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Early stress leads to effects on estrous cycle and differential responses to stress

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ABSTRACT

V. Mourlon, L. Naudon, B. Giros, M. Crumeyrolle-Arias and V. Daugé. Early stress leads to effects on estrous cycle and differential responses to stress. Physiol Behav—Women are more susceptible than men to stress-related mental disorders. However, few animal studies have been conducted on females. Given the interactions between gonadic hormones and the hypothalamo–pituitary–adrenal (HPA) axis, we hypothesized that the effects of early stress may be different between males and females depending on the state of their estrous cycle.

Using adult Long–Evans rats of both genders, the effects of maternal deprivation were investigated on the estrous cycle length, corticosterone levels after food deprivation or restraint stress procedures, and the negative feedback efficiency of dexamethasone on the HPA axis. The individual length of the estrous cycle was evaluated using vaginal smears. Non-deprived (AFR) females mainly exhibited regular 5-day cycles (40% of the population) and 4–5-day cycles (26%), with fewer 4-day cycles (18%) and irregular cycles (16%). Comparatively, deprived (D) females displayed a significant decrease of 5-day cycles (24%) and a significant increase of irregular cycles (28%). After the restraint stress procedure, D females exhibited higher corticosterone level than AFR females during proestrous. After the food deprivation procedure, D and AFR females maintained dose–response sensitivity to the negative feedback induced by dexamethasone but only during proestrous. No differences were observed between D and AFR males under these experimental conditions. These data highlight the importance of early environmental factors in regulating the spontaneous pattern of the estrous cycle as well as gender- and stressor-dependent sensitivity of the HPA axis according to steroid levels.

1. Introduction

Early-life adverse events, such as childhood abuse or neglect, may alter normal brain development and cause a subsequent increase in the vulnerability to a variety of mental disorders in adulthood [1,2]. Women are more susceptible than men to stress-related mental disorders [3]. However, few animal studies have been conducted on females, although the influence of estrous cycle on stress-induced corticosterone and CRF receptor mRNA levels has been demonstrated [4–7]. Several investigators have used long maternal separation procedures (≥3 h) in male rats and have shown long-lasting effects on the hypothalamo–pituitary–adrenal (HPA) axis during adulthood [8–11, for review 12, but see also 13]. However, few studies have examined the effects of maternal separation in both male and female rats. One study has shown that separated males (3 h per day from postnatal days 3–10) exposed to the elevated-plus maze test display a potentiation of ACTH release compared to separated females, but not in the forced swimming test [14]. In contrast, an increase of HPA axis activity in females subjected to an acute swim stress and attenuated recovery of corticosterone levels in females submitted to restraint stress were observed after long maternal separation (3 h per day from postnatal days 2–14 and 6 h per day from postnatal days 2–10, respectively) [15,16]. It therefore appears that long-lasting alterations in the HPA axis activity are gender-dependent and sensitive to both the type of stressor and the model of maternal separation used. Surprisingly, the effects of maternal separation on HPA responsiveness according to the state of the estrous cycle have not been investigated. Given the interactions between gonadic hormones and the HPA axis, we hypothesized that the effects of early stress may be different between male and female rats, depending on the estrous cycle state of the females. Thus, the present study focused on the long-term effects of maternal deprivation, which consisted of daily

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separation of newborn pups from their mothers as well as littermates from each other for 3 h per day from postnatal days 1 to 14. This maternal deprivation procedure is a more severe postnatal manipulation than the separation of the intact litter from the dam [17]. In adult animals, we examined: (1) the estrous cycle under regular housing conditions and after the transfer to new housing near the experimental room to examine the possible consequences of transfer stress and (2) the corticosterone response of male and female rats to different stressful situations. Restraint stress is a mixture of physical and psychological stressors, with the maximal response of stress effector systems usually seen within the first 30 min following the immobilization stress [8,18–21]. Overnight food deprivation is both a metabolic and psychological stressor [22–26] and recalls the lack of nutrients available during postnatal period in maternally deprived rats. We also assessed the sensitivity of the HPA axis to the glucocorticoid negative feedback by administration of dexamethasone. Special attention was paid to the estrous cycle state of the female rats. We previously showed that there are no differences in baseline levels of corticosterone or in baseline levels of CRF mRNA between D female or D male and AFR rats [11, Bluet MT and Daugé V: unpublished results]. These groups were therefore not studied in the present study in order to simplify the experimental protocol and to use the minimum number of animals required to examine stress reactivity in D and AFR rats.

2. Materials and methods

2.1. Animals

Three series (n = 13, 22, 25) of pregnant Long–Evans rats (Janvier, Le Genest ST. Isle, France) were purchased on day 14 of gestation. Dams gave birth 1 week after inclusion and simultaneously in a 12 h interval. Litters were housed in clear plastic cages in a well-ventilated environment, with constant temperature and humidity (24 ± 1 °C; 50 ± 5%), and a 12 h light/12 h dark cycle (lights on at 0800 h). They received food and tap water *ad libitum*. Cages and sawdust were changed only once per week to avoid excessive handling.

