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Spontaneous failure of the estrous cycle induces anxiogenic-related behaviors in middle-aged female mice

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HIGHLIGHTS

- Middle-aged mice display increased duration of the estrous cycle.
- Middle-aged mice in diestrus display increased anxiety.
- The perimenopausal period affects anxiety related behaviors in mice.

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ABSTRACT

Clinical studies have shown that women during perimenopause and menopause have a higher incidence in the diagnoses of psychiatric problems compared with men. However, little literature information about the influence of spontaneous perimenopause on anxiety- and mood-related behaviors in mice is available. To this aim, we compared the behavioral responses of middle-aged and young adult female mice both in the diestrus phase in the elevated plus-maze, open field and forced swimming tests. In middle-aged mice, the duration of the estrous cycle was significantly prolonged compared to young adults, thus indicating that our middle-aged mice are in the perimenopausal period. In the elevated plus-maze test, middle-aged mice explored less the open arms when compared to young adults, suggesting an anxiogenic-like phenotype. No significant differences were observed in the estrogen plasma levels and emotional behavior in the forced swim and open field tests. In conclusion, the spontaneous failure of the estrous cycle increased anxiety in middle-aged females. These data suggest that the perimenopausal period has a significant influence on anxiety-related behaviors in female mice.

1. Introduction

The perimenopausal period concerns the interval between the onsets of symptoms around menopause. It begins at the early menopausal transition and ends 12 months after the final menstrual period, thus resulting from the ovarian aging and follicle depletion [1]. This transition period is marked by the appearance of irregular menstrual cycles, besides endocrine and metabolic alterations [2–6]. Increased depressed mood and anxiety are common complaints during menopause and in postmenopause [7–11]. However, a lot of controversy about menopausal transition and mood and anxiety symptoms in midlife women is found in the literature [12–14].

Substantial hormone fluctuations are physiologically found during perimenopause but these alterations are also quite often accompanied by social changes, such as in personal, professional and/or familiar [14]. Estrogen plays an important modulatory role in mood, cognitive regulation and anxiety, hence the effects noted when midlife women are exposed to significant estrogen fluctuations or to estrogen-based therapies (use or withdrawal) [14].

The interaction between menopausal transition and emotional behavior remains unclear, particularly considering the preclinical findings. Two main strategies have been used to investigate the impact of the perimenopause hormonal changes on anxiety- and mood-related behaviors in female rodents: i) administration of drugs which deplete ovarian follicles; and ii) spontaneous aged rodents. Regarding the depletion of ovarian follicles, a recent study showed that the administration of 4-vinylcyclohexene diepoxide (VCD) results in endocrine and anxiety-related changes [15].

Senescent rodents provide a useful but less studied model to investigate the effects of fluctuations in endogenous estrogen levels on

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anxiety- and depression-related behavior. Despite the massive clinical information about the relationship between psychopathologies and menopausal transition, few preclinical studies have shown the effects of spontaneous failure of the estrous cycle on anxiety- and mood-related behaviors in mice. To this aim, behavioral tests, such as open field, elevated-plus maze and forced swimming, were used to evaluate the influence of perimenopause on spontaneous locomotion, anxiety- and mood-related behaviors, respectively. Middle-aged females were compared to young adult mice (both at the diestrus phase) for behavioral assessment and quantification of estrogen levels. Overall, these findings can give support to the influence of spontaneous perimenopausal transition on psychopathologies.

2. Material and methods

2.1. Animals

Twenty five adult (3 months old) and thirty two middle-aged (14 months old) female Swiss mice that had never had a litter were employed in the present study. These animals were obtained from the breeding colony of the Federal University of Rio Grande do Norte. Animals were housed in plastic cages (41 × 34 × 16 cm), 12–15 per cage, under a 12-hour light/dark cycle (lights on 6:00 AM) at a constant room temperature of 23 ± 1 °C, with water and food ad libitum. Experiments were performed between 13:00 and 17:00 h in a sound attenuated room. Animal behavior was assessed by a video camera connected to a personal computer placed in another room. Experiments were analyzed by an expert observer. All experiments were conducted in accordance with Brazilian Law No. 11.794/2008 for animal experimental use. The protocol was approved by the Ethics Committee for Animal Use of the Federal University of Rio Grande do Norte (License No. 024/2013). The observer was blind to the animal condition. This study is reported following the ARRIVE guidelines [16].

