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Active maternal phenotype is established before breeding and leads offspring to align growth trajectory outcomes and reflex ontogeny ☆

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HIGHLIGHTS

- Active maternal phenotype presents intra-individual responses to environmental stimulus.
- Maternal investments on growth trajectory overlap perinatal physical activity.
- Phenotipic plasticity influences reflex ontogeny of the pups based on matrilineal experience.

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ABSTRACT

The main goals of this study were to classify dams according to the level of voluntary physical activity before breeding and during pregnancy/lactation and to evaluate the effects on growth trajectory and reflex ontogenesis of offspring. Voluntary physical activity was ranked by traveled distance, time and daily estimated calorie burned. Thirty-five female Wistar rats were classified as control (C, n = 5), inactive (I, n = 10), active (A, n = 8) and very active (VA, n = 12). During 30 d before breeding, traveled distance, average speed, time and calorie burned were daily recorded for active and very active groups. Traveled distance was recorded each 2 h every day of adaptation. Body weight, food intake and fasting glycemia were measured throughout the experiment. During lactation, litters were evaluated in terms of physical features and reflex ontogeny. VA showed a progressive increase in the traveled distance and time while A dams presented constant values. VA rats showed lower body weight and higher food intake. During pregnancy, both groups performed less than 1 km/day. Pups from A and VA dams showed higher lateral–lateral axis of the skull, longitudinal axis, tail length, and anticipation of the pavilion and auditory canal opening, and erupting incisors. I, A and VA groups showed a delay of righting, cliff aversion and vibrissae placing reflexes. In conclusion, active maternal phenotype is established before breeding allowing mothers to fit ecological and influencing growth trajectory outcomes and reflex ontogeny of the offspring based on matrilineal experience.

1. Introduction

Maternal stimulus has been used to assess how organisms adapt to different ecological conditions and how they establish investment strategies on offspring during development [1]. A poor maternal nutrient environment early in life is considered a predisposing factor for the development of obesity, hypertension, diabetes type 2 and related diseases in adult offspring [2–4]. Likewise, maternal high-fat milieu induced a higher body weight gain in mothers and their offspring developed larger adipocytes during growth, insulin resistance in muscle and adipocyte, and altered cholesterol profile at adulthood [5,6]. In rats, a controlled moderate to low-intensity exercise before and during gestation (5 days/week, 60 min/day, 40% to 70% of VO_{2max}) increased the mothers' resting oxygen consumption and altered growth trajectory of the offspring [7].

In the offspring, differential ontogeny of physiological system, such as faster postnatal growth, childhood obesity or behavioral changes can be predicted by perinatal background (nutrition, drugs, stress and physical activity) [3]. For example, faster postnatal growth is related with undernutrition during pregnancy [8] and childhood obesity is related with maternal obesity and excessive body weight gain during pregnancy [9,10]. Our previous studies have shown that maternal

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pharmacological manipulation of the serotoninergic system is associated with delayed reflex ontogeny, growth retard, reduction of intraspecific aggression, and deficits in locomotor activity [11–13]. Previous studies using animal models have shown that maternal physical training on a treadmill (5 days/week, progressive reduction of duration and intensity 50–20 min/day, 65–30% VO_{2max}) resulted in reduced body weight gain, insulin secretion, and high resting oxygen consumption (resting VO_2) and attenuated the delayed reflex ontogeny induced by undernutrition [14–16].

The biological phenomenon underlying those associations is termed phenotypic plasticity and refers to the ability of a phenotype associated with a single genotype to produce changes in the organism in terms of morphology, physiology and/or behavior in response to any environmental circumstances [17]. In this context, it is plausible to consider maternal voluntary physical activity as a proactive prediction on the part of the mother in order to buffer an eventual injured growth trajectory on the part of the offspring. The term "voluntary physical activity" is used when locomotion is not related with survival or directly motivated by any external factor [18]. In humans, voluntary physical activity affects the energetic cost and can be motivated by psychological (behavioral) or physical (sports and physical fitness) rewarding [19]. In animals, motivation for voluntary exercise can be measured by wheel running, which is rewarding and represent the classic self-motivated behavior [18].

Previous studies have reported the effects of maternal physical activity in rats [20–22]. For example, an enriched housing, including wheel running during pregnancy, transiently enhances memory and hippocampal neurogenesis in the offspring [20,21]. Voluntary physical activity in the pregnant rat attenuated the loss of mineral bone over the pregnancy/lactation period [22] and improved insulin sensibility in the adult offspring [23]. It was also seen that maternal voluntary physical activity provided long-lasting protection from neurodegeneration and brain plasticity [24]. However, less is known about the variation of the effects of physical activity according to the maternal fitness as well as the timing of the exercise during pregnancy [7,25].

