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Variations in the neonatal environment modulate adult behavioral and brain responses to palatable food withdrawal in adult female rats☆

Juliana Barcellos Colman[a], Daniela Pereira Laureano[b], Tatiane Madeira Reis[a], Rachel Krolow[c], Carla Dalmaz[b,c], Carla da Silva Benetti[a], Patrícia Pelufo Silveira[a,b,*]

[a] Programa de Pós-Graduação em Saúde da Criança e do Adolescente, Faculdade de Medicina, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[b] Programa de Pós-Graduação em Neurociências, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[c] Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

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ABSTRACT

Background/objectives: Early handling alters adult behavioral responses to palatable food and to its withdrawal following a period of chronic exposure. However, the central mechanisms involved in this phenomenon are not known. Since neonatal handling has persistent effects on stress and anxiety responses, we hypothesized that its involvement in the aforementioned association may be associated with differential neuroadaptations in the amygdala during withdrawal periods.
Methods: Litters were randomized into two groups: handled (H, removed from their dam for 10 min per day from the first to the tenth postnatal day and placed in an incubator at 32 °C) and non-handled (NH). Experiment 1: on PNDs 80–100, females were assigned to receive palatable food + rat chow for 15 or 30 days, and these two groups were compared in terms of palatable food preference, body weight and abdominal fat deposition. In Experiment 2, H and NH rats were exposed to a chronic diet of palatable food + rat chow for 15 days, followed by (a) no withdrawal, (b) 24 h withdrawal from palatable food (receiving only rat chow) or (c) 7-day withdrawal from palatable food (receiving only rat chow). Body weight, 10-min rebound palatable food intake, abdominal fat deposition, serum corticosterone as well as TH and pCREB levels in the amygdala were then compared between groups.
Results: Experiment 1—chronic exposure to palatable food induces comparable metabolic effects after 15 and 30 days. Experiment 2—neonatal handling is associated with a peculiar response to palatable food withdrawal following chronic exposure for 15 days. Rats exposed to early handling ingested less of this food after a 24 h withdrawal period, and displayed increased amygdala TH and pCREB levels.
Conclusions: Variations in the neonatal environment affect both behavioral responses and amygdala neuroadaptation to acute withdrawal from a palatable diet. These findings contribute to the comprehension of the mechanisms that link early life events and altered feeding behavior and related morbidities such as obesity in adulthood.

1. Introduction

Eating is essential to survival, and the consumption of palatable foods is associated with additional advantages in the form of pleasurable sensations, which lead to the overindulgence on this type of food beyond homeostatic needs. The consumption of tasty foods can be triggered by exposure to specific cues (Grosshans et al., 2012; Wang et al., 2009) or stress (Adam and Epel, 2007; Epel et al., 2001). Both of these mechanisms are especially evident in obese individuals (Davids et al., 2010; Gibson, 2012). Currently, the increased

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* Corresponding author at: Hospital de Clinicas de Porto, Departamento de Pediatria, Faculdade de Medicina, Ramiro Barcelos 2350, Largo Eduardo Zaccaro Faraco, 90035-003 Porto Alegre, RS, Brazil. Tel.: +55 51 3359 8000; fax: +55 51 3359 8001.
E-mail addresses: 00032386@ufrgs.br, raty@cpovo.net (P.P. Silveira).

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consumption of energy dense, high-fat, high-sugar 'junk'/palatable foods is considered a major environmental contributor to weight gain and fat deposition.

The phenomenon whereby animals and humans continue to consume high-fat, high-sugar foods even after satiety and despite their negative health consequences is similar to behaviors associated with drug dependence (Erlanson-Albertsson, 2005; Kelley and Berridge, 2002). In fact, studies have revealed substantial overlap between the brain circuitry underlying addictive behaviors and overeating; for instance, both substance-dependent and obese subjects exhibit decreased reward circuit activation in response to the drug of choice or palatable foods (Stice et al., 2008; Tomasi and Volkow, 2013; Volkow et al., 2013). Rats have also been found to self-administer sugar in ways which resemble substance abuse, involving loss of control, cross tolerance, failed attempts to quit, and the spontaneous production of withdrawal signs and symptoms following opiate antagonist administration (Avena et al., 2005; Gold and Avena, 2013; Hoebel et al., 2009).

One factor which has been recently found to influence food preferences and eating behavior over the life-course is fetal/neonatal history (Portella et al., 2012; Silveira et al., 2006b, 2004, 2008). In addition to its known long-term effects on the programming of the hypothalamus–pituitary–adrenal (HPA) axis (Ader and Grota, 1969; Meaney et al., 1985a; Plotsky and Meaney, 1993), the early postnatal environment has been found to have several other effects on food intake. Neonatal handling is a form of early life stress induced in the pups by brief separations from their dam (Raineki et al., 2014) and has been found to increase the intake of palatable foods (Silveira et al., 2004), reduce plasma ghrelin levels (Silveira et al., 2006b) and dopamine metabolism in the nucleus accumbens in adult rats (Brake et al., 2004; Silveira et al., 2010). Although these animals show an increased intake of highly palatable foods, rich in sugar and fat (Benetti et al., 2007; Silveira et al., 2004), neonatally handled rats have also been shown to have better caloric efficiency, decreased levels of triglycerides and smaller abdominal fat depots when chronically exposed to this type of diet (Benetti et al., 2007). Additionally, after periods of chronic exposure followed by sudden withdrawal of the palatable diet, neonatally handled females display fewer behavioral signs of withdrawal (Benetti et al., 2010) but higher rebound intake in relation to controls (Benetti et al., 2013). Therefore, although such neonatal interventions are known to alter the adult behavioral response to palatable food withdrawal after periods of chronic exposure to this type of diet, the central mechanisms involved in this association are not known.

