

Multi-level analysis of mouse behavior in a home cage environment using PhenoLab®

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Abstract

To contribute to the refinement of behavioral phenotyping methods for inbred and mutant mice, we developed an automated tool for observing and analyzing behavior in a home cage-like environment (PhenoLab®, Noldus Information Technology, Wageningen, The Netherlands). Testing animals in their home cage environment holds several advantages; it allows continuous observations over consecutive days and the evaluation of both challenge-induced and baseline behaviors. Home cage testing also minimizes human intervention (such as handling) and reduces interactions with other environmental factors not related to the behavioral test (such as animal transport). Here, data will be presented of studies in inbred strains of mice (C57BL/6 and DBA/2) on locomotor activity and anxiety-related behavior in the PhenoLab® system. We found that different aspects of activity behave differently over time. Anxiety-related behavior was studied by introducing an aversive light stimulus in the cage after an adaptation period of 4 days. The light stimulus illuminated the area around the feeding station, inducing pronounced avoidance behavior in the animals. Possibilities and limitations of PhenoLab® as a tool for the behavioral phenotyping of mice are discussed.

Keywords

Automated home cage observations, mice, locomotor activity, anxiety.

1 Introduction

The continuous increase in mouse models for central nervous system functioning demand extensive behavioral assays to elucidate the impact of genetic alterations [2,4,7]. Current methods for behavioral phenotyping of mice often involve batteries of individual tests, each addressing different motivational systems, such as activity in novel environment, anxiety, spatial cognition, etc [1,3]. Although most tests are pharmacologically validated and relatively easy to perform, some limitations are evident. First, long term development of behavior as a response to stimuli (e.g. novelty or drug effects) and circadian processes are ignored due to the limited testing period. Second, information on the complex interactions between motivational systems is often difficult to acquire.

We developed a new tool to study mouse behavior in a home cage like environment [5]. This approach has several advantages; it allows continuous observations over days and the evaluation of both challenge-induced and baseline behavior. Furthermore, the confounding influence of stress caused by handling and transport is minimized.

In this paper we present data from two separate experiments to demonstrate some of the possibilities of automated home cage observations. The first experiment addresses time-courses of specific aspects of locomotor activity as mice gradually become habituated to the novel

home cage system. In the second experiment, an aversive stimulus is used to create an approach-avoidance conflict, which allows for the detection of parameters indicative of anxiety.

2 Methods

2.1 Animals

Female mice of the C57BL/6OlaHsd (n=6) and DBA/2OlaHsd (n=10) strain were used in the first experiment. Male mice of the C57BL/6OlaHsd strain (n=10) were used in the second experiment. All mice were purchased from Harlan (Horst, The Netherlands). Upon arrival at the animal facility, mice were either housed in pairs (females) or single (males) and allowed to acclimatize for two weeks under a reversed light/dark cycle (lights on: 19.00 hrs). All mice were provided with a shelter, tissue and paper shreds as enrichment. Humidity was kept at a constant level and room temperature was maintained at 21.0 ± 2.0 °C. The Animal Ethical Committee of Utrecht University approved the experiments.

2.2 Automated home cage observations

Locomotor activity was automatically recorded with video tracking in specially designed home cages (PhenoLab®, Noldus Information Technology, Wageningen, The Netherlands, see Figure 1). Each PhenoLab® system consists of four cages, connected to a PC that runs EthoVision 3.0 (Noldus Information Technology) for videotracking. On top of each cage a unit is placed containing a digital infrared sensitive camera and infrared lights. This allows continuous recordings during both light and dark period of the day. The top unit further contains a bright white light stimulus that could be switched on automatically by programming EthoVision 3.0. This lightspot illuminates approximately one quarter of the cage with a light intensity of 1000 lux.

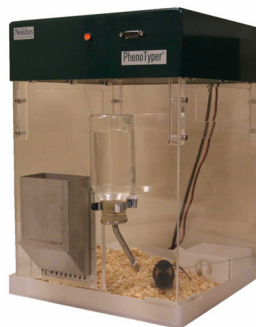


Figure 1. Single cage with top unit of the PhenoLab® system for automated home cage observations. Each cage contains a fixed water bottle, feeding station and shelter.

