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

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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 (Regens 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 is in turn provides information that helps to both optimize the assessment of genetically engineered animal strains and to establish appropriate endpoints for the assessment of GE animals. More specifically, comprehensive phenotyping can strongly influence the outcome behavior of experiments. It is of particular concern for genetically engineered mice that will be used for phenotyping (Würbel, 2000). Since appropriate environmental enrichment promotes the expression of normal behavior, minor variability between animals, and promotes breeding success, flow refinement (could be particularly important to ensure accurate, valid phenotyping using the host number of animals. We are interested in the impact the neonatal environment has on genetically engineered animal behavior and its environmental enrichment in mouse colonies represents a significant opportunity for refinement of the neonatal environment.

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).

These are often necessary to assess: the effect of different mating systems (such as monozygotic vs. dizygotic) on the phenotype of pups (Wright & Brown, 2000). 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 (Regens et al., 1999). Comprehensive phenotyping can strongly influence the outcome behavior of experiments. It is of particular concern for genetically engineered mice that will be used for phenotyping (Würbel, 2000). Since appropriate environmental enrichment promotes the expression of normal behavior, minor variability between animals, and promotes breeding success, flow refinement (could be particularly important to ensure accurate, valid phenotyping using the host number of animals. We are interested in the impact the neonatal environment has on genetically engineered animal behavior and its environmental enrichment in mouse colonies represents a significant opportunity for refinement of the neonatal environment.

Why do comprehensive phenotyping?

- Establishes what is "normal" for the GE strain.
- Detects subtle alterations in phenotype (important for scientific validity and welfare) (La Peres, 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?

We need research on the effects of the neonatal environment on comprehensive phenotyping.

Standardizing comprehensive phenotyping protocols

Jegstrup et al. (2001) 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 desire of parameters to assess. In order to develop standardized phenotyping protocols are underway:

- U.S. National Institutes of Health (Moldin, 2001; Battey, 1999).
- EUROMIFIA, a consortium of European research institutes. (http://www.euromifia.org/EMIF/index.html). (please see Table 1).

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

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

<table>
<thead>
<tr>
<th>Issue</th>
<th>Background</th>
<th>Questions/Concerns</th>
<th>Gaps</th>
<th>Found</th>
<th>Recommendations</th>
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<tbody>
<tr>
<td>Effect of the breeding system</td>
<td>Familiarity with the environment</td>
<td>- Use of breeding pairs father present with litter (several breeding litters?) (father absent?)</td>
<td>- Does paternal behavior affect the phenotype of offspring?</td>
<td>- Does paternal care vary between strains?</td>
<td>- Can paternal care be altered genetically? (yes, use the other phenotype of parents)</td>
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<tr>
<td>Environment of the neonate</td>
<td>GE mouse reared in an enriched environment has profound effects on cognition and the anatomy of the brain (learning, memory or anxiety) are assessed whether the neonatal environment would be beneficial to ensure the mouse expresses the fullest range of their behavioral repertoire; and Which enrichment protocol is likely to be best?</td>
<td>- Differences in maternal care contribute to differences in cognitive development in many species, eg., rodents, humans and primates (references in handout)</td>
<td>- Research needs to determine Whether this enrichment protocol is likely to be best?</td>
<td>- Research is needed to determine How might the enrichment protocol be best?</td>
<td></td>
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<tr>
<td>Complexity &amp; environmental research</td>
<td>- Environmental enrichment items: commercially available housing (please see Table 1).</td>
<td>- Could the environment affect the phenotype of the animals?</td>
<td>- No literature found on phenotypic differences between heterozygotic GE mice reared with other heterozygotic mice compared to the same strain of inbred litter.</td>
<td>- More research is needed to determine the effects of different housing environments on phenotyping results.</td>
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References

Regens et al. (1999) Comprehensive phenotyping - a useful tool for optimizing the care and use of genetically engineered (GE) mouse strains, since the neonatal environment can have profound effects on the subsequent behavior of many mammalian species.
Priestnall & Young (1978): no difference in litter size or breeding pairs (father present or absent) (mother presence or absence). Comprehensive phenotyping results.
Wright & Brown (2000) confirmed that newborn mice reared in the presence of the father had uniformly homozygous offspring, whereas those reared with the mother alone (father absent) had different mating systems, the effect on phenotype should be further investigated in this area.

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