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Impact of chronic stressors on the anxiety profile of pregnant rats

Soliani Flaviane Cristina de Brito Guzzo [a], Cabbia Rafael [a], Batistela Matheus Fitipaldi [a], Almeida Amarylis Garcia [a], Kümpel Vinícius Dias [a], Yamauchi Junior Luiz [a], Frei Fernando [b], Andrade Telma Gonçalves Carneiro Spera de [a,*]

[a] UNESP — Univ Estadual Paulista, FCL, Department of Biological Science, Laboratory of Physiology, Avenida Dom Antônio 2100, 19806-900, Assis, SP, Brazil
[b] UNESP — Univ Estadual Paulista, FCL, Department of Biological Science, Laboratory of Statistic, Avenida Dom Antônio 2100, 19806-900, Assis, SP, Brazil

HIGHLIGHTS

- The manifestation of anxiety in pregnant rats is greater at the end of gestation.
- Social separation causes anxiolysis in the third gestational week.
- Unpredictable chronic stress increases anxiety, especially at the end of 2nd week.

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ABSTRACT

The manifestation of anxiety during pregnancy can be caused by multiple factors and may have emotional and physical consequences for both the mother and the fetus. The prevalence of gestational anxiety has grown in recent years, making the development of studies for its comprehension essential. Thus, the aim of this investigation was to evaluate the effects of predictable and unpredictable chronic stressors on the anxiety profile of rats in three distinct stages of pregnancy (1st, 2nd and 3rd weeks). *Wistar* dams were divided into three groups: control, social separation and unpredictable chronic stress. Behavioral assessments were conducted in the Elevated Plus-Maze at the end of the 1st, 2nd and 3rd weeks of gestation. The results showed that there was increased anxiety in the proximity of parturition in control dams. Chronic stressors differentially affected the behavior of pregnant rats according to the gestational period where they were applied: social separation decreased anxiety at the end of the 3rd week, while unpredictable chronic stress caused increased anxiety, especially at the end of the 2nd gestational week. These results show that there is a critical time during pregnancy for the onset of anxiety in control rats, depending on the gestational stage. The exposure to different types of chronic stressors may result in distinct behaviors related to this disorder.

1. Introduction

Mental disorders afflict approximately 450 million people worldwide [1]. Among them, anxiety has a lifetime prevalence of 31.2% in the general population: 36.4% for women and 25.4% for men [2]. Consequently, there has been an alarming increase in the consumption of drugs used for treatment of this disorder [3]. It is known that women are more susceptible to the development and manifestation of different types of anxiety, especially in developmental periods (puberty and climacteric), during the female hormonal cycle (premenstrual period) or as a result of bilateral ovariectomy, i.e., conditions where estrogen and progesterone concentrations are at low or unstable levels [4–10].

Besides, pregnancy is also a critical period for the manifestation of this disturbance. The prevalence of anxiety disorders during pregnancy varies according to the evaluated period, in addition to factors like socioeconomic status, ethnicity, age, personal resources (self-esteem, optimism and self-control), relationship and social support, previous deliveries, and medical risks, among others (see [11], for review). A study conducted by Lee and colleagues [12] showed that 54% of women reported anxiety in pregnancy for at least one trimester.

Pregnancy is a time of crisis, characterized by profound changes in social, professional, and emotional life and the body of a woman. Moreover, it is well-known that modern women, in addition to incubating the conceptus throughout the gestational period, delivering and breastfeeding their children, play other social roles, as wife, mother, and professional, among numerous other functions they perform. This set of functions, added to the anatomical, physiological and psychological changes inherent in pregnancy can enhance the impact of stressors, impairing life quality. All this stress may also result in drastic consequences for the mother's body, such as suppression of the immune response [13], changes in eating habits [14] and in sleep pattern [15], predisposing her even more

* Corresponding author.
E-mail address: raica@assis.unesp.br (A. Telma Gonçalves Carneiro Spera de).

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to a manifestation of anxiety [16,17]. The exposure to stressors and consequently the manifestation of anxiety during pregnancy (either in women or rats) may also culminate in the occurrence of prematurity [18–20], as well as in changes that might cause psychophysiological problems in the offspring (see [21], for review).

It is already known that stressors activate the hypothalamic–pituitary–adrenal (HPA) axis, which triggers secretion of glucocorticoid hormones (cortisol for humans and corticosterone for rats) in order to induce systemic changes in the physiological pattern, aiming to return the body to homeostasis [14,15,22]. The exposure to high levels of glucocorticoids can lead to changes in fetal programming [23], as well as affecting the development of glucocorticoid receptors in the fetal brain [24]. For the mother, glucocorticoids induce physiological and behavioral changes that can impair the care of offspring [25–28].

Attenuation of HPA axis activity thus becomes essential to confer protection to the adverse effects of stress [29]. The functional change of this neuroendocrine axis is an important protection for the body, decreasing its perception and response to physical and psychological stressor stimuli [30,31]. This protection takes place at strategic moments in life, such as right after birth, in both sexes [32], and during the gestational and puerperal periods of females [25,26]. Attenuation of the HPA axis becomes essential for the success of pregnancy, parturition and rearing, protecting the mother and her progeny from the exposure to the adverse effects of stress [27]. In fact, some experimental studies in rats have suggested that pregnancy is tranquilizing and decreases anxiety, as well as increasing cognitive and memory functions [33].

