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The free-exploratory paradigm as a model of trait anxiety in female rats: Test–retest reliability

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HIGHLIGHTS

- The free-exploratory paradigm (FEP) has been proposed as a model of trait anxiety.
- FEP has been proven stable over time, but only for male rats.
- There are sex influences at all levels of the nervous system.
- This study assessed the stability over time of FEP for female rats.
- Stability over time was found for three of the four evaluated parameters.

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ABSTRACT

The free-exploratory paradigm has been proposed as a model of trait anxiety and as such has been proven stable over time, which is a *sine qua non* condition for any model proposing to evaluate a personality trait. However this stability has only been shown for male rats. Considering that anxiety disorders are most prevalent in women, it's crucial that females are tested in animal models. With this in mind, the stability over time of female rats in the free-exploratory paradigm was evaluated using a test-retest procedure. The behaviour of drug-naive, adult, female, Wistar rats was measured in the free-exploratory apparatus on two occasions two months apart. The following parameters were evaluated: percentage of distance travelled in the novel compartment; number of attempts to enter the novel compartment; percentage of time spent in the novel compartment; and percentage of time rearing in the novel compartment. Subsequently, the intraclass correlation coefficient (ICC) and the kappa index (κ) were calculated for each of these parameters. The "percentage of time spent in the novel compartment" (ICC = 0.727; κ = 0.457), the "percentage of distance travelled in the novel compartment" (ICC = 0.680; κ = 0.370), and the "percentage of time rearing in the novel compartment" (ICC = 0.648; κ = 0.309) were found to be stable over time. Analysis of these parameters indicated fair to substantial reliability over time in two-month inter-trial interval. Therefore, our results support the idea of the free-exploratory paradigm as an animal model of trait anxiety for female rats.

1. Introduction

In the field of anxiety research, animal models have mainly been used for two purposes: (1) for the pre-clinical evaluation of new drugs with a potential anxiolytic effect, and; (2) for the study of mechanisms underlying emotional behaviour [1–3]. There are currently a number of animal models of anxiety [4], however they may not all measure the same psychophysiological state, as anxiety is not a unitary phenomenon [5], something evidenced by the heterogeneity of anxiety disorders (DSM-IV-R). There is also an important distinction to be made between trait and state anxiety. State anxiety is the anxiety a subject experiences at a particular moment in time, it is transitory and may be affected by external stimuli; whereas trait anxiety is considered to be an enduring feature of an individual and is relatively stable over time [4,6].

Most animal models of anxiety confront the animals with an anxiety provoking situation, either through anxiogenic chemicals (β-carbolines, yohimbine, caffeine), conflict tests (Geller and Seifter box, light/dark chamber, elevated plus-maze) or exposure to aversive stimuli (defensive burying) [3], thus modelling state anxiety.

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While this approach is practical and convenient, it must be remembered that a drug that is effective in these animal models, and which may therefore reduce state anxiety in humans in threatening situations, might not be effective in reducing long-term anxiety in chronically anxious patients, as the underlying biological mechanisms of state and trait anxiety may not be the same [4]. An example of this are β-blockers, which act in the central nervous system [7] and are useful in the prevention of performance anxiety, but perform poorly in controlled clinical trials of patients suffering from social anxiety disorder (for a review see [8]). Furthermore, it has been shown that the anxiety response to a threatening stimulus involves brain structures such as amygdala, bed nucleus of the stria terminalis, septo-hippocampal system, median raphe nucleus, ventral periaqueductal grey matter and locus coeruleus [9–11]; while the anxious trait is thought to be related to the orbitofrontal cortex [12,13].

