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Assessment of mouse anxiety-like behavior in the light–dark box and open-field arena: Role of equipment and procedure

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HIGHLIGHTS

- Effect of start side in light–dark box and floor brightness in open field was investigated.
- Placing the mice in the light compartment promoted an approach-behavior in the light–dark box.
- Black floor promoted an approach-behavior in the open field arena.
- Strain rankings and exploratory patterns were not affected by the modifications of the procedure or equipment.

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ABSTRACT

Light–dark box and open field are conventional tests for assessment of anxiety-like behavior in the laboratory mice, based on approach–avoidance conflict. However, except the basic principles, variations in the equipment and procedures are very common. Therefore, contribution of certain methodological issues in different settings was investigated. Three inbred strains (C57BL/6, 129/Sv, DBA/2) and one outbred stock (ICR) of mice were used in the experiments. An effect of initial placement of mice either in the light or dark compartment was 6 May 2014 studied in the light–dark test. Moreover, two tracking systems were applied — position of the animals was detected either by infrared sensors in square box (1/2 dark) or by videotracking in rectangular box (1/3 dark). Both approaches revealed robust and consistent strain differences in the exploratory behavior. In general, C57BL/6 and ICR mice showed reduced anxiety-like behavior as compared to 129/Sv and DBA/2 strains. However, the latter two strains differed markedly in their behavior. DBA/2 mice displayed high avoidance of the light compartment accompanied by thigmotaxis, whereas the hypoactive 129 mice spent a significant proportion of time in risk-assessment behavior at the opening between two compartments. Starting from the light side increased the time spent in the light compartment and reduced the latency to the first transition. In the open field arena, black floor promoted exploratory behavior — increased time and distance in the center and increased rearing compared to white floor. In conclusion, modifications of the apparatus and procedure had significant effects on approach–avoidance behavior in general whereas the strain rankings remained unaffected.

1. Introduction

Anxiety disorders are highly prevalent in human population [1]. Therefore, more efforts are needed for understanding the mechanisms of the disorders and for developing new treatments. Animal models are widely used in basic research and during the last two decades the mouse has emerged as a model of choice in genetic research [2,3].

Tests and/or models used for assessment of anxiety-like behavior in rodents can be divided into unconditioned (ethologically-based) tests and conditioned models [4,5]. The most popular unconditioned tests include the open field, elevated plus maze and light–dark box. These methods are based on measuring approach–avoidance behavior. However, the material and methods used for performing these tests vary enormously between the laboratories and therefore, the interpretation and comparison of the results is not always easy and straightforward [6–8]. Moreover, even rigorous standardization is not sufficient for avoiding inter-laboratory differences [9] and modifications of equipment are needed in order to achieve similar results [10,11]. On the other hand, inbred strains of mice with proven phenotypes provide a good resource for validation of the tests and in fact, it has been shown that differences between these strains can be rather stable over time [12].

It has been stated that ethological parameters should always be measured in ethologically-based tests [4,13]. With increasing number of mutant mice there is a clear need for automation of the tests and

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high-throughput methods for behavioral phenotyping [14]. However, often such approaches do not favor recording of ethological parameters. On the other hand, the automated monitoring systems allow precise spatiotemporal analysis of animal's location and movement. Despite these options, often only a limited set of data has been analyzed or presented in the research reports. Therefore, we believe that the read-out of the conventional methods for assessment of anxiety-like behavior in rodents can be significantly refined.

Light–dark test is one of the few paradigms originally developed for use with the mice [15]. The test is based on the innate aversion of rodents to the brightly illuminated and open areas and on the spontaneous novelty-induced exploratory behavior. It has been one of the most popular methods for behavioral phenotyping of the mutant mice and for screening of the potential anxiolytic compounds. The advantages of the test are being quick and easy to use without requiring prior training of the mice.

In the present study we sought to investigate the effect of start compartment by placing the mice either in dark or light part of the arena in the beginning of the experiment. In addition, two methods (differing in animal detection method and size of the arena) previously used in our laboratory for testing the light–dark exploration, were applied and compared [16–18]. Testing of anxiety-like behavior was completed by applying the open field with black or white floor. Experiments were carried out in mouse strains known to exhibit different levels of anxiety-like behavior.