2.2. Vaginal cytology

Vaginal smears were analyzed a fresco, and the following criteria were used to identify the estrous stage: estrus was characterized by a predominance of cornified cells; diestrus by the predominance of leukocytes; proestrus by the predominance of nucleated epithelial cells; and metestrus by the presence of cornified cells, leukocytes and cornified cells [17]. Aiming to provide a good standard of comparison, we took into consideration in performing statistical analysis of behavioral tests only those middle-aged and young adult mice which were in diestrus. In order to avoid the effects of manipulation in the behavioral responses, vaginal smears were performed immediately after tests. However, for the assessment of estrogen circulating levels, vaginal smears were done immediately before blood collection. Estrus cycles were followed day to day during approximately 15 days before experiments.

2.3. Behavioral tests

2.3.1. Elevated plus maze

The elevated plus maze consisted of two open (30 × 5 cm) and two wall enclosed arms (30 × 5 × 15 cm) connected by a central platform (5 × 5 cm) as previously described [18]. The apparatus was elevated 40 cm above the floor. The floor and the walls of the enclosed arms of the maze were constructed of brown wood. Each mouse was placed on the central platform, facing a closed arm, and observed for a 5 min time period. The frequency of entry into and time spent in either open or enclosed arms, entries and time spent at the end of open arms, as well as ethological parameters, such as head dippings, stretch attend posture and rearings were recorded. An entry was scored as such only when the animal placed all four limbs into any given arm. Drugs with anxiolytic like activity usually increase the percentage of time spent in and/or frequency of entries into open arms, whereas the reverse holds true for anxiogenic-like drugs. In a previous study from our research group, the effects of intraperitoneally (i.p.) injected diazepam 1 mg/kg, 15 min before the behavioral test were used for validating our experimental conditions [19]. Furthermore, the number of entries into closed arms was used as an index of general activity. The ratio "time spent in open arms/time spent in all (open and closed) arms" was calculated and multiplied by 100 to yield the percentages of time spent in open arms. The elevated plus-maze apparatus was placed in a small closed room lit by a red light, with an intensity of 100 lx in open and 30 lx in closed arms. After each mouse, the apparatus was cleaned with ethanol 10%.

2.3.2. Open field test

The spontaneous locomotor activity of mice was measured using the open field test. During the test, animals were allowed to freely explore the apparatus during 30 min. The apparatus, made of wood covered with impermeable formica, had a black floor of 40 × 40 cm and black walls of 40 cm high. Light intensity of 100 lx in the center of the open field apparatus was used. Each mouse was placed in the center of the open field and the distance traveled during 30 min was registered, through automatic observation (Anymaze, Stoelting Co., Wood Dale, IL, USA).

2.3.3. Forced swimming test

The forced swimming test was performed as previously described [20]. Briefly, mice were dropped individually into a glass cylinder (height: 25 cm; diameter: 15 cm) containing 18 cm of water, maintained at 23–25 °C, and left there for 6 min. A mouse was judged to be immobile when it floated in an upright position, and made only small movements to keep its head above water. Previously, the effect of nortriptyline (a tricyclic antidepressant drug) orally injected 60 min before the test was evaluated [19]. The duration of immobility was recorded during the last 4 min of the 6-min testing time period.

2.4. Electrochemiluminescence assay for the serum total estrogen level

Serum total estrogen levels were measured in some female adult and middle-aged mice. The blood was collected with cardiac puncture in anesthetized animals (thiopental 80 mg/kg, i.p.). Afterwards, blood was allowed to clot at 4 °C, and serum was collected after centrifugation (3000 rpm, 3 min). The serum was stored at −20 °C until assays were performed. The lower limit of detection for total estrogens was 5 pg/mL. A commercial laboratory (Instituto Hermes Pardini — Divisão de veterinária, Vespasiano, MG, Brazil) was contracted to perform these assays.

2.5. Statistical analysis

Data are presented as the mean ± SEM. Differences among groups were detected by Student's t test. Differences were considered significant when $P < 0.05$. Results were analyzed by the INSTAT version 3.06 software (La Jolla, CA, USA).

3. Results

Fig. 1 shows the change in the duration of the estrous cycle of middle-aged and young adult female mice. The duration of the estrous cycle was significantly prolonged in middle-aged compared to young adults (Fig. 1A; $t = 4.46$; df = 21; $P = 0.0002$). This data support the view that our middle-aged mice are in perimenopause.

The total estrogen levels were assessed in the cardiac blood of mice collected during diestrus in young adult and middle-aged female mice. No significant differences in the serum concentrations of estrogens in young adult and middle-aged female mice were observed (Fig. 1B; $t = 1.01$; df = 11; $P = 0.33$). These findings argue in favor of similar