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CONCURRENT MATERNAL AND PUP POSTNATAL TOBACCO SMOKE EXPOSURE IN WISTAR RATS CHANGES FOOD PREFERENCE AND DOPAMINERGIC REWARD SYSTEM PARAMETERS IN THE ADULT MALE OFFSPRING

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Abstract—Children from pregnant smokers are more susceptible to become obese adults and to become drug or food addicts. Drugs and food activate the mesolimbic reward pathway, causing a sense of pleasure that induces further consumption. Here, we studied the relationship between tobacco smoke exposure during lactation with feeding, behavior and brain dopaminergic reward system parameters at adulthood. Nursing Wistar rats and their pups were divided into two groups: tobacco smoke-exposed (S: 4 times/day, from the 3rd to the 21th day of lactation), and ambient air-exposed (C). On PN175, both offspring groups were subdivided for a food challenge: S and C that received standard chow (SC) or that chose between high-fat (HFD) and high-sucrose diets (HSDs). Food intake was recorded after 30 min and 12 h. Offspring were tested in the elevated plus maze and open field on PN178–179; they were euthanized for dopaminergic analysis on PN180. SSD (self-selected diet) animals presented a higher food intake compared to SC ones. S-SSD animals ate more than C-SSD ones at 30 min and 12 h. Both groups preferred the HFD. However, S-SSD animals consumed relatively more HFD than C-SSD at 30 min. No behavioral differences were observed between groups. S animals presented lower tyrosine hydroxylase (TH) content in the ventral tegmental area, lower TH, dopaminergic receptor 2, higher dopaminergic receptor 1 contents in the nucleus accumbens and lower OBRb in hypothalamic arcuate nucleus. Tobacco-smoke exposure during lactation increases preference for fat in the adult progeny possibly due to alterations in the dopaminergic system.

Key words: lactation, programming, smoke, feeding preference, behavior, reward system.

INTRODUCTION

Several reports have shown that nutritional and hormonal influences in critical windows of development can permanently alter physiological parameters in adult life. This process, known as metabolic programming, is associated with several chronic adult diseases such as obesity, dyslipidemia, diabetes and cardiovascular disease (Barker, 2003; De Moura et al., 2008). Nowadays this phenomenon is also called developmental plasticity (Gluckman and Hanson, 2007). Besides, it is known that environmental aspects, such as exposure to cigarette smoke, can also act as programming factors, contributing to obesity development for example. Indeed, epidemiological data show that maternal smoking can be a risk factor for child and teenager obesity and hypertension (von Kries et al., 2002; Wideroe et al., 2003; Goldani et al., 2007).

Smoking during pregnancy is associated with prematurity, lower birth weight, lower child growth, and higher infant mortality (Hegaard et al., 2006; Fenercioglu et al., 2009). During lactation, our group showed that smoking is associated with short and long-term alterations in rat pups (Santos-Silva et al., 2011, 2013). The offspring exposed to cigarette smoke during lactation showed lower body weight, lower retroperitoneal fat mass and increase of adrenal function (catecholamine and corticosterone). On the other hand, at adulthood (6 months-old), offspring were programed for hyperphagia, higher central adiposity, hyperleptinemia, hyperglycemia, dyslipidemia, decreased adrenal function and increased thyroid function (Santos-Silva et al., 2013).

It is known that children from mothers who smoked during pregnancy have changes in food preference in adulthood. In a study cohort, individuals consumed more carbohydrates than protein, compared to those whose mothers did not smoke during pregnancy (Ayres

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Abbreviations: ARC, arcuate nucleus; DAT, dopaminergic transporter; EDTA, ethylenediaminetetraacetic acid; FPLSD, Fisher's Protected Least Significant Difference; HEPES, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); HFD, high-fat diet; HSD, high-sucrose diet; mANOVA, multivariate analyses of variance; NAc, nucleus accumbens; OBRb, leptin receptor; OF, open field arena; pJAK2, phosphorylated janus kinase 2; pSTAT3, phosphorylated signal transducer and activator of transcription 3; RIA, radioimmunoassay; SC, standard chow; SOCS3, suppressor of cytokine signaling 3; SSD, self-selected diet; TH, tyrosine hydroxylase; uANOVA, univariate analyses of variance; VTA, ventral tegmental area.

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et al., 2011). In addition, experimental studies have shown that exposure to tobacco smoke during pregnancy increases the offspring's consumption of palatable food (Al Mamun et al., 2006; Franke et al., 2008). It has also been shown that children whose parents smoke are more vulnerable to becoming addicted to drugs and comfort food (Vargas et al., 1999).

