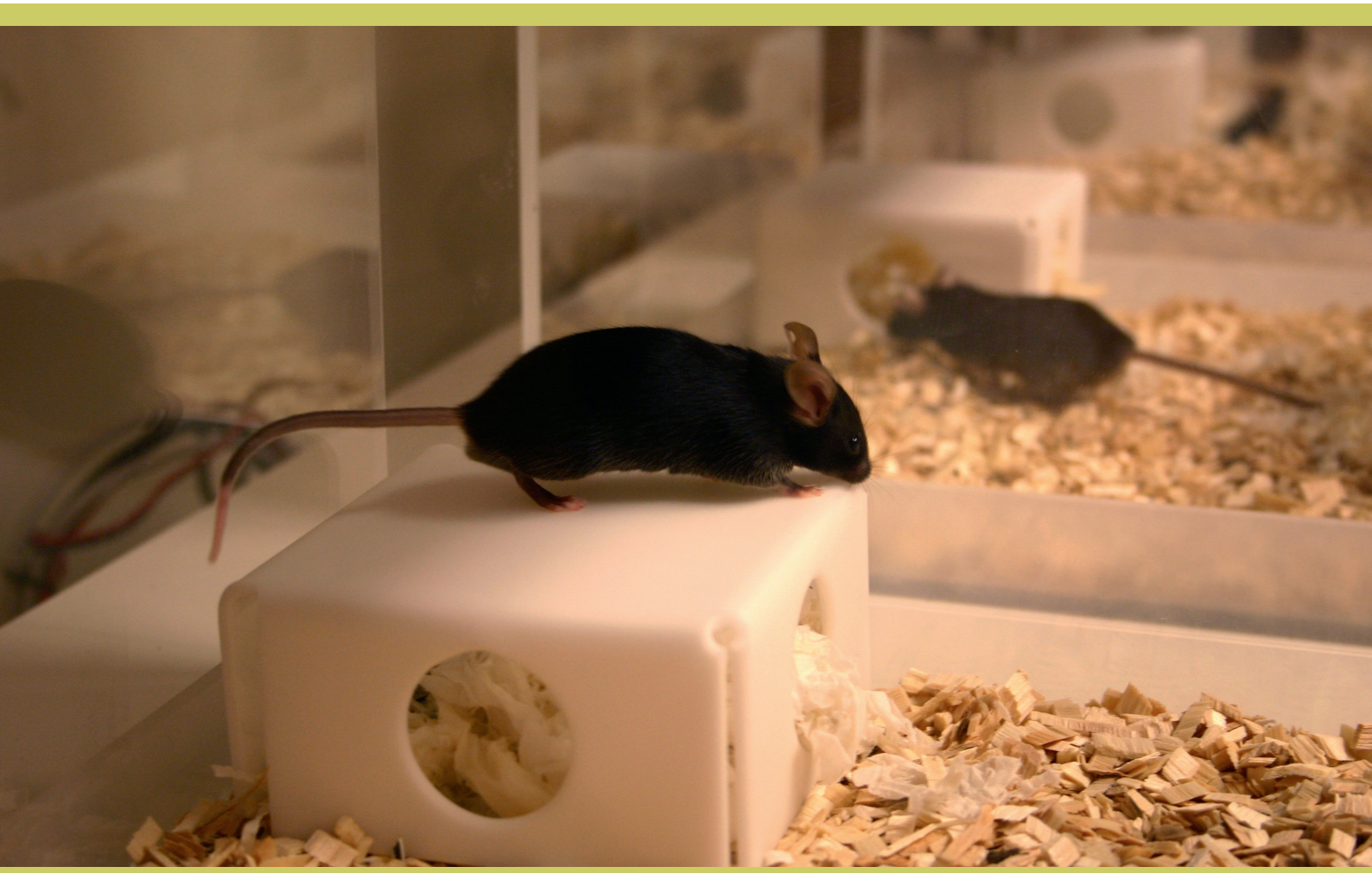
A PUBLICATION FROM NOLDUS INFORMATION TECHNOLOGY

Why study rodent behavior in the home cage



This is a chapter from the e-book:

Basic behavioral neuroscience in rodents.

