Mouse Phenotyping in the IntelliCage: From Spontaneous Behavior to Cognitive Function

D.P. Wolfer[1,2], V. Voikar[3], E. Vannoni[1], G. Colacicco[1], H.P. Lipp[1]

[1]*Institute of Anatomy, University of Zurich, Zurich, Switzerland. dpwolfer@anatom.uzh.ch*

[2]*Institute of Human Movement Sciences, ETH Zurich, Zurich, Switzerland. dwolfer@ethz.ch*

[3]*Neuroscience Center, University of Helsinki, Helsinki, Finland. vootele.voikar@helsinki.fi*

Introduction

Increasingly sophisticated and efficient methods of mutagenesis produce an exploding 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, there is a large demand for comprehensive behavioral phenotyping of genetically modified mouse lines. During the past 25 years our laboratory has been involved in the behavioral phenotyping of >11,000 mice and >130 different mutations. 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, and repeated handling by humans. The resulting stress responses introduce artifacts and reduce test reliability. 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, whereas observation times of traditional tests are typically short, often too short. Several systems permitting to test mouse behavior in the home cage have appeared on the market in recent years. The IntelliCage (IC), used by my lab since 5 years, is peculiar in the sense that it permits to phenotype mice in a social home cage setting. As shown in Figure 1, IC is a large home cage designed for 10-16 mice with four computer-controlled learning corners that provide space for one mouse at a time and contain two drinking bottles each. Water access can be blocked by motorized doors that open in response to nosepokes. Because the mice carry RFID tags, all corner visits, nosepokes and licking events can be attributed to individual mice. Each corner can present light stimuli, deliver air puffs as punishment, and is fully programmable as an operant conditioning chamber.

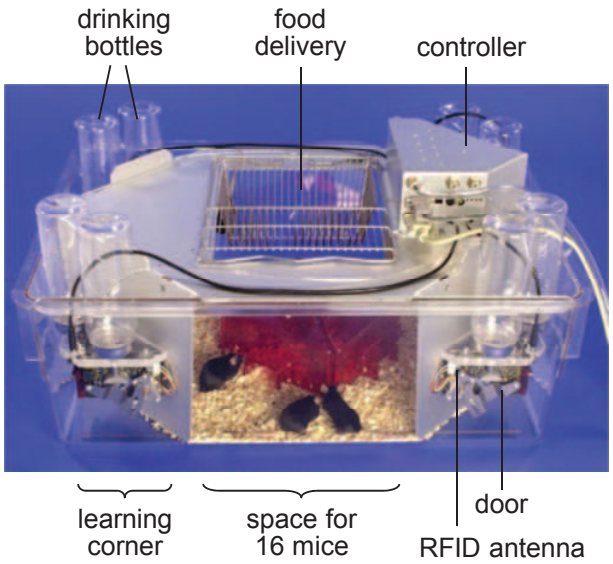

Figure 1. The IntelliCage apparatus.

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Spontaneous behavior

While many specialized protocols have been developed for IntelliCage to test learning and memory, attention, impulsivity and emotional responses (see below), all mice begin testing with about one week of *free adaptation*, during which all water bottles are freely accessible at all times. Spontaneous corner visits, nosepoking patterns and licking activity are monitored 24/7 during this phase. Since the IC system was last presented at the Measuring Behavior conference in 2008, we have collected data on 50 behavioral parameters of >800 mice. These parameters include duration, content, spatial distribution and temporal patterning of visits, nosepokes, as wells as licking episodes. Subsequent factor analysis of these parameters extracted 12 orthogonal factors accounting for 81% of total variance. Comparison of factor scores of C57BL/6, DBA/2, BALB/c and 129S2 mice revealed a unique profile for each strain. Analysis of mice with hippocampal, prefrontal and striatal lesions also yielded unique profiles for each condition. Monitoring of mutant mice with known deficits in hippocampus-dependent tests produced profiles very similar to those of hippocampal lesions [1, 4]. On the other hand, this analysis revealed strikingly similar changes of diurnal activity distribution in the 5xFAD mouse which over-expresses β-amyloid precursor protein (βAPP) and a βAPP hypomorphic mutant [6]. Thus, already the monitoring of spontaneous behavior during a week of free adaptation in IC permits high throughput prescreening of mutant mice. On the other hand, our data indicate that tight control of genetic background remains essential also if behavioral testing occurs in the home cage. In order to familiarize the mice with the operant properties of IC used in most learning tasks, free adaptation is followed by *nosepoke adaptation*, during which doors are closed but can be opened at any times once per corner visits by performing a nosepoke. This is followed by a *drinking session adaptation* protocol in which the mice learn to adapt their activity to a regime of scheduled drinking sessions. This form of learning seems highly and specifically sensitive to hippocampal lesions.

