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Spontaneous behavior in the social homecage discriminates strains, lesions and mutations in mice

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HIGHLIGHTS

- IntelliCage captures 11 dimensions of spontaneous behavioral variation in mice.
- Mouse strains have unique and reproducible behavioral profiles in IntelliCage.
- Spontaneous behavior in IntelliCage supports high throughput prescreening of mutants.

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ABSTRACT

Background: Modern molecular genetics create a rapidly growing number of mutant mouse lines, many of which need to be phenotyped behaviorally. Poor reliability and low efficiency of traditional behavioral tests have prompted the development of new approaches to behavioral phenotyping, such as fully automated analysis of behavior in the homecage.
New method: We asked whether the analysis of spontaneous behavior during the first week in the social homecage system IntelliCage could provide useful prescreening information before specialized and time consuming test batteries are run. To determine how much behavioral variation is captured in this data, we performed principal component analysis on free adaptation data of 1552 mice tested in the IntelliCage during the past years. We then computed individual component scores to characterize and compare groups of mice.
Result: We found 11 uncorrelated components which accounted for 82% of total variance. They characterize frequency and properties of corner visits and nosepokes, drinking activity, spatial distribution, as well as diurnal time course of activity. Behavioral profiles created using individual component scores were highly characteristic for different inbred strains or different lesion models of the nervous system. They were also remarkably stable across labs and experiments.
Comparison with existing methods: Monitoring of mutant mice with known deficits in hippocampus-dependent tests produced profiles very similar to those of hippocampally lesioned mice.
Conclusions: Taken together, our results suggest that already the monitoring of spontaneous behavior during a week of free adaptation in the IntelliCage can contribute significantly to high throughput prescreening of mutant mice.

1. Introduction

Increasingly sophisticated and efficient methods of mutagenesis produce a rapidly growing number of genetically modified mouse lines, many of which are inducible or pharmacologically controllable. Additional models are produced using viral vectors and RNA interference techniques. Because many of these mouse models address questions related to nervous system function and disease,

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there is a large demand for comprehensive behavioral phenotyping of genetically modified mouse lines. Traditional behavioral tests for mice are inefficient, labor intensive, and often difficult to interpret because they were originally developed and validated for rats. Traditional tests typically involve social isolation, sensory deprivation, exposure to unfamiliar apparatus with very short observation time, and repeated handling by humans. The resulting stress responses introduce artifacts and reduce test reliability (Bohannon, 2002; Chesler et al., 2002; Crabbe et al., 1999). These shortcomings have created an urgent need to develop new more efficient approaches to behavioral phenotyping of mutant mice. A new approach with the potential to eliminate most of these problems is to automate behavioral experiments and to run them in the home cage of the animals. Home cage behavior can be monitored continuously over extended periods of time without human intervention. Several systems permitting to test mouse behavior in the home cage have been established in recent years (Richardson, 2012; Spruijt and De Visser, 2006). The IntelliCage is peculiar in the sense that it permits to phenotype mice in a social home cage setting while still collecting data of individual mice.

Since the introduction of the IntelliCage system, specialized protocols have been developed to test learning and memory, attention, impulsivity and emotional responses (Albuquerque et al., 2013; Codita et al., 2010, 2012; d'Isa et al., 2011; Endo et al., 2011; Galsworthy et al., 2005; Knapska et al., 2006; Kobayashi et al., 2013; Krackow et al., 2010; Ramakers et al., 2012; Safi et al., 2006; Voikar et al., 2010; Weyer et al., 2011). Even though these protocols are fully automated, require no or minimal intervention of the experimenter and are often shorter than comparable tasks in classical operant chambers, the mice still require a considerable amount of time to learn them. In the interest of phenotyping efficiency, the test battery needs to be selected carefully and unnecessary tests with negative outcome should be avoided.

Free adaptation is the mandatory first stage of any test battery for mice in IntelliCage but is typically not analyzed in detail. However, given that minimal restrictions are imposed on the behavior of the mice during this stage and that visits, nosepokes and licks are recorded continuously in multiple locations of the cage, one would expect this data to contain valuable information about spontaneous behavior of the tested mice. This prompted us to ask whether this data could be exploited for a prescreening of mutant lines or other experimental models. That is, could the sensitivity of behavioral screening be increased by detecting behavioral changes already during this early stage? Could the comparison of profiles of spontaneous behavior in IntelliCage with those resulting from well characterized experimental manipulations help to identify affected brain structures and support the selection of tasks for subsequent testing? To address these questions, we (i) sought to determine how much behavioral diversity is captured in the data recorded during the free adaptation stage; (ii) investigated whether different inbred strains and lesion models have distinct and reproducible behavioral profiles during free adaptation in IntelliCage that would permit to discriminate between these conditions; and finally (iii) compared behavioral profiles of selected mutant lines with those of mice with hippocampal lesions, asking whether mutations known to affect hippocampal function would reproduce at least part of the behavioral profile of a hippocampal lesion during free adaptation in IntelliCage.

