, a score of 1-5 is assigned based on the presence or absence of fat and muscle in key anatomic areas (see figure). A BCS provides a more accurate reflection of health status than body weight and is useful to define an experimental endpoint. Body condition scores are also unaffected by variability due to age or strain of the animal, so that they can be compared between disparate groups. Disadvantages of BCSs are that they are only semi-quantitative and precision between individuals may require additional training and practice.

A combination of body weight and BCS provides the most information about an animal's general well being. The two parameters can easily be collected and recorded at the same time. Please contact RARC's Veterinary Services if you would like to receive further training on conducting body condition scoring.

An inadvertent influence?

Cont. from pg. 1

they had male and female lab members sleep in cotton t-shirts and then placed them in the mouse room after administering the painful stimulus. The findings were similar to those observed in the earlier experiment; the shirts worn by men resulted in a reduced facial grimace. The same result was observed when they exposed mice to gauze soaked in volatile acidic and steroidal compounds found in male axillary secretions.

To confirm that the effect was actually the result of stress-induced analgesia and not just a behavioral change, they correlated these effects with an increase in serum and fecal corticosterone levels. Corticicosterone, not cortisol, is the principal murine stress hormone. Increased anxiety was also confirmed using the open field test. Anxious mice are more likely to demonstrate wall hugging behavior.

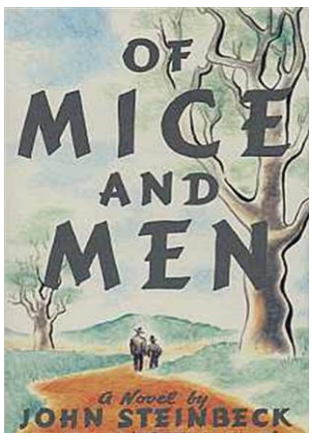
The authors noted that the concentrations of volatile acidic and steroidal compounds used in many of their tests far exceeded what is normally found in human sweat, but that the affects of male researchers still produced statistically significant quantitative and qualitative changes.

In conclusion, experimenter sex may have an effect on the baseline for behavioral and pain related research. As we strive to make research more reproducible, it may be advisable to perform video surveillance instead of direct observation.

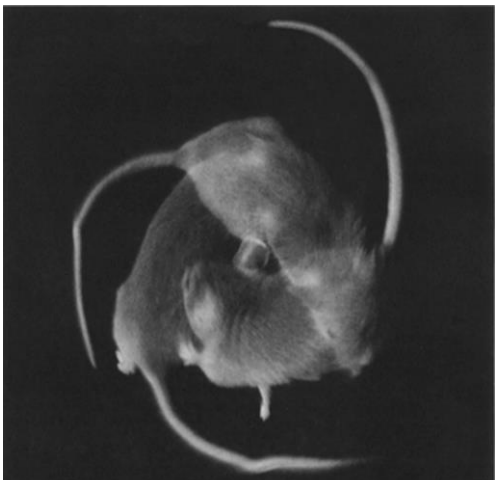
References:

1. Sorge RE, Martin LJ, Isbester KA, Sotocinal SG, Rosen S, Tuttle AH, Wieskopf JS, Acland El, Dokova A, Kadoura B, Leger P, Mapplebeck JCS, McPhail M, Delaney A, Wigerblad G, Schumann AP, Quinn T, Frasnelli J, Svensson CI, Sternberg WF & Mogil JS. (2014). Olfactory exposure to males, including men, causes stress and related analgesia in rodents. Nature methods.

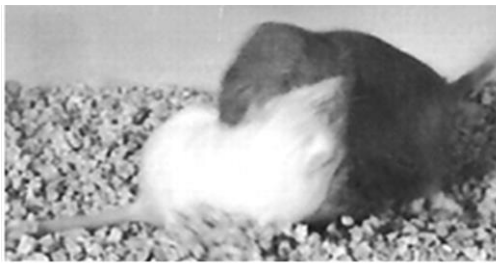
2. Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, LaCroix-Fralish ML, Matsumiya L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, van den Maagdenberg AMJM, Ferrari MD, Craig KD, & Mogil JS. (2010). Coding of facial expressions of pain in the laboratory mouse. Nature methods, 7(6), 447-449.

