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Research report

Gene–environment interaction influences anxiety-like behavior in ethologically based mouse models

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ABSTRACT

Ethologically based animal models are widely used; however, results from different laboratories vary significantly which may partly be due to the lack of standardization. Here, we examined the effects of circadian rhythm, lighting condition and mouse strain (BALB/c and C57BL/6, known to differ in measures of avoidance and risk assessment behavior) on two well established behavioral tests in mice: the Elevated Plus Maze (EPM) and the Open Field (OF). Parameters from both paradigms are commonly used as indices of anxiety-like behavior. BALB/c mice and C57BL/6 mice were independently tested in the morning and at night, in regular laboratory lighting and in the dark. We developed a novel method based on infrared lighting from below, coupled to respective video-tracking equipment, which facilitates standard testing of behavior interference-free in complete darkness. The two mouse strains differed in anxiety-related variables for the EPM in the dark, and for the OF in regular laboratory lighting. Moreover, BALB/c displayed greater anxiety-like behavior than C57BL/6 in the OF but less anxiety-like behavior than C57BL/6 in the EPM. Lighting condition has a major influence on both behavioral tests and this to a considerably larger extent than circadian rhythm. In addition, the lighting condition interacts strongly with the genetic background, producing discriminative differences in the anxiety-related variables depending on mouse strain and lighting condition. These results challenge the comparability of not sufficiently standardized tests of anxiety-like behavior and emphasize the need for controlling environmental variables in behavioral phenotyping.

1. Introduction

Animal models of behavioral traits or phenotypes associated with neuropsychiatric disorders are essential for the understanding of the neurobiological basis of these phenomena, as they allow experimental manipulation and multidisciplinary research strategies to identify their neural correlates [30]. Elevated Plus Maze (EPM) and Open Field (OF) are two useful ethologically based paradigms of anxiety-like behavior, which require minimum apparatus and are easy to conduct. Both tests are widely used for assessing anxiety-like behavior by measuring exploratory behavior as an index thereof [27], based on the hypothesis that behavior in a novel situation results from the two competitive forces exploration and avoidance [28]. Anxiety-like behavior in the EPM is reflected by the animals' preference for an enclosed space during a 5 min interval [26]. Unlike the EPM, the OF was originally not intended as a test of anxiety-like behavior, but rather as a test of locomotion and emotionality [39]. All the same, the animal's reluctance to leave the safe retaining wall and enter the central area is frequently scored as an anxiety index (see for example [21,22]).

Although both tests seem to yield similar data, there are also contradictory results. For example, the two mouse strains BALB/c and C57BL/6 have been frequently compared. One recent study [23] concluded that C57BL/6 are more active in the OF and show less anxiety-related behavior in the EPM. This is in accordance with many other studies, as BALB/c mice are described as an animal model for high trait anxiety [6] exhibiting a five-fold decrease in benzodiazepine receptor density in the amygdala [18]. Other studies report no differences in baseline EPM performance [14] and that both strains exhibit similar, intermediate levels of anxiety-like behavior [35]. Ambiguously, there are studies demonstrating *attenuated* anxiety-like behavior in BALB/c in the EPM [17,36] in combination with *greater* anxiety-like behavior in the OF [4]. Although some of the studies mentioned above may refer to substrains different from the ones used in this experiment, it is apparent that the two genotypes more generally differ in mea-

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sures of anxiety, which makes them a likely target when comparing different genetic backgrounds in behavioral tests.

Apart from the use of different substrains, one possible reason for contradictory results is that testing procedures vary considerably across laboratories [19,34] and only few studies specify all the relevant experimental details. Procedural variables (according to ref. [8]) impacting on behavioral tests are housing condition [33], pre-test handling [7], apparatus construction [3], circadian rhythm [21] and illumination level in either testing [20] or housing [11]. Strain and gender can also bring about different effects [15,33]. Even though, or because these influences have increasingly been taken into account, the standard procedures vary among laboratories, especially concerning the appliance of light during the test. In the OF, commonly used lighting conditions range from rather dim lighting (about 50 lx, see for example [29,36]) to very bright lighting (540 lx, see [32]). In the EPM things are more or less the same, with lighting conditions ranging from dim red light (<50 lx, see [32]) to regular laboratory lighting [24].

An important reason for the varying materials and colors from which apparatuses are made is that sufficient contrast between animal and background is essential for the proper functioning of video-tracking systems [38]. Thus, animals of different color need to either be measured on different apparatuses or behavior has to be recorded manually. Either way, a confounding variable is introduced. Another problem of conventional video-tracking systems and their need for sufficient contrast is that testing in complete darkness, which is most rodents' naturally active phase, has not been possible with this method.

To resolve these problems we constructed an EPM and an OF from PERSPEX XT, a black opaque material which is semi-permeable to infrared light, and illuminated both apparatuses with bright infrared light from below (visible for neither man nor mouse, but easily detectable by a CCD camera). Thus, animals create an infrared shadow and can easily be tracked, regardless of fur color and background lighting (Fig. 1).

Making use of this new possibility, we compared the two mouse strains BALB/c (white) and C57BL/6 (black) on the EPM and the OF. We also varied the lighting condition, measuring at regular laboratory lighting and in the dark, and tested in the morning and at night.

