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Original article

Assessing anxiety in C57BL/6J mice: A pharmacological characterization of the open-field and light/dark tests

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ABSTRACT

Introduction: In order to assess anxiety in mammals various tests and species are currently available. These current assays measure changes in anxiety-like behaviors. The open-field and the light/dark are anxiety tests based on the spontaneous behavior of the animals, with C57BL/6J mice being a frequently used strain in behavioral studies. However, the suitability of this strain as a choice in anxiety studies has been questioned. In this study, we performed two pharmacological characterizations of this strain in both the open-field and the light/dark tests. **Methods**: We examined the changes in the anxiety-like behaviors of C57BL/6J mice exposed to chlordiazepoxide (CDP), an anxiolytic drug, at doses of 5 and 10 mg/kg, picrotoxine (PTX), an anxiogenic drug, at doses of 0.5 and 1 mg/kg, and methylphenidate (MPH), a psychomotor stimulant drug, at doses of 5 and 10 mg/kg, in a first experiment. In a second experiment, we tested CDP at 2.5 mg/kg, PTX at 2 mg/kg and MPH at 2.5 mg/kg. **Results**: Results showed an absence of anxiolytic-like effects of CDP in open-field and light/dark tests. Light/dark test was more sensitive to the anxiogenic effects of PTX than the open-field test. Finally, a clear anxiogenic effect of MPH was observed in the two tests. **Discussion**: Although C57BL/6J mice could not be a sensitive model to study anxiolytic effects in pharmacological or behavioral interventions, it might be a suitable model to test anxiogenic effects. Further studies are necessary to corroborate these results.

1. Introduction

Anxiety disorders are among the most common psychiatric disorders in high-income countries. Recent epidemiological studies have shown a high 12-month prevalence of anxiety disorders in the USA and Europe (Kessler & Wang, 2008; Wittchen et al., 2011). At present, it is thought that anxiety is a complex and multidimensional phenomenon, with many studies being conducted to separate their behavioral components (Ennaceur, 2012; Henderson, Turri, DeFries, & Flint, 2004; Ramos, 2008; Ramos, Pereira, Martins, Wehrmeister, & Izidio, 2008). Animal models were developed to facilitate the discovery of genetic and neurobiological substrates of anxiety, and to assess anxiolytic drugs. A number of different tests to evaluate anxiety-like behavior are currently available. In this study, we performed two pharmacological validations of two widely used tests for anxiety-like behavior; the open-field test and the light/dark test. The open-field test is used to assess anxiety, as well as exploration and locomotor activity (Archer, 1973; Walsh & Cummins, 1976). This is an unconditioned test based on the spontaneous behavior of the animals. The test allows the animals to exhibit a wide range of behaviors. The distance traveled over the maze is typically interpreted as an activity-like measure (Vanmeer & Raber, 2005). Moreover, the tendency of mice to avoid the central area is an indicator of anxiety-like levels, under the assumption that the central area is more threatening for rodents than the peripheral area (Angrini & Leslie, 1997; Montgomery & Monkman, 1955). On the other hand, the light/dark test is based on the innate aversion of rodents to brightly illuminated areas and on their spontaneous exploratory behaviors. In the original validation study, an increase in the number of transitions between the two compartments of the apparatus is suggested as an index of anxiolytic activity (Crawley & Goodwin, 1980). Moreover, animals with high anxiety-like levels tend to avoid the illuminated compartment and to spend more time in the dark compartment of the box (Crawley, 1996). Hascoet and Bourin (1998) suggested that the most consistent and useful measurement for assessing anxiolytic-like action, is the time spent in the light compartment, as this parameter provides the most consistent dose-effect results.

The choice of the strain used in anxiety studies is a crucial decision because a clear gene-environment interaction has been observed in ethologically based animal models, which alter the capability of the tests to detect anxiety-related changes (Post et al., 2011; Vanmeer & Raber, 2005). In relation to this, C57BL/6J mice are among the most commonly used strain in anxiety studies (Bouwknecht & Paylor, 2008;

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Hart et al., 2010). However, this strain has shown an unusual anxiety-related behavior (Crawley, 1996; Tang, Xiao, Parris, Fang, & Sanford, 2005). In fact, a low level of anxiety-like behavior in these mice, compared to other strains, has been shown (Crawley et al., 1997). In a recent investigation, we did not find any change in anxiety-related behavior in the zero maze test, after chlordiazepoxide administration (Heredia, Torrente, Colomina, & Domingo, 2013). Since this strain is commonly used in toxicological studies and as background of the genetic models of mice, in the present investigation we decided to use these animals in the characterization of the two mazes (Crawley et al., 1997). Three pharmacological compounds: chlordiazepoxide (CDP), picrotoxine (PTX) and methylphenidate (MPH) were used. CDP is a non-selective classical benzodiazepine used in the treatment of anxious states, which has been administered in pharmacological characterizations as an anxiolytic drug (Kulkarni, Singh, & Bishnoi, 2007; Lalonde & Strazielle, 2012; Mathiasen, Mirza, & Rodgers, 2008). PTX is a $GABA_A$ receptor antagonist, which blocks the chloride channel. It has been used as anxiogenic drug in pharmacological characterizations (Clenet, Hascoet, Fillion, Galons, & Bourin, 2005; Kulkarni et al., 2007). Finally, MPH is a psychomotor stimulant used for the treatment of the attention deficit hyperactivity disorder (ADHD) (Niculescu, Ehrlich, & Unterwald, 2005). Although MPH blocks dopamine and norepinephrine reuptake transporters, it exerts a minimal effect on serotonin transporters (Brookshire & Jones, 2012; Kuczenski & Segal, 1997). Niculescu et al. (2005) showed that MPH could increase the activity-like levels in mice, while a similar conclusion was also reached in another recent study (Fitzgerald et al., 2010). Therefore, we used MPH to control 'false positives' related to increases in the activity levels, which might be interpreted as changes in anxiety-like levels.