Both drugs and food activate the mesolimbic reward pathway, causing a sense of pleasure that further induces consumption of these substances. The intake of these substances stimulates neurons of the ventral tegmental area (VTA) to release dopamine in the nucleus accumbens (NAc). Dopamine binding on dopaminergic receptors mediates sensations of reward or pleasure (Mark et al., 1991; Bassareo and Di Chiara, 1997; Hajnal et al., 2004; Rada et al., 2005; Liang et al., 2006). In addition, it has been shown that individuals with binge eating disorder and obesity exhibit dysfunctions in the brain reward system that lead to food addiction (Johnson and Kenny, 2010). Besides, hormones such as insulin and leptin may modulate the brain reward system stimulating reward, reducing the preference for food intake or drug use (Figlewicz, 2003; Figlewicz et al., 2007). Conversely, animals with insulin and/or leptin resistance tend to consume more palatable food or drugs (Figlewicz et al., 2007).

Considering early evidence for metabolic changes in offspring exposed to cigarette smoke during early postnatal development (Santos-Silva et al., 2013), it is possible that this obese progeny presents changes in the brain reward system, making them more susceptible to food addiction. Thus, the aim of this study is to evaluate whether exposure to cigarette smoke during lactation is associated with changes in dopaminergic signaling in the brain reward system in adult offspring as well as alterations in the leptin signaling pathway in the hypothalamic arcuate nucleus (ARC), and whether these alterations are associated with changes in offspring's food preference. Based on evidence that nicotine, considered the main psychoactive substance present in tobacco smoke, has significant effects on anxiety levels (Brioni et al., 1993; Cohen et al., 2009; Kupferschmidt et al., 2010) and induces hyperactivity (O'Neill et al., 1991; Vaglenova et al., 2004), we also assessed anxiety-like behavior and locomotor activity in the present study to verify if tobacco smoke exposure during lactation has significant long-term behavioral effects.

EXPERIMENTAL PROCEDURES

Our experimental design was approved by the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro (CEUA/066/2012; CEUA/019/2014), which based its decision on the principles promulgated by Brazilian Law n° 11.794/2008. The experiment was conducted to minimize the number of animals and any suffering, following the ethical doctrine of the three ''Rs'' (reduction, refinement and replacement).

Wistar rats were kept in a temperature-controlled room (25 ± 1 °C) with artificial light–dark cycle (lights on 7:00 a.m., lights off 7:00 p.m.). Three-month-old, female rats were caged with male rats (ratio of 3:1). After mating, each female was placed in an individual cage with free access to food and water until delivery.

Experimental model of direct tobacco smoke exposure during lactation

Three days after birth, nursing rats with their litters were randomly assigned into the following groups:

(1) Smoke-exposed (S group, $n = 8$ dams) – dams and offspring were placed into a smoking machine (TE-10, Teague Enterprises, Davis, CA, USA), 4 times per day (1 h each exposure). This machine generated tobacco smoke from 1 research cigarette type 3R4F at a time (nicotine = 0.73 mg/cigt; Total Particulate Matter = 11.0 mg/cigt; Tar = 9.4 m g/cigt; Carbon Monoxide = 12.0 m g/cigt; Reference Cigarette Program, University of Kentucky, Lexington, KY, USA). A smoke mixture containing 89% sidestream smoke (smoke released from the burning end of a cigarette) and 11% mainstream smoke (smoke from the puff stream), as a surrogate for active smoking (Abreu-Villaça et al., 2010, 2013), was generated by the smoking machine in a staggered manner at the rate of a single 35 ml puff of 2-s duration each min. During exposure, the total suspended particulate was measured by weighing Teflon-coated fiber filters (TX40H120-WW, Pallflex Products Co., Putnam, CT, USA) before and after a 5-min period, when air was collected from the chamber. There were 12 periods of collection, which generated levels of 24.0 ± 2.4 mg/m^3 (mean ± SEM).

(2) Control (C group, $n = 10$ dams) – dams and offspring were exposed to ambient air in a chamber similar to the one used for tobacco smoke exposure.

At birth, litter adjustments were performed and six male pups were kept per S or C dam to maximize lactation performance. Weaning occurred at PN21. Two pups per litter were removed and used in a different study while the remaining four pups from the same litter were kept in the same cage. Body mass was monitored every 4 days until PN180 and data were expressed as sum of body mass from PN21 until PN180. We used 32 S and 40 C offspring, four animals per litter.

Feeding study

We used 32 S and 40 C offspring, four animals per litter. At PN175, two randomly selected animals per litter from both S and C groups were submitted to the food challenge test, while the other two offspring remained in the standard diet. Offspring were fasted from 9 a.m. until 9 p.m. Offspring were fasted because food deprivation increases the compulsion to drugs and palatable foods (Avena et al., 2005). The food challenge was offered at night, during the active period for rodents, as conducted by Avena et al. (2008). After the fasting period, the diets described below were offered for one continuous period of 12 h. The first record of food intake occurred at