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Pharmacological validation of the free-exploratory paradigm in male Wistar rats: A proposed test of trait anxiety

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The free-exploratory paradigm (FEP) has been proposed as a model of trait anxiety for both mice and rats. However, its pharmacological validation has only been carried out for the mice. Thus, the aim of the present study was to pharmacologically validate FEP for Wistar rats, by testing the effects of clinically established anxiolytic and anxiogenic drugs, in four different experiments. In all experiments, male Wistar rats were first tested in FEP to be categorized according to their levels of trait anxiety (high, medium and low). Then, only medium trait anxiety rats were selected to be tested again in FEP, two weeks later, after being pharmacologically treated, according to each experiment as follows: Experiment I: 0.5 mg/kg of diazepam (DZP) or vehicle; Experiment II: 20 mg/kg of pentylenetetrazole (PTZ) or vehicle; Experiment III: 5 mg/kg of fluoxetine (FLX5) or vehicle: and Experiment IV: 0.5 mg/kg of fluoxetine (FLX0.5) or vehicle. As a group, the results showed that PTZ and FLX5 increased levels of trait anxiety and reduced locomotor activity, whereas DZP and FLX0.5 decreased levels of trait anxiety, without impairing locomotor activity. These results demonstrate that FEP for rats is able to predict clinical anxiolytic and anxiogenic activities of different drugs, including fluoxetine, which is believed to present a dual effect on anxiety. Therefore, this paradigm can be proposed as an effective method for testing potential trait anxiety-reducing drugs, in rats.

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1. Introduction

Anxiety disorders are associated with significant impairment in quality of life and negative interferences on occupational, academic and social contexts (Olatunji et al., 2007), being the most prevalent class of psychiatric disorders in the general population (Kessler et al., 2005) and the third most costly brain disorder in Europe, with an economic impact of billions of euros (Olesen et al., 2012). However, its treatment is still challenging, as the drugs used for the relief of anxiety symptoms can have important side-effects, promote therapeutic dependence, or present a delay in their onset of action (Starcevic, 2005). Furthermore, not all patients benefit from the available treatments, and only a few of them have a response near to complete recovery (Ravindran and Stein, 2010). These facts justify the growing number of studies in order to develop more effective drugs, with fewer side-effects, for the control of anxiety disorders.

For the experimental evaluation of new drugs with a potential anxiolytic effect, the scientific community relies on various animal models. However, most of these models 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; Garner et al., 2009; Martin, 1998; McGonigle, 2014; Treit, 2010), thus modelling state anxiety, which is the anxiety a subject experiences at a particular moment in time, in response to a threatening situation. However, in the study of anxiety there is another important concept: trait anxiety, which describes individual differences, related to a tendency to present state anxiety; it is relatively stable overtime (Spielberger, 1970; Treit, 2010) and elevated in anxiety disorder patients (Bieling et al., 1998; Kennedy et al., 2001).

The underlying biological mechanisms of state and trait anxiety may not be the same (Treit et al., 2010). 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 (Brandão et al., 2003; Davis, 2006; Gray and Mcnaughton, 2000); while the anxious trait is thought to be related to the orbitofrontal cortex (Blackmon et al., 2011; Kalin et al., 2007). Therefore, it is reasonable to believe that a drug that is effective in animal models of state anxiety, and which may therefore reduce state

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anxiety in humans in threatening situations, might not be effective in reducing long-term anxiety in chronically anxious patients.

Taking all this into consideration, it becomes clear that pre-clinical tests for the development of new anxiolytic drugs should include animal models of trait anxiety.

To the best of our knowledge, the only test that has been proposed as an animal model of trait anxiety is the free-exploratory paradigm (FEP) (Griebel, 1993). 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.

FEP was first described, in the 1960s, by Hughes (Hughes, 1965, 1968), who observed that rats preferred the novel environment, spending more time in it. Later, Misslin and collaborators (Misslin et al., 1982; Misslin and Cigrang, 1986) observed that Swiss mice, in this situation, did not present physiological signs of fear unless they were forced into the novel environment, indicating that there was no change in state anxiety during this test situation. Notwithstanding, Griebel and collaborators (1993), testing two strains of mice, BALB/c and C57BL/6, known, respectively, as “emotional” and “non-emotional”, observed that BALB/c mice presented a marked preference for the familiar environment, while C57BL/6 mice exhibited a preference for novelty, which showed that FEP could differentiate predisposition to anxiety. Together, these results indicated that this paradigm was able to evaluate traits rather than states of anxiety. This was further evidenced by the fact that Belzung and Le Pape (1994), using a principal component analysis, demonstrated that variables measured in FEP 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. Moreover, it was recently demonstrated, using both male (Teixeira-Silva et al., 2009) and female (Oliveira et al., 2014) Wistar rats, 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 (Spielberger et al., 1970).