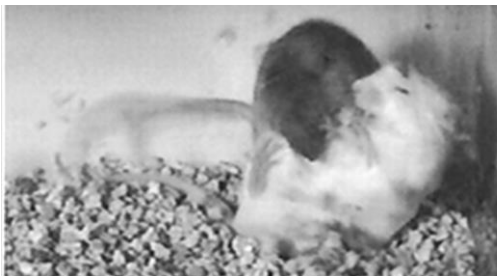


Environmental Enrichment, *Cont. from pg. 1*

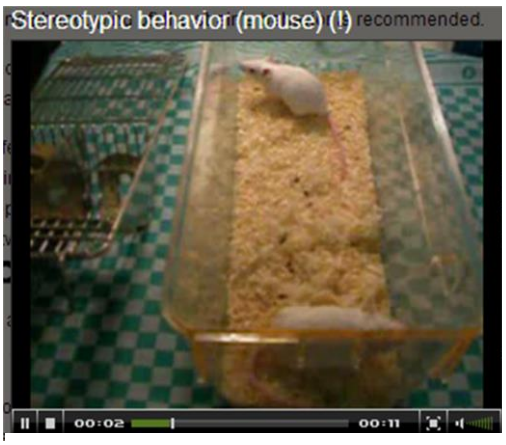


Mouse circling stereotypy is an example of a malfunctional behavior
Image credit:
http://wiki.ubc.ca/File:Bne_105_5_755_fig1a.gif





Maladaptive behaviors such as excessive aggression can be addressed by altering group size.
Image credit excerpt from:
http://www.pnas.org/content/96/22/12887/F2.expansion.html



To view videos demonstrating stereotypy see: http://www.humane-endpoints.info/eng/index.php?option=com_content&view=article&id=232&Itemid=248&lang=en

same natural and instinctive behaviors displayed by wild mice. Laboratory mice do not exhibit different behaviors than wild mice when provided the opportunity to express them[1]. Therefore, it is important to understand the natural history and behavior of wild mice to provide biologically relevant materials that will be enriching. Mice are a social, omnivorous, nocturnal prey species. They avoid brightly lit open spaces and are cautious when exploring new environments. They will burrow if provided an appropriate substrate, and build nests for protection and warmth. They typical live in demes composed of 1 male, 1-2 breeding females, pre-weaning mice and pups. Although their territory belongs to the single male in the deme, the other mice will also vigorously defend it by ambushing intruders from various vantage points. Activities performed by wild mice include socializing, exploring, foraging, grooming, digging, nest building and seeking shelter. These behaviors can be assessed to evaluate the utility of enrichment provided to laboratory mice.

In addition to increasing the frequency of species typical behaviors, other criteria must be examined to determine enrichment suitability. Enrichment should be safe for the mice and animal care staff, pose no biosecurity risks, be biologically inert so as not to interfere with ongoing research and have a positive effect on animal health, behavior and well-being[1-3]. Poorly designed enrichment can be detrimental to mice and research. It is the responsibility of the IACUC, veterinary and animal care staff, and researchers to review and continually evaluate whether the enrichment provided is beneficial to animal well-being and suitable for the research being performed[3]. Consistent observation by all staff is essential to assess the effects of enrichment.

Poorly designed enrichment can induce the expression of undesirable behaviors. These behaviors can be classified into two major categories: malfunctional and maladaptive[1]. Malfunctional behaviors occur when the environment induces a pathological change in the animal's neurophysiology and neuroendocrinology[1]. Examples of this include ulcerative dermatitis and stereotypies. In some of these cases, additional enrichment is not beneficial. There is no enrichment strategy that can be used to alleviate discomfort caused by ulcerative dermatitis. However, in the case of stereotypies such as route tracing or back-flipping, provision of extra nesting material can reduce the need for animals to perform these behaviors. As stereotypies have been associated with higher levels of frustration, using enrichment to reduce them benefits the animal's well-being. Maladaptive behaviors are the result of abnormal or uncontrollable environmental stimuli acting on a normal animal to produce unexpected (i.e., excessive or inappropriate) behavioral responses[1]. Examples of this include excessive aggression and infanticide. Excessive aggression can be managed by controlling group size, altering ambient temperature and maintaining odor cues during cage changing. This prevents mice from needing treatment for fight wounds, thus providing a health benefit to the animals. Infanticide may be reduced by providing a foraging substrate. In the latter case, if the pups are valuable or the strain has difficulty reproducing, providing enrichment may help maintain the line, thus benefiting the researcher. Maladaptive behaviors can frequently be managed or eliminated by providing suitable enrichment.

