Behavioural Brain Research 283 (2015) 53–60

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

A 1-night operant learning task without food-restriction differentiates among mouse strains in an automated home-cage environment

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HIGHLIGHTS

- Operant learning can be studied in a 1-night automated home-cage task.
- Food restriction is not necessary in this protocol.
- BALB/cJ shows a larger dynamic range than C57BL/6J and DBA/2J.

ARTICLE INFO

Article history:
Received 27 October 2014
Received in revised form 8 January 2015
Accepted 10 January 2015
Available online 17 January 2015

Keywords:
Operant conditioning
Instrumental learning
PhenoTyper
Automated home-cage
Mice

ABSTRACT

Individuals are able to change their behavior based on its consequences, a process involving instrumental learning. Studying instrumental learning in mice can provide new insights in this elementary aspect of cognition. Conventional appetitive operant learning tasks that facilitate the study of this form of learning in mice, as well as more complex operant paradigms, require labor-intensive handling and food deprivation to motivate the animals. Here, we describe a 1-night operant learning protocol that exploits the advantages of automated home-cage testing and circumvents the interfering effects of food restriction. The task builds on behavior that is part of the spontaneous exploratory repertoire during the days before the task. We compared the behavior of C57BL/6J, BALB/cJ and DBA/2J mice and found various differences in behavior during this task, but no differences in learning curves. BALB/cJ mice showed the largest instrumental learning response, providing a superior dynamic range and statistical power to study instrumental learning by using this protocol. Insights gained with this home-cage-based learning protocol without food restriction will be valuable for the development of other, more complex, cognitive tasks in automated home-cages.

1. Introduction

Humans, and other animals, have the cognitive skills to adjust their behavioral repertoire in the face of novel situations. The ability to change behavior based on its consequences, also known as instrumental learning [1], or operant conditioning [2], can be considered fundamental to many forms of cognitive functioning. Studying this form of learning in mice, in, for instance, panels of inbred lines or mutant mouse lines, is important for our understanding of the genetic mechanisms underlying this elementary aspect of cognitive functioning.

Appetitive operant conditioning is a form of instrumental learning in which the reinforcing stimulus is palatable. This is traditionally studied in mice by using an operant conditioning chamber in which the animals have to learn to respond with a lever press or nose poke to a stimulus in order to receive a food or liquid reward,

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http://dx.doi.org/10.1016/j.bbr.2015.01.020
0166-4328/

delivered at a specific location. Tasks for more complex forms of cognition that are performed in operant chambers, like a reversal learning task that measures flexibility, or the five choice serial reaction time task that measures attention and impulsivity, use the same principle albeit with more complex schedules of reinforcement. Although operant testing provides in-depth insights into cognition, it unfortunately requires labor-intensive animal handling, which may confound task outcome and can cause handling stress [3–6]. Another drawback is that food-restriction protocols, regularly used in operant testing to motivate mice to perform, can affect behavioral responses differentially in different mouse strains [7–9]. In particular, food restriction can be perceived as a stressor, as it has been shown to increase stress hormone levels [10–12], and can in this way influence task outcome. Additionally, food restriction can change circadian and task-related activity patterns in rodents [11–14].

To increase throughput and reproducibility of behavioral screening, new fully automated testing strategies are desirable [15–18], e.g., testing mice in their home-cage with subsequent automated data analysis. Automatic tracking of spontaneous behavior of mice in their home-cage for extended periods without human interference can provide comprehensive and detailed analysis of naturalistic behavior [19–24]. Additionally, testing mice in an automated home-cage produced consistent strain differences across laboratories [25].

In this study, the main experimental question was to design an operant conditioning procedure, without food restriction. In our effort toward enhancing the efficiency of behavioral testing, we have included this task in a 1-week automated home-cage protocol that combines observations of spontaneous behavior during a habituation period [26], with two other tasks: an avoidance learning task [27] and an anxiety task (unpublished data). Together, this protocol allows for the study of multiple behavioral domains, i.e. locomotor activity, learning and anxiety, in an environment that does not require experimenter intervention.

In this report, we describe the design and analysis of the 1-night operant conditioning procedure in this 7-day protocol that exploits the advantages of automated home-cage testing with diminished interfering effects of handling or food-restriction stress on task outcome and activity. These advantages also allow for increased reproducibility and scalability.

