Behavioural Brain Research 240 (2013) 160–170

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Ethopharmacological evaluation of the rat exposure test: A prey–predator interaction test

Kelciane Ferreira Caetano Campos[a,b], Vanessa Cristiane Santana Amaral[a,b,c], Javier Leonardo Rico[a,d], Tarciso Tadeu Miguel[a,b,e], Ricardo Luiz Nunes-de-Souza[a,b,e,*]

[a] Laboratory of Pharmacology, School of Pharmaceutical Sciences, Universidade Estadual Paulista, UNESP, Araraquara, SP 14801-902, Brazil
[b] Joint Graduate Program in Physiological Sciences UFSCar/UNESP. Rod. Washington Luís, Km 235, São Carlos, SP 13565-905, Brazil
[c] Unidade Universitária de Ciências Exatas e Tecnológicas, UnUCET-UEG Br 153 n.3105 CEP: 75132-903 Anápolis, GO, Brazil
[d] Laboratory of Animal Behavior, Fundación Universitaria Konrad Lorenz, Bogotá, Colombia
[e] Institute for Neuroscience and Behavior-IneC, USP, Ribeirão Preto, SP 14040-901, Brazil

HIGHLIGHTS

- Factor analysis of the behavior of mice in the rat (predator) exposure test (RET).
- The defensive behavior factor is attenuated by the potent benzodiazepine, alprazolam.
- The panicogenic-like drug, caffeine, facilitated the defensive behavior.
- The predator-induced defensive behavior is impaired by fluoxetine given chronically.
- The RET: a useful test to assess the effects of panicolytic and panicogenic drugs.

ARTICLE INFO

Article history:
Received 26 September 2012
Received in revised form
14 November 2012
Accepted 16 November 2012
Available online 27 November 2012

Keywords:
Rat exposure test
Prey–predator interaction
Panicolytic drugs
Panicogenic drugs
Factor analysis
Mice

ABSTRACT

The rat exposure test (RET) is a prey (mouse)–predator (rat) situation that activates brain defensive areas and elicits hormonal and defensive behavior in the mouse. Here, we investigated possible correlations between the spatiotemporal [time spent in protected (home chamber and tunnel) and unprotected (surface) compartments and frequency of entries into the three compartments] and ethological [e.g., duration of protected and unprotected stretched-attend postures (SAP), duration of contact with the rat's compartment] measures (Experiment 1). Secondly, we investigated the effects of systemic treatment with pro- or anti-aversive drugs on the behavior that emerged from the factor analysis (Experiment 2). The effects of chronic (21 days) imipramine and fluoxetine on defensive behavior were also investigated (Experiment 3). Exp. 1 revealed that the time in the protected compartment, protected SAP and rat contacts loaded on factor 1 (defensive behavior), while the total entries and unprotected SAP loaded on factor 2 (locomotor activity). Exp. 2 showed that alprazolam (but not diazepam) selectively changed the defensive factor. Caffeine produced a mild proaversive-like effect, whereas yohimbine only decreased locomotor activity (total entries). Fluoxetine (but not imipramine) produced a weak proaversive-like effect. 5-HT_{1A}/5-HT_2 receptor ligands did not change any behavioral measure. In Exp. 3, chronic fluoxetine (but not imipramine) attenuated the defensive behavior factor without changing locomotion. Given that the defensive factor was sensitive to drugs known to attenuate (alprazolam and chronic fluoxetine) and induce (caffeine) panic attack, we suggest the RET as a useful test to assess the effects of panicolytic and panicogenic drugs.

1. Introduction

In the field of animal research, rodents have played a pivotal role in the study of the neurobiology of emotional states such as fear and anxiety. Although most studies have used rats to identify physiological, neurochemical and neurotransmitter systems underlying defensive behavior, a growing number of laboratories have emphasized the use of mice to investigate potential substrates of psychiatric disorders. In this context, it has been shown that

* Corresponding author at: Laboratory of Pharmacology, School of Pharmaceutical Sciences, Universidade Estadual Paulista, UNESP, Araraquara, SP 14801-902, Brazil. Tel.: +55 16 3301 6983; fax: +55 16 3301 6980.
E-mail address: souzarn@fcfar.unesp.br (R.L. Nunes-de-Souza).

