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Increased corticosterone levels in mice subjected to the rat exposure test

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ABSTRACT

In recent years, there has been a notable interest in studying prey–predator relationships to develop rodent-based models for the neurobehavioral aspects of stress and emotion. However, despite the growing use of transgenic mice and results showing important differences in the behavioral responses of rats and mice, little research has been conducted regarding the responses of mice to predators. The rat exposure test (RET), a recently developed and behaviorally validated prey–predator (mouse-rat)-based model, has proven to be a useful tool in evaluating the defensive responses of mice facing rats. To further validate the RET, we investigated the endocrine and behavioral responses of mice exposed to this apparatus. We first constructed a plasma corticosterone secretion curve in mice exposed to a rat or to an empty cage (control). Rat-exposed mice showed a pronounced rise in corticosterone levels that peaked 15 min from the beginning of the predator exposure. The corticosterone levels and behavioral responses of mice exposed to a rat or to a toy in the RET apparatus were then measured. We observed high plasma corticosterone levels along with clear avoidance behaviors represented by decreases in tunnel and surface area exploration and increases in risk assessment behaviors and freezing. This strongly suggests that the test elicits a repertoire of behavioral responses compatible with an aversion state and indicates that it is a promising model for the evaluation of prey-predator interactions. However, more physiological, neurochemical, and pharmacological studies are needed to further validate the test.

Introduction

In recent years, a notable interest in the study of prey–predator relationships has emerged for a number of reasons. First, predator exposure elicits defensive behaviors that are innate and easily evoked in the laboratory. Second, the prey–predator interaction model provides an excellent tool to elucidate the neurobehavioral aspects of stress and emotion. Finally, there is continuing interest in the development of rodent-based models of anxiety for the preclinical testing of anxiolytic drugs.

Although most reports on the effects of predator stress on prey reactions have used cats as predators (Canteras and Goto, 1999; Belzung et al., 2001; Adamec and Shallow, 1993; Adamec et al., 2004; 2006; Kavaliers and Colwell, 1991), recent studies have made use of another natural predator, the rat, to assess defensive reactions in mice (Blanchard et al., 2001; Yang et al., 2004; Beekman et al., 2005; Carvalho-Netto et al., 2007, 2009; Litvin et al., 2007). In nature, as well as in the laboratory, rats have been observed to kill and consume mice (Karli, 1956; O'Boyle, 1974, 1975; Malick, 1975, Rylov, 1985). Additionally, when confronted by a rat, both laboratory and wild mice show clear innate defensive behaviors such as flight, avoidance, freezing, and risk assessment (Blanchard et al. 1998a; Anisman et al., 2001; Hayley et al., 2001; Beekman et al., 2005) reduced locomotion and decreases in nondefensive behaviors such as eating, drinking, and exploration (Calvo-Torrent et al., 1999; Dalm et al., 2009). Moreover, mice appear to recognize rats as dangerous without contact or prior experience (e.g., rat exposure causes disruption of pregnancy in mice) (De Catanzaro, 1988).

There are several advantages to using a mouse and rat model instead of a rat and cat model to study prey–predator interactions. First, difficulties involved in maintaining and handling cats in laboratory facilities are avoided. Second, transgenic mice can be used to study the genetic basis of prey–predator interactions, a growing area of research. Third, risk assessment behaviors occur much more frequently in mice than in rats when confronted with predators (Blanchard et al., 1997, 1998a, 2001). Based on studies of laboratory and wild rats and mice, Blanchard et al. (1998a) argued that these differences may be due to domestication processes in rats that can render this animal inappropriate for studying some types of defensive behaviors. Therefore, Blanchard's group developed an ethological prey–predator-based model with rats and mice called

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the rat exposure test (RET) (Yang et al., 2004). This model has been satisfactorily used to investigate the effects of anxiolytic and anxiogenic compounds on defensive behavior in mice (Litvin et al., 2007; Carvalho-Netto et al., 2007, 2009). Furthermore, a recent study conducted by Martinez et al. (2008) using the RET showed a marked increase in Fos-like immunoreactivity in medial hypothalamic regions involved in predatory threat modulation as well as in autonomic and parvicellular parts of the paraventricular hypothalamic nucleus in mice exposed to the RET.

A series of experiments to behaviorally validate the RET has previously been carried out (Yang et al., 2004). The results showed that the RET elicits a singular pattern of avoidance in mice that is characterized by high levels of risk assessment that are markedly enhanced in this model when compared to other commonly used mouse anxiety models such as the elevated plus maze (EPM), the light/dark test and the Mouse Defense Test Battery (MDTB) (Yang et al., 2004). Risk assessment can be described as information-gathering behaviors displayed in potentially threatening situations which function to optimize the adaptive behavioral strategy (Blanchard and Blanchard, 1989). It has been shown that risk assessment behaviors are very sensitive to the effects of anxiolytic drugs, suggesting that they may play a critical role in anxiety-related psychopathologies (Blanchard et al., 2001). Assuming that risk assessment behaviors are an important index of anxiety/fear reactions, the RET could be considered an ethologically relevant model to study this disorder in mice. However, several authors have argued that animal models of anxiety/fear should be examined much more closely from a behavioral perspective. Specifically, a valid animal model should demonstrate a resemblance to the clinically defined symptoms of the disorder. Therefore, it is crucial that this model elicit both physiological and behavioral responses.

