



## Perinatal undernutrition stimulates seeking food reward

Amanda Alves Marcelino da Silva<sup>a</sup>, Tássia Karin Ferreira Borba<sup>b</sup>, Lívia de Almeida Lira<sup>b</sup>,  
Taisy Cinthia Ferro Cavalcante<sup>a</sup>, Manuela Figueiroa Lyra de Freitas<sup>d</sup>,  
Carol Gois Leandro<sup>a,c</sup>, Elizabeth do Nascimento<sup>a,d</sup>, Sandra Lopes de Souza<sup>a,\*</sup>

<sup>a</sup> Postgraduate Nutrition, Universidade Federal Pernambuco – UFPE, Recife, PE, Brazil

<sup>b</sup> Postgraduate Neuropsychiatry and Behavioral Sciences, Universidade Federal Pernambuco – UFPE, Recife, PE, Brazil

<sup>c</sup> Centro Acadêmico de Vitória, Universidade Federal de Pernambuco, Vitória de Santo Antão, PE, Brazil

<sup>d</sup> Department of Anatomy, Universidade Federal de Pernambuco – UFPE, Recife, PE, Brazil

### ARTICLE INFO

#### Article history:

Received 15 December 2012

Received in revised form 6 April 2013

Accepted 2 May 2013

#### Keywords:

Feeding behavior

Motivation

Rats

Protein c-Fos

Low-protein diet

### ABSTRACT

Experiments in animals have revealed that perinatal nutritional restriction, which manifests in adulthood, increases food intake and preference for palatable foods. Considering this, we aimed to evaluate the effects of perinatal malnutrition on hedonic control of feeding behavior. In this study, we divided Wistar rats into two groups according to the diet provided to their mothers during pregnancy and lactation: the control group (diet with 17% casein) and low-protein group (diet with 8% casein). We assessed the animals' motivational behavior in adulthood by giving them a stimulus of food reward. We also assessed their neuronal activation triggered by the stimulus of palatable food using FOS protein labeling of neurons activated in the caudate putamen, paraventricular, dorsomedial, ventromedial, and lateral hypothalamic nuclei and amygdala. Evaluation of body weight in malnourished animals showed reduction from the 6th day of life until adulthood. Analysis of feeding behavior revealed that these animals were more motivated by food reward, but they had delays during learning of the task. This finding correlated with the number of c-FOS-immunoreactive neurons, which indicated that malnourished animals had an increase in the number of neurons activated in response to the palatable diet, especially in the amygdala and caudate putamen. The study therefore confirmed our hypothesis that early nutritional insults promote changes in encephalic control mechanisms, especially those related to food intake and search for reward.

© 2013 ISDN. Published by Elsevier Ltd. All rights reserved.

### 1. Introduction

Control of energy balance is regulated by a complex integration of the central nervous system, peripheral signal acquisition, and use and storage of energy, altogether resulting in sensations of hunger and satiety (Wynne et al., 2005). In addition to this homeostatic component, which is organized particularly by the hypothalamus, the expression of feeding behavior is under the influence of reward system and is stimulated by palatable properties (flavor) of food (Berthoud, 2006). This involves the hedonic control structures of the limbic system (Berridge and Kringelbach, 2008) (ventral tegmental area, nucleus accumbens, amygdala, hippocampus,

caudate putamen) and regions of the cortex (orbitofrontal cortex, cingulate gyrus) along with neurotransmitters, the main messengers being dopamine (Barbano et al., 2009), serotonin (Pratt et al., 2009), opioids (Barbano et al., 2009), and endocannabinoids (Andrade et al., 1995, 1996; Mahler et al., 2007). Among these brain structures, the amygdala, caudate putamen, and some hypothalamic nuclei are involved in related events, especially by search for reward (DiLeone et al., 2012). The amygdala is related to the stimulus emotional pleasure or aversive reward provided to an individual. The amygdala processes events, basically incentive value of reward, which can result in a positive or negative stimulus front of reward (Baxter and Murray, 2002). The caudate putamen, in turn, receives dopaminergic projections arising from the ventral tegmental area and the substantia nigra, which regulate motivational aspects and organoleptic properties of food (Farooqi et al., 2007). While the lateral hypothalamus mediate behavioral responses to search for reward (Kelley et al., 2005).

The pleasant sensations promoted by palatable foods can temporarily suppress the homeostatic satiety signals, allowing continued consumption of such foods beyond the energy needs of the body (Erlanson-Albertsson, 2005). The hedonic control of

\* Corresponding author at: Av. Prof. Moraes Rego, 1235, Cidade Universitária, Recife 50670901, PE, Brazil. Tel.: +55 81 21268567.