0166-4328/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2012.11.023

the defensive behavior of mice differs from that exhibited by rats in both magnitude and coping strategy. Moreover, regarding the expression of behavioral response to aversive stimuli, laboratory mice exhibit more resemblance with wild mice than laboratory rats with their wild type [1,2]. Therefore, it has been suggested that laboratory mice would be more suitable subjects to evaluate the neurobiology of defensive reactions than laboratory rats [2,3].

Based on this assumption, Blanchards' group developed the rat exposure test (RET), an ethological model of prey–predator interactions, in which a mouse (prey) is exposed to a rat (predator) [4]. Rats have been shown in nature, as well as in the laboratory, killing and consuming mice [5–8]. The apparatus allows the mouse to avoid the predatory stimulus by hiding either in a home chamber or in a tunnel, or to regulate its own exposure by approaching or avoiding the source of the threat [9,10]. During the rat exposure test, various types of spatiotemporal and ethological defensive and non-defensive behavior can be assessed in the mouse, such as frequency of entries in the three compartments (i.e., home chamber, tunnel and surface) and time spent in these compartments; time spent displaying self-grooming, freezing, burying and risk assessment (stretched-attend posture and flat-back posture). Given this wealth of behavioral variables, it seems relevant to identify potential strong correlations among those variables that are, for instance, closely related to defensive behavior, differentiating them from those that are related to non-defensive behavior. The identification of markedly correlated behavioral measures would lead to a smaller number of variables and simplify the behavioral analysis in the RET.

Furthermore, in the behavioral validation of the RET, Yang et al. [4] showed that this animal model elicits a remarkable risk assessment response in the prey, a particularly important mode of behavior that has been related to the defense reaction state in various animal tests of anxiety and especially notable for its sensitivity to anxiolytic- or anxiogenic-like drugs [11,12]. This feature has been explored in subsequent experiments to identify neurotransmitters and neural systems potentially involved in the modulation of behavioral defensive responses in mice exposed to the RET [10,13–17]. However, as far as we know, there are no studies showing the effects of systemic administration of anxiolytic- and anxiogenic-like compounds on the behavior of mice exposed to the RET.

In parallel to the behavioral validation of the RET, previous studies have assessed the physiological responses and brain circuitry in the mouse exposed to the predator in the RET [18,19]. Briefly, these studies have shown that the exposure of mice to the RET increases both plasma corticosterone levels [19] and Fos protein labeling in limbic brain areas (e.g., anterior hypothalamic nucleus, the dorsomedial part of the ventromedial hypothalamic nucleus and the dorsal premammillary nucleus) [18]. A similar profile of c-Fos activation was observed in previous studies on rats exposed to a cat or a cat odor [20,21], supporting the concept that a specific defensive circuit, involving similar brain areas, is present in different species. Altogether, these studies have shown that the RET is a promising experimental paradigm for the investigation of prey–predator interactions and can be extended, as a naturalistic animal model, to the study of the neurobiology of anxiety.

Thus, in view of the increasing use of the RET and its potential validity as an animal model to assess defensive reactions, in this study we investigated the behavior of the mouse during prey–predator interaction, by factor analytic techniques (related to principal components analysis). Given that strongly correlated measures are expected to respond similarly to pharmacological manipulations such as treatment with anxiolytic- or anxiogenic-like drugs, in this study the effects of acute treatment with anxiolytic/panicolytic- or anxiogenic/panicogenic-like drugs, as well as those of chronic treatment with antidepressant-like drugs, on the main behavioral parameters emerged from the factor analysis were investigated.

