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Anxiety-like, novelty-seeking and memory/learning behavioral traits in male Wistar rats submitted to early weaning

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HIGHLIGHTS

- The breastfeeding period has been shortening progressively.
- Models of early weaning show that adult rats present several endocrine alterations.
- Maternal deprivation and pharmacological early weaning affect behavior in adult rats.
- Early weaning by wrapping dams with a bandage does not affect behavior at adulthood.
- Hypercorticosteronemia at early weaning may be a relevant imprinting factor.

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ABSTRACT

The most frequently used animal models of early weaning (EW) in rodents, maternal deprivation and pharmacological inhibition of lactation, present confounding factors, such as high stress or drug side effects, that can mask or interact with the effects of milk deprivation per se. Given these limitations, the development of new models of EW may provide useful information regarding the impact of a shortened period of breastfeeding on the endocrine and nervous systems, both during development and at adulthood. Using a model of EW in which lactating Wistar rat dams are wrapped with a bandage to block access to milk during the last three days of lactation, we have recently shown that the adult offspring presented higher body mass, hyperphagia, hyperleptinemia, leptin as well as insulin resistance, and higher adrenal catecholamine content at adulthood. Here, we used this EW model, which involves no pharmacological treatment or maternal separation, to analyze anxiety-like, novelty-seeking and memory/learning behavioral traits in the adult male offspring. To that end, animals were tested in the elevated plus maze, in the hole board arena and in the radial arm water maze. Except for an increased number of rearing events (a measure of vertical activity), no other behavioral differences were observed between EW and control animals. The contrasting behavioral results between the three EW models may be associated with differences in HPA axis function in the offspring at weaning, since it has been observed that bandaging does not affect corticosteronemia while maternal separation and pharmacological EW increase it.

1. Introduction

According to the World Health Organization (WHO), "exclusive consumption of breast milk and no other food or drink, not even water for 6 months of life" is defined as exclusive breastfeeding [88]. Exclusive and prolonged breastfeeding has been associated with protection against long-term chronic diseases, such as obesity and diabetes [33]. Because breast milk mediates unequaled beneficial effects regarding nutritional, immunological and cognitive outcomes in infants, a global public health recommendation is that infants should be solely breastfed for the first 6 months of life [45,46]. On the other hand, women are currently becoming more active in the labor market and, for many new mothers, the breastfeeding period has been shortening progressively [31]. In fact, no more than 35% of infants worldwide are exclusively breastfed during the first four months of life [88].

Epidemiological and experimental studies have shown that nutritional alterations during early periods of life can imprint epigenetic changes, which lead to disorders such as obesity, diabetes, hypertension

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[3,19] and vulnerability to psychopathological disorders such as anxiety and depression at adulthood [13]. This phenomenon, which was initially denominated programming [3] and now is also known as developmental plasticity [32], has been defined as a biological phenomenon that determines the relationship between physical and chemical stimuli during critical periods of early life (gestation and lactation) with the future functional status of the adult offspring.

Experimental models that address the immediate, early and late-emerging repercussions of shortened lactation may be useful to assess its long-lasting effects on the nutritional, metabolic, endocrine and behavioral status of the progeny. Dos Santos et al. [23] observed, for example, that animals weaned at postnatal day (PN) 15 by physical separation from the progenitors are more likely to prefer high-fat diets when compared to animals weaned at PN30, showing a behavioral programming caused by early weaning. Other behavioral changes associated with early weaning through maternal deprivation (MD) include higher anxiety [42,61], higher motor activity [43] and reduced cognitive performance [68].

Since maternal deprivation can, by itself, be an imprinting factor, it is conceivable that its effects on the development of the offspring can mask or interact with the effects of milk deprivation. In addition, it is a less representative model of early weaning in humans since mothers do not usually separate completely from their babies, and when separation is inevitable, a caregiver is frequently present. Thus, our laboratory developed two different models of early weaning (EW) without maternal deprivation. In the first EW model, lactation is interrupted during the last three days (PN19–PN21) of the lactation period through maternal treatment with bromocriptine, a prolactin (PRL) inhibitor [6–8,18,28]. We have shown that, at weaning (PN21), the offspring presented hyperleptinemia [8], hypothyroidism and hypercorticosteronemia [28]. At adulthood, in addition to the development of alterations associated with the metabolic syndrome (MS), such as obesity and insulin and leptin resistance [7], adult rats programmed by maternal hypoprolactinemia presented lower levels of serum TSH, T3 and T4, characterizing a central hypothyroidism [6], hypoadiponectinemia, hypoprolactinemia, hypercorticosteronemia and higher total adrenal catecholamine content [18]. Moreover, we observed that maternal hypoprolactinemia resulted in long lasting effects on behavioral parameters: The adult EW offspring displayed higher anxiety-like behavior and poorer memory/learning performance. No differences were observed regarding novelty-seeking behavior [28]. In the second model, EW is caused by a physical barrier — a breast bandage that prevented pups from suckling from PN19 to PN21 [50,51]. In previous reports, we have shown that, at weaning (PN21), the offspring presented hypoleptinemia [51], hypothyroidism, but normal adrenal hormones [50]. At adulthood, these EW animals presented obesity, hyperphagia, hypoadiponectinemia, hyperglycemia, leptin and insulin resistance, hypoprolactinemia [51] and higher total adrenal catecholamine content [50].