Based on these aspects and being aware that hormonal fluctuation during the gestational period may make the behavioral pattern inconsistent [25], this study aimed to investigate the anxiety profile of rats in three stages of pregnancy. We also evaluated whether exposure to predictable and unpredictable chronic stressors applied at different gestational periods modified the behavior related to the manifestation of anxiety. The detection of emotional disorders during pregnancy, such as anxiety, will certainly benefit the physical and mental health of the progenitress, and possibly of the next generation [21].

2. Material and methods

2.1. Animals

Virgin female *Wistar* rats with an average age of 75 days, from UNESP Central Vivarium (Botucatu/SP), were randomly grouped (5 rats per box, identified by different pen markings made on their tails) in polypropylene boxes (41 × 34 × 17 cm), using sawdust as bedding material, in the Female's Vivarium of the Physiology Laboratory, maintained under controlled conditions of temperature (21 °C ± 2 °C), and lighting (50 lx at the center of the room and 12h:12h light–dark cycle, with lights on at 07:00 am), and receiving chow and water *ad libitum*. The animals were handled only during box cleaning and at specific moments in the experiment. The study was approved by the local Ethics Committee on the Use of Animals (CEUA 011/2012). All procedures were conducted in accordance with international ethical standards concerning animal experimentation.

2.2. Mating and weighing

After a minimum period of 7 days in the Vivarium of the Physiology Laboratory, the estrous cycle of rats was monitored by vaginal smear. When presenting proestrus or estrus, the rats were individually allocated with an experienced male (one couple per box). The criterion for statement of mating and determination of gestational day (GD) 1 was the presence of a vaginal plug. When not found, a vaginal smear was taken to observe the presence of sperm. After mating, all female rats were relocated to their boxes, remaining grouped (5 per box) or not, according to each experimental group. The dams were weighed 3 times a week, in order to verify whether the procedures caused weight change in the gestational week in which they were applied and to monitor weight gain throughout gestation. The analysis was made on the basis of relative weight in the evaluated week and the total, where:

$$\text{Relative weight gain in week}(\%) = \frac{\text{Last weighing in evaluated week(g)}-\text{First weighing in evaluated week(g)} \times 100}{\text{First weighing in evaluated week(g)}}$$

$$\text{Total relative weight gain } (\%) = \frac{\text{Last gestational weighing (g)}-\text{First gestational weighing(g)} \times 100}{\text{First gestational weighing(g)}}.$$

2.3. Groups and experimental design

The dams (a total number of 70) were divided into three major groups: control (C), social separation (SS) and unpredictable chronic stress (UCS). Whereas pregnancy in rats lasts about 21 days (3 weeks), there was still a division into subgroups according to the gestational period: 1st gestational week — total n = 19 (C n = 6; SS n = 7 and UCS n = 6); 2nd gestational week — total n = 27 (C n = 8; SS n = 11 and UCS n = 8); and 3rd gestational week — total n = 24 (C n = 7; SS n = 9 and UCS n = 8). Dams of the three groups (C, SS and UCS), at the end of each gestational week under analysis, were submitted to the Elevated Plus-Maze (EPM) to assess their profile of anxiety. Fig. 1 summarizes the sequence of procedures developed throughout the study.

2.4. Chronic stress

Control dams were not submitted to any kind of manipulation after mating except for weighing and box cleaning (3 times a week) and evaluation in the EPM. Dams belonging to SS and UCS groups were submitted to their respective stressor schemes. Stressors were applied only during one of the gestational weeks: first, second or third week of gestation (1st GW, 2nd GW or 3rd GW), as shown in Fig. 1. Dams of SS groups were only separated from others (one animal per box) during 7 days of one of the gestational weeks. It is important to note that dams were kept in the same vivarium and therefore maintained olfactory and auditory contact with the other animals. Dams of UCS groups were socially separated (one animal per box) and also submitted, on each day of the gestational week, to a different stressor, following the adapted model from Echandía and colleagues [34] and González and colleagues [35], as shown in Table 1.

The stressors of unpredictable chronic stress were applied randomly (the order presented in Table 1 is an example of a possible schedule and, therefore, the order was not the same for every animal), but the same stressor was not performed on two consecutive days (for example, sleep deprivation). “Forced swim and exercise” was settled as the last stressor, aiming to have the same procedure on the day of behavioral assessment. The stressors were applied in the morning, except for sleep deprivation, which started in the morning and lasted until the next morning.

2.5. Elevated Plus-Maze (EPM)

All dams were assessed on the last day of the gestational week to which their subgroup belonged (1st GW — assessment on GD 7, 2nd GW — assessment on GD 14 and 3rd GW — assessment on GD 21).

Assessment of the anxiety profile was conducted in the EPM, an animal model of anxiety. The EPM was made of wood, having two open arms (50 × 10 cm) perpendicular to two closed arms (50 × 10 × 40 cm), elevated 50 cm from the floor, according to the model of Pellow and colleagues [36]. A 3-mm wood rim surrounded the open arms, preventing animals from falling. The illumination intensity at the center of the apparatus was 50 lx, avoiding shadow over the arms. The assessment was conducted between 2:00 pm and 5:00 pm. Each dam was individually carried in a polypropylene box lined with sawdust to the experimental room. The test period was 5 min and began when the