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SCIENCE DIRECT
Behavioural Brain Research 169 (2006) 282–288
BEHAVIOURAL BRAIN RESEARCH
www.elsevier.com/locate/bbr

Research report

Comparisons between anxiety tests for selection of anxious and non anxious mice

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Received 2 December 2005; received in revised form 10 January 2006; accepted 17 January 2006
Available online 21 February 2006

Abstract

Male Swiss albinos mice were submitted to two behavioural tests intended to determine their anxiety level: the elevated plus-maze test as well as the black and white compartments test. In addition they were submitted to the hole-board test. It was observed: (i) that the correlation between scores in the two first tests was weak, suggesting that they explore different components of anxiety; (ii) that the score on the latter test was better correlated with the response in the elevated plus-maze test than in the black and white compartments test. From these data three groups of animals were constituted, considered, respectively, as anxious, non anxious and intermediates. It was observed that both horizontal and vertical locomotion in an unfamiliar environment differed between groups, with higher activity in non anxious than in anxious. In the hole-board test, only animals classified as anxious displayed an obvious response to the anxiolytic drug diazepam (0.5 mg/kg). Finally in the forced-swimming test, the three groups demonstrated a similar immobility time, suggesting that the operated segregation was not depending on a helpless component. It is proposed that the selection of mice from a combination of either elevated plus-maze and black and white compartments tests or a combination of hole-board test and black and white compartments test, allows to distinguish high or low anxiety animals among a population of mice.

Keywords: Anxiety; Mice; Elevated plus-maze test; Black and white compartments test; Hole-board test; Locomotor activity; Diazepam

1. Introduction

The experimental testing of psychotropic agents, aimed at relieving psychologic or psychiatric troubles, is generally carried out in non selected rodents. It appears more and more clearly that several psychiatric or psychological troubles can be modeled in rodents [1,9,15], thus offering the opportunity to test the effects of psychotropic agents on behavioural perturbations. This may be illustrated by some studies carried out in the field of depression and antidepressant drugs. Thus, on the tail suspension test [19,23], the reference antidepressant drug imipramine revealed no effect in non selected CD1 albino mice. However, when operating in mice previously selected as helpless in this test (i.e. displaying a longer immobility period than the majority of mice), the antiresignation effect of imipramine clearly appeared [24,25].

In the field of anxiety and phobias, it seems a priori important to test the anxiolytic potential of the tested molecule in mice presenting a high level of anxiety and thereby selected to this purpose. The aims of the present study were:

(i) to compare the same mice on two classical tests aimed at evaluating anxiety in rodents and at screening drugs with potential anxiolytic effects, the so-called elevated plus-maze test [10,11] and black and white compartments test [2,18];
(ii) to check whether the hole-board test, originally developed to assess the curiosity level in rodents [3,4] might have, as claimed by several authors, the ability to evidence anxiety and to detect anxiolytic properties of drugs [6,16,21];

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0166-4328/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2006.01.018

(iii) to determine the most appropriate test(s) for distinguishing anxious and non anxious mice among a whole population;
(iv) and finally to consider whether mice selected as anxious differ from non anxious as regards their horizontal and vertical locomotor activity in an unfamiliar environment, and as regards their immobility time in the forced-swimming test, commonly used to evaluate helplessness of mice and to screen antidepressant drugs.

2. Materials and methods

2.1. Animals

Male Swiss albinos CD1 mice (IFFA-CREDO/Charles River, Saint-Germain sur L'Arbresle, France), weighing 20–22 g, were housed 20 in Makrolon cages (*L*: 40 cm, *W*: 25 cm, *H*: 18 cm), with free access to water and food (U.A.R., Villemoisson sur Orge, France). The animals were kept in a ventilated room, at a temperature of 22 ± 1 °C, under a 12-h light/12-h dark cycle (light on between 7:00 a.m. and 7:00 p.m.).

All the experiments were carried out between 9:00 a.m. and 6:00 p.m. in testing rooms adjacent to the animal rooms. Animal testing was performed according to the European Communities Council Directive of 24 November 1986 (86:609:EEC) and conducted by authorized investigators.

2.2. Elevated plus-maze test

The elevated plus-maze test was performed according to Pellow et al. [11]. The apparatus for mice consisted of a wooden Greek cross, painted black and placed 60 cm above the floor in a dimly illuminated room. The four arms (dimension of each arm *L*: 18 cm, *W*: 6 cm) were interconnected by a central platform (7 cm × 7 cm). Two opposite arms were surrounded by walls (*H*: 6 cm; closed arms) while the two others were devoid of enclosing walls (open arms). The light intensity at the level of the elevated plus-maze was 30 lx. Fifteen minutes before the experiments, the animals were isolated in small individual cages (*L*: 25 cm, *W*: 9 cm, *H*: 8 cm) at an ambient temperature of 22 ± 1 °C. Each mouse was placed at the center of the maze, head facing a closed arm. The time spent in open and closed arms and in the central area during a 5 min period was recorded using an automated image analysis system (Videotrack MV 45 system, Viewpoint, Lyon, France). Mice were considered as anxious (Am) when the time spent in the open arms was below the mean − 2S.E.M. of the whole population. They were considered as non anxious (NAm) when this time was above the mean + 2S.E.M. of the whole population. In all other cases mice were considered as intermediate (Im).

