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Validation of the dimensionality emergence assay for the measurement of innate anxiety in laboratory mice

Apar Jain [a], Anna Dvorkin [b], Ehud Fonio [b], Ilan Golani [b], Cornelius T. Gross [a,*]

[a] *Mouse Biology Unit, European Molecular Biology Laboratory (EMBL), 00015 Monterotondo, Italy*
[b] *Department of Zoology, Tel-Aviv University, Tel Aviv 69978, Israel*

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Abstract

The open field test is a common tool to measure innate anxiety in rodents. In the usual configuration of this test the animal is forced to explore the open arena and its behavior includes both anxiety and non-anxiety responses. However, the open arena is generally small and allows only limited expression of exploratory behavior. The recently developed dimensionality emergence assay in which an animal is housed in a home cage with free access to a large circular arena elicits graded exploration and promises to serve as a more ethological test of anxiety. Here we examined the predictive validity of this assay for anxiety-related measures in mice. First, we compared their behavior in the presence or absence of access to the home cage and found that mice with access to the home cage exhibited a gradual build-up in exploration of the arena while those without did not. Then we identified behavioral measures that responded to treatment with the anxiolytic drug diazepam. Diazepam altered several classical measures of innate anxiety, such as distance traveled and thigmotaxis, but also led to a dose-dependent acceleration of the build-up as reflected in a significantly reduced latency to attain several exploratory landmarks. Finally, we tested the utility of the dimensionality emergence assay in assessing alterations in innate anxiety reported in mice carrying a knockout allele for the serotonin 1A receptor (Htr1a). Our findings support the validity of the dimensionality emergence assay as a method to extract an expanded repertoire of behavioral measures for the assessment of anxiety in laboratory mice.

1. Introduction

Anxiety is a mental state that is commonly elicited by the anticipation of a threatening experience. Anxiety is associated with a variety of defensive behaviors that can be readily measured in both humans and other animals. One of the most common tests used to measure anxiety-related behavior in rodents is the open field test where the animal is forcibly exposed to an unfamiliar open arena (Crawley, 1985; Stone, 1932). Typical anxiety-related measures in this test include time spent in the center, latency to enter the center, and

* Corresponding author.
E-mail address: gross@embl.it (C.T. Gross).

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distance travelled in the center relative to total distance. Treatment of rodents with benzodiazepines, GABAergic allosteric agonists with anxiolytic activity in humans, leads to a dose-dependent increase in total distance travelled, time spent in the center, and relative distance in center (Marriott and Smith, 1972) confirming the predictive validity of this test.

However, because the animal is forced to explore the open field in this test its behavior is likely to reflect a complex combination of anxiety and non-anxiety-related factors and discriminating between these is not straightforward. Several modifications to the test have been developed to address this issue. The light-dark test (Crawley and Goodwin, 1980) offers the animal access to a darkened enclosure within the open arena, while the emergence test (Paré et al., 2001; Prickaerts et al., 1996) gives the animal access to a familiar home cage. In the free exploration test (Cigrang et al., 1986; Griebel et al., 1993) the animal is habituated in one part of the test apparatus before being given access to a novel portion. Unlike in the classical open field test, in these tests the animal is offered a clear choice between a more or less safe area of the apparatus, and anxiety-related measures are based on relative time and distance in the exposed or novel compartment. These measures have increased ethological validity compared to those derived from the classical open field, and at least for the light-dark and free exploration tests, these measures have been pharmacologically validated (Chaouloff et al., 1997; Crawley, 1981; Griebel et al., 1993).

Another issue is that both the classical and modified open field tests generally use small open enclosures (typically <50 cm across) and thus provide minimal space for the expression of exploration. Furthermore, exploration of the exposed or novel arena is generally captured as total distance travelled and/or time spent in a particular part of the arena, measures that may be insensitive to more subtle variations in exploratory strategy. In an attempt to address these limitations, Fonio et al. (2009) developed the dimensionality emergence assay. In this assay, the animal is habituated in a home cage from which it is then given access to a large (here 180 cm diameter) circular arena by the opening of a small shutter. Exploratory behavior in the open arena shows a gradual build-up over a period of 20–40 min, with the animal first exploring a small area close to the home cage opening (called "garden") before venturing along the border of the circular arena, and then finally moving away from the walls to enter the center.