Finally, enrichment can influence mouse behavior, physiology and even neuroanatomy[2]. Despite potentially affecting research outcomes, providing enrichment to laboratory mice is a necessary component of animal care. Because adequate enrichment may reduce anxiety and stress, it can improve research results[4]. Furthermore, in mice, various studies have shown that overall, providing enrichment does not compromise or diminish the ability of investigators to create experimentally reproducible results[3].

The second part of this article will be published in the Fall edition of InFocus and will focus on specific types of enrichment that may be provided to laboratory mice.

References

1) Winnicker C, Gaskill BG, Garner J, Pritchett-Corning K. 2012. A guide to the behavior and enrichment of laboratory rodents. Boston (MA): Charles River Laboratories.

2) Baumans V. 2005. Environmental enrichment for laboratory rodents and rabbits: requirements of rodents, rabbits and research. ILAR journal 46: 162-170.

3) National Research Council. 2011. Guide for the care and use of animals, 8th Ed. Washington (DC): National Academies Press.

4) Smith AL, Corrow DJ. 2005. Modifications to husbandry and housing conditions of laboratory rodents for improved well-being. ILAR Journal 46: 140-147.

Some enrichment is more fun than others!

Image credit:
http://smekker.hu/category/ xbox/

Preclinical animal models, *Cont. from pg. 1*

A high percentage of drugs fail in human clinical trials when they were shown to be effective in animal models. How can we improve experimental design to reverse that trend?

Image credit: http://courses.washington.edu/z490/

clinical trials based on preclinical animal models. The founding principle supporting the use of animals as models for human disease is that basic processes are similar enough across species to allow for extrapolation. According to a recently published study, an animal model is considered valid when it "resembles the human condition in etiology, pathophysiology, symptomatology and response to therapeutic interventions"[6]. It is not that the foundation of this concept is flawed, but the execution which warrants refinement. Animal studies are hindered by subject sample homogeneity and imperfect control measures[11]. For example, most studies in mice are conducted on a young, healthy, and homogenous population of animals, which is a poor match for the heterogeneity of the human population. Other challenges lie in the short lifespan of mice and the inability to fully recapitulate the course of human disease over time. Finally, comorbidities may be present in many human diseases.

Recent review articles cite numerous areas in which improvement is needed when designing and publishing experiments using animal models for preclinical trials. Below is a summary of these findings[3-11]:

- Exclude irrelevant animals: Human subjects that die for reasons unrelated to disease are routinely excluded from clinical trials. The same standard should hold for preclinical trials and reasons for exclusion should be well documented
- Balance for gender: For example, in SOD1G93A mice analysis of 5429 mice found that there is a statistically significant difference in survival of males versus females[5]. Even very small differences in gender can confound modest drug effects
- Monitor genotype: As in-house breeding colonies of animals are maintained, fragile genes that induce disease may not be reliably inherited. Regular genotyping assays are essential to ensure the same number of copies of a transgene are present before commencing experimentation
- Split littermates among groups
- Ensure proper sample size: Highly variable animal models could require hundred of animals per group
- Each study should be blinded to both animal technicians and investigators

The FDA publishes a Guidance for Industry: Animal Models – Essential Elements to Address Efficacy Under the Animal Rule on the development of preclinical animal models for efficacy testing[9]. Included are basic, but important guidelines for testing, such as "the disease manifestations, including clinical signs and their known time course, laboratory parameters, histopathology, gross pathology, and the outcome (morbidity or mortality), should be compared between untreated animals and untreated humans." Additional resources are listed in the references, including Henderson et al which contains a systematic review of guidelines for in vivo animal experiments and lists 55 recommendations for general, neurobiological, cardiovascular, pain, and many other types of studies[12].