2.3 Experiment 1

For the first experiment, mice were introduced to the PhenoLab[®] cages and locomotor activity was recorded for six consecutive days. Cages were equipped with a fixed water bottle and feeding station, and shelter, tissue and paper shreds for enrichment. For details on parameter settings in EthoVision 3.0, see [6]). Parameters presented in the present paper are 'duration of movement' as a percentage of total observation time and 'velocity' in cm/s. Values for each parameters are calculated for each individual in 1-hour bins and subsequently averaged over 12 hours to differentiate between dark and light period of the day.

2.4 Experiment 2

In the second experiment, mice were introduced to the PhenoLab[®] cages following the procedure of experiment 1, but now the bright white light stimulus was programmed to switch on, on day 5 immediately at the onset of the dark period. The light spot was directed on the feeding station for three hours continuously. This created an approach-avoidance conflict for the animals. Again, locomotor activity was recorded for six consecutive days in total. Parameters analyzed for this paper were 'duration of movement' in seconds per hour and the 'time spent in feeding zone'. The feeding zone was defined as one body length around the feeding station. During the time the light spot was on, this zone was illuminated.

2.5 Statistical analysis

Statistical analysis was conducted using SPSS 10.0 for Windows. Repeated measures ANOVA were performed to test for overall effects of within-subjects factor 'day' and between-subjects factor 'strain' on duration of movement and velocity in experiment 1. *Posthoc* independent samples t-tests were used to compare strains on each experimental day. Levels of significance were assigned at $p=0.05$.

3 Results

3.1 Experiment 1

Results from experiment 1 are modified from the study described in [5]. Figure 2 presents the 'duration of movement' during the dark period of each experimental day. Overall, C57BL/6 mice showed higher levels of movement (repeated measures ANOVA between-subjects factor 'strain' $F_{1,14}=6,663$; $p=0.022$) compared to DBA/2 mice. *Posthoc* comparisons per day revealed significant higher duration of movement in C57BL/6 on day 1, 5 and 6. Duration of movement changed over time (repeated measures ANOVA within-subjects factor 'day' $F_{5,70}=41,559$; $p<0.001$) but this was independent of strain (repeated measures ANOVA 'day' x 'strain': $F_{5,70}=1,227$; $p=0.310$). In Figure 3 strain differences on the parameter 'velocity' are shown. Overall, DBA/2 mice moved with higher velocity compared to C57BL/6 (repeated measures ANOVA between-subjects factor 'strain': $F_{1,14}=7,060$; $p=0.019$). However, when tested per experimental day differences were only significant on day 5 and 6. Velocity changed significantly over time (repeated measures ANOVA within-subjects factor 'day': $F_{5,70}=5,041$; $p=0.008$) independent of strain (repeated measures ANOVA 'day' x 'strain': $F_{5,70}=2,339$; $p=0.100$).

3.2 Experiment 2

The mice showed a strong cyclic activity pattern with high levels of movement duration during the dark phase as compared to the light phase.

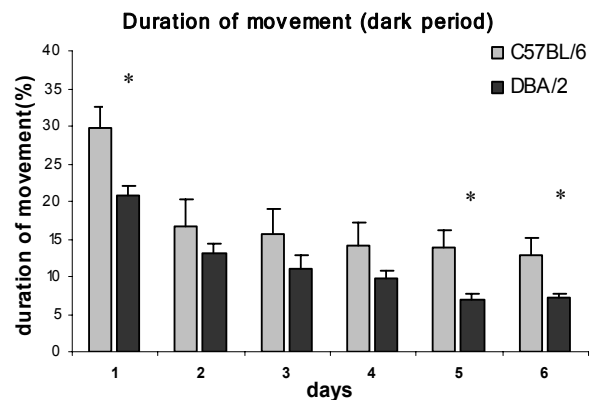


Figure 2. Duration of movement (as a percentage of time) during the dark period for each experimental day (1-6). Means + SEM per 12-hour period are used. * $p<0.05$ for differences between strains; independent sample t-test.

