Behavioural Brain Research 221 (2011) 172–181

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Automated test of behavioral flexibility in mice using a behavioral sequencing task in IntelliCage

Toshihiro Endo[a], Fumihiko Maekawa[c], Vootele Võikar[d], Asahi Haijima[a], Yukari Uemura[b], Yan Zhang[a], Wataru Miyazaki[a], Shigetomo Suyama[c], Kuniko Shimazaki[c], David P. Wolfer[e,f], Toshihiko Yada[c], Chiharu Tohyama[a], Hans-Peter Lipp[d], Masaki Kakeyama[a,*]

[a] Laboratory of Environmental Health Sciences, Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, The University of Tokyo, Japan
[b] Department of Biostatistics, School of Public Health, The University of Tokyo, Japan
[c] Department of Physiology, Division of Integrative Physiology, Jichi Medical University, School of Medicine, Japan
[d] Institute of Anatomy, University of Zurich, Switzerland
[e] Institute of Anatomy and Zurich Center for Integrative Human Physiology, University of Zurich, Switzerland
[f] Institute of Human Movement Sciences, ETH Zurich, Switzerland

ARTICLE INFO

Article history:
Received 6 September 2010
Received in revised form 23 February 2011
Accepted 28 February 2011

Keywords:
Mouse
Automated analysis
Serial reversal learning
Behavioral flexibility
IntelliCage

ABSTRACT

There has been a long-standing need to develop efficient and standardized behavioral test methods for evaluating higher-order brain functions in mice. Here, we developed and validated a behavioral flexibility test in mice using IntelliCage, a fully automated behavioral analysis system for mice in a group-housed environment. We first developed a "behavioral sequencing task" in the IntelliCage that enables us to assess the learning ability of place discrimination and behavioral sequence for reward acquisition. In the serial reversal learning using the task, the discriminated spatial patterns of the rewarded and never-rewarded places were serially reversed, and thus, mice were accordingly expected to realign the previously acquired behavioral sequence. In general, the tested mice showed rapid acquisition of the behavioral sequencing task and behavioral flexibility in the subsequent serial reversal stages both in intra- and inter-session analyses. It was found that essentially the same results were obtained among three different laboratories, which confirm the high stability of the present test protocol in different strains of mice (C57BL/6, DBA/2, and ICR). In particular, the most trained cohort of C57BL/6 mice achieved a markedly rapid adaptation to the reversal task in the final phase of the long-term serial reversal test, which possibly indicated that the mice adapted to the "reversal rule" itself. In conclusion, the newly developed behavioral test was shown to be a valid assay of behavioral flexibility in mice, and is expected to be utilized in tests of mouse models of cognitive deficits.

1. Introduction

The behavioral characterization of genetically modified mice as well as wild-type strains has become a powerful tool for investigating not only the molecular bases of normal brain functions but also the pathogenesis and treatment of neuropsychological disorders [1–9]. However, the levels of efficiency, standardization, and reproducibility of the testing methods for mouse behavioral assessment have been considered still inadequate [10–14]. More importantly, the limited number of established "higher-order" cognitive test paradigm for mice makes it difficult for researchers to determine the accurate neurobehavioral phenotypes of both wild-type and pathological mice [15].

To overcome this problem, a number of computer-assisted technologies for automatically capturing rodent behavior over long periods have become available [16–18]. Among them, IntelliCage (New Behavior AG; http://www.newbehavior.com/) used in the present study is a unique approach in the sense that this system is specially designed for the cognitive assessment of group-housed mice. The IntelliCage system can be a powerful tool for the behavioral characterization of mice by at least fivefold. First, its use makes it possible to achieve a sensitive and highly standardized experiment by minimizing the artifacts that arise from unavoidable differences among experimenters or other laboratory-specific conditions. Second, long term monitoring of mouse behavior can be performed in a familiar and thus less stressful environment. Third, high-throughput testing is possible by analyzing a maximum of 16 mice simultaneously. Fourth, experimenters can design and use their own original cognitive task depending on their research objective. Fifth, IntelliCage can be run in a fully automated manner,

* Corresponding author. Tel.: +81 3 5841 1415; fax: +81 3 5841 1434.
E-mail address: kake@m.u-tokyo.ac.jp (M. Kakeyama).