Recent research has shown that heightened preference for high-sucrose and high-fat foods and increased anxiety following the withdrawal of palatable high-fat diets was accompanied by a reduction in tyrosine hydroxylase (TH) and phospho-CREB (pCREB) expression in the amygdala (Sharma et al., 2013). Similar neuroadaptations have been observed following nicotine withdrawal (Pandey et al., 2001), suggesting that decreased CREB transcriptional activity in this region may be important in restoring palatable food intake after withdrawal. Given the persistent effects of neonatal handling on stress responses and anxiety (Meaney et al., 1993), we hypothesized that early-handled animals may display distinct neuroadaptation processes in the amygdala, which could be responsible for their altered behavioral responses to withdrawal after chronic exposure to palatable diets.

2. Methods

Pregnant Wistar rats bred at our animal facility were randomly selected and housed individually in Plexiglas (65 × 25 × 15 cm) cages with sawdust-covered floors, which were kept in a controlled environment (lights on between 07:00 h and 19:00 h, temperature at 22 ± 2 °C, cage cleaning twice a week, food and water provided) until parturition. Litters were kept intact save for handling procedures, which were carried out between 10:00 h and 11:00 h. During this period, the incubator was set up, cages were transported and dams were allowed to acclimate to the new room, pups were carefully removed from the nest, then handled for a period of time until they were returned to their dams. After a brief period, the cages were returned to the animal facility. Researchers also changed gloves before handling each litter to avoid the spread of any odors between nests.

The day of birth was considered postnatal day (PND) 0, and after weaning occurred on PND 21, rats were placed in home cages similar to those described above, each of which housed two to three animals. A hundred and forty experimental female rats derived from 24 different litters, were used in the present experiments. Although no more than 2 pups from the same litter were used in the same experiment, all female pups were used either in Experiment 1 or in Experiment 2. The number of animals used was estimated from previous experiments (Benetti et al., 2013, 2010; Silveira et al., 2004). After weaning, rats had free access to food (standard lab rat chow or standard lab rat chow + palatable food, see below) and water. The rat chow used was Nuvilab®, having 2.95 kcal/g, 15% protein, 12% fat, 73% carbohydrate. All animal procedures followed national and international ethics guidelines (Brazilian Law 11.794/08, the Universal Declaration on Animal Welfare issued on January 27th, 1978, and the Council for International Organizations of Medical Sciences—CIOMS/WHO standards), and were approved by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre (GPPG/HCPA, project number 11-0025). The study was performed in climate-controlled rooms within our animal research facility (Unidade de Experimentação Animal/HCPA).

2.1. Neonatal stress model

Non-handled group (NH): pups were left undisturbed with their dams until weaning. Dirty sawdust was carefully removed from one side of the cage, without disturbing the mother or the pups, and replaced by clean sawdust by the principal researcher.

Neonatal handled group (H): pups were gently removed from their home cages and placed in a clean cage lined with clean paper towels, inside an incubator set to 32 °C. After 10 min, pups were returned to their dams. This procedure was carried out in the first 10 days of life, after which pups were left undisturbed until PND 21.

2.2. Habituation to the palatable diet

Starting at PND 60 days, rats were habituated to the palatable diet (4.82 kcal/g, 14% protein, 34% fat, 30.2% carbohydrate, 20% of which was derived from sucrose, manufactured by Prag Soluções®, Jaú, SP, Brazil). To decrease the neophobia to the new food, a previously weighted amount of this food was placed in a clean cage (similar to the animal's own home cage), in which rats were individually placed for 3 min every day for 5 consecutive days (Silveira et al., 2004). For the rest of the day, rats were kept under very mild food restriction (receiving approximately 80% of the usual rat chow intake over 24 h).

2.3. Experiment 1—Comparison between 15 and 30 days of exposure to palatable food

All animals were weighed between PNDs 80 and 100, before being randomly distributed between the following groups according to body weight:

(a) 15 days of chronic exposure to palatable food: (a1) NH + rat chow + palatable food and (a2) H + rat chow + palatable food for 15 days in the home cage;
(b) 30 days of chronic exposure to palatable food: (b1) NH + rat chow + palatable food and (b2) H + rat chow + palatable food for 30 days in the home cage.

During this period, food intake was monitored by placing known quantities of the diets in each cage and measuring the amount remaining after each day. Since food intake was measured in each cage, data is represented as mean intake per rat, per cage (n = number of cages in each group), while preference for palatable food was measured by dividing the palatable food intake by total intake in kcal. Body weight was measured once a week using a scale with 0.01 g precision (Marte®, Canoas, Brazil). After decapitation, the two major portions of abdominal fat (gonadal and retroperitoneal adipose tissue depots) were dissected and weighed using a scale with 0.01 g resolution (Marte®, Canoas, Brazil). Results were expressed as percentage of body weight.

2.4. Experiment 2—Withdrawal from palatable food—Comparison between 0, 24 h and 7-day withdrawal periods

Only the animals who underwent chronic exposure to the palatable food for 15 days were used in Experiment 2. After the 15-day exposure period, rats in the H and NH groups were assigned to one of the following: (a) no withdrawal (b) 24 h withdrawal from palatable food (receiving only rat chow) and (c) 7-day withdrawal from palatable food (receiving only rat chow). Each animal's body weight as well as 10-min palatable food intake were measured in a manner identical to that described in Section 2.2. Rats then fasted for 4 h before being decapitated for tissue collection.