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Research report

Evaluation of two genetic animal models in behavioral tests of anxiety and depression

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Abstract

Anxiety- and depression-related disorders often appear associated and may be affected by common genetic factors. The inbred rat strains Lewis (LEW) and spontaneously hypertensive rats (SHR) and the outbred rat lines Floripa H and L, which were selectively bred for high and low locomotion in the central area of the open field (OF) test, respectively, have been proposed as experimental tools to study anxiety. The main goal of the present study was to characterize the behavior of these animals in two models of anxiety, elevated plus-maze (EPM) and OF, in two models of depression, forced swim test (FST) and tail suspension test (TST) and in their home-cages. Emotionality-related differences between LEW and SHR rats and between Floripa H and L rats were found in the EPM, OF and FST. Those lines showing low anxiety-like profiles in the EPM and OF (SHR and Floripa H) also showed low immobility in the FST. The TST failed to unveil any line differences. Factor analysis involving all tests revealed three independent factors with one of them associating anxiety-related measures from the OF and EPM to immobility in the FST. When observed in their home-cages, LEW and SHR rats showed no differences in general activity, but when acutely treated with imipramine (15 mg/kg), only LEW rats were sensitive to its antidepressant effects. These results suggest the existence of a genetic link between two tests used in the screening of anxiolytic drugs and one test of antidepressant activity. Moreover, the LEW and SHR rat strains were shown to be an interesting model to study the comorbidity between anxiety- and depression-related disorders.

Keywords: Anxiety; Depression; Animal models; Rat strains; Imipramine; Home cage; Principal components analysis

1. Introduction

Anxiety- and depression-related disorders are the most prevalent psychiatric conditions and frequently appear associated in the population [29,47]. Some studies provide indirect evidence that these disorders may be influenced, at least in part, by common genes, i.e., genetic pleiotropy (for a review see [22]). For the past few decades, animal models have been widely used as tools to develop new therapeutic drugs as well as to study the neurobiological and genetic bases of each of those disorders. However, little attention has been given to the potential usefulness of these experimental models to study the relationship between anxiety and depression.

The elevated plus-maze (EPM) test, which is based on the conflict displayed by rodents between the drive to explore a new environment and the fear of open elevated areas [37] and the open field (OF) test, which consists of a novel large arena containing an aversive central area, are two widely used models of emotionality/anxiety [44]. Some classical anxiolytic drugs can increase the exploration of both aversive environments, the open arms of the EPM and the center of the OF [24,37,41]. Other stressful behavioral paradigms have been proposed as animal models of depression. One of the most widely used ones is the forced swim test (FST) [38], where the subject is exposed twice to a stressful and inescapable situation represented by a small tank filled with water. After a period of struggling, the animal shows a state of immobility which is reduced by antidepressant compounds [10]. The tail suspension test (TST) is another depression model proposed by Stéru et al. [49] and adapted by Chermat et al. [12], where the animal is suspended by the tail

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and the time of immobility is also used to screen antidepressant drugs [49,52].

Many studies have shown important interindividual variations in the responses of rodents to tests of anxiety and depression. This variability is partially caused by genotypic variations, since different strains react differently when submitted to these tests [36,39,44]. The comparison of genetically distinct populations in a series of behavioral tests helps us to identify genetic relationships between different types of measures, either through the simple comparison of group means or through more complex factorial analyses [42,53]. Further genetic investigation can then be used to corroborate and dissect the biological link between distinct variables [35].

Ramos et al. [42,43,45] have shown that the inbred rat strains Lewis (LEW) and spontaneously hypertensive rats (SHR), although not selected for emotionality, are a useful genetic model for the study of anxiety. The LEW/NIco and SHR/NIco rats, commercialized by IFFA CREDO in France, showed contrasting responses to different stressful stimuli, including the EPM, the OF and the black/white box tests, with LEW rats displaying more anxious-like behaviors that their SHR counterparts [42,43]. More recent studies [45] have shown that Brazilian substrains of LEW and SHR rats present the same anxiety-related differences. Regarding depression models, LEW and SHR rats have been compared in the FST, with some authors showing no strain differences [5,31,34] and some showing LEW rats to be more immobile than SHRs [9,30]. Such an inconsistency may result from the fact that different LEW and SHR substrains have been used in these different studies [21,28,50,51].

Another genetic model of anxiety was recently developed through the bidirectional selective breeding of rats for high and low locomotor activity in the center of the OF, which generated the rat lines Floripa H and L, respectively [46]. Initial studies have shown that these lines contrast not only for the selected trait but also for emotional behaviors from two other tests of anxiety, the EPM and black/white box [46]. These new rat lines have never been submitted to any model of depression.

