



2008 Johns Hopkins Phenotyping Symposium
Johns Hopkins University School of Medicine; Baltimore, MD 21205

PROGRAM & Poster abstracts

Tuesday February 19, 2008

- 9:00-9:15 Introduction to Phenotyping and the Phenotyping Core ~ *Cory Brayton*
- 9:15-9:30 What Strain for What Studies? ~ *Cory Brayton*
- 9:30-10:00 Environment and Phenotype ~ *Julie Watson*
- 10:00-10:30 Embryonic Stem Cell to Mouse ~ *Roger Reeves*
- 10:45-11:15 Why Fish are Better Than Mice ~ *Shannon Fisher*
- 11:15-11:45 Non-Invasive Initial Phenotyping ~ *Cory Brayton, Nadine Forbes*
- 11:45-12:15 Motor Phenotyping ~ *E Hess*

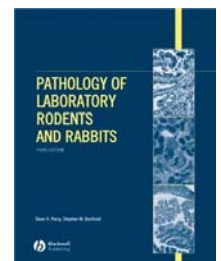
LUNCH – VISIT EXHIBITS AND POSTERS*

- 1:00-1:45 Resource and Repositories for Genetically Engineered Mice**
Franziska Grieder – NIH, NCRR
- 1:45-2:15 Auditory Phenotyping ~ *Bradford May*
- 2:30-3:00 Emotional/Cognitive/Social Phenotyping ~ *Mikhail Pletnikov*
- 3:00-3:30 Small Animal Imaging ~ *Martin Pomper*
- 3:30-4:00 Phenotyping by Ultrasound with Histopathology ~ *Kathleen Gabrielson*
- 4:00-4:30 Pulmonary Phenotyping ~ *Wayne Mitzner*
- 4:30-5:00 Pathology in Phenotyping and Other Translational Research ~ *Baktiar Karim*
- 5:00-6:00 Poster Session and Reception – Visit EXHIBITS AND POSTERS***

**Poster presenters should set up by 9am, and should attend their posters at the reception.*

Wednesday February 20 -- Confirm your reservations at registration.

- 9am -12** – Slide Conference - BRB G05 - Moderator Cory Brayton, Nadine Forbes
 Chance to win **Pathology of Laboratory Rodents and Rabbits -3rd ed**
 Thank you Wiley Liss !
- 9am** - SAIRP Imaging Tour –Meet in G03 - Coordinator James Fox
- 11am** - NBC Tour - Meet in G03 - Coordinator Dani Smith



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Poster Abstracts

01

Vestibular Syndrome due to Brainstem Infarction in Swiss Mice*

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Spontaneous vestibular syndrome in mice, characterized clinically by head tilt, circling or rolling, can be attributed to otitis media, arteritis or central nervous system lesions. Post mortem examination on seven non-inbred Swiss mice (one Hsd:ND4 and six Hsd:ICR(CD-1)) submitted for pathology due to acute onset of vestibular signs revealed similar brainstem lesions. The lesions were characterized by unilateral, well-demarcated areas of necrosis, malacia and gliosis, with variable amounts of hemorrhage, in the lateral aspect of the medulla and caudal pons. The affected area includes the medial, lateral and superior vestibular nuclei, the facial nucleus and the spinal trigeminal nucleus. In one mouse, the patency of the vascular supply to the brainstem was investigated. Intracardiac injection of black ink immediately after euthanasia revealed a filling defect in the rostral segment of the vertebral artery, at the level of C1, on the side of the brainstem lesion. These findings suggest that the unilateral brainstem necrosis is secondary to occlusion or rupture of the vertebral artery. Unilateral brainstem infarction represents another potential cause of vestibular phenotype in mice and shares features with Wallenberg's Lateral Medullary Syndrome, the most common brainstem stroke in humans