0166-4328/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2011.02.037

utilizing sensors and four operant conditioning units placed in each corner of the cage.

Recent studies have already shown the advantages of using this IntelliCage system in the spontaneous and learning behavioral analysis of mice [19–27]. By contrast, there are still few established protocols for IntelliCage that focus on higher-order cognitive skills typified by executive brain functions. The executive brain function is a shorthand description of a set of cognitive processes that are responsible for appropriately organizing, performing, and maintaining goal-directed actions under ever-changing environmental contexts [28–32]. The quality of life based on intellectual and mental integrity is largely dependent on such brain function, and its dysfunction is widely seen in people with aging-associated cognitive decline and various neuropsychological disorders [29,33–37] as well as patients with frontal lobe damage caused by traumatic brain injury and cerebrovascular disease [38,39]. Although researchers have recently established executive function tests for mice such as attentional set-shifting task [40–43] and selective attention [44], few reports that assessed the executive functions of group-housed mice in a computerized, high-throughput manner are available.

Thus, the aim of this study is to establish a behavioral test protocol for mice that enables one to evaluate behavioral flexibility as one of the executive brain functions using IntelliCage. The test is composed of a newly developed "behavioral sequencing task" followed by its serial reversals. The test paradigm was originally developed on the basis of the idea of the Brixton Spatial Anticipation Task [45] which has been utilized as one of the clinical assessment methods of human executive functions using a visuospatial sequencing task [45–52]. By using the IntelliCage-based test in this study, it became possible to address not only acquisition of spatial and temporal pattern of rewarded places but also behavioral flexibility of mice in various time scales in a fully automated manner. The reproducibility of the protocol was verified by different experimenters at three different laboratories located in the University of Tokyo (UT), Tokyo Japan, Jichi Medical University (JMU), Tochigi, Japan and the University of Zurich (UZH), Zurich, Switzerland, using three strains of mice (C57BL/6, DBA/2 and ICR) and of different ages (from 2 to 8 months old).

2. Materials and methods

2.1. Animals and facilities

The experiments described in this study were conducted using identical type of IntelliCage systems at three different laboratories located at the University of Tokyo (UT), Tokyo, Japan, Jichi Medical University (JMU), Tochigi, Japan and the University of Zurich (UZH), Zurich, Switzerland. All the animal experiments were performed in a humane manner in accordance with the local guidelines of each institution.

At UT, male C57BL/6 (B6-UT, 6 months old, $n = 8$) and DBA/2 (D2-UT, 8 months old, $n = 8$) mice were used. All mice were purchased from CLEA Japan (Tokyo, Japan) and housed in $22 \pm 1\,^{\circ}C$, $50 \pm 10\%$ humidity, 12 h LD cycle (lights on 8:00–20:00). At JMU, male C57BL/6 (B6-JMU, 3 months old, $n = 15$) and ICR mice (ICR-JMU, 2 months old, $n = 11$) were used. All mice were purchased from CLEA Japan and Japan SLC, Inc. (Shizuoka, Japan) and housed in $22 \pm 1\,^{\circ}C$, $60 \pm 10\%$ humidity, 12 h LD cycle (lights on 7:00–19:00). At UZH, young (3 months old) and aged (12 months old) male mice of C57BL/6 and DBA/2 strains were used (Young B6-UZH, Aged B6-UZH, Young D2-UZH and Aged D2-UZH, $n = 11$, 12, 12 and 11, respectively). All mice were purchased from Charles-River Laboratories (Sulzfeld, Germany) and housed in $21 \pm 1\,^{\circ}C$, $50 \pm 5\%$ humidity, 12 h LD cycle (lights on 20:00–8:00 as reversed cycle). In total, eight cohorts of male mice were analyzed using the IntelliCage system as described below.

All the animals were subcutaneously implanted with a glass-covered transponder with unique ID codes (Datamars SA) for radio-frequency identification (RFID)-based animal identification before the start of the experiments under light-anesthesia with diethyl ether or isoflurane.