Given our previous behavioral results in the experimental model in which EW was caused by maternal treatment with bromocriptine [28], the aim of the present study was to analyze behavior in the adult offspring of dams in which breastfeeding was interrupted by a physical barrier, that is, lactation interruption that does not involve the use of pharmacological substances or maternal deprivation. Here we focused on anxiety-like and novelty-seeking behaviors, as well as on memory/learning performance. Since we have already published the hormonal profile of the animals used in the current study [50,51], we will discuss our behavioral results in light of the previously reported hormonal and metabolic alterations.

2. Materials and methods

This study was conducted under the institutional approval of the Universidade do Estado do Rio de Janeiro (protocol: CEUA/017/2009). All experiments were carried out in accordance with the declaration of Helsinki and with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health, and followed the principles described in the guide for the care and use of laboratory animals [4]. All animals (Wistar rats) used in the present study were kept in a temperature-controlled (23 ± 1 °C) vivarium on a 12 h light/dark cycle (lights on at 7:00 a.m.).

2.1. Experimental model of programming by early weaning

Three-month-old, nulliparous female rats were caged with male rats (3:1). After mating, each female was placed in an individual cage with free access to food (standard chow for rodents, Nuvilab CR1 — São Paulo, SP, Brazil) and water until delivery. Pregnant rats in our vivarium usually have litters consisting of 10 to 12 pups. In order to avoid the influence of litter size on the programming effect, we only used litters consisting of 10 pups and, at birth, litters were adjusted to 6 male pups per dam to maximize lactation performance [62].

In the 1st postnatal (PN) day, 20 lactating rats were randomly assigned to the two following groups: EW (early weaning, n = 10) – dams were lightly anesthetized with thiopental (0.06 mg/ml/100 g) and wrapped with a breast bandage (physical barrier) to interrupt suckling during the last 3 days of the lactation period – from PN19 to PN21 [50,51]; and C (control, n = 10) — no barrier was used to avoid suckling. After weaning (PN21), all EW and C animals had free access to food and water. Siblings were kept together throughout the experiment since single housing could influence behavior (cage size: 39 cm long × 32 cm wide × 14 cm high) [81].

2.2. Behavioral studies

From PN160 to PN178, 32 EW and 38 C offspring were submitted to the behavioral tests (pups per litter — EW: 3.2 ± 0.1; C: 3.8 ± 0.2). The following tests were used: 1) elevated plus-maze (EPM); 2) hole board arena (HB); 3) radial arm water maze (RAWM). A full description of these testing equipments and protocols can be found elsewhere [27–30]. All testing sessions were performed between 1:00 p.m. and 6:00 p.m. in a sound attenuated room. All tests were video recorded and all behavioral analyses were carried out using the video images of the tests.

Anxiety-like behavior was assessed at PN160 in the EPM. Rats were allowed 10 min to explore the maze. The number of entries and the total time spent in open (respectively: Time OA and Entries OA) and closed (respectively: Time CA and Entries CA) arms and in the central area (Time CN and Entries CN) of the EPM were recorded. The percentage of open arms entries [%Entries OA: Entries OA / (Entries OA + Entries CA)] and the percentage of time spent in the open arms [%Time OA: Time OA / (Time OA + Time CA)] were used as anxiety measures. In addition, Entries CA was used a measure of locomotor activity and the percentage of time spent in the center of the maze (%Time CN: time spent in center divided by total time) was used as an independent measure of decision-making [55,69]. In addition, ethological measures such as head-dipping, grooming and return CA were analyzed [12,15,38]. All animals were returned to their home cages after the EPM test, where they remained until they were tested in the HB. The EPM was cleaned with paper towels soaked in 50% ethanol and dried before each trial.

Novelty-seeking behavior was assessed at PN163 in the HB. Rats were allowed 10 min to explore the arena. The total number of holes explored (nose-pokes) by each animal in the center and in the periphery of the equipment (respectively CH and PH) was noted and used as a measure of exploratory activity [27–30]. The %CH [CH / (CH + PH)] was also calculated. By using a 4 × 4 grid of same-sized rectangles drawn on a transparent overlay positioned on top of the video-images of the tests, it was possible to measure locomotor activity and anxiety-like behavior [1]. The total number of rectangles crossed was used as a measure of locomotor activity. The number of rectangles crossed in