In summary, FEP seems to allow the evaluation of anxious profiles and, as a consequence, the assessment of drugs with potential effects on trait anxiety. An anxiolytic drug is expected to increase novelty exploration, whereas an anxiogenic drug should do the opposite. As this paradigm does not provoke an anxious reaction, an anxiolytic effect cannot be easily observed in low trait anxiety individuals, because of a floor effect, as it has been demonstrated by Griebel and collaborators (1993). In the same way, an anxiogenic effect may not be observed in high trait anxiety subjects, due to a ceiling effect. Yet, both anxiolytic and anxiogenic effects can be observed in medium trait anxiety individuals, as they can more easily increase or decrease novel side exploration.

Although FEP has been performed in both mice (Misslin et al., 1982; Misslin and Cigrang, 1986; Griebel et al., 1993; Belzung and Le Pape, 1994) and rats (Hughes, 1968; Matos et al., 2011; Goes et al., 2013), and various different psychotropic drugs have already been tested in it, including physostigmine, scopolamine, methylscopolamine, methylphenidate, methamphetamine and imipramine (Horsburgh and Hughes, 1981; Hughes et al., 1975; Hughes and Greig, 1976; Hughes and Pither, 1987; Hughes, 1992), its pharmacological validation as an animal model of trait anxiety has only been carried out for mice (Griebel et al., 1993; Belzung and Berton, 1997; Belzung et al., 2001).

With this in mind, the aim of the present study was to pharmacologically validate FEP as an animal model of trait anxiety in male Wistar rats. For that, the following drugs were used: 1) diazepam, benzodiazepine full agonist — typically used for the pharmacological validation of animal models (Andreatini et al., 2001); 2) pentylenetetrazole, a convulsant compound GABA-benzodiazepine receptor blocker (Huang et al., 2001), which induces anxiety in lower doses (subconvulsant; Pellow et al., 1985); and 3) fluoxetine, a selective serotonin reuptake inhibitor, which has been reported as the most prescribed drug for the relief of anxiety symptoms (Marshall et al., 2009), and that in animal models present controversial effects, varying from anxiolytic to anxiogenic (Borelli et al., 2004; Drapier et al., 2007; De Vry et al., 2004; Griebel et al., 1999; Robert et al., 2011).

2. Material and methods

2.1. Animals

One hundred thirty-four adult (2–3 months), male Wistar rats were obtained from our own colony. 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. The use of Wistar rats was due to the fact that, currently, this is one of the most popular strains used in medical and biological research, including nonclinical drug discovery and development.

All procedures were approved by the local ethical committee (Universidade Federal de Sergipe) and complied with both national (Brazilian National Council on the Control of Animal Experimentation — Law 11.794, of October 8, 2008) and international guidelines (Council Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010) for the care of animals.

2.2. Free-exploratory paradigm

The free-exploratory paradigm was set up as described by Antunes et al. (2011). The apparatus (Fig. 1) consisted of a wooden box, divided into two equal rectangular compartments, with each of these further subdivided into three equal square exploratory

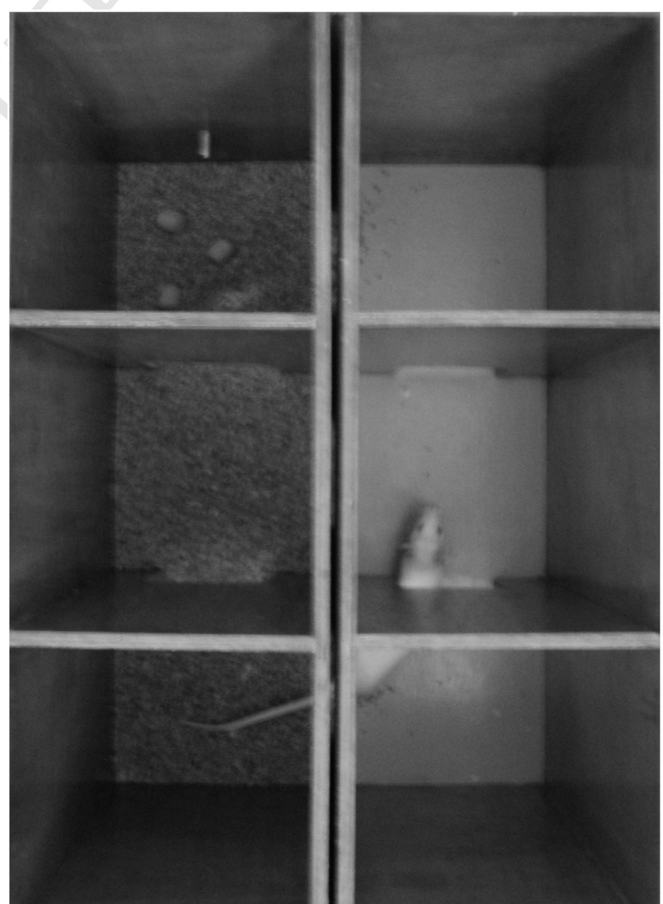

Fig. 1. Free-exploratory box with a rat in the novel side (right compartment). The left side corresponds to the familiar environment, which has fresh zeolites covering the floor and free access to food and water. Each compartment is subdivided into three equal exploratory units, interconnected by small openings.

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