Phenotyping Mouse Models

Investigate Phenotypes in Inbred Mouse Strains or Mouse Models of Disease

INTRODUCTION

The rapid growth of systems biology approaches in preclinical research, such as whole-genome sequencing and genome editing, has contributed to the need for high-throughput and reproducible phenotypic screening of genetically engineered animals (1,2). The relationship between genotype and phenotype is complex: targeted genes of interest interact with background genes and unknown mutations, as well as epigenetic and environmental factors, to exert specific or collective effects on health and behavior (1-3).

The availability of different strains and a wealth of genetic and phenotyping resources make the laboratory mouse an excellent genetic model. However, despite collective awareness and improvements in phenotyping pipelines and procedures, reproducibility and translatability remain constant challenges in rodent phenotypic screening (4,5). Due to time, labor, and cost constraints, mutant alleles are typically studied in a single inbred strain and sex or during limited time points. Furthermore, trained technicians perform traditional behavioral assays outside of the home cage, where inadvertent human impact or environmental factors can provide additional sources of variability (6). In this study, we investigate how continuous monitoring of animals in their home cage using automated metrics can reveal different longitudinal patterns of behavior among four commonly used inbred mouse strains.

METHODS

ANIMALS

Four-week old male (n=15) and female (n=15) C57Bl/6J, C3H/J, BALBc/J, and DBA /1J mice housed in Vium Digital Smart Housing™ were acclimated to the vivarium for three days, then single-housed, and evaluated until ~24 weeks of age. Animals were weighed every two weeks. Experiments were conducted in Vium’s AAALAC-accredited facility in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Vium.

MOTION AND BREATHING RATE

Subjects were housed within the Vium Digital Vivarium, where intelligent sensors and HD cameras allow for 24/7 continuous and minimally invasive monitoring of animals, as well as collection of automated metrics, including motion and breathing rate, in the home cage. All study data is available in real-time and accessible via the online Research Suite.

STATISTICAL ANALYSIS

Bi-monthly body weight measurements and daily motion and breathing rates were binned into months for analysis. Two-way ANOVAS with Tukey’s and Dunnett’s multiple comparisons test were used to compare between and within strains, respectively. P values less than 0.05 were considered statistically significant.
RESULTS

We evaluated changes in night-time motion (Fig. 1A and 1B) across time and among strains. Male C57Bl/6J and C3H/J mice, as well as female C57Bl/6J and BALBc/J mice, showed increased motion over time. In contrast, DBA/1J males and females showed decreased motion over time. Regardless of sex, C57Bl/6J displayed the highest night-time activity. In contrast, DBA/1J males and females, as well as BALBc/J females had a tendency towards lower night-time activity.

Figure 1. Monthly bins of night-time motion in four inbred strains of mice.
(A) Males: C57Bl/6J and C3H/J males showed increased motion, while DBA/1J males showed decreased motion over time (#P<0.05 vs. Month 1). Among the four strains, C57Bl/6J males were generally the most active (*P<0.0001 vs. All strains), while DBA/1J males were the least active (*P<0.01 vs. BALBc/J and C57Bl/6J). Motion was generally as follows: DBA/1J < C3H/J < BALBc/J < C57Bl/6J. (B) Females: C57Bl/6J and BALBc/J females showed increased motion over time, while DBA/1J females showed decreased motion over time (#P<0.05 vs. Month 1). Among the four strains, C57Bl/6J females were generally the most active (*P<0.0001 vs. All strains), followed by C3H/J (*P<0.001 vs. BALBc/J and DBA/1J). Motion was generally as follows: BALBc/J < DBA/1J < C3H/J < C57Bl/6J. N=15 per group. Values represent Means ± SEM.

For breathing rates (Fig. 2A and 2B), all groups, with the exception of C57Bl/6J males showed decreased breathing rates over time. Regardless, of sex, C57Bl/6J mice displayed the highest breathing rates, followed by BALBc/J, DBA/1J, and lastly C3H/J.

Figure 2. Monthly bins of breathing rate in four inbred strains of mice.
(A) Males: DBA/1J, C3H/J, and BALBc/J males showed decreased breathing rates over time (#P<0.05 vs. Month 1). Among the four strains, C57Bl/6J males generally possessed the highest breathing rates (*P<0.0001 vs. All Strains), followed by BALBc/J (*P<0.0001 vs. C3H/J and DBA/1J), DBA/1J (*P<0.0001 vs. C3H/J), and C3H/J. (B) Females: All mice showed decreased breathing rates over time (#P<0.05 vs. Month 1). Among the four strains, C57Bl/6J females generally possessed the highest breathing rates (*P<0.0001 vs. All Strains), followed by BALBc/J (*P<0.0001 vs. C3H/J and DBA/1J), DBA/1J (*P<0.0001 vs. C3H/J), and C3H/J. For both males and females, breathing rate was generally as follows: C3H/J < DBA/1J < BALBc/J < C57Bl/6J. N=15 per group. Values represent Means ± SEM.
We demonstrate that continuous monitoring of animal behavior in the home cage allows researchers to observe longitudinal differences among two sexes in four inbred strains of mice. We observed strain and sex-specific patterns in night-time motion and breathing rate, which was generally consistent over the five-month study duration. In general, differences among genetic backgrounds were greater than differences between sex. Consistent with previous research, C57Bl/6J mice were the most active and possessed the highest breathing rates, while C3H/J mice showed the lowest breathing rates (1,2,7).

Regardless of strain and sex, body weight increased over time for all groups (Fig. 3A and 3B). In general, C3H/J mice weighed the most, while DBA/1J mice weighed the least during a five-month study duration.

**DISCUSSION**

We also observed changes in behavior over time (Table 2). While motion increased and breathing rate moderately decreased over time for C57Bl/6J mice, motion and breathing rate both generally decreased over time for DBA/1J mice. Interactions between motion, breathing rate, and body weight may serve as functional read-outs for changes in metabolism and energy expenditure as animals age (8).

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**Table 1.** Summary of behavioral and physiological differences across four inbred strains of male and female mice (1 to 5 months old)

<table>
<thead>
<tr>
<th>Metric</th>
<th>Sex</th>
<th>Lowest to Highest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Night-time Motion</td>
<td>Male</td>
<td>DBA/1J &lt; C3H/J &lt; BALBc/J &lt; C57Bl/6J</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>BALBc/J &lt; DBA/1J &lt; C3H/J &lt; C57Bl/6J</td>
</tr>
<tr>
<td>Breathing Rate</td>
<td>Male</td>
<td>C3H/J &lt; DBA/1J &lt; BALBc/J &lt; C57Bl/6J</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>C3H/J &lt; DBA/1J &lt; BALBc/J &lt; C57Bl/6J</td>
</tr>
<tr>
<td>Body Weight</td>
<td>Male</td>
<td>DBA/1J &lt; C57Bl/6J &lt; BALBc/J &lt; C3H/J</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>DBA/1J &lt; C57Bl/6J &lt; BALBc/J &lt; C3H/J</td>
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A challenge in preclinical research is performing high-throughput, long-term, and unbiased phenotypic screening on mouse models of disease. By monitoring animals in the home cage environment, researchers can continuously assess natural behaviors in the animals without the additional stress of removing them from their cage to perform additional assays. This approach allows researchers to perform larger-scale phenotypic screens to better complement the growing needs and rapid advancements in preclinical research.

REFERENCES