2. Materials and methods

2.1. Subjects

Subjects were male Swiss mice (from the animal facility at UNESP, Araraquara, SP, Brazil) weighing 25–35 g at testing. They were housed in groups of 10 per cage (41 cm × 34 cm × 16 cm) and maintained under a normal 12-h light cycle (lights on at 7:00 h) in a temperature-controlled environment (23 ± 1 °C). Food and water were freely available except during the brief test periods. Male Long-Evans rats of approximately 600 g were used as predator stimuli during the study.

2.2. Drugs

All drugs were dissolved in physiological saline or vehicle (saline + 2% Tween-80). Drugs used were: diazepam (Formil Química Ltd., Brazil) and alprazolam (Formil Química Ltd., Brazil); the 5-HT_{1A} receptor partial and full agonist, respectively, buspirone hydrochloride (Sigma–Aldrich, USA) and 8-OH-DPAT [(±)-8-hydroxy-2-(di-*n*-propylamine) tetralin hydrobromide (Sigma–Aldrich, USA)]; the 5-$HT_{2B/2C}$ receptor antagonist, SDZ SER-082 fumarate [(+)-*cis*-4,5,7a,8,9,10,11,11a-octahydro-7-H-10-methyindol O[1,7-bc] [2,6]-naphthyridine fumarate (Tocris, USA)], the 5-HT_{2C} receptor agonist, MK-212 [6-chloro-2-(1-piperazinyl) pyrazine hydrochloride (Tocris, USA)]; the α_2-adrenergic receptor antagonist, yohimbine hydrochloride (Sigma–Aldrich, USA); the adenosine receptor antagonist, caffeine (Sigma–Aldrich, USA); the selective serotonin reuptake inhibitor, fluoxetine hydrochloride (Tocris, USA) and serotonin and noradrenaline reuptake inhibitor imipramine hydrochloride (Sigma–Aldrich, USA). All drugs were injected intraperitoneally (i.p.) in a volume of 10 ml/kg body weight (b.w.). All doses used in the current study were based on previous studies [22–25].

Apomorphine (Siegfried Zofinger, Switzerland) was dissolved in physiological saline and subcutaneously (s.c.) administered to Long-Evans rats at a single dose of 3.0 mg/kg b.w., 20 min prior to placement into the rat exposure chamber. This procedure was used to keep the stimulus rats uniformly active during and across test sessions.

2.3. Apparatus

The rat exposure test (see Fig. 1) was developed and behaviorally validated by Yang et al. [4] in order to facilitate the measurement of risk assessment behavior

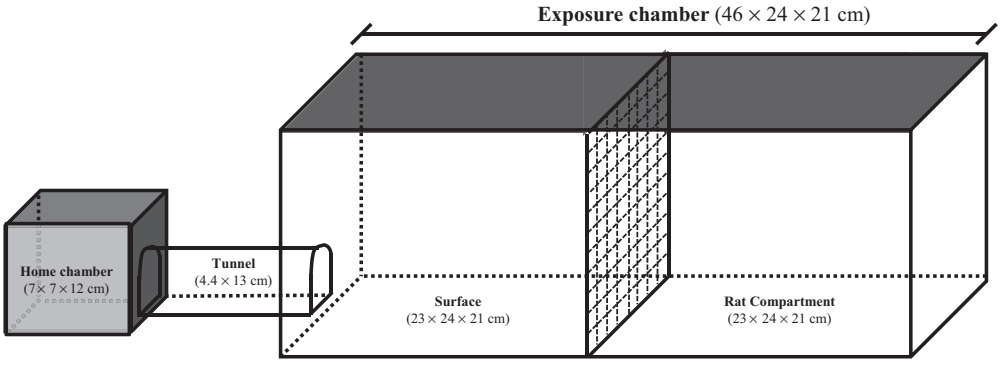

Fig. 1. A schematic side view of the rat exposure test (RET).