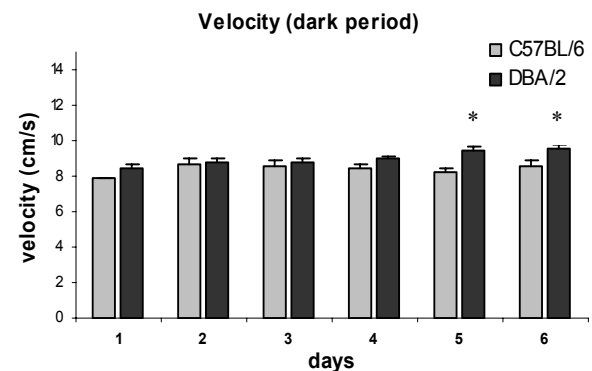


Figure 3. Velocity (in cm/s) during the dark period for each experimental day (1-6). Means + SEM per 12-hour period are used. * $p<0.05$ for differences between strains; independent sample t-test.

The light spot induced a small decrease in overall activity as reflected by lower duration of movement (Figure 5). Furthermore, there was a shift in activity towards the end of the dark phase on day 5, compared to day 4. 24 hours after the light spot (day 6), duration of movement was still lower than what would have been expected from day 4.

The light spot induced pronounced avoidance behavior in male C57BL/6 mice (see Figure 4). Time spent in the feeding zone decreased markedly during the time the light spot was switched on (day 5) compared to the day before (day 4). Some degree of habituation to the stimulus occurred, which was reflected by the increase in time spent in the feeding zone within the three hours the light spot was on. Notably, after the spot was switched off, there was a marked increase in the time spent in the feeding zone during the fourth hour of the dark phase. Values exceeded the duration in feeding zone during the fourth hour of the dark phase of day 4 and day 6.

24 hours after the light spot (day 6), time spent in feeding zone was still decreased compared to day 4. However, during the first three hours of day 6, time in the feeding zone increased faster compared to this time period on day 5.

Time in feeding zone

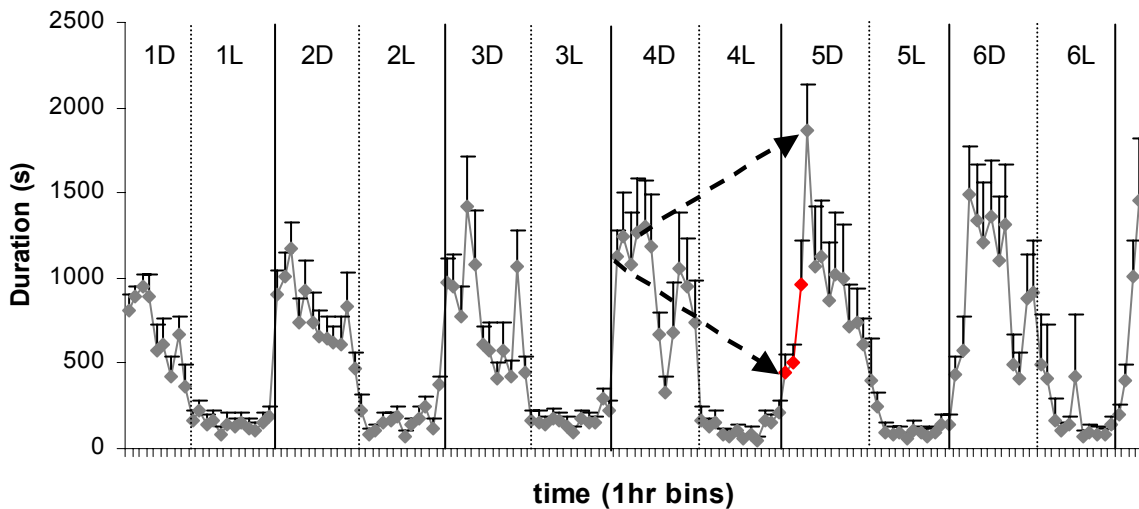


Figure 4. Time spent in feeding zone during six consecutive days. Means and SEM of 1hr bins are used. Days are indicated by numbers (1-6) and dark and light periods are distinguished by either D (dark period) or L (light period). Black data points on day 5 represent time window when light spot was switched on. Arrows indicate the main findings, see text for further explanation.