2. Methods

2.1. Animals

Ninety-nine male C57BL/6J mice (Charles River, CRIFFA, Barcelona, Spain) were used in this study. All mice weighed between 25 and 30 g, being three months old (90 ± 5 days) at the beginning of the experimental procedure. Animals were housed in standard plastic cages (4–5 animals/cage) in a climate-controlled facility at a temperature of 22 ± 2 °C, a relative humidity of 50 ± 10%, and a constant day–night cycle (light: 08:00–20:00 h), with free access to food (Panlab rodent chow, Barcelona, Spain) and municipal tap water. At the end of the experimental period, all mice were sacrificed by cervical dislocation. The use of animals and the experimental protocol were approved by the Animal Care and Use Committee of the Universitat Rovira i Virgili (Tarragona, Catalonia, Spain) following the 'Principles of Laboratory Animal Care' and were carried out in accordance with the European Union Directive 2010/63/EU for animal experiments.

2.2. Experimental groups

The drugs used in this study were CDP (Teofarma Iberica, Barcelona, Spain), PTX (Sigma-Aldrich Química, Madrid, Spain) and MPH (Laboratorios Rubió, Barcelona, Spain). Mice were randomly assigned to ten experimental groups. In experiment I (pharmacological validation with the initial doses of the anxiolytic (CDP) and anxiogenic (PTX) drugs, and a psychomotor stimulant (MPH)), the groups were the following: control (0.9% saline, n = 9), CDP groups (5 mg/kg, n = 9, and 10 mg/kg, n = 9), PTX groups (0.5 mg/kg, n = 9, and 1 mg/kg, n = 9), and MPH groups (5 mg/kg, n = 9, and 10 mg/kg, n = 9). In the experiment II (complementary pharmacological validation with refined doses of the anxiolytic (CDP) and anxiogenic (PTX) drugs, and a psychomotor stimulant (MPH)), animals were distributed into four groups: control (0.9% saline, n = 9), CDP group (2.5 mg/kg, n = 9), PTX group (2 mg/kg, n = 9) and MPH group (2.5 mg/kg, n = 9). This protocol was based on previous studies carried out in our laboratory. Animals were not handled by the experimenters previously to the testing because they were grouped housed (4–5 mice for cage). Previous studies showed no effects due to handling when they are grouped housed (Heredia, Torrente, Domingo, & Colomina, 2012; Heredia et al., 2013; Kwak, Lee, & Kaang, 2009). The behavioral testing was performed at the same time of the day for the two tests; from 9:00 to 11:00 AM. All mice in each group were tested in the open-field at three months old (90 ± 5 days). The testing period for the open field test was 5 days, in which four animals were tested in four open fields at the same time for 30 min. Animals were tested in the light/dark box four weeks after the open-field test in order to minimize carryover effects (Paylor, Spencer, Yuva-Paylor, & Pieke-Dahl, 2006). Animals were four months old (118 ± 7 days) when they were assessed in light/dark test and the testing period for this test was two days. The groups were balanced and randomized across the days of experiments. Thirty minutes before each test, the mice were given either an intraperitoneal (i.p.) injection of the respective drug (according to the treatment group dissolved in 0.9% saline solution), or 0.9% saline solution only (control group) (Kulkarni et al., 2007; Shepherd, Grewal, Fletcher, Bill, & Dourish, 1994). The selection of the initial drug doses was based on the results of previous investigations with these drugs showing their effects on anxiety in rodents (Kulkarni et al., 2007; Mathiasen et al., 2008; Niculescu et al., 2005). The refined doses were based on our previous results in Experiment I, as well as recent results in our laboratory using the zero maze test (Heredia et al., 2013).

2.3. Open-field test

The open-field test consisted in a 60 cm × 60 cm wooden square surrounded by a 50 cm high wall. The area of the maze that was within 15 cm from the wall was considered as peripheral. The rest of the open-field was considered as the central area (Christakis, Ramirez, & Ramirez, 2012; Hess, Jinnah, Kozak, & Wilson, 1992; Takahashi, Kato, Makino, Shiroishi, & Koide, 2006). At the beginning of the test period, mice were placed in the center of the arena. They were allowed to move freely around the maze and to explore the environment for 30 min (Paylor et al., 2006). Between a mouse and the next one, the open-field was cleaned with clothes dampened with 50% ethanol. The video tracking software Ethovision 3.0© (Noldus Information Technologies, Wageningen, The Netherlands) was used to measure the following parameters: distance traveled over the maze and over the central area, time spent in the central area, total movement time (time in which the mouse actively explores the maze, as any period where there is any movement of the mouse) and number of rearings (vertical standing of mice on two hind legs). From these measured parameters, the distance ratio (distance traveled in central area/total distance traveled in the maze) and the rearings ratio (rearings made in central area/total number of rearings) were calculated. During the behavioral testing, indirect lighting was used and the lighting levels were maintained at ~100 lx in the testing room (Lalonde & Strazielle, 2012).

2.4. Light/dark test

The apparatus used for the light/dark test was based on that described by Crawley and Goodwin (1980). A closed top arena (45 × 27 × 27 cm) with methacrylate walls and opaque floor, comprised two compartments. One compartment was painted black (dark compartment) and another white (light compartment). The compartments were separated by a methacrylate partition with a centrally-positioned 7.5 × 7.5 cm opening at floor level. The light/white compartment was illuminated by a lamp in the ceiling (~350 lx at the floor of light/dark apparatus) (Bourin & Hascoet, 2003), while the black/dark compartment was not illuminated. Mice were individually placed in the center of the dark compartment and allowed 5 min to