E-mail addresses: [amanda\\_biomedica@hotmail.com](mailto:amanda_biomedica@hotmail.com) (A.A.M. da Silva), [ftassiakarim@yahoo.com.br](mailto:ftassiakarim@yahoo.com.br) (T.K.F. Borba), [ilalira@hotmail.com](mailto:ilalira@hotmail.com) (L. de Almeida Lira), [taisyferro@yahoo.com.br](mailto:taisyferro@yahoo.com.br) (T.C.F. Cavalcante), [manuelaff@uol.com.br](mailto:manuelaff@uol.com.br) (M.F.L. de Freitas), [carollleandro22@yahoo.com.br](mailto:carollleandro22@yahoo.com.br) (C.G. Leandro), [nlizbeth@gmail.com](mailto:nlizbeth@gmail.com) (E. do Nascimento), [sanlopesufpe@gmail.com](mailto:sanlopesufpe@gmail.com) (S.L. de Souza).

eating behavior is composed of three distinct neurobiological processes: learning, liking, and wanting (Berridge and Kringelbach, 2008). “Learning” reflects past experiences and is related to long-term memory and to the processes of binge eating (Berridge and Kringelbach, 2008). “Liking” is a conscious reaction related to cognitive mechanisms, and palatability is its main stimulus. “Wanting” is a mechanism of motivation that is implicated in the desire to eat a particular type of food, and it may be either unconditioned or learned (Berridge and Kringelbach, 2008). The Wanting is basically the process of incentive value that is a behavioral expression in response to stimuli involving the desire for reward (Berridge et al., 2009).

Changes in these components can result in an increase in the consumption of palatable or rewarding foods, which are often high in energy (Lee et al., 2012). The hedonic stimulus therefore results in a positive energy balance. Excessive consumption of food is an important factor in the etiology of eating disorders, particularly those that lead to obesity. Interestingly, evidence from experimental studies in rodents indicates that nutritional stimuli in early life can permanently affect feeding behavior. Notably, malnutrition in utero and/or during neonatal development results in hyperphagia and a stronger preference for energy-dense foods in adulthood (Bellinger et al., 2004; Archer et al., 2005; Bellinger and Langley-Evans, 2005; Desai et al., 2007a,b; Lopes de Souza et al., 2008; Orozco-Solis et al., 2010). Undernourished rats have been shown to have increased food intake during the perinatal period, with a reduced latency time before eating, intake of larger quantity, and delayed onset of satiety (Orozco-Solis et al., 2009); this is associated with alterations in the expression levels of several hypothalamic neuropeptides that regulate food intake (Plagemann et al., 1999, 2000a,b; Ikenasio-Thorpe et al., 2007) and with decrease in the number of neurons that express galanin and neuropeptide Y (NPY) (Plagemann et al., 2000a,b; Yura et al., 2005). Furthermore, the anorexic action of insulin (Sardinha et al., 2006), leptin (Sardinha et al., 2006), and serotonin (Desai et al., 2007a,b; Lopes de Souza et al., 2008) has been shown to be reduced in the offspring of rats subjected to restriction of proteins or calories. Collectively, these observations indicate that perinatal malnutrition induces a long-lasting dysfunction of the hypothalamic homeostatic control circuitry for food intake. However, it has not been determined whether the regulation of hedonic control is also changed by restriction of nutrients during childhood.

Consequently, we investigated the long-term effects of protein restriction during pregnancy and lactation on the neurobiological processes driven by food reward. Given the relationship between malnutrition in early life and a greater preference for energy-dense foods in adulthood, we hypothesized that increased consumption of palatable foods by such animals occurs as a result of increased motivation for food reward for compromising neural pathways present in potentially hedonic brain areas.

## 2. Materials and methods

### 2.1. Animals

All experiments were approved by the Ethics Committee on Animal Experiments of the Center for Biological Sciences, Federal University of Pernambuco and were performed in accordance with the recommendations of the Brazilian Committee for Animal Experimentation (COBEA).

Virgin female Wistar ( $n = 10$ ) weighing 250–300 g were obtained from their birth vivarium (Department of Nutrition, Federal University of Pernambuco) and were kept on a reversed 12-h light/dark cycle (lights on at 1800 h), under controlled conditions of temperature ( $22 \pm 2^\circ\text{C}$ ), with water and standard diet (Purina, Campinas, SP, Brazil S/A) provided ad libitum. After an adaptation period of 15 days, the rats were mated at a ratio of one male to one female. After confirmation of pregnancy through visualization of sperm in vaginal smear, females were moved to individual cages and fed either a normal-protein diet (17% of casein,  $n = 5$ ) or a low-protein diet (8% of casein,  $n = 5$ ) pregnancy and lactation (Crnic and Chase, 1978; Wiener and Levine, 1983; Falcao-Tebras et al., 2012) (Table 1). Birth was recorded as postnatal day

zero (P0) for the pups. Sexing was performed at 24 h after birth, and the numbers of pups were adjusted to give 8 pups per mother with an equal ratio (4:4) of males and females. In this paper, female pups were used only to complete the litters maintaining the same male:female ratio. The experimental groups consisted of two male rats from each litter and, at the end, a total of 10 animals of each control and low-protein restrict groups were used. Weaning took place at P22 and, from P22 to P35, pups from both groups were fed a diet with a higher energy density ( $>1.2$  kcal/g) than that of the standard diet (Table 1) to stimulate the catch-up growth characteristic of this period (Bieswal et al., 2006). This phenomenon was observed for the first time in undernourished rats, who showed raised growth rate during the re-feeding phase (Osborne, 1914). Initially, the catch-up growth was named growth compensation period (Bohman, 1955). The manipulation of a high-energy diet during this period was a tool used to stimulate the rapid growth of animal in impairment of prenatal undernutrition. From P35, all animals were fed a standard diet (Labina<sup>®</sup>; Purina). Pups were weighed during the whole lactation period (at 1, 6, 16, and 21 days of age) and at 35, 60, and 180 days of age.