2.2. IntelliCage apparatus

IntelliCage (NewBehavior AG; http://www.newbehavior.com) is a computer-based, fully automated testing apparatus used to analyze the spontaneous and learning behavior of RFID-tagged mice in a home cage (Fig. 1A). (For further descriptions, see [19–27]). In short, a large standard plastic cage ($55 \times 37.5 \times 20.5\,cm^3$) equipped with four triangular operant learning chambers (corners, hereafter) ($15 \times 15 \times 21\,cm^3$) that fit into each corner of the cage, RFID readers, and other types of sensor allows simultaneous monitoring of up to 16 transponder-tagged mice living in the same cage. Mice were allowed to enter the corner (corner visit, hereafter) through a short narrow tunnel that functions as an RFID antenna. In this unit, only one mouse can enter a corner at a time because of the limited size of the corner and tunnel. In the inner space of the corner, mice can find two nosepoke holes with an infrared beam-break response detector. The "correct" nosepoke triggers the opening of a motorized access gate to water-bottle nipples (gate, hereafter). In IntelliCage, the time and duration of each behavioral event (corner visit, nosepoke and lick), mouse ID and corner ID were automatically recorded through RFID readers, infrared sensors and lickometers.

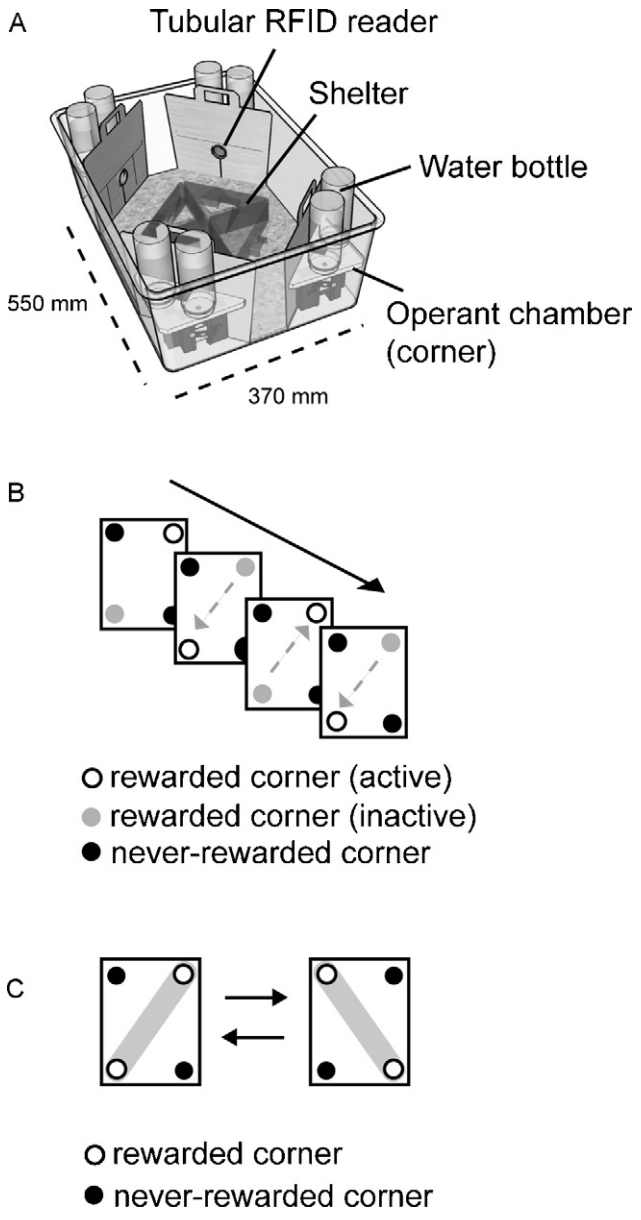

Fig. 1. Apparatus and task paradigm. (A) Overview of IntelliCage apparatus. Mice were group-housed and their behavioral responses (corner visits, nosepokes, and lickings) were monitored in a fully automated manner. The tubular RFID reader can record an implanted ID number when a mouse visits a corner where it can receive water as a reward. (B) Diagrams of behavioral sequencing task. Mice obtained a reward by alternately visiting the two distantly positioned rewarded corners (open circle). A visit to the never-rewarded corners (black circles) was counted as an "error" choice. (C) Serial reversal learning of the behavioral sequencing task. The patterns of corner conditions (rewarded or never-rewarded) were reversely switched every several sessions. The thick grey line in (C) indicates the expected shuttling path on which mice shuttle between the distantly positioned rewarded corners.