The task protocol builds on experimental procedures previously developed by de Heer et al. [28] and uses food reward without prior food restriction. After 3 days of habituation and analysis of spontaneous behavior [26], on the fourth day mice can receive a food reward by performing an instrumental response, i.e., climbing on their shelter. The task was distributed over multiple sessions to prevent satiety. The task started after a habituation period of 3 days because our previous home-cage experiments [26], as well as those of others [22,29], showed that it can take up to three days, depending on the mouse strain used, for activity parameters to stabilize.

We compared the behavior of C57BL/6J, BALB/cJ and DBA/2J mice on this task and were able to detect instrumental learning within C57BL/6J and BALB/cJ mice by analyzing their locomotor patterns. Differences in the magnitude of the instrumental learning response were found between BALB/cJ mice and C57BL/6J mice.

2. Materials and methods

2.1. Mice

61 C57BL/6J, 27 BALB/cJ and 32 DBA/2J male mice were obtained from Charles River Laboratories (L'Arbresle, France; European supplier of Jackson Laboratories) and maintained in the facilities of the NeuroBSIK consortium (VU University, Amsterdam, The Netherlands or at Harlan Laboratories, Horst, The Netherlands). At an age of 8–12 weeks, mice were single housed on sawdust in standard Makrolon type II cages enriched with cardboard nesting material for at least one week prior to experiments, with water and food (2018 Teklad, Harlan Laboratories, Horst, The Netherlands) ad libitum (7:00/19:00 lights on/off; providing an abrupt phase transition). Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), and with approval of the Animal Experiments Committee of the VU University.

2.2. Automated home-cage and testing protocol

Testing was performed in an automated home-cage environment (PhenoTyper model 3000, Noldus Information Technology, Wageningen, The Netherlands) in which behavior was tracked by video and where hardware actions were triggered by the location of the mouse, as described in detail in Maroteaux et al. [27]. Cages ($30 \times 30 \times 35$ cm) were made of transparent Perspex walls with an opaque Perspex floor covered with bedding based on cellulose and were equipped with a water bottle and a feeding station. A triangular-shaped shelter with two entrances was fixed in one corner ($H \times D = 10 \times 9$ cm), (non-transparent material). In the opposite corner, an aluminum tube of a reward dispenser protruded into the cage. Food and water were provided ad libitum.

The top unit of each cage contained an array of infrared LEDs and an infrared-sensitive video camera used for video-tracking. The *X*–*Y* coordinates of the center of gravity (COG) of mice, sampled at a resolution of 15 coordinates per second, were acquired using EthoVision software (EthoVision HTP 2.1.2.0, based on EthoVision XT 4.1, Noldus Information Technology, Wageningen, The Netherlands [30]) and processed to generate behavioral parameters using AHCODA^{TM} analysis software (Synaptologics BV, Amsterdam, The Netherlands) and R, version 2.15.0 [31]. Two zones were digitally defined: an OnShelter zone on top of the shelter and a RewardZone in the corner where the pellet dispenser was positioned.

Mice were introduced to the PhenoTyper during the light phase (14:00–16:00 h) and housed in this cage without any further human interference. Video tracking and the testing protocol started at the onset of the first dark phase (19:00 h). The protocol (Fig. 1) started off with 3 days of habituation where spontaneous behavior was tracked. At the start of the 4th dark phase, the operant conditioning task became active. In this task, mice had to learn that a visit to the top of the shelter (i.e. an OnShelter visit) led to a reward (14 mg Dustless Precision Pellets F05684, 14 mg, Bio-Serve, Frenchtown, NJ, USA) being dropped by the reward dispenser in the corner opposite to the shelter (i.e. RewardZone). We refer to this task as an operant conditioning task and not a Pavlovian cue approach learning or autoshaping task, because mice had to make a specific operant response (OnShelter visit) to receive a reward. Furthermore, neither the cue light nor the shelter was at the location of the reward and therefore approach behavior, such as first described by Brown and Jenkins [32], could not be studied.

The operant protocol was not continuously active during the night, but split up in 10 sessions of 15 min, with session intervals of 1 h to prevent satiety for the rewards. Each session started off with a 'free' reward delivery. Mice could earn additional rewards by making OnShelter visits. These visits were rewarded according to a fixed ratio (FR1) schedule, but only when an OnShelter visit was followed by a visit of the reward zone (i.e. a RewardZone visit). There were no temporal restrictions for this RewardZone visit to occur. The start and duration of each session was indicated by a yellow cue light in the top unit that was on for the full duration of each session, which was intended to signal the possibility to earn