### 2.2. Motivation test: runway task incentive

At 60 days of age, rats in both the control and malnourished groups underwent a runway task incentive test (Silveira et al., 2010). This test assesses the motivation of the animals to obtain a stimulation reward, evaluating the time necessary for the animal to get through a center runway to the end, where a box containing the stimulation reward is placed (Pecina et al., 2003). The runway task incentive is used as tool to evaluate this component through a training aimed at searching for a specific target (reward). The food serves as salient incentive for motivation in animals. As rats consume most of their food during the night, behavioral assessments were performed 6–8 h after the onset of the dark phase of the light/dark cycle.

The apparatus used consisted of a tunnel with three compartments: A start box (19 cm  $\times$  14 cm  $\times$  30 cm), a center runway (150 cm  $\times$  14 cm  $\times$  30 cm), and a target box (19 cm  $\times$  14 cm  $\times$  30 cm). The boxes were made of transparent acrylic, and the center runway was made of opaque polypropylene. The images were captured with a video camera positioned in the center runway to allow view of the entire apparatus. The starting box could be moved along the tunnel so as to attain 15–150 cm distant from the target box. The stimulus was placed inside the target box and consisted of 5 g of chocolate-flavored cookies (Chocookies; Nabisco<sup>®</sup>, East Hanover, NJ, USA).

The test consisted of 11 training sessions of 5 min each, held on alternate days for a total period of 22 days. The animals were deprived of food for 4 h before each training session. During the adaptation period (1–3 session), the animal was placed directly in the closed-target box and allowed to access the reward for 5 min. Session 4 the starting box was placed 15 cm away from the target box, and the rats were placed in the start box for 30 s with the door closed. After 30 s, the door was opened, and the animal was allowed to enter the center runway. If the rat did not leave the start box within 3 min, it was gently pushed into the target box. For each subsequent session, the target box was moved further away from the start box (i.e., 30 cm away for session 5, 60 cm for session 6, 75 cm for session 7, 90 cm for session 8, 120 cm for session 9, and 150 cm for sessions 10 and 11). The sessions were defined as previously described by Pecina et al. (2003), with sessions 1–3 considered as the adjustment phase (in which the animal is exposed to a new environment and reward so that the natural neophobic behavior disappears), sessions 4–6 as the pre-exposure phase (in which the animal is exposed to the target box and execution center), sessions 7–9 as the learning-incentive or reinforcement phase (encouraged by the stimulus of reward), and sessions 10 and 11 as the fully trained phase (during which the animal confirms their learning by showing total familiarity with the tests and directly accessing the reward). The speed of task completion for each session was calculated by dividing the latency time taken to reach the target box by the length of the track. The speed for searching the reward in the end provides the motivation to the animal. The rat was considered to have left the starting box when all four limbs were out of the box and was considered to have entered the target box when all four limbs were within the target box. Once the rat entered the target box and started eating, it was allowed to consume the palatable food for 30 s before being removed.

The following parameters were analyzed: (1) latency time before leaving the start box, (2) number of pauses on the track, (3) number of times the rat reversed direction away from the target (this behavior involved turning round toward the start box, and was usually accompanied by sniffing), (4) latency before beginning intake of the palatable food, and (5) time take to carry out the complete task.

### 2.3. c-Fos immunohistochemistry

At 180 days of age, the animals were fasted for 4 h and were given palatable food stimulus (30 g of chocolate cookies–Chocookies; Nabisco<sup>®</sup>, East Hanover, NJ, USA) by placing them on the cages 90 min before transcardiac perfusion to stimulate Fos expression in both control ( $n = 5$ ) and low-protein restrict ( $n = 5$ ) groups. After 90 min, the animals were deeply anesthetized with a combination of ketamine and xylazine (50 mg/kg), followed by transcardiac perfusion, first with saline (0.9% NaCl) and then with 4% paraformaldehyde (PFA). The brains were removed and post-fixed in 4% PFA plus 20% sucrose for 4 h, and then transferred to 0.1 mol/l phosphate buffer plus 20% sucrose for 48 h at  $4^\circ\text{C}$ . Coronal sections of 40- $\mu\text{m}$  thickness were cut on a cryostat. Five series of sections were collected for each animal in 25-well acrylic

Download English Version:

<https://daneshyari.com/en/article/2785983>

Download Persian Version:

<https://daneshyari.com/article/2785983>

[Daneshyari.com](https://daneshyari.com)