2. Materials and methods

2.1. Animals

In order to base our retrospective analysis on a population as large and diverse as possible, we included all mice whose spontaneous behavior in the IntelliCage had been monitored using the free adaptation protocol described below, a total of 1552 subjects. Of these, 619 mice were obtained directly from Harlan Laboratories (Füllinsdorf, Switzerland or Venray, Netherlands) or Charles River Laboratories (Sulzfeld, Germany). The remaining 933 were shipped to us from various partner labs in Europe or from overseas. The analyzed population comprised various genetic backgrounds and included mutant lines, lesion models, as well as inbred and hybrid strains (Table 1). Because we have not tested male mice in IntelliCage until recently, a majority of 1408 mice were females, and only 144 were males. The median age at begin of testing was 4.27 months, with 80% of the animals falling within the range of 2.33–8.97 months. 86 mice were older than one year. Available information on genetic background is summarized in Table 1. Unless in IntelliCage, mice were housed in same sex groups in standard mouse cages under a 12/12 light cycle (lights on at 20:00) with food and water available ad libitum. All experiments were approved by the Veterinary Office of the Canton of Zurich.

In the present study we also reanalyzed in more detail free adaptation data of female mice used in previously published lesion studies. Full descriptions of the lesion models are given in the respective publications. In brief, bilateral dorsolateral striatal lesions were generated by stereotactic injection of NMDA in C57BL/6NCrl mice from Charles-River Laboratories (Sulzfeld, Germany) with sham lesions serving as controls (Voikar et al., 2010). Complete bilateral exitotoxic hippocampal lesions ("hippocampal lesion 1" in Fig. 4) were generated in C57BL/6JRccHsd mice (Harlan Laboratories, Füllinsdorf, Switzerland) using the same approach (Voikar et al., 2010). Later unpublished hippocampal lesion experiments using the same methods in C57BL/6NCrl mice from Charles-River Laboratories (Sulzfeld, Germany) are also included for comparison ("hippocampal lesion 2" and "hippocampal lesion 3" in Fig. 4). A selective postnatal ablation of the medial habenula had been achieved by conditional expression of diphtheria toxin A on a C57BL/6 J background in the mHb:DTA mouse line (Kobayashi et al., 2013). Spared nerve injury of the sciatic nerve (SNI) was generated by ligating and distally transecting the common peroneal and tibial nerve while leaving the sural nerve intact. This was performed on C57BL/6 J mice as well as in progranulin deficient mice previously backcrossed into C57BL/6 J (Albuquerque et al., 2013).

Further, detailed reanalysis is presented for female subjects of five previously published mutant lines. These include the βAPPsα/sα-DM line, a double mutant on a mixed genetic background with contributions from C57BL/6 and 129S2 resulting from a cross between βAPPsα knock-in mice expressing solely the secreted ectodomain of βAPP instead of the complete protein and APLP2-null mice (Weyer et al., 2011). The Thy1/5xFAD mouse line expresses 3 human amyloidogenic βAPP mutations together with 2 presenilin 1 mutations as a transgenic package under control of the Thy1 promoter on a mixed genetic background with contributions from C57BL/6 and SJL (Oakley et al., 2006). These mice were tested in the context of a collaboration with Erich Wanker (Max Delbrück Center for Molecular Medicine, Berlin, Germany) and originally purchased from the Jackson Laboratory (Bar Harbor, ME, USA). On a C57BL/6 background, PDGF/EPO-tg6 mice overexpress human erythropoietin ubiquitously from a transgene driven by the human platelet-derived growth factor B-chain promoter. They have a strongly increased hematocrit and suffer from progressive multiple organ degeneration (Heinicke et al., 2006; Ruschitzka et al., 2000). SNAP-25 mutant mice have a C57BL/6 background and are heterozygous for a constitutive deletion of the *Snap25* gene and express reduced levels of synaptosomal-associated protein of 25 kDa (Corradini et al., 2014; Washbourne et al., 2002). Finally, Munc18-1 mutant mice are heterozygous for a constitutive