* *Young Investigator Award Poster ACVP 2007*

02

Lung Mechanics in Heterozygous Cystic Fibrosis Mice after Repeated LPS Dosing

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Mice that are homozygous for Cystic Fibrosis Regulator (CFTR) mutations are often difficult to breed and maintain. Others have shown increased inflammatory cytokine response in CFTR heterozygous cells and mice compared to wild type (WiTy). Therefore, heterozygous CF mice may be a useful surrogate. Hypothesis: Mice heterozygous (Het) for the S489X CFTR mutation would have greater change in lung mechanics and wt loss with repeated dosing of endotracheal LPS compared to wild type. Methods: Under an JHU ACUC protocol, 12 week old (3) WiTy and (3) CF heterozygous littermates were identified by PCR. Mice were weighed and sedated with ketamine/xylazine IP, and intubated with a 20 GA IV catheter orally. Aliquots of *Pseudomonas Aeruginosa*, LPS Serotype 10 (Sigma), 5 micrograms/40 micro liters PBS were warmed to 37C and directly instilled while the mice breathed spontaneously. This was repeated every 3 days for a total of 6 doses. Prior to the first dose and 3 days after the last dose, each mouse underwent end inflation lung mechanics. The mice were ventilated with a solenoid controlled ventilator with appropriate Vt, rate, and PEEP of 3 cmH2O. Before measurement, spontaneous breathing was stopped with succinyl choline IM and a 3 sec inflation given. Wt, Resistance and Elastance of respiratory system (Rrs, Ers) were recorded. Resistance (R) [cmH2O/mL/sec] values minus the resistance of the ET tube were calculated. The lungs were excised for later analysis. Results: Wt(g) decrease and (R) increase was greater in the CF Het mice. Results follow and means were compared by Paired t-test. Wt nadir occurred for both group after 3 doses. Mean Day 1 Wt. (WiTy)=22.4, (Het)=20.3, p>0.69, after 3 doses (WiTy)=20.6, (Het)=17.19, p<0.02. Mean Day 1 (R). (WiTy)=1.9, (Het)=1.5, p=0.02, after 6 doses (WiTy)=1.6, (Het)=3, p=0.001. Conclusion: Our initial data support the hypothesis that CF carrier state (heterozygous) in mice results in enhanced lung mechanics response to inflammatory stimuli.



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03

Mouse Phenome Database

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Large-scale phenotyping projects have greatly increased the power of inbred strains for the study of human disease. The phenome approach captures complexities of entire biological pathways that are simply not accessible through conventional approaches. The Mouse Phenome Project, an international collaborative effort, was launched in 2000 to complement the mouse genome sequencing effort. The Project promotes and facilitates phenotyping projects in a strain survey format following a set of recommendations proposed by members of the research community in order to standardize testing across laboratories and over time, and ultimately to maximize data reproducibility and value. Phenotypic and genotypic data on the laboratory mouse collected and curated by the Project is made available through the Mouse Phenome Database (MPD; www.jax.org/phenome). MPD currently contains about 1000 phenotypic measurements contributed by research teams worldwide. These include measurements for phenotypes relevant to human diseases, including cancer susceptibility, aging, obesity, susceptibility to infectious diseases, cardiovascular diseases, and neurosensory disorders. Additionally, MPD provides analysis tools for comparing strains, correlating phenotypes, and linking phenotype and genotype. With electronic access to centralized strain data, investigators can choose appropriate strains for modeling human diseases and many systems-based research applications. This functionality, in turn, accelerates research and leverages existing community resources.

04

Phenotyping Visual, Ocular Motor and CNS Function in Mice

Hugh Cahill and Jeremy Nathans

Department of Neuroscience; Johns Hopkins University; Baltimore, MD

We present a rapid behavioral assay for phenotyping visual, ocular motor and CNS function in mice. The optokinetic reflex (OKR) is a visually guided eye movement that serves to stabilize a moving image on the retina. The OKR requires no training, assesses the function of diverse CNS circuits, can be induced repeatedly with minimal fatigue or adaptation, and produces an electronic record that is readily and objectively quantifiable. We describe a new type of OKR test apparatus in which computer-controlled visual stimuli and streamlined data analysis facilitate a relatively high throughput behavioral assay. We used this apparatus, in conjunction with infrared imaging, to quantify basic OKR stimulus-response characteristics for C57BL/6J and 129/SvEv mouse strains and for genetically engineered lines a number of known retinal and CNS phenotypes. These experiments show that the mouse OKR is well suited for neurologic testing in the context of drug discovery and large-scale phenotyping programs.

05

Gait Analysis in Animal Models of Muscular Weakness.

Thomas G. Hampton^{1,2}, Ajit Kale^{1,2}, Ivo Amende³, Case Van Dongen⁴

¹Mouse Specifics, Inc., Boston, MA; ²The CuraVita Corporation, Boston, MA; ³Caritas St. Elizabeth's Medical Center – Tufts School of Medicine, Brookline, MA; ⁴BioBreeders, Inc., Watertown, MA

Animal models are used by researchers of musculoskeletal diseases, such as muscular dystrophy. Clinically, muscle diseases result in gait disturbances, and gait analysis is routinely used to aid diagnosis and treatment. The dystrophin-deficient [mdx] mouse, and the delta-sarcoglycan-deficient [BIO TO2] hamster are excellent models to study muscular dystrophy and the efficacy of novel therapies. Yet, little is known about how skeletal muscle weakness affects gait. Here, we examine treadmill gait in neonatal and young adult B6 mice, and BIO TO2 and BIO F1B control hamsters to identify physio-markers of muscular weakness. Decreases in hind limb propulsion duration and increases in hind paw eversion are



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characteristic of muscle weakness in BIO TO2 dystrophic hamsters compared to BIO F1B controls.