The free exploratory paradigm has been proposed as a model of trait anxiety [14]. In this situation, animals are given the opportunity to move around freely within an environment containing both familiar and novel parts. This approach allows the evaluation of neophobic responses. As the animals have a choice between novelty and familiarity, it is expected that individuals with low trait anxiety would exhibit a preference for novelty, whereas high trait anxiety subjects would prefer familiarity. This free choice paradigm was first described by Hughes [15,16], who observed that Wistar rats actually preferred the novel environment, spending more time in it. Subsequently, Griebel and collaborators [14], comparing two strains of mice, BALB/c and C57BL/6, known, respectively, as "emotional" and "nonemotional", observed that BALB/c mice presented a marked preference for the familiar environment, while C57BL/6 mice exhibited a preference for novelty. This result suggests that the free-exploratory paradigm can differentiate traits of anxiety. There is also some evidence showing that there is no change in state anxiety during this test situation. Misslin and colleagues [17,18] observed that Swiss mice did not present physiological signs of fear unless they were forced into the novel environment, while Belzung and Le Pape [5], using a principal component analysis, demonstrated that variables measured in the free-exploratory paradigm were not described by the same factors as variables measured in models where the animals were forced into a novel environment, i.e., confronted with an anxiety provoking situation. In addition, Teixeira-Silva et al. [19], using Wistar male rats, demonstrated that FEP is stable over time, a *sine qua non* condition for any model proposing to measure trait anxiety, which, by definition, does not vary from moment to moment [6]. However, this stability has never been shown for female animals. It is evident that there are sex influences at all levels of the nervous system, from genetic to behavioural levels [20]. Considering that anxiety disorders are most prevalent in women, it's crucial that females are tested in animal models.

With this in mind, the aim of the present study was to evaluate the stability over time of female rats in the free-exploratory paradigm, using a test–retest procedure.

2. Animals, materials and methods

2.1. Animals

Adult (2–3 months) female Wistar rats (200–250 g b.w.) bred at our laboratory were used. The animals were kept five per cage (41 × 34 × 18 cm), in a temperature (22–24 °C) and light (12 h/12 h light/dark cycle; lights on at 06:00 a.m.) controlled room, with water and food ad libitum. To assess the progression of the oestrous cycle, vaginal washes were taken once a day, starting 14 days prior to experimentation. The pipette smear technique was applied: a small amount of distilled water was gently introduced into the vagina, using a pipette, and then a drop of the resulting cell suspension was placed onto a slide, which was immediately examined.

The oestrous cycle phases were determined according to vaginal cellular population, epithelial, cornified and leukocytes, in the colpocytological examination. Only those animals showing 3 consecutive 4 to 5-day cycles were used.

All procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Procedure

The free-exploratory paradigm was set up as in a similar study, with male rats [19], and automated as described by Antunes et al. [21]. The apparatus consisted of a wooden box, divided into two compartments, with each compartment further subdivided into three exploratory units (20 × 20 cm), interconnected by small openings. The two compartments were separated by a removable partition. The box was placed on a stand in the rat home room. Approximately 24 h before testing, the partition was installed and an animal was put into one-half of the apparatus and left there until the test time, in order to become familiarized with it. This familiar half had fresh zeolites (Zoocel Biotério®—Celta Brasil, Cotia, BR) covering the floor and the animal had free access to food and water. On the test day the partition, between the familiar and the novel compartments, was removed and the animal was observed for 15 min, under infra-red light.

Thirty naive animals were tested on two occasions, two months apart. In both occasions, the rats were put into the apparatus on the night of proestrous, being therefore tested in the oestrous phase. During the 15 min evaluation the following parameters were measured: total distance travelled number of attempts to enter the novel compartment and, for each compartment, the time spent in it and the time spent rearing. Between each use, the free-exploratory boxes were emptied and then cleaned using a 10% ethanol solution.

After the first evaluation, the animals were put back into their home cages, and kept under standard conditions (described in the "Animals" section) until the retest. During the inter-trial interval, there was no collection of vaginal material, which was reinstated on the week of the retest.

The oestrous phase was chosen for behavioural observation due to technical reasons. Although vaginal smears are widely used for rat's oestrous cycle classification, the time span of each phase is controversial. Yet, proestrous phase is usually described as lasting 12–14 h, while oestrous lasts at least 12 h [22,23]. Therefore, an animal put into the apparatus on the night of proestrous would be almost guaranteed to be in oestrous 24 h later.

All tests were performed in the dark phase of the light cycle, between 6:00 and 7:00 p.m.

2.3. Statistical analyses

The time spent in and the time rearing in the novel compartment, as well as the distance travelled in this compartment, were calculated as percentages of: the total test time, the total time rearing and the total distance travelled in the whole apparatus, respectively (i.e. novel compartment/total × 100). The total number of stretched postures towards the novel compartment followed by avoidance of novelty (attempts) was also analyzed. These measurements were considered parameters of anxiety-like behaviour, while the total distance travelled in the apparatus was considered a parameter of locomotor activity. This classification was in accordance with previous studies performed in mice [5,14,24] and male rats [19,21,25].

In order to examine the evolution of behaviour throughout the test and the retest, the observation period was divided into 15