2. Methods and materials

2.1. Animals

BALB/cAnNCrl (male, $n = 36$) and C57BL/6NCrl (male, $n = 36$) were obtained from Charles River Laboratories (Sulzfeld, Germany) and housed in groups of four per cage (Polysulfone type 3 standard cage, wood-chip bedding) under controlled temperature (21.6 °C ± 0.1 °C) and humidity (51.5% ± 0.5%) conditions, under a 12/12 h light–dark cycle (lights on at 7 AM and lights off at 7 PM). Animals had unrestricted access to food and water. They were allowed to habituate to the testing facility for 2 weeks before being subjected to the EPM test (at the age of 8–10 weeks), remained on the premises till the conclusion of the OF test 9 weeks later (at the age of 17–19 weeks) and were regularly handled by the experimenter throughout this time. Animals from the different EPM conditions were randomly assigned to conditions in the OF. All animal protocols have been reviewed and approved by the review board of the District Government of Lower Franconia and conducted according to the Directive of the European Communities Council of November 24th, 1986 (86/609/EEC).

2.2. Behavioral tests

2.2.1. Elevated Plus Maze

A plus-shaped maze made of black opaque PERSPEX XT (semi-permeable to infrared light, TSE Systems, Inc., Bad Homburg, Germany) was used. The device comprised two opposing open arms (30 cm × 5 cm, with 0.5 cm wide boundaries elevated 0.2 cm) and two opposing closed arms (30 cm × 5 cm) that had 15.5 cm high, opaque walls. The four arms were connected by a central platform (5 cm × 5 cm). The maze was elevated to a height of 60 cm above floor level and illuminated by infrared LEDs from below.

In the dark condition, the testing chamber was completely devoid of visible light (0 lx). In the light condition, the open arms were illuminated with an intensity of 113 lx, the central area with 77 lx and the closed arms with 24 lx. Mice were initially placed in the center area facing one of the open arms and then were allowed to investigate the maze for 5 min. Their behavior was recorded using an infrared sensitive CCD camera and video-tracking software (VideoMot2, TSE Systems, Bad Homburg, Germany). Entry into an arm was defined as the moment when the mouse placed all four of its paws onto the arm. None of the mice fell from the EPM during testing. As no mice fell off the apparatus during the experiment, there was no need for testing further animals.

2.2.2. Open Field

The OF consisted of a quadratic black opaque PERSPEX XT box (50 cm × 50 cm × 40 cm, semi-permeable to infrared light, TSE Systems, Inc., Bad Homburg, Germany). The apparatus was illuminated by infrared LEDs from below. Activity monitoring was conducted using an infrared sensitive CCD camera and the computer-based video-tracking software VideoMot 2 (TSE Systems, Bad Homburg, Germany). In the dark condition, the testing chamber was completely devoid of visible light (0 lx). In the light condition, illumination at floor level was 125 lx. The area of the OF was divided into a 36 cm × 36 cm central zone (such that any thigmotaxis with the surrounding walls could be ruled out) and the surrounding periphery. An entry into either central zone or periphery was defined as the moment when the mouse placed all four of its paws into the respective zone. Mice were individually placed against a predetermined retaining wall and behavior was registered for an interval of 5 min.

2.3. Procedure and statistical analysis

A 2 × 2 × 2 between subjects factorial design was used in both behavioral tests ($n = 9$ per group). The factors were mouse strain (M, BALB/c or C57BL/6), time of day (T, 2 h into the light phase or 2 h into the dark phase/AM or PM, respectively) and lighting condition (L, light or dark/120 lx or 0 lx, respectively). 9 BALB/c and 9 C57BL/6 animals were randomly assigned to one combination of T and L and tested

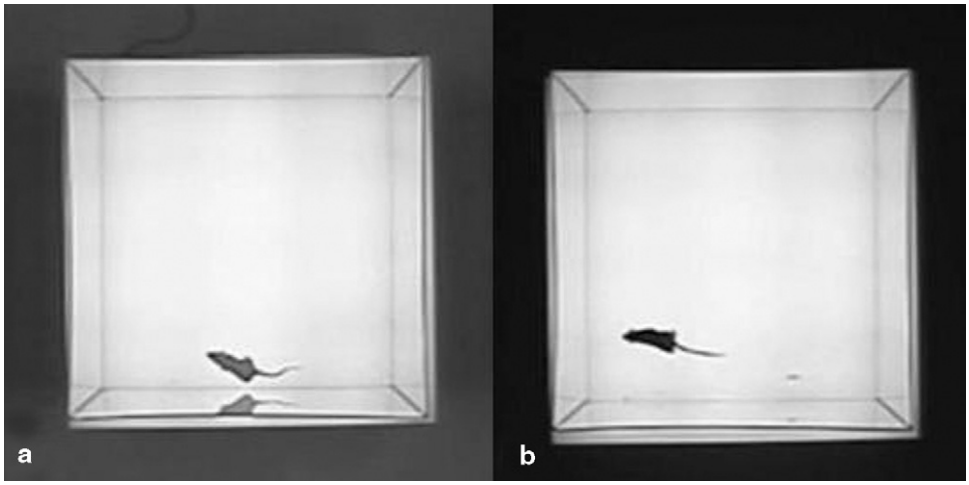

Fig. 1. Screenshots of Open Field testing; (a) BALB/c mouse at 120 lx and (b) C57BL/6 mouse at 0 lx.