In this context, it has been established that stressful situations such as the presence of a predator elicits a physiological stress response in animals that is characterized by robust activation of the hypothalamus–pituitary–adrenocortical (HPA) axis (Blanchard et al., 1998b; Anisman et al., 1998; 2001; Roseboom et al., 2007). Activation of the HPA is proportional to the perceived risk. The increased plasma levels of catecholamine and glucocorticoids (e.g., corticosterones) are directed to energy mobilization, which in turn is used to display a behavioral response (Sapolsky et al., 2000). Therefore, corticosterone is a well-accepted biological marker of stress (Korte, 2001; Herman et al., 2005).

To further demonstrate the validity of the RET as an animal model of anxiety/fear, we investigated the endocrine and behavioral responses of mice exposed to this apparatus. For this purpose, we performed two experiments. In the first test, we assessed the magnitude and duration of predator stress-induced corticosterone secretion in mice exposed to the RET, and in the second, we compared behavioral responses and corticosterone secretion in mice exposed either to a toy or to a rat in the RET.

Materials and methods

Animals

Male adult Swiss mice (Sao Paulo State University/UNESP, SP, Brazil) weighing 28–35 g at testing were used in the study. Mice were housed in groups of 10 per cage (size: 41×34×16 cm) and maintained under a normal 12-h light cycle (lights on at 07:00 h) in a temperature- and humidity-controlled environment (23±1 °C/55±5%). Food and water were freely available except during the brief test periods. All mice were naïve at the beginning of the experiments. A total of 5 male Long-Evans rats weighing approximately 600 g were used as predator stimuli during the course of the study.

Drugs

Apomorphine (Siegfried Zofinger, Switzerland) was dissolved in physiological saline (NaCl 0.9%) and administered s.c. to Long-Evans rats at a single dose of 3.0 mg/kg 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.

Apparatus

The rat exposure test, which was designed to facilitate the measurement of risk assessment behaviors in mice, was developed and behaviorally validated by Yang et al. (2004). Testing procedures were conducted in a 46×24×21 cm clear polycarbonate cage (i.e., the exposure chamber) covered with a black polycarbonate lid. The exposure chamber was divided into two equal-sized compartments (the surface and the predator compartment) by a wire mesh screen. The home chamber was a 7×7×12 cm box made of black Plexiglas on three sides and clear Plexiglas on the fourth side to facilitate videotaping. The home chamber was connected to the exposure cage by a clear Plexiglas tube tunnel (4. 4 cm in diameter, 13 cm in length, and elevated 1. 5 cm off the floor of the two chamber see Fig. 1).

Corticosterone radioimmunoassay

The radioimmunoassay for corticosterone was conducted as described previously by our research group (Marin et al., 2007). Briefly, the assay was performed using antibodies obtained from Sigma (St. Louis, MO) and (^{3}H)-corticosterone obtained from New England Nuclear (Boston, MA). The method was adapted from that described by Sarnyai et al. (1992). Briefly, 20 µl of plasma was diluted 50 times with 0.01 M PBS and placed in a water bath at 75 °C for 1 h heat inactivation of corticosteroid-binding globulin. One hundred microliters of a solution of antibody and (^{3}H)-corticosterone (10,000 to 20,000 cpm/ml) were added to each sample, mixed, and incubated overnight at 4 °C. Dextran-coated charcoal was used to adsorb free steroid after incubation. Tubes were centrifuged at 2000×*g* for 10 min at 4 °C, and the supernatant from each tube was transferred to scintillation vials. Radioactivity was quantified by liquid scintillation spectrometry. Standard curves were constructed using 31.25, 62.5, 125, 250, 500, and 1000 pg/100 µl (triplicates) of corticosterone (Sigma). After dilution, all the concentrations of the corticosterone samples were within the linear range of the standard curve. The lower limit of detection was 0.23 µg/dL and inter- and intra-assays variations were 4.0% and 8.1%, respectively.

General procedure

All testing was conducted between 8:00 h and 12:30 h in the light phase of the light/dark cycle under illumination of a 100 W red light bulb (2 lx at the surface area of the RET). Each apparatus was cleaned with 20% alcohol and dried with paper towels between trials. Prior to each trial, the individual cage bedding of each subject was poured into the home chamber and the surface of the RET so as to cover the entire floor of the apparatus. The procedure consisted of two parts: the habituation phase and the exposure test. In the habituation phase, each mouse was placed on the center of the surface and was allowed to freely explore the apparatus for 10 min in the absence of the rat. This protocol was carried out each day for 4 consecutive days, and the exposure test was conducted on the subsequent day (inter day interval: 24 h). During the test, each mouse was placed on the center of the surface in the presence of an apomorphine-treated Long-Evans rat placed behind the wire mesh (see Fig. 1). Sessions of experiment 2 (see below) were filmed by a camera linked to a monitor and a DVD recorder in an adjacent laboratory. The scored behaviors comprised