The animal models for voluntary exercise during pregnancy present a methodological limitation since dams are submitted to voluntary physical activity in the beginning of gestation without a representative period of physiological adaptation-induced exercise. Moreover, previous studies did not consider that maternal phenotype for physical exercise can vary according to the load of activity (distance traveled), intensity (average speed), frequency (hours per day or times per week) and duration (minutes per session) [7]. In addition, there is no information of a comprehensive description of the pattern of voluntary physical activity behaviors that are established before breeding, the consequences during pregnancy and lactation and the effects on earlier time points of ontogenesis of the offspring.

In the present study, our hypothesis was built on the theoretical model of phenotypic plasticity that considers the existence of an intra-individual variation which brings out the dual influence of the environment and genes during development. Thus, maternal physical activity previously to pregnancy, as an environment responsible for variation in its phenotype over the time, enables the maternal investment on the energy stores during gestation. This strategy of investment will lead changes in the growth patterns and reflex ontogeny of the offspring. Hence, the main goal of this study was to classify dams according to the level of voluntary physical activity before breeding. In addition, we described the physical activity during pregnancy/lactation and the effects on growth trajectory and reflex ontogenesis of the offspring.

2. Material and methods

The experimental protocol was approved by the Ethical Committee of the Biological Sciences Center (protocol no. 23076.022745/2011-11), Federal University of Pernambuco, Brazil, and followed the Guidelines for the Care and Use of Laboratory Animals [26].

Table 1
Composition of the diets.

Ingredients	AIN-93G* g\100 g	AIN-93M* g\100 g
Corn starch (87% carbohydrates), g	39.74	46.47
Casein (protein ≥80%), g	20.00	14.10
Dextrinized starch (92% tetrasaccharides), g	13.20	15.50
Sucrose, g	10.00	10.00
Soya oil, g	7.00	4.00
Cellulose, g	5.00	5.00
Mineral mixture (AIN-93G-MX)*, g	3.50	3.50
Vitamin mix (AIN-93-VX)*, g	1.00	1.00
L-Methionine, g	0.30	0.18
Choline bitartrate (41.1% choline), g	0.25	0.25
Tert-butylhydroquinone (TBHQ), g	0.014	0.008
Macronutrients		
Total energy (cal/g)	3.56	3.44
Protein	18%	14%
Lipids	18%	11%
Carbohydrates	64%	75%

* Reeves et al. [27].

2.1. Animals

Thirty-five 12-wk-old virgin female albino Wistar rats (*Rattus norvegicus*) were obtained from the Department of Nutrition, Federal University of Pernambuco, Brazil and were maintained at a room temperature of 22 ± 1 °C with a controlled light–dark cycle (dark 08.00 am–8.00 pm). Standard laboratory chow (Table 1) and water were given ad libitum according to the period of life: AIN-93G (gestation/lactation) and AIN-93M (maintenance) [27]. Special cages were built with a stainless steel wheel running and dams were allowed to run for a period of four weeks. After the period of adaptation (four weeks), females were placed into a standard cage and mated (1 female for 1 male) for a period of 2–4 days. Females had no access to the running wheel during mating. The day on which spermatozoa were present in a vaginal smear was designated as the day of conception, day 0 of pregnancy. Pregnant rats were then transferred to their original cages with free access to the running wheel throughout pregnancy, and up to postnatal day 15. Dam's body weight and food intake were measured twice a week during the four weeks before breeding, pregnancy and nursing. On postnatal day 1, litters were reduced to 8 pups per mother, ensuring only males per litter when possible. Eventually, litters were completed to 8 pups with 2–3 females when necessary. Wheels were locked on postnatal day 15 to prevent the pups from running and/or being injured. Pups from dams in the pilot study ($n = 10$) were not evaluated. Not all animals successfully performed the exercise required by their group. Two of the twelve-five animals were excluded in the final analysis for failing to have eight or more live offspring (1 control and 1 very active). The litters of eight pups from each mother represent the sample that was evaluated: control ($n = 4$); inactive ($n = 6$); active ($n = 6$); very active ($n = 7$). The evaluation of physical features, growth, and reflex ontogeny of male pups was daily performed during the suckling period. The collection of behavioral data was performed under blind conditions.

2.2. Voluntary physical activity measurements

Female Wistar rats (initial body weight 226 ± 0.8 g) were singly housed into an acrylic cage (cage size: 34 cm height, 27 cm width and 61 cm length). A stainless steel wheel (27 cm diameter) was placed into the cage for running physical activity with food and water ad libitum. A wireless cyclocomputer (Cataye, model CC-AT200W, Colorado, USA) was attached in the wheel to calculate and display trip information, such as average speed, trip distance, trip time, total distance traveled, estimated calorie burned, and the current time. Distance was determined by counting the number of rotations, which was translated