Canadian Council on Animal Care
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Good Animal Practice in Science
Bonnes pratiques animales en science

# Implications of the neonatal environment on comprehensive phenotyping of genetically modified mice

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courtesy of Charles River Laboratories

## Abstract

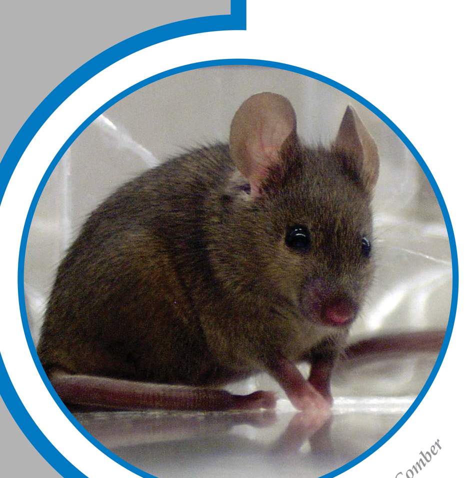
Comprehensive phenotyping involves subjecting a statistically valid sample of animals to a battery of clinical, anatomical and neurological tests to fully characterize the strain. Some types of comprehensive phenotyping are so sensitive, they can distinguish between different inbred strains of mice (Rogers *et al.*, 1999). Comprehensive phenotyping of genetically engineered animals is particularly important to detect the subtle effects that modification of the genome can have on the phenotype of genetically engineered animals. This in turn provides information that helps to both optimize the assessment of genetically engineered animal welfare and to establish appropriate endpoints for the genetically engineered strain. Neonatal experiences (such as maternal influences and the cage environment) can strongly influence the resultant behavior of offspring. This is of particular concern for genetically engineered mice that will be used for phenotyping (Würbel, 2002). Since appropriate environmental enrichment promotes the expression of normal behavior, reduces variability between animals, and promotes breeding success, this refinement could be particularly important to ensure accurate, statistically valid phenotyping using the least number of animals. We are interested in the impact the neonatal environment has on genetically engineered animal behavior since environmental enrichment in mouse colonies represents a significant opportunity for refinement for large numbers of animals.

## Introduction

The Canadian Council on Animal Care (CCAC) is revising the *guidelines on: transgenic animals* (1997). One issue under consideration is the effect of the neonatal environment on the use of comprehensive phenotyping to characterize genetically engineered (GE) mouse strains, since the neonatal environment can have profound effects on the subsequent behavior of many mammalian species.

There are two types of phenotyping:

- Targeted phenotyping:
  - Only the specific tissue or organ system of interest is assessed.
- Comprehensive phenotyping:
  - Assesses the entire animal (including the targeted organ).
  - Uses a battery of clinical, anatomical and neurological tests on a statistically valid sample of animals.
  - Can result in the detection of subtle differences, even between different inbred strains (Rogers *et al.*, 1999).



courtesy of Julie Comber

## Why do comprehensive phenotyping?

- Establishes what is "normal" for the GE strain.
- Detects subtle alterations in phenotype (important for scientific validity and welfare) (La Perle, 2004).
- Optimizes the care and use of GE animals by answering the following questions:
  - Does the GE line require more frequent welfare assessment?
  - When in the lifecycle would this extra monitoring be required?
  - Are there mitigation factors that can be implemented?
  - Do appropriate endpoints need to be established and implemented? (see CCAC's *guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing* (1998))

## Standardizing comprehensive phenotyping protocols

Jegstrup *et al.* (2003) reviewed phenotyping protocols, and concluded there are good protocols already available for characterizing GE mouse strains, but that they vary greatly in their comprehensiveness and choice of parameters to assess. Initiatives to develop standardized phenotyping protocols are underway:

- US National Institutes of Health (Moldin *et al.*, 2001; Battey *et al.*, 1999).
- EUMORPHIA, a consortium of European research institutes (http://www.eumorphia.org/EMPReSS/servlet/EMPReSS.Frameset).

Jackson Laboratories has established a searchable online database (the Mouse Phenome Database, http://aretha.jax.org/pub-cgi/phenome/mpdcgi) for mouse strain characterization data being generated by the scientific community.

courtesy of Charles River Laboratories

*An example of a mouse with enrichment items: commercially available nesting material and a "house".*

Photo courtesy of Julie Comber

## Effects of neonatal environment on subsequent behavior

- Rearing animals in an enriched environment has profound effects on cognition and the anatomy of the brain of the adult animal (reviewed in Cirulli *et al.*, 2003, more references in handout).
- Human handling of rodent neonates enhances cognition and delays age-related learning impairments, while maternal deprivation in infancy diminishes cognitive functioning and exacerbates age-related learning deficits.
- Individual variation in maternal care contributes to differences in cognitive development in many species, eg., rodents, humans and primates (references in handout).

## Research needs on the effect of the neonatal environment on comprehensive phenotyping

The neonatal environment can affect the behavior of adult mice; however, information about the rearing environment is absent in publications on behavioral phenotyping of mice (van der Staay & Steckler, 2002). Therefore, questions remain about the effects of routine husbandry, cage furnishing (such as houses) and breeding systems used (please see Table 1).

## Conclusions

- Comprehensive phenotyping can be a useful tool for optimizing the care and use of GE animals.
- There are unanswered questions about what effect the neonatal environment could have on the offspring, and whether this could affect the results of comprehensive phenotyping.
- Research is needed to determine:
  - Whether a more enriched neonatal environment would be beneficial to ensure that the mice express the fullest range of their behavioral repertoire; and
  - Whether an enriched neonatal environment improves the validity and reproducibility of comprehensive phenotyping protocols.
- In the meantime, it is suggested that publications and database entries which include phenotyping results should provide more information about the neonatal environment of the mice that were phenotyped.