Decreases in hind limb propulsion duration and hind paw eversion are also characteristic of neonatal B6 mice that are just able to support their weight and walk on the treadmill [~ 16 days old], as compared to young adult mice [~5 weeks old]. Taken together, two distinct gait metrics are common to two different animal models of muscle weakness, indicating that gait analysis in rodent models may provide phenotypic information regarding muscle strength and functional gait phenotypes due to muscular weakness.

06

ECG Disturbances in a Mouse Model of Stroke

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The availability of genetically mutated mice facilitates the identification of genes and drugs that worsen or improve outcome from stroke. Clinically, electrocardiographic changes, autonomic nervous system [ANS] disturbances, and myocardial damage have been observed in patients with acute cerebrovascular accidents. Here, we tested whether occlusion of the middle central artery [MCA] in mice would result in electrocardiographic changes or ANS disturbances. Methods: Electrocardiograms were recorded non-invasively in conscious male B6 mice 2 days following occlusion of the MCA [MCAO, n=6], and littermates that underwent surgery but no occlusion [SHAM, n=5]. Results: Heart rate was lower in MCAO than in SHAM [665 ± 13 bpm vs. 773 ± 5 bpm, P<0.05], and heart rate variability was higher [64 ± 7 bpm vs. 20 ± 2 bpm, P<0.05]. Power spectral analysis demonstrated significantly higher total power in the variability of the R-R intervals in the MCAO compared to SHAM [38.1 ± 8.8 ms² vs. 1.4 ± 0.3 ms²], due to a higher sympathetic low frequency component [29.9 ± 7.1 ms² vs. 1.0 ± 0.3 ms²], and a higher parasympathetic high frequency component [4.9 ± 1.8 ms² vs. 0.2 ± 0.1 ms²]. Conclusion: The results demonstrate that neuronal injury caused by brain ischemia affects ANS regulation of the heart in a murine model of stroke.

07

Advances in instrumentation and behavioral technology in research using mouse models

Dani R. Smith, Martin J. Schmidt and Michela Gallagher

Neurogenetics and Behavior Center, Department of Psychological and Brain Sciences, Johns Hopkins Univ., Baltimore, MD.

The Neurogenetics and Behavior Center (NBC) is a resource on the Johns Hopkins University campus for advancing experimentation using mice, both genetically-defined inbred strains and experimentally generated mutants. As part of our mission, the NBC has developed instrumentation and behavioral technology to provide sensitive measurement systems and make the most efficient use of space and staff personnel. This presentation will feature examples from a wide range of such activities. Tours of the facility will be given February 20, 2008 (email daniro@jhu.edu for free registration). We show integrated, rack mounted test systems which can be operated at high density for efficient use of space and personnel. The Center has designed an automated fiber optic lick detector system and custom-designed Lixit nozzles that can be fitted directly in the home cage for high accuracy in consumption tests over short and long intervals. The presentation will also include an example of versatility in test equipment, using a commercially available apparatus to implement a range of behavioral protocols with ease of adaptation (< 5 min prep). Altogether, the NBC offers over 80 protocols for mouse behavioral assessment as described on the website at nbc.jhu.edu. Supported by NCCR (RR017688)



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Background strain phenotypes: what you know could help you

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Genetically Engineered Mice (GEM) are created from a variety of inbred strains and non-inbred stocks. The most commonly used inbred mice are FVB, C57Bl/6 and various 129 substrains, but other strains or stocks are used intentionally and inadvertently. Genetically homogeneous inbred mice are valuable genetic tools in dissecting and understanding gene function. Wild derived mice and non inbred mice also can be used to advantage. Sophisticated and expensive phenotyping efforts on genetically mixed GEM can yield interesting and important results, but knowledge of the background strains and of expected phenotypes in those strains can be critical to interpretation of the results. In addition, background phenotypes that are a liability in one study may confer great advantage to another area of research and may justify back crossing a genetic manipulation into another strain. Inbred strains, and even substrains, can vary substantially with regard to immune function, metabolism, physiology, behaviour, anatomic variation (including CNS, cardiovascular, pulmonary, musculoskeletal characteristics), susceptibilities to infectious diseases, incidences of spontaneous neoplasms (with hematopoietic, mammary, lung and liver tumours being most common in most strains) and other phenotypes. Some important phenotypes of 129, A, AKR, BALB, C3H, C57, DBA, FVB and SJL mice will be summarized and demonstrated. Such phenotypes may frustrate or obfuscate, but also can be used to advantage when we are aware of them.