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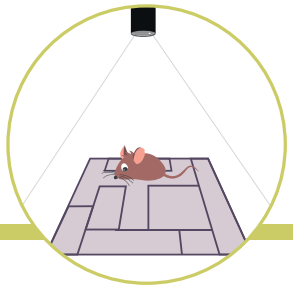
INTRODUCTION



Standard tests, such as the open field, elevated plus maze only provide brief snapshots of animal behavior at pre-determined time points. This way you can specifically evoke a type of behavior that is of interest. For example when you specifically want to look at acute anxiety. However a more ethologically relevant approach is to look at behavior in the home cage.

WHY DO WE TEST IN A HOME CAGE?

Monitoring behavior in the home cage eliminates stress in the animal that can be induced by for example handling and exposure to a new environment. Home cage monitoring also adds the option of monitoring behavior over time, be it food/water intake or behavioral changes over time after a (novel) manipulation (e.g. pharmacological or genetic). This can also be utilized to directly increase resolution in behavioral models that tend to show subtle abnormalities.

Also reproducibility is essential, though often still an overlooked aspect. All the behavioral tests described in this e-book rely on standardizing testing and assay protocols for reproducible data. A lot of variation still exists between labs, but also within labs which can be attributed to many factors (even the people that execute the test). The more variables that can be controlled, and standardized within the scientific community, the more reproducible the data becomes. This reproducibility can be increased in standardized home cage setup

HOW DO WE TEST IN A HOME CAGE

Home cage monitoring can be done with a self-constructed setup, like mounting cameras above each cage. Also systems with IR sensors are implemented, or beam breaks to determine whether the animals has crossed a specific zone and even under-cage capacitive plates. The difficulty in these setups arises when the data, specifically the timing, needs to be integrated. This is where home cage monitoring systems come in. These systems, like Noldus' PhenoTyper, have specifically been developed for this purpose and contain a number of hardware modules that can be controlled and integrated within EthoVision XT.

WHAT MAKES A CAGE A HOME CAGE

The definition, and ultimately design, of a home cage monitoring system is key to approaching a more *natural relevant* environment to test in. Key features include:

- Bedding material
- Chow and drinking water identical to vivarium conditions
- Environmental enrichment
- Adequate cage ventilation
- Cage size

Additionally the home cage monitoring system can be expanded with things like running wheels to measure activity, continuous food and water intake measurements, recording ultrasonic vocalizations and a cognition wall to measure learning and memory in a home cage environment.

HOW LONG CAN WE TEST IN A HOME CAGE

Continuous monitoring of behavior in a home cage monitoring system can range anywhere from 3 days to 4 weeks. When opting for longer periods of observation, it can be beneficial to use larger cages as they permit less frequent cage cleaning. Prolonged observation of behavior, for example continuous monitoring and/or longitudinal studies has proven to be indispensable to gain insights in the interplay of genetic factors and time. For example, studies of locomotor activity in genetic mouse models for autism uncovered consistent hypoactivity in the dark phase (the perceived active phase of rodents) with multiple-week home cage monitoring. This is in line with abnormalities in rest and activity rhythms in Autism Spectrum Disorder patients. Previous studies in acute testing situations like the open field for example however reported either hyperactivity or hypoactivity. This shows that home cage monitoring proves to be of great value in such delicate behavioral models.

Home cage monitoring systems, and the benefits they bring to reproducibility, is extensively reviewed by Grieco *et al.* in a large collaborative study.

WHAT TO MEASURE?

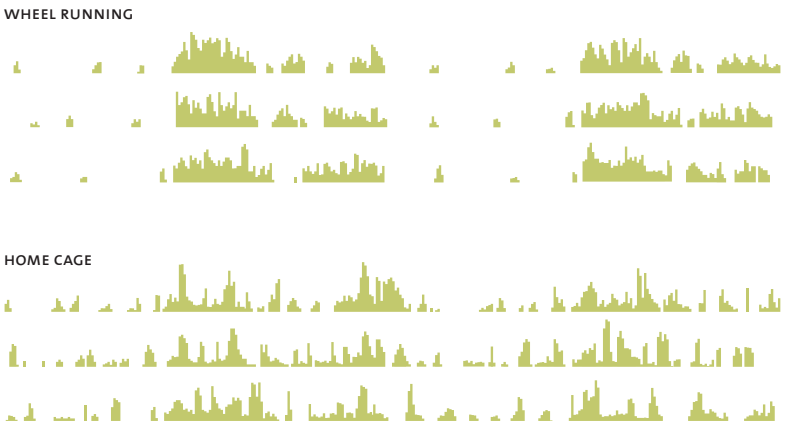
ACTIVITY, MOBILITY, CIRCADIAN RHYTHMICITY

Locomotor activity is a key component in many behavioral tests, suggesting that genetic differences in activity levels may be a critical consideration when comparing mouse strains.

