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Effects of early weaning on the circadian rhythm and behavioral satiety sequence in rats

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ABSTRACT

The objective of this work was to study the effect of early weaning on circadian rhythm and the behavioral satiety sequence in adult rats. Male Wistar rat pups were weaned for separation from the mother at 15 (D15), 21 (D21) and 30 (D30) days old. Body weight and food intake was measured every 30 days until pups were 150 days old. At 90 days of age, the circadian rhythm of food intake was evaluated every 4 h for three days. Behavioral satiety was evaluated at 35 and 100 days of age. This work demonstrated that body weight and food intake were not altered, but the behavioral satiety sequence demonstrated that the D15 group delayed satiety compared with the D30 group at 100 days of age. In the circadian rhythm of the food intake study, early weaning (D15) changed food intake in the intermediary period of the light phase and in the intermediary period of the dark phase. In conclusion, our study showed that early weaning may alter the feeding behavior mainly in relation to satiety and the circadian rhythm of feeding. It is possible that the presence of other environmental stimuli during early weaning can cause hyperphagia and deregulate the mechanisms of homeostasis and body weight control. This study supports theories that depict insults during early life as determinants of chronic diseases.

1. Introduction

Some studies demonstrate that epigenetic factors occurring in critical periods of life can affect the development of organs and tissues, provoking permanent structural and physiological alterations (Hales and Barker, 2001; Orozco-Solis et al., 2009; Ravelli et al., 1976). The biological phenomenon that establishes the relationship between these stimulations in the critical period of development, particularly during pregnancy and lactation, and the future functional state is called programming (Barker, 2004; de Moura and Passos, 2005; Lucas, 1994).

Lactation is a critical period because in this period important neurobehavioral development occurs and mother–pup interaction is strong during this process (Caldji et al., 2000; Liu et al., 2000; Plotsky and Meaney, 1993). Environmental changes, malnutrition (Orozco-Solis et al., 2009), hormonal alterations (Passos et al., 2007), or the neonatal stress of maternal separation (Hancock and Grant, 2009) during lactation can provoke permanent functional alterations. Weaning is one of the most important events in the beginning of life for mammals (Nakamura et al., 2008). After weaning, the young mammals become nutritionally and behaviorally independent from their mothers. In rats, spontaneous weaning begins around the third week of life and continues until 30 days of age when the pups cease milk intake completely (Henning, 1981; Krecek and Kreckova, 1957). Interactions between the mother and the pup during the end of the lactation period are important for behavioral development in rodents (Kanari et al., 2005; Kikusui et al., 2005). The reduced maternal care in this phase also has been associated with alterations in the behavioral and physiological responses to stress in adult progeny (Liu et al., 1997, 2000).

Another behavior that can be programmed by nutrition and stress at the beginning of the life is the feeding behavior (Orozco-Solis et al., 2009). Malnutrition at the beginning of life can modify the behavioral satiety sequence (BSS) (Orozco-Solis et al., 2009) and can lead to the development of a greater preference for high fat foods (Cambraia et al., 2001). On the other hand, stress from maternal separation in the lactation period increases palatable food intake (Silveira et al., 2004, 2005). The BSS is an efficient method for the analysis of feeding behavior (Halford et al., 1998). The effects of pharmacological and nutritional manipulations on the natural physiological regulation of food intake can be evaluated by using

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the BSS (Halford et al., 1998; Orozco-Solis et al., 2009). Although a reduction or an increase in food ingestion reflects an effect on appetite, the measurement of food intake alone does not allow to determinate whether these changes are due to an alteration of the physiological mechanisms regulating food intake, or are due to non specific effect such as sedation, hyperactivity, nausea, palatability or enhance of the satiety. This type of behavioral analysis can improve our understanding of the complex psychological and physiological process involved in the regulation of feeding behavior (Halford et al., 1998).

In different mammalian species, mechanisms that control feeding behavior are influenced by circadian rhythm. The light cycle (light–dark) is an effective signal that synchronizes the biological rhythm with the environment (Ohta et al., 2008) and is directly related with feeding behavior (Tallett et al., 2009). The principal structure responsible for generation and the synchronization of biological rhythm to 24 h environmental cycles is the suprachiasmatic nucleous of the anterior hypothalamus (Moore and Eichler, 1972). An imbalance between the rhythm of different compartments differentially controlled by the autonomous nervous system and in turn by the system central nervous may be the cause of metabolic syndrome (Kreier et al., 2003a,b). It is possible that, similar to malnutrition, aggression in the initial periods of life can result in the alteration of the circadian rhythm of the feeding behavior (Orozco-Solis et al., 2009).