09

Microbe-related phenotypes, good and bad, its good to be aware

Cory Brayton, Nadine Forbes, Teresa Southard

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Infectious or infesting agents may be introduced intentionally into genetically engineered mice (GEM) for the purpose of eliciting phenotypes that elucidate gene function, but often are examples of environmental (nurture) factors that can impact a wide variety of phenotypes. Induced pathology, disease or phenotypes frequently are strain dependent, and vary with other factors including sex and age of the host, strain and dose of the agent, route of infection, copathogens, spontaneous mutations and genetic manipulations. Infectious agents are more common in laboratory mouse colonies than many of us realize. The most prevalent agents do not cause overt clinical disease in most of the mice we use. They are neither obvious nor uncommon in colonies that are termed 'SPF' or 'barriers'. Worldwide the most prevalent agents include Mouse Hepatitis Virus (MHV, a Coronavirus), mouse parvoviruses, Theilers mouse encephalitis virus (TMEV or GDVII, a Picornavirus), mouse Rotavirus (EDIM), helicobacters, *Pasteurella pneumotropica*, pinworms and mites. While these agents may not cause significant morbidity or mortality, they should be expected to immunomodulate and to cause or impact a variety of other phenotypes, including but not limited to CNS and neurobehavioral phenotypes, enterohepatic and metabolic phenotypes, fecundity, growth, physiology, cancer susceptibility and progression. Infections are not all bad, and their phenotypic effects may improve our understanding of gene functions. However, we should be aware of the agents in our colonies, and of their potential impacts on phenotypes. Phenotypes related to some of the most common agents in our colonies will be discussed briefly.



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Non Invasive In Vivo Phenotyping for Mice

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Phenotype can be defined as any manifestation of a trait as determined by an organism's genetic constitution (nature) and its environment (nurture). Phenotypes may be morphological (anatomical), biochemical/molecular, physiological, or behavioral. Phenotyping, defined as any (all) evaluations that assess or measure phenotypes, is applicable to all areas of research that involve living organisms, although the term has become widely used for its relevance to genetically engineered organisms. Clinical in vivo (survival) phenotyping assessments need to be relatively low cost and non-invasive in order to preserve the animal and resources for further assessments. The core has obtained equipment and developed testing protocols that are especially suited to small subjects and to diverse projects.

Link to additional phenotyping resources and to test request page from the Core home page: <http://www.hopkinsmedicine.org/mcp/PHENOCORE/index.html> . Also contact us at phenocore@jhmi.edu to suggest instruments you would like to have, resources or expertise you would like to share.

Currently Available Minimally Invasive Testing in the phenocore

Measurement	Equipment / Instrument
Total Body Fat, Lean Mass, Body Water	EchoMRI-100™
Blood Glucose	Accu-Chek ® (Roche) / OneTouch® (LifeScan)
Hematology CBC	Hema Vet 950 Hematological Analyzer
Plasma/Serum Chemistry	VetACE™ Clinical Chemistry System
Gastrointestinal hemorrhage	Hema Occult ®
Urinalysis	Specific Gravity & Dipstick
Physical Characteristics (Dysmorphology)	SHIRPA – type
Clinical/Behavioral	SHIRPA – type

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JHMI GEM Database

Cory Brayton, Nadine Forbes, Julie Watson,

<http://www.hopkinsmedicine.org/JHUphenotyping/JHMIGEM/GEMhome.html>

or follow links from the core home page (Google: Hopkins phenotyping).

The site and information are for JH use to inform colleagues about Genetically Engineered (or spontaneous mutant) mice (GEM) in the JH community, to provide assistance with nomenclature, surveillance and husbandry information for publication, peer review and grant submission, and to facilitate access to testing and interdisciplinary collaboration.

The web based submission form, requires only contact information, mutant gene name, background strain(s) if known, for a GEM to be included. There is NO obligation to share or distribute mice as in a repository. Your contact information need not be posted (phenocore can be listed as the contact). Additional name, origin and breeding information helps to determine correct nomenclature per MGNC (Mouse Genome Nomenclature Committee <http://www.informatics.jax.org/mgihome/nomen/index.shtml>) as required by some publications and reviewers. Housing and husbandry details and pathogen status (as required/requested in protocol descriptions) can be determined from the room number for most JH facilities. Note that room numbers are NOT posted in the database.

Contact Dr. Brayton cbrayton@jhmi.edu or phenocore@jhmi.edu , for additional information or assistance, or if you are interested in participating in this or related database initiatives at JHU.