The first chapter of this e-book described exploratory and locomotor behavior as a tool to gain an understanding of the (behavioral) phenotype of a rodent model. A test like the open field approaches this in an acute situation for a short period of time, which has benefits such as to measure the acute response to a novel environment. However to add translational value long term measurements prove to be an ideal tool. The example of Autism Spectrum Disorder, as studied in a home cage by Angelakos *et al.*, shows this.

Long term measurements of activity, mobility and the circadian rhythmicity simply give an insight in the daily routine of the animal. In a standard test we look at the spontaneous reaction to a specific situation. You can imagine this also translates to different situations considering humans.

Wheel running in a home cage has been the *original* measure for home cage activity as wheel running counters were easy to implement, and provided data files that were not very large in size, while being able to accumulate data over the period of weeks or even months. Wheel running however presents a problem when stating the want or need to measure normal home cage activity, since this tends to increase activity and food intake, while also being able to modulate the circadian regulation of metabolism. Bains *et al.* present the differences between wheel running and home cage activity in a very insightful review.



Wheel running behavior versus standard home cage behavior do not show the same activity patterns. This is why wheel running behavior cannot be considered a proxy of standard home cage behavior.

ANXIETY

Anxiety is an extremely important parameter to assess, as also explained in the second chapter of this e-book. It is however a very sensitive readout, since a rodent can easily be stressed. Often a rodents' response to a specific situation can be hard to interpret and/or classify as anxiety (or another behavioral response). This is the reason why, with anxiety, we tend to want to stick to validated test setups and protocols. The elevated plus maze is the prime example of this.

Anxiety in the home cage is however also measurable. The most popular approach to this is the *light spot test*. During this test a bright light is programmed to be targeted at the drinking and feeding zone during the dark phase. Rodents are nocturnal animals, they want to avoid lit environments. Control animals versus animals that have been treated with Diazepam, an acute anxiolytic drug, show decreased exploration outside of the shelter (present in the home cage).

FOOD AND WATER CONSUMPTION

Food and water consumption are important biomarkers of general wellbeing, but can also be linked to spontaneous behavior that may vary with genetic and pharmacological interventions. Traditionally food and water has been assessed either for a short period of time, or by fasting animals and assessing intake shortly after this period. Home cage observation offers the advantage of continuous monitoring of this intake, without the need of handling the animals or having to move them to a novel cage.

Food intake assessment is also important in metabolic and/or reward studies. Does a specific intervention prompt animals to eat more/less? Also the response to a novel (high fat) diet can be closely monitored, or even things such as stress eating. Water consumption can be used to, for example, perform a sucrose preference test. Here two water sources are presented, one plain water, the other sucrose water, and hedonic-like behavior can be assessed. The integration with lick-o-meters makes data integration extremely robust.

SOCIAL BEHAVIOR

Classically social behavior is tested in a three chamber arena. More recent studies have performed prolonged observation in social groups of rodents using a home cage recording. Discriminating between animals can be realized with RFID chips combined with video tracking. Peleh *et al.* found that, with this method, BTBR mice (a mouse model for Autism Spectrum Disorder) engaged in fewer social interactions compared with C57BL/6J mice.

COGNITION, LEARNING AND MEMORY

Although cognition, learning and memory are classically measured in standard, short term, test setups, recent advancements has also seen the use of *cognition walls* in home cage setups. Previously learning and memory testing was quite restricted to operant condition tasks, such as the pressing of a lever for a food reward, this however often requires either labor-intensive animal handling, or food

restriction in order to motivate the animals to press such a lever. The Sylics CognitionWall can be placed in front of a traditional reward dispenser, after initial assessment of basal behavior. Animals then need to learn to earn a reward by passing through one of the holes, while entering through the other two holes does not result in a reward. The difficulty of this task can be varied by adjusting the number of entries required to receive the reward.

PhenoTyper home cage monitoring system can come equipped with a number of hardware solutions. Including a Cognition Wall (by Sylics) and a food pellet dispenser. Photo credits: Sylics.