The main goal of the present study was to investigate the profile of the four above-mentioned rat lines (LEW, SHR, Floripa H and L) in four behavioral tests thought to predict the effects of either anxiolytic (EPM and OF) or antidepressant (FST and TST) drugs. Line comparisons followed by a principal component analysis should help us to understand the genetic relationship between the different measures as well as to evaluate the usefulness of these lines to study the comorbidity between anxiety and depression. Because LEW and SHR rats showed the most consistent differences in both anxiety- and depression-related behaviors, they were also evaluated in their home-cage and in the FST following the administration of imipramine, an antidepressant drug.

2. Materials and methods

2.1. Animals

A total of 165 individuals from four rat lines (LEW, SHR, Floripa H and L) and both sexes were used in the first set of experiments, which involved two models of anxiety (EPM and OF) and two models of depression (FST and TST). LEW and SHR strains are inbred and therefore have been kept in our laboratory under a system of brother–sister mating. The Floripa H and L rats have been selectively bred for high and low locomotion in the central aversive area of the OF, respectively, according to the method described by Ramos et al. [46]. Briefly, three rat lines were intercrossed to produce a heterogeneous population named S0. Starting with S0, throughout generations, all rats were submitted to the OF test. Males and females with highest scores of central OF locomotion were selected as progenitors of the following generation of the Floripa H line, whereas animals with lowest scores for this same variable were selected to produce the following generation of Floripa L. The rats used in the present study were from generations S6 and S7. The animals were weaned and separated by sex at 4 weeks of age and thereafter kept in collective plastic cages (four to six rats per cage) with food and water available ad libitum, under a 12 h light/dark schedule (lights on at 7:00 a.m.), at $22 \pm 2\,^{\circ}C$.

At 7–8 or 8–9 weeks of age (for LEW/SHR or Floripa H/L, respectively), rats started to be tested, first in the EPM and then in the OF. In the subsequent week they were tested in the FST and TST. For each rat, there was an interval of 2 days between the EPM and OF and of 7 days between the FST and TST. Before being submitted to the EPM all rats were naïve to behavioral testing, having been manipulated only during regular cage cleaning. Because no strain differences were found in the TST applied after the FST, the order of these two models was inverted for the testing of additional groups of naïve rats. Consequently, the first set of experiments was divided in four blocks according to the rat lines and the sequence of the tests: (i) LEW/SHR tested in the EPM, OF, FST and TST; (ii) LEW/SHR in the EPM, OF, TST and FST; (iii) Floripa H/L (S6) in the EPM, OF, FST and TST; (iv) Floripa H/L (S7) in the EPM, OF, TST and FST.

A total of 139 naïve male and female rats of the LEW and SHR strains were used, at 7–8 weeks of age, in the second set of experiments, in which the spontaneous behavior of the animals inside their home-cages was observed. Finally, 60 naïve LEW and SHR rats of both sexes were tested in the FST at 9–10 weeks of age following pharmacological treatment with an antidepressant drug. Males and females were always tested in alternate days, with all tests being carried out between 1:00 and 6:00 p.m. All procedures were carried out according to the guidelines of the local Committee for Animal Care in Research (CEUA/UFSC) and had the valid permission number 23080.001871/2003-54.

2.2. Elevated plus-maze (EPM)

The apparatus, described by Ramos et al. [45], was made of wood covered with black formica and consisted of a plus-shaped platform elevated 52 cm from the floor. Two opposite arms (50 cm × 10 cm) were enclosed by 40 cm high walls whereas the other two arms had no walls, only a surrounding ledge (1 mm thick and 5 mm high) to avoid rats falling off the arms. The four arms had at their intersection a central platform (10 cm × 13.5 cm), which gave access to any of the four arms. The illumination in the central platform was of about 70 lx. At the beginning of each test, the rat was placed in the central platform facing an open arm. The behaviors were registered for 5 min by a video camera mounted above the apparatus and monitored on a TV set by an experimenter located in an adjacent room. The variables observed were: the number of entries and the time spent (with all four paws) in each type of arm. The apparatus was cleaned with water using a wet sponge and a paper towel before the introduction of each animal.

2.3. Open field (OF)

The apparatus, described by Ramos et al. [45], consisted of a large arena made of wood, covered with white formica and surrounded by white walls that were 40 cm high. The floor of 100 cm × 100 cm was divided by black lines into 25 squares of 20 cm × 20 cm. The illumination in the test room was controlled to provide about 7 lx inside the apparatus. Each rat was placed in the center of the open field and its behavior was registered for 5 min. The variables observed were: number of peripheral (adjacent to the walls) squares crossed (outer locomotion), number of central (away from the walls) squares crossed (central locomotion) and total number of faecal boli (defecation). Methods of