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Enriched environment restricted to gestation accelerates the development of sensory and motor circuits in the rat pup

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ABSTRACT

The effects of stimulating environments on the neural plasticity of the adult brain have been well explored; however, how an enriched environment (EE) affects the mother-fetus interaction is poorly understood. We hypothesized that an enriched environment restricted to pregnancy will succeed in accelerating the development of sensory and motor circuits in the offspring. Pregnant Wistar rats were maintained either under a standard condition – two animals per standard cage- or an enriched environment – eight subjects in larger cages with different physical configurations-. After birth, litters from both groups ($n = 16$ per group) were cross-fostered with mothers that were simultaneously maintained under standard environment during pregnancy. Sensory and motor development were studied in the pups of both groups with a battery of reflex and physical tests. Auditory and gait reflexes appeared two days earlier in the offspring of EE rats as compared to control subjects ($p < 0.05$). In addition, EE pups displayed a better performance in righting reflex, inclined board and geotaxis tests ($p < 0.05$). Differences were found even three weeks after birth. We conclude that EE limited to the phase of pregnancy stimulates the development of pups *in utero* so that they are born with a higher grade of development.

1. Introduction

During pregnancy, the mother is the source of oxygen, nutrients and a variety of hormones and trophic factors, providing the progeny of a favorable microenvironment for growth and development. To obtain an adequate uterine environment, which is critical for pups' long-term physiology and health, the mother undergoes a variety of metabolic adaptations to meet the needs of the litter and placenta. Changes in the dynamics of several maternal hormones and growth factors play an essential role on the pattern of fetal growth and differentiation. Therefore, external environmental factors that induce modifications in the mother's metabolism can considerably affect the embryonic and fetal development by inducing changes in hormonal and growth factor levels (Langley-Evans, 1997). In this sense, the effects of different stressors during pregnancy on the physiology and behavior of the progeny have been largely studied. For example, exposing the pregnant mother to restraint or a predator's odor alters cognitive performance, neuronal integrity and hormonal neuromodulation of the pups (Lordi et al., 2000; Saboory et al., 2011). Thus, evidence shows that the development *in utero* can be highly sensitive to be altered by environmental factors that modify the physiological and metabolic adaptations of the dam during pregnancy.

Enriched environment (EE), on the other hand, is an experimental condition designed to study the effects of sensory experience on the brain and behavior. Given that animals in EE are housed in large cages containing different interacting objects such as tunnels, balls, nesting material, running wheels and usually a high number

Abbreviations: EE, enriched environment; STD, standard; PD, postnatal day; IGFs, insulin-like growth factors; GLM, general lineal model.

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of subjects per cage, they receive a higher and continuous sensory, cognitive and motor stimulation, compared to subjects living in standard laboratory conditions (for a review, see Baroncelli et al., 2010). EE has been shown to exert profound morphological, physiological and behavioral changes in the juvenile, adult and aged brain. Increased dendritic branching and length, augmented neurotrophic factors and improvement in learning and memory are some consequences of exposing animals to EE (Connor et al., 1982; Dahlqvist et al., 1999; Ickes et al., 2000; Soffié et al., 1999).

The effects of gestational EE on the development of the nervous system of the progeny are much less known. Recently, it was observed that maternal EE accelerated the temporal dynamics of cell migration and cell death in the retina of the prenatal rat (Sale et al., 2007). Similarly, a precocious eye opening was observed in mice pups when mothers were housed in EE conditions from the last week of pregnancy to the first postnatal weeks (Cancedda et al., 2004). Physiologically, cell proliferation in the dentate gyrus of the hippocampus was observed to be modified in fetus of mice housed in EE conditions during pregnancy (Maruoka et al., 2009). Since trophic factors are involved in the development of neuronal systems, a plausible hypothesis is that the EE restricted to the gestation period will modify the reproductive physiology in the mother in such a way that *in utero* development of sensorial and motor circuits in the progeny is accelerated.

To test this hypothesis, we maintained pregnant rats either under standard conditions or under EE during the whole pregnancy. After birth, litters were cross-fostered with timed mothers maintained in a standard environment during pregnancy. Litters with foster mothers were maintained under standard condition during lactation. Given that maturation of neurological reflexes and motor coordination are hallmarks of nervous system development (Altman and Sudarshan, 1975), we used a battery of tests to quantify the sensory and motor function of the pups, which comprised measurements of physical development, reflex testing and locomotor maturation (Fox, 1965). These tests are fairly noninvasive and require very little time to complete.