Although an important paper has shown that aggression in the neonatal period can exert changes on the programming of feeding behavior, there are few studies concerning the effect of precocious weaning on the programming of this behavior. The objectives of this work are to investigate the effect the manipulation of the weaning period exerts on feeding behavior through the study of the BSS and to investigate if this manipulation can interfere with the circadian rhythm of food intake.

2. Materials and methods

2.1. Animals

Wistar rats, aged between 0 and 150 days old, originated from the Department of Nutrition's colony from the University Federal de Pernambuco – Brazil. Virgin female Wistar rats weighing 250–300 g were obtained and maintained in the laboratory with an inverted light/dark cycle of 12 h (lights on at 6:00 PM) for 15 days for adaptation, with water and a standard diet (Purina do Brasil S/A) ad libitum. After the adaptation period, females were assigned in a proportion of two females for one male. The day of the birth was considered day zero. Day one after birth, pups from different mothers were mixed and the male pups were separated. After, only the male pups were distributed, eight male pups per dam. The experimental groups were classified in accordance with the weaning period. The pups from the D15 group (early weaning, $n = 10$) were separated from the mother on the 15th postnatal day. The pups from the D21 group (control 21 days, $n = 10$) were separated from the mother on the 21st postnatal day. The pups from the D30 group (control 30 days, $n = 10$) were separated from the mother on the 30th postnatal day. After the separation from their mothers, the younglings from D15 or D21 group were maintained together in their cage original (eight pups per cage) from day weaning to day 30. On the 30th postnatal day, animals from all groups were housed in individual acrylic cages (54 cm × 30 cm × 20 cm) for realization of the experimental procedures. All experiments were performed in accordance with recommendations from the Comitê Brasileiro de Experimentação Animal – COBEA, and were approved by the Comissão de Ética em Experimentação Animal from Centro de Ciências Biológicas from the Universidade Federal de Pernambuco.

2.2. Measurement of body weight and food intake

The body weight (g) of each pup was recorded daily from the 15th until the 30th postnatal day. Each pup was removed from home cage individually. After the measurement of the body weight it was returned to its home cage immediately. After the 30th postnatal day, the body weight was recorded every 30 days until pups reached 150 days of life. Food intake was measured on the 35th, 90th, 120th and 150th days of life. For the circadian rhythm study, food intake was measured, beginning on the 90th day, for three consecutive days at 4 h intervals.

2.3. Behavioral satiety sequence

The BSS study occurred on the 36th and 100th day of life. The analysis of the behavioral satiety sequence was performed essentially as described by Halford et al. (1998). Feeding and non-feeding behaviors during a 60 min test meal were continuously scored by a highly trained experimenter, blind to the nutritional status of the animals, and recorded on a videotape to be re-examined by a second skilled observer. Behaviors were categorized as: eating (ingesting food, gnawing, chewing or holding food in paws), grooming (body care movements with the mouth or forelimbs), and resting (sitting or lying in a resting position or sleeping). Other measures scored from the behavioral observation of feeding were: food intake (food consumed (g) during the time of observation of the BSS), meal duration (time (s) over the entire monitoring period the animal was actually eating food), local feeding rate (amount of food consumed (g)/meal duration (min)) and global feeding rate (amount of food consumed (g)/analysis of BSS duration (min)). To promote feeding, food was removed from home cages 3 h before the onset of the test and the presentation of food took place 1 h before the onset of the dark cycle. Food was weighed at the beginning and end of each session.

2.4. Data analysis

Experimental results are expressed as means ± S.E.M. All data were analyzed using a SigmaStat 2.03 demo program. Body weight, feeding behavior and data from the BSS were analyzed using a one-way ANOVA followed by the Tukey test for multiple comparisons between groups. Before using the ANOVA test, data were submitted to variance and normality tests with 5% tolerance.

3. Results

3.1. Body weight and food intake

In the period after weaning, a reduction ($p < 0.05$) of body weight of animals from the D15 group was observed. Between the 16th and 18th day of life, the D15 group presented a minor body weight reduction in relation to the D21 and D30 groups. In the 19th day of life, the body weight of the D15 group was less than the D30 group. Beginning on the 20th day of life, the body weight recovered and did not differ between groups (Fig. 1A and B). No difference between groups in food intake was observed until 150 days of life.

3.2. Behavioral satiety sequence

Behavioral satiety sequence in the progression of feeding behavior, cleaning and resting place without interruption of behavioral sequence was observed. Each 5 min period was quantified for the duration of each behavior and there was no difference between the experimental groups in the 12 evaluation periods.The point of satiety occurred at 41 min in the D15 group, at 39 min in the