Adequate measures were taken to minimize pain or discomfort. The experimental procedures and care of animals were in accordance with local committee guidelines and the European Communities Directive of November 24, 1986 (86/609/EEC).

2.2. Maternal deprivation

The day of birth was defined as day 0. On postnatal day 1 (PND 1), litters were cross-fostered and culled to 10–12 pups with 1/3 of the litter consisting of male pups and the other 2/3 consisting of female pups. Random redistribution of pups among dams was done to control for possible effects of genetic and prenatal factors, and to obtain similar litter size. Each pup received similar handling during this procedure. It cannot be excluded that litters may have suffered from prenatal stress due to the transport of pregnant rats and that cross-fostering may change maternal behavior. However, the same procedure was applied to all pups from the AFR and D groups before handling, allowing valid data comparison.

The litters were randomly divided into two experimental groups. One group underwent the maternal deprivation procedure from PND 1 to 14 (D group), and one control group (AFR, Animal Facility Rearing) was not separated from the dams but pups were briefly handled once a week during the changing of the bedding. The maternal deprivation procedure consisted of daily separation of the rat pups from their mothers for 3 h, from PND 1 to 14. The deprivation was always performed from 1400 to 1700 h. The mothers were removed from their home cage and placed in a new cage, which was the same at each separation. The pups were then individually placed in temperature- (30/33 °C) and humidity-controlled cages that were divided into compartments, in a room separated from their mothers. These cages contained sawdust covered with absorbing paper. At the end of the deprivation period, each litter was placed in the housing cage and the dam was transferred back to the housing cage. From PND 15 to 21, all pups remained with their mothers. On PND 22, pups were weaned from their mothers and separated according to sex. Males and females were housed in groups of 3 or 4 rats per cage and in two separated ventilated cabinets according to sex until 2.5–3 months of age. For each stressful situation, the same number of proestrus and diestrous females from each litter was used, and rats from each litter received all treatments to avoid any litter effects.

2.3. Estrous cycle

Beginning at 2 months of age, vaginal smears were gently performed each morning in order to monitor the estrous cycle. The smears were obtained throughout a 4-week period prior to the start of experiments, as well as during the 3 weeks of the experiment. Females (n = 296) and males (n = 69) were briefly and gently handled each day only during these 7 weeks. Male rats were handled in a similar manner as the female rats and their underbellies were massaged. The occurrence of 4- and 5-day cycles was analyzed during the first 2 weeks under regular housing conditions. Males and females were housed in independent ventilated cabinets, under the same temperature, humidity and light/dark cycle. After that, males and females were transferred to independent ventilated rooms close to the experimental room that were more exposed to noise and odors than previously, but under the same light/dark cycle, and temperature and humidity conditions. Female cyclicity was examined for 2 additional weeks. The vaginal smears were laid on a Superfrost™ slide, in a drop of methylene blue, and dried at room temperature. Slides were examined under a microscope (10× objective, Axioskop 2 plus, Carl Zeiss, Le Pecq, France) after addition of a drop of water. The state of estrous cycle was identified by the observation of nucleated cells, cornified cells and leukocytes. Proestrous and estrous states were determined by the predominance of nucleated cells or cornified cells respectively. Post-estrous was characterized by a mixture of leukocytes and cornified cells. Diestrous was identified by the main presence of leukocytes. Finally, the classification of the estrous cycle state was based on (I) vaginal smear history, (II) vaginal smear on the day of experiment and (III) observation of physiological criteria after dissection: (a) aspect of uterine horns (ballooned at proestrous, thin at diestrous) and (b) eventual presence of oocytes in the oviduct (oocytes within cumulus-corona radiata at estrous and nude oocytes at post-estrous). The normal cycle is an ovulatory cycle, which can be 4- and/or 5-days in length [27].

2.4. Experimental designs

Experimental procedures used to evaluate stress reactivity are described in Fig. 1.

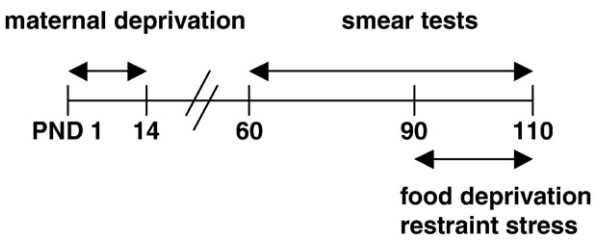

Fig. 1. Experimental procedure used to evaluate stress reactivity. Maternal deprivation started 1 day after birth (PND1) 3 h daily for 14 days. Weaning occurred at PND 21–22. Vaginal smears started at PND 60 for 7 weeks. Food deprivation or restraint stress occurred at PND 90 for 3 weeks.