2.3. Black and white compartments test

The apparatus consisted of a Plexiglas enclosure divided in two compartments, each measuring *L*: 32 cm, *W*: 22 cm, *H*: 18 cm, and placed on an infrared floor. One compartment was dark (painted black and covered with a black Plexiglas lid) and the other compartment (not covered) was white and illuminated by a 100 W light bulb (200 lx intensity at the level of the white compartment), set 50 cm above the floor. The compartments communicated through an opening (*W*: 5 cm, *H*: 5 cm) located at the base, in the middle of the partition wall. Fifteen minutes before the experiments, the animals were isolated in small individual cages (*L*: 25 cm, *W*: 9 cm, *H*: 8 cm) at an ambient temperature of 22 ± 1 °C. Each mouse was placed in the black compartment (head facing a corner) and the compartment was covered. The total time spent, respectively, in the black and white compartments was recorded during a 5 min period by using an automated image analysis system (Videotrack MV 45 system, Viewpoint, Lyon, France). Mice were considered as anxious (Abw) when the time spent in the white compartment was below the mean − 2S.E.M. of the whole population. They were considered as non anxious (NAbw) when this time was above the mean + 2S.E.M. of the whole population. In all other cases mice were considered as intermediate (Ibw).

2.4. Exploratory behaviour

Exploratory behaviour was assessed using the hole-board test, according to Boissier and Simon [3]. The apparatus consisted of a square plastic plate, 40 cm × 40 cm, 1 cm thick, with 16 holes (diameter 2 cm), regularly spaced on the surface, at 3.5 cm from the edges. The apparatus was elevated to the height of 100 cm, in a dimly illuminated room. Mice were placed in the center of the plate and the number of head dips was immediately counted during two or three consecutive periods of 5 min each. Mice were considered as anxious (Ah) when the number of head dips was below the mean − 2S.E.M. of the whole population. They were considered as non anxious (NAh) when this number was above the mean + 2S.E.M. of the whole population. In all other cases mice were considered as intermediate (Ih).

2.5. Selection of anxious and non anxious mice

Mice, previously isolated in individual cages (*L*: 27, *W*: 13, *H*: 13 cm) 15 min before testing, were tested in an elevated plus-maze, followed (2 days later) by black and white compartments test and (4 days later) hole-board test. Mice which satisfied the selection criteria either as anxious, non anxious or intermediate in all the three tests were retained for the study and constituted three groups: anxious mice (A), non anxious mice (NA) and intermediate mice (I).

2.6. Measurement of locomotor activity

Locomotor activity was assessed using a Digiscan actimeter (Omnitech Electronics, Colombus, OH, USA), placed in a dimly lit, sound-attenuated room. The animals were placed individually in 20 cm × 20 cm × 30 cm compartments. The recording apparatus was connected to a computer to process the data. Horizontal locomotor activity (the number of infrared beams crossed) and vertical locomotor activity (the number of infrared beams broken up to a height of 9 cm) were measured during four consecutive 15 min periods.

2.7. Forced-swimming test

The forced-swimming test was essentially similar to that described by Porsolt et al. [14], but used an apparatus with a larger Plexiglas cylinder (14 cm diameter instead of 10 cm) similar to that employed by Semba and Takahashi [17], since Sunal et al. [20] have established that a cylinder with a higher diameter decreases the number of false positive responses. The apparatus consisted of two Plexiglas cylinders (20 cm height, 14 cm internal diameter) placed side by side in a Makrolon cage (38 cm × 24 cm × 18 cm), filled with water, maintained at 22 ± 1 °C, to a height of 12 cm instead of 6 cm suggested by Porsolt et al. [14], since, according to Petit-Demouliere et al. [12], the depth of water is an important parameter to be considered as mice should not sense a limit under the level of water. The behaviour of the mice would indeed be altered if their tails touch the bottom of the cylinder. Fifteen minutes before the experiments, the animals were isolated in small individual cages (*L*: 25 cm, *W*: 9 cm, *H*: 8 cm) at an ambient temperature of 22 ± 1 °C. Two mice were tested simultaneously for a 6 min period inside vertical Plexiglas cylinders; an opaque screen placed between the two cylinders prevented mice from seeing each other. The total duration of immobility was measured during three consecutive periods of 2 min each, with an automated image analysis system (Videotrack MV 45 system).

2.8. Drug and solution

Diazepam (5 mg/ml, Valium®, Roche for injections) was diluted 1:100 in 0.9% NaCl just before intraperitoneal (i.p.) administration (0.5 mg/kg).

2.9. Statistical analysis

Results are expressed as means ± S.E.M. Data were tested for conformity to normality and homogeneity of variance prior to parametric analysis. The links between plus-maze test, black and white compartments test and hole-board test were determined by Pearson's correlation test. Differences between groups were assessed by an one-way analysis of variance (ANOVA). Diazepam effects