Duration of movement

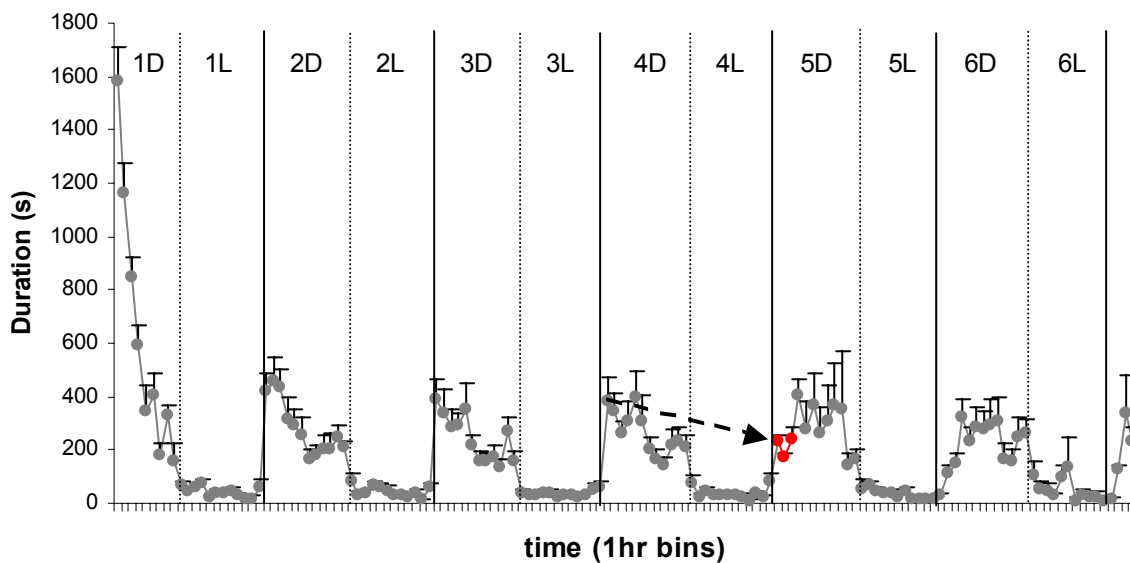


Figure 5. Duration of movement during six consecutive days. Means and SEM of 1hr bins are used. Days are indicated by numbers (1-6) and dark and light periods are distinguished by either D (dark period) or L (light period). Black data points on day 5 represent time window when light spot was switched on. Arrow indicates the main finding, see text for further explanation.

4 Discussion

Automated home cage observations offer several possibilities for the behavioral characterization of mice. Novelty induced exploration can be distinguished from activity in a familiar environment using a single test. It appeared that some aspects of locomotor activity, such as duration of movement, are more dependent on familiarity with the environment than others, such as velocity. Furthermore, by presenting an aversive stimulus when the animals were habituated to the environment, we were able to create an approach-avoidance conflict. This allowed evaluation of possible anxiety-related behavior, while avoiding the confounding influences of novelty, handling

and transport. Without these external stress factors it is possible to distinguish “state” from “trait” characteristics. Currently, experiments are being performed with the anxiolytic drug diazepam to find further evidence for the use of the aversive light spot as an anxiety test.

However, limitations of the presented method are in the time-consuming process of data handling and analysis. The vast amounts of data generated by the system demand sophisticated data handling software and statistical tests. Moreover, a functional interpretation of this computer output in terms of ethologically relevant profiles is a challenge in itself. To facilitate this, statistical tools for data reduction and clustering, such as Principal

Components Analysis, are adopted [6]. With these tools, the interrelation of parameters can be studied to reveal underlying motivational systems. Pharmacological experiments can further prove the predictive validity of parameters that showed differential potential between e.g. inbred mice.

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