In summary, it is unlikely that animal models will become obsolete any time soon. Therefore we must strive to ensure that the design of studies involving animal models is appropriate and repeatable. This is especially important in the case of animal models for preclinical trials as the consequences of failure may set back a potential new drug or treatment for years.

References:
1.Kolata G. [Internet]. 2013. Mice Fall Short as Test Subjects for Some of Humans' Deadly Ills. New York Times. [Cited 30 April 2013]. Available at:- http://www.nytimes.com/2013/02/12/science/testing-of-some-deadly-diseases-on-mice-mislead-report-says.html?_r=0
2.Seok J et al. 2013. Genomic responses in mouse models poorly mimic human inflammatory diseases. PNAS. 110:3507-3512
3.Perrin S. 2014. Make Mouse Studies Work. Nature. 507: 423-425.
4.Arrowsmith J. 2011. Phase II failures: 2008-2010. Nature Rev. Drug Discov. 10:328-329
5.Scott S et al. 2008. Design, power, and interpretation of studies in a standard murine model of ALS. Amyotrophic Lateral Sclerosis. 9:4-15
6.Van Dam D and De Deyn PP. 2006. Drug discovery in dementia: The role of rodent models. Nature Rev. Drug Discov. 5:956-970
7.Begley CG and Ellis LM. 2012. Raise standards for preclinical cancer research. Nature. 483:531-533
8.Varga OE, Hansen AK, Sandoe P, Olsson IAS. 2010. Validating Animal Models for Preclinical Research: A Scientific and Ethical Discussion. ATLA. 38:245-248.
9.Food and Drug Administration. [Internet]. 2009. Guidance for Industry: Animal Models – Essential Elements to Address Efficacy Under the Animal Rule. [Cited 30 April 2013]. Available at: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm078923.pdf
10.Francia G and Kerbel RS. 2010. Raising the bar for cancer therapy models. Nature Biotech. 28:561-562
11.Markou A, Chiamulera C, Geyer MA, Tricklebank M, Steckler T. 2009. Removing obstacles in neuroscience drug discovery: The future path for animal models. Neuropsychopharmacology. 34:74-89.
12.Henderson VC, Kimmelman J, Fergusson D, Grimshaw JM, Hackam DG. 2013. Threats to Validity in the Design and Conduct of Preclinical Efficacy Studies: A Systematic Review of Guidelines for In Vivo Animal Experiments. PLOS Medicine. 10:e1001489

Why do I need a Special Husbandry Card?

WCMC is required to prepare a detailed written description of its animal care and use program including describing the housing and environmental conditions provided to all research subjects. This description is provided to regulatory agencies on request. Provision of husbandry that differs from that described is considered "non-standard" and requires approval in an IACUC animal care and use protocol.

In EnCCoMPass Protocol, these conditions are described in the "Special Considerations" (AU.10-19) section. Conditions to be listed include, feed/water restrictions, the provision of nonstandard or medicated diet and/or water and special housing conditions that may be required such as the use of hazardous materials, human xenograft models, special caging, and/or alterations to standard environmental enrichment. Once IACUC approved, these nonstandard housing conditions can be requested for individual cages by initiating a request and/or printing/requesting a "Special Husbandry" card using EnCCoMPass Census, which will be available to investigators in the next few months.

Altering an animal's environment can affect the animals' health and your research outcomes. Often large populations of rodents need to be provided oral medications and compounds, which can be efficiently provided in either the food or water. RARC recommends providing chemicals/drugs in feed whenever possible due to their proven stability[3]. Studies have demonstrated that select compounds can rapidly degrade in water[3]. Additionally, rodents may be averse to the taste of the medicated water, which limits the water consumed and the effectiveness of the compound. RARC stocks many special diets such as those containing doxycycline, Sulfatrim, and amoxicillin.

Next stop Brazil!
Image credit: http://www.redbubble.com/people/ellen/portfolio