Cognitive function

In order to asses spatial learning and memory, we have developed a suite of tasks that are based on a drinking session protocol but make the additional restriction, that for each individual mouse only one of the four corners delivers water reward in response to nosepokes. The rule which the mice must learn in order to predict where water will be available next changes between tasks and becomes increasingly difficult. The simplest implementation is a *corner preference* task in which each mouse can obtain water in the same corner during a week. So far, this task was learned successfully by all tested mouse models, indicating that this is a simple control procedure (sort of an IC equivalent of the cue navigation protocol in the water-maze) useful to verify that the mice possess the sensory and motor abilities needed to perform the other tasks in this suite. Next, the mice proceed to a *reversal learning* phase in which the reward location is moved to the opposite corner, and finally to a *serial reversal protocol*, in which the location of reward changes between drinking sessions in a random fashion. A multi-lab study has demonstrated that these protocols deliver consistent results independently of lab environment [3]. Finally, the mice learn two protocols in which the location of reward changes between individual visits. In the *chaining task*, the animals must learn to visit corners in a clock- or anti-clock-wise sequence. The *patrolling task*, is similar but more difficult to learn because the reference that determines location of reward is not the most recently visited corner, but the one in which reward was last obtained. In both tasks, acquisition of the rule is followed by a reversal phase in which the direction is reversed. Patrolling reversal appears to be extremely difficult for mice to learn, even intact C57BL/6 learn extremely slowly. Mice take about 2 months to proceed through all the tasks of this suite. In our validation studies, we have found that mice with bilateral complete hippocampal (but not prefrontal or dorso-lateral striatal) lesions are strongly impaired in reversal, serial reversal as well in as chaining, patrolling and other forms of sequence learning. Since the last presentation of IC at the Measuring Behavior conference in 2008, this suite of tasks has revealed strong impairments in mutant mouse models with deficient function or connectivity of the hippocampus, for example in mice with a null mutation of alphaPix/Arhgef6, a gene associated with X-linked intellectual disability [4], as well as in a mouse model of Alzheimer's disease [1]. Massive deficits were also revealed in a βAPP hypomorphic mutant due to motor deficits cannot be tested in many of the conventional spatial learning tasks [6]. The suite of spatial tasks is complemented by a *corner avoidance* protocol in which the animals learn to avoid one of the four corners in which nosepokes are punished by air puffs during a training period of 24h hours. In order to test long-term memory, the animals can be reintroduced into IC for a probe trial after varying

retention intervals outside IC. If left for several days in the IC, the mice show *extinction learning*, that is gradually resume drinking in the no longer punished corner. Performance in this test is sensitive to hippocampal manipulations as well [1, 5]. Together with other tests, this paradigm has revealed that mice lacking Ras-GRF1 are selectively impaired in fear conditioning but have intact spatial memory [2]. More recent developments whose validation is still in progress include an IC version of the *conditioned taste aversion* and *social transmission of food preferences* tests.

Adaptive decision making and inhibitory response control are important aspects of cognition that are often neglected in mouse phenotyping because their testing in conventional operant setups is extremely time consuming and resource intensive. Therefore, we have recently developed a simple *reaction time task* for IntelliCage. At any time and in any corner a mouse can start a trial by entering the corner and making a first nosepoke. After a variable delay, stimulus lights will go on and the mouse is allowed to drink at the door where the first poke was made. Principal readouts are the number of premature responses as a measure of motor impulsivity and the latency of correct responses (reaction time). In a strain comparison this test recapitulated the results obtained in conventional operant reaction time tasks, such as the five-choice serial reaction time task. Mice with lesions of the medial habenula show increased impulsivity in this IC task as well as in the traditional 5-choice serial reaction time task. In order to assess how well mice can control their response to reward, we have also developed a *delay discounting task* for IC. In all corners the mice are given a choice between a bottle with plain water and one with 0.5% saccharin solution. In a training phase, the mice are allowed to learn about the existence of this choice and to establish their (usually exclusive or almost exclusive) preference for the saccharin solution. In the following test phase, access to saccharin is increasingly delayed at a rate of 0.5s per day. As a consequence of this imposed delay, the mice will eventually abandon their preference for the saccharin solution. Reduced delay tolerance in this test can provide evidence for increased cognitive impulsivity, whereas excessive delay tolerance in conjunction with saccharin overconsumption and compulsive nosepoking at the saccharin door are indicators of a poorly controlled response to reward. The latter observation was made when we tested mice with lesions of the medial habenula in this test, as well as in a transgenic mouse line over-expressing erythropoietin selectively in the CNS.

Thus, the fully automated IC system permits efficient prescreening of socially housed mutant mice by recording profiles of spontaneous individual behavior, as well as assessment of specific aspects of cognitive function using specialized operant tasks.

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