2. Materials and methods

2.1. Experimental design

At the beginning of the experiment, virgin female rats were randomly assigned to either environmental enrichment (EE, $n = 16$) or standard (STD, $n = 16$) housing. After seven days in these conditions, one Wistar adult male rat and one female were housed together for a maximum of two days. This facilitated mating, which was confirmed by the presence of sperm in a vaginal smear. Then, each female was returned to its respective housing condition for the entire pregnancy. On delivery day, each litter was removed from its mother, culled to 10 pups (five of each sex, as possible) and housed with a foster mother, which was maintained simultaneously in standard conditions during her pregnancy. With this procedure, we ensured that the effects of EE conditions on the pups were obtained exclusively from their biological mother during pregnancy. Timed-pregnant foster mothers were also nulliparous rats with similar weights at the beginning of the study. Thus, each foster mother with its respective adopted litter was housed in the standard condition from birth until the end of the experiments.

2.2. Enriched and standard housing

Every EE cage ($120 \times 100 \times 70$ cm) housed eight animals per cage, four pregnant rats (EE rats) and four additional filler rats, and consisted of three floors with lid ramps, a plastic tube, small plastic balls and nest material. In order to increase environmental novelty, other three procedures were conducted: interacting objects were rearranged every four days, location of food was daily moved and the same eight animals per cage were translated to a second EE cage with a similar size but with a different spatial configuration, during the second week of gestation, and returned them to the first EE cage for the third gestational week. On the other hand, STD subjects were housed two animals per standard cage, containing only bedding material.

2.3. Experimental animals and ethics statement

Female Wistar rats weighting 225 ± 20 g at the beginning of the experiments were reared in our animal facility. Subjects were housed with an artificial 12/12 light/dark cycle at $22\,^{\circ}C \pm 2$, adequate ventilation and food pellets and water *ad libitum*. Cage cleaning was done every five days. Animal care was carried out in accordance with the procedures of the National Guide for the Production, Care and Use of Laboratory Animals (Norma Oficial Mexicana NOM-062-ZOO-1999), which complies with international guidelines of the Society for Neuroscience on the ethical use of animals.

2.4. Assessment of pup development

2.4.1. Litter features and physical development

The weight of each mother and each litter was recorded at birth, as well as the number of pups per litter. The days each pup opened both eyes and their two incisors erupted were also recorded and results obtained for each litter were averaged. Litters aimed to study eye opening and incisor eruption (eight litters per group) were different from litters for reflex tests (eight litters per group).

2.4.2. Sensorimotor maturation and reflex testing

Entire litters were removed from their does and one male and one female pup from each litter were randomly selected, marked, and individually tested in a different temperature controlled room ($32\,^{\circ}C \pm 2$), with a very low level of ambient noise. At the end of the daily tests, the two selected pups per litter were returned to the litter and then simultaneously returned to the respective doe cage. All tests were conducted blindly by the same experimenter.

2.4.2.1. Auditory startle. A clicker was used at a distance of 30 cm above each pup and we observed if the animal displayed a startle response immediately after the click. The day of appearance of this reflex was recorded.

2.4.2.2. Negative geotaxis. Each pup was placed on an inclined board with its head facing downwards. The time taken by the pup to turn around 180° to put its head upwards was recorded. If the pup took longer than 60 s, the test was stopped and recorded as 60 s. From postnatal day (PD) 2 to 8, the board had an inclination of 20° and from PD 10 to 16, an inclination of 50°. The day of appearance of this reflex was also recorded.

2.4.2.3. Grasp reflex. Each pup was held in the air and the forelimbs were gently stroked with a thin rod. The reflex was considered present when the animal closed the stimulated paw around the rod, and the first day of grasping was recorded.

2.4.2.4. Crossed extensor reflex. Each pup was held in the air, the left hindlimb was pinched and we observed if extension of the right hindlimb was present. The day of disappearance of this reflex was recorded.

2.4.2.5. Gait. Each pup was placed in the center of a paper circle of 13 cm in diameter and the time taken to the animal to move