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Table 1. Phenotyping Issues Specific to the Breeding of GE mice

| Issue | Background | Questions/Concerns | Gaps | Recommendation |
|---|---|---|---|---|
| **Effect of the breeding system: Paternal care** | • Use of breeding pairs (**father present with litter**) versus females raising litters alone (**father absent**).<br>• Behavior of dam affects the behavior of offspring.<br>• Priestnall & Young (1978): no difference in rate of development of litters raised by a lone female compared to a breeding pair, BUT suggested should check for more subtle changes.<br>• Wright & Brown (2000) confirmed that male mice in breeding pairs exhibit paternal care (such as huddling). | • Does paternal behavior affect the phenotype of offspring?<br>• Does paternal care vary between strains?<br>• Can paternal care be affected by genetic engineering? If so, could this affect the phenotype of pups? | • **No literature found that assessed whether the presence of the father affects the subsequent behaviour of *Mus musculus* pups**.<br>• California mouse: paternal presence had variable, gender-specific effects on spatial and nonspatial learning in adult offspring (Bredy *et al.*, 2004). | Since institutions have different mating systems, the paternal effect should be further investigated in *Mus musculus*. |
| **Genotype of littermates** | GE lines maintained in a homozygous state will have uniformly homozygous offspring, while lines maintained with hemizygous (or heterozygous) parents will have mixed litters with wildtype, hemizygotic and homozygotic pups. | • Could the genotype of littermates affect the phenotype of the homozygote?<br>• What is the effect of rearing by a homozygotic mother versus a hemizygotic mother? | **No literature found on phenotypic differences between homozygotic GE mice reared with other homozygotes compared to those raised in mixed litters.** | Research is needed to determine if the genotype of littermates affects the phenotype of GE mouse homozygotes. |
| **Complexity & environmental mismatch** | Würbel (2000; 2001; 2002): providing complexity in the environment leads to more "standard" results, because it allows the expression of normal capacity.<br>• Relevant to the phenotyping of GE mouse strains since complex behaviors strongly modulated by environmental factors (learning, memory or anxiety) are assessed.<br>Sherwin (2004): mismatch between the relatively barren neonatal environment and the more varied and complex adult environment can jeopardize the validity of research results. | • What impact does the relatively barren, standard neonatal caging environment have on subsequent behavior? | Würbel (2002): systematic variation of genetic and environmental backgrounds, instead of excessive standardization, is needed to understand the biologically relevant interactions between the GE mutation and the genetic and environmental background of GE mice.<br>**No literature found on comparing effect of different rearing environments on phenotyping results.** | The impact of the complexity of the neonatal environment on subsequent behavior should be further investigated. |

### References

Battey J., Jordan E., Cox D. & Dove W. (1999) An action plan for mouse genomics. *Nature Genetics* 21(1):73-75.

Bredy T.W., Lee A.W., Meaney M.J. & Brown R.E. (2004) Effect of neonatal handling and paternal care on offspring cognitive development in the monogamous California mouse (*Peromyscus Californicus*). *Hormones and Behavior* 46(1):30-38.

Canadian Council on Animal Care (CCAC) (1997) CCAC *guidelines on: transgenic animals*. 12pp. Ottawa ON: CCAC. Available at http://www.ccac.ca/english/gui_pol/GUFRAME.HTM

Canadian Council on Animal Care (CCAC) (1998) CCAC *guidelines on: choosing an appropriate endpoint in axperiments using animals for research, teaching and testing*. 30pp. Ottawa ON: CCAC. Available at http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GDLINES/ENDPTS/APPOPEN.HTM

Cirulli F., Berry A. & Alleva E. (2003) Early disruption of the mother-infant relationship: effects on brain plasticity and implications for psychopathology. *Neuroscience and Biobehavioral Reviews* 27(1-2):73-82.

Jegstrup I., Thon R., Hansen A.K. & Hoitinga M.R. (2003) Characterization of transgenic mice - a comparison of protocols for welfare evaluation and phenotype characterization of mice with a suggestion on a future certificate of instruction. *Laboratory Animals* 37(1):1-9.

La Perle K. (2004) *Approaches for evaluating phenotype*. Electronic document, http://www.aalas.org/education/conferences/2004_NM/webinars/La%20Perle/index.html

Moldin S., Farmer M. & Battey J.Jr. (2001) Trans-NIH neuroscience initiatives on mouse phenotyping and mutagenesis. *Mammalian Genome* 12(8):575-581.

Priestnall R. & Young S. (1978) An observational study of caretaking behavior of male and female mice housed together. *Developmental Psychobiology* 11(1):23-30.

Rogers D.C., Jones D.N., Nelson P.R., Jones C.M., Quilter C.A., Robinson T.L. & Hagan J.J. (1999) Use of SHIRPA and discriminant analysis to characterise marked differences in the behavioural phenotype of six inbred mouse strains. *Behavioural Brain Research* 105(2):207-217.

Sherwin C.M. (2004) The influences of standard laboratory cages on rodents and the validity of research data. Science in the Service of Animal Welfare. Proceedings of the UFAW International Symposium, Edinburgh, 2-4 April 2003. *Animal Welfare* 13:S9-15.

van der Staay F.J. & Steckler T. (2002) The fallacy of behavioral phenotyping without standardisation. *Genes, Brain and Behavior* 1(1):9-13.

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

Würbel H. (2001) Ideal homes? Housing effects on rodent brain and behaviour. *TRENDS in Neurosciences* 24(4):207-211.

Würbel H. (2002) Behavioral phenotyping enhanced -- beyond (environmental) standardization. *Genes, Brain and Behavior* 1(1):3-8.

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