2. Material and methods

2.1. Animals

Female mice were used in this study as follows: 32 C57BL/6NHsd (B6) and 30 DBA/2OlaHsd (D2) were purchased from a commercial breeder (Harlan, The Netherlands) and arrived in the laboratory at the age of 8 weeks; additional groups of mice were obtained from the local animal facility: 129S2/SvHsd (129, n = 35), C57BL/6NHsd (B6Hel, n = 20) and Hsd:ICR (ICR, n = 24). Starting from the age of 8 weeks all animals were in the same animal facility and received similar handling. All animals were living in groups of 3–6 with food and water available ad libitum under controlled temperature (21 ± 1) and humidity (50–60%). The bedding (aspen chips 5 × 5 × 1 mm, Tapvei Oy, Finland) was changed weekly. Nesting material (aspen wool, PM90L/R, 3 mm × 20 cm, Tapvei Oy, Finland) and wooden tube were provided as environmental enrichment. The lights were on between 6:00 and 18:00, and the experiments were carried out between 9:00 and 15:00. The mice were randomly allocated to different test conditions as shown in Table 1. Behavioral testing started at the age of 10 weeks. The mice were transferred to testing room at least 30 min before the beginning of the experiment. All animal experiments were carried out in accordance with the Guidelines laid down with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the County Administrative Board of Southern Finland (license number ESAVI-2010-09011/Ym-23).

Table 1
The number of animals used in different experimental settings.

	Light–dark: infrared		Light–dark: video	
Strain	Light	Dark	Light	Dark
129	12	11	6	6
B6	8	8	8	8
D2	7	8	8	7
B6Hel	10	10		
ICR	11	13		

	Open field	
	Black	White
129	13	13
B6	15	14
D2	15	15
B6Hel	8	7
ICR	9	10

2.2. Light–dark box with infrared sensors (IR)

The test was carried out in the open field arena with white floor (30 × 30 × 20 cm, Med Associates, St. Albans, VT) equipped with infrared light sensors (at 1.5 cm intervals) detecting horizontal and vertical activity (1.5 and 6 cm above the floor level, respectively). The dark insert (with black walls and lid, non-transparent for visible light) was used to divide the arena into two parts of equal size (15 × 15 cm). An opening (width 5.5 cm and height 7 cm) in the wall of the insert allowed animal's free movement from one compartment to another. The light side was illuminated by two 40 W light bulbs 50 cm above the floor (illumination in the center of the light compartment ~1000 lx). Animal was released in the center of the light or dark compartment (facing away from the opening) and allowed to explore the arena for 10 min. Distance traveled, number of rearings, and time spent in different compartments were recorded. For analysis, the following additional zones were defined in both compartments (Fig. 1a): door zone (8 × 4.5 cm) and corners (4.5 × 4.5 cm).

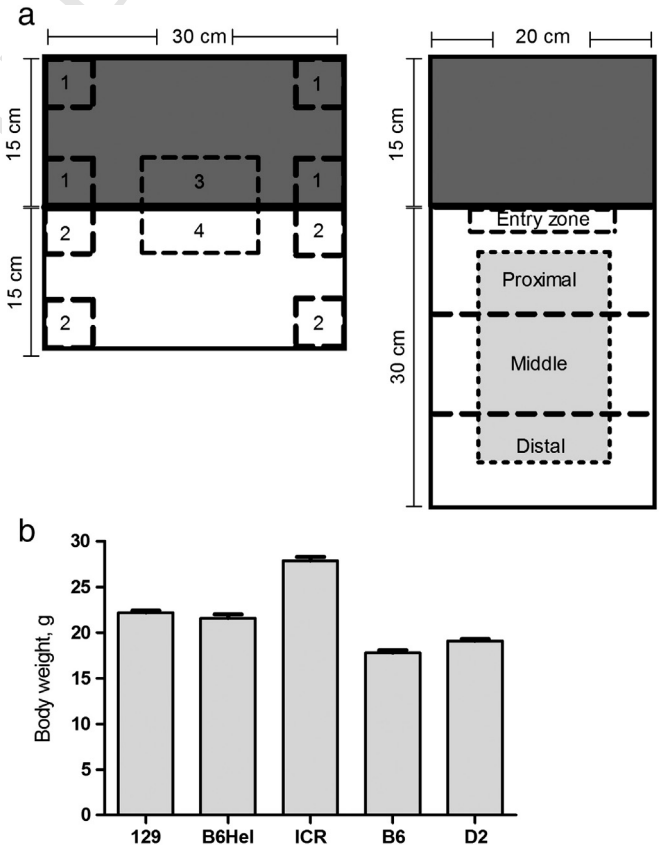

Fig. 1. a) Zones used for spatial analysis in the light–dark box (area of dark compartment is shaded). Square box (30 × 30 cm) used with infrared sensors: Zone 1 — corners in the dark compartment; Zone 2 — corners in the light compartment; Zone 3 — door zone in the dark; Zone 4 — door zone in the light. Rectangular arena (45 × 20 cm) used for videotracking: entry zone for detecting animal appearance in or leaving from the light compartment; proximal, middle, distal zones — 10 cm wide zones from the opening between two compartments; light-gray area in the middle of light compartment — center zone. b) Average body weight of the mice (age 10 weeks) used in the experiments.