The dimensionality emergence assay has several advantages for measuring rodent exploratory behavior. First, the large diameter of the open arena uncovers an expanded exploratory repertoire that follows a stereotyped progression of landmarks (including *cross and retreat*, *exit garden*, *enter center*, *full circle*, *home-related shuttle*, and *cage skip*) that can be readily quantified and have high ethological validity. Second, the large size and circular geometry of the open arena is ideal for applying unbiased videotracking analysis of locomotion using the Software for the Exploration of Exploration (SEE) package (Hen et al., 2004; Drai et al., 2000; Golani et al., 1993). SEE extracts movement episodes and moment-by-moment speed and acceleration data from whole body trajectories in an unbiased, animal-by-animal approach. Using SEE, Lipkind et al. (2004) were able to extract a set of sixteen putative anxiety-related variables from videotracking data of mice in a large (200 cm) forced open field test. Thus, SEE appears to be a promising method to extract additional anxiety-related measures from rodent exploration tests, at least for tests using large circular open arenas.

Here, we present data supporting the validation of the dimensionality emergence assay as a test of innate anxiety in mice. First, we made a direct comparison of exploratory behavior in the forced versus the free version of the test and confirmed the importance of home cage access to the gradual exploratory build-up. Second, we identified exploratory measures that were significantly and dose-dependently modified by treatment with the benzodiazepine diazepam. Third, we examined the impact of lighting on exploratory behavior in the test. Finally, we sought to assess the utility of the dimensionality emergence assay in assessing alterations in innate anxiety reported in mice carrying a knockout allele for the serotonin 1A receptor (Htr1a). Mice lacking Htr1a displayed decreased total locomotion and relative locomotion and time spent in the center in the open field test (Parks et al., 1998; Ramboz et al., 1998; Heisler et al., 1998). These findings demonstrate that the dimensionality emergence assay is a robust and ethological assay of innate anxiety in rodents.

2. Experimental procedures

2.1. Mouse husbandry

Male C57BL/6 J mice (10–12 weeks of age) were purchased from Charles River Laboratories (Calco, Italy) and housed 5 per cage in individually ventilated cages (Thoren Caging Systems, Hazleton, PA) with food and water provided *ad libidum* under controlled temperature (21 ± 0.5 °C), humidity (55–75%), and lighting (lights on: 07:00–19:00) conditions. Htr1a knockout mice (Gross et al., 2002) were backcrossed onto the C57BL/6 J background for 10 generations. Weaning and tail biopsy for genotyping was performed at postnatal day 21 (P21) and after weaning mice were group housed (three to five per cage). Male mice were used for all experiments. Mice were singly housed 8–10 days before the experiment to reduce variation in behavior associated with dominance hierarchies known to form among group-housed male mice.

2.2. Drug treatment

Animals were injected with vehicle (1–2% Tween-80 in saline, i.p.; Sigma-Aldrich, Milan, Italy) or diazepam (0.5 or 1.5 mg/kg, i.p.; Sigma-Aldrich) 15 min before being transferred to the experimental apparatus.

2.3. Dimensionality emergence assay

In our version of this assay we used a large wooden arena (180 cm diameter, 40 cm high, painted white) connected to a wooden shelter (28 cm long, 21 cm wide, 16 cm high, painted black) via a small opening (4.5 cm wide, 4 cm high, inverted-U shape) that could be opened or closed with a removable shutter. The shelter floor was covered with fresh bedding material and the cage top holding food and water as well as some soiled bedding (70% of total) were transferred to the shelter from the home cage together with the animal. The arena was surrounded by a black curtain extending to the ceiling that allowed different lighting in the shelter (<0.1 lux red light) and arena (Low: 2–7 lux red light, High: 25–28 lux white light, periphery to center, respectively). All tests were conducted under Low light conditions unless otherwise specified. Animals were tested during the light period between 10:00 and 18:00. Fifteen minutes after transferring the animal to the shelter the shutter was removed