



Severe maternal undernutrition and post-weaning behavior of rabbits[☆]



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HIGHLIGHTS

- Effect of severe prenatal undernutrition (50%) on rabbits' behavior was examined.
- Prenatally undernourished (PU) rabbits were less active compared to the others.
- The effects were more obvious in PU rabbits, during the final third of pregnancy.

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ABSTRACT

The objective of the present experiment was to investigate the implications of severe maternal undernutrition on the post-weaning behavior of rabbits. Thirty two does were randomly assigned to four groups: the control group (C) that was fed 100% of the recommended energy maintenance requirements throughout pregnancy and lactation and the U1, U2 and U3 groups that were fed 50% of the recommended energy maintenance requirements between the 6th and the 19th day of pregnancy, between the 20th and the 27th day of pregnancy and between the 3rd and the 10th day of lactation, respectively. At the age of 50 and 65 days, behavior of rabbits in cages was recorded and rabbits were further subjected to an open-field test; a paradigm used as an indicator of fear and emotional distress.

Significant differences were found between the U2 and the other experimental groups for the duration of eating and drinking, and duration of locomotory and investigatory behaviors ($P < 0.05$). At the age of 65 days, duration of comfort behaviors was also lower in the U2 compared to the other groups ($P < 0.05$). On the other hand, duration of resting appeared to be the highest in the group of rabbits born from undernourished does between the 20th and the 27th day of pregnancy ($P < 0.05$). Duration of resting was significantly increased, although frequency and duration of eating and drinking were significantly decreased at the age of 65 days ($P < 0.05$). Male rabbits had greater values for locomotory and investigatory behaviors, and duration of eating and drinking compared to the females ($P < 0.01$). A decreased activity of rabbits during the light period was observed (as expected), whereas an increase in duration of locomotory and investigatory behaviors was observed during the first four and the last 4 h of the light and dark periods, respectively ($P < 0.05$).

Rabbits born from the U1 and U2 group of does displayed increased rates of latency to leave the start position compared to the other groups during the implementation of the open-field test ($P < 0.05$). No other significant differences in the exhibition of the examined behaviors were found between the treatment groups, with the exception of 'moving forelegs' activity that had the lowest value in the U3 group of rabbits.

It is concluded that severe maternal undernutrition (50% of the recommended maintenance requirements) especially between the 20th and the 27th day of pregnancy has significant effects on the exhibition of rabbits' post-weaning behavior, since the offspring from the U2 undernourished does were significantly less active compared to the other experimental groups.

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[☆] This paper is dedicated to the loving memory of Prof. Zafeiris Abas, scientific supervisor of this project, who has so unexpectedly passed away during the writing process of this paper.

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1. Introduction

Maternal nutrition can program long-term effects on the offspring without necessarily affecting size at birth, since a stimulus or insult at a critical, sensitive period of early life can have permanent

consequences on structure, physiology, metabolism and behavior (fetal programming) [1]. This discovery of a link between the in utero experiences and the later metabolic and behavioral abnormalities or disorders is one of the most important advances in epidemiology research of recent years [2].

Maternal stress during gestation can influence the physiological development of the offspring, although these effects vary considerably, depending on the nature, intensity and duration of the stressor as well as on the stage of gestation at which the stressor is effective [3]. There are critical periods in development when the organism is particularly vulnerable to these malnutrition effects. The type and magnitude of these consequences depend on the stage of brain development at the time when the malnutrition is imposed and its duration. Scientific data clearly demonstrate that the earlier the dietary insult, the more severe and more permanent is its effect [4].

Prenatal stress has also programming effects on the hypothalamic–pituitary–adrenocortical (HPA) axis, brain neurotransmitter systems and cognitive abilities of the offspring, leading to an increase in the exhibition of anxiety and emotionality, especially during stressful situations [5–7]. In general, stressors, such as malnutrition, appear to impose a limitation on the complexity of neuronal circuits and on the functional capabilities of the brain. As a result, this brain dysfunction can ultimately influence attentional processes and interactions of the organism with the environment, i.e. a decreased responsiveness to environmental stimuli and functional isolation from the environment, leading to various types of learning disabilities [4]. Generally, the undernourished animal has difficulty coping with novel situations that require adaptive responses and accommodations to the new environmental demands, as a result of the inadequate hippocampal formation, which is a prominent archicortical structure of the limbic system that comprises a large number of neural components involved in the regulation of autonomic and somatic behaviors [8,9].

Rabbit can be classified as a perinatal brain developer like the human infant, meaning that the spurt in brain growth and development occurs close to the time of birth [10] and it was therefore chosen as an animal model in the present experiment. According to previous studies, alterations in the fetal environment, such as severe maternal undernutrition, can have crucial long-term consequences on offspring development, physiology and behavior in rat [4]. As a result, we carried out the present study to determine possible effects of severe prenatal undernutrition (50% of the recommended energy maintenance requirements) during different time periods in does on the exhibition of their offspring behavior at the age of 50 and 65 days.

2. Materials and methods

2.1. Animals

Thirty-two does (*Oryctolagus cuniculus*) of a hybrid commercial breed (Hyla Nouvelle Generation), of similar age (7.5 months old) and body weight (4338 ± 134 g) were purchased from a breeding farm. Upon arrival at the experimental facilities, does were kept indoors in individual cages ($0.40 \times 1.00 \times 0.39$ m) and seven days later does were artificially inseminated (sperm from Hylamax bucks). Seven days after the artificial insemination, does were randomly allocated to four groups: the control group (C) ($n = 8$) that was fed 100% of the recommended energy maintenance requirements throughout pregnancy and lactation and the three nutrient restricted groups U1 ($n = 8$), U2 ($n = 8$) and U3 ($n = 8$) offered 50% of the recommended energy maintenance requirements between the 6th and the 19th day of pregnancy, between the 20th and the 27th day of pregnancy and between the 3rd and the 10th day of lactation, respectively. According to the experimental design, 100% of the recommended energy maintenance requirements of the feed were also provided to the undernourished groups before and after the examined time periods, until weaning (during lactation the does were also offered the appropriate amount of feed to cover the

needs for milk production). Maintenance and milk production requirements were estimated according to Xiccato and Trocino [11]. The diet consisted of a commercial concentrate mixture (in pellets) containing barley (25%), wheat bran (14%), soybean meal (9%), sunflower meal (10%), alfalfa meal (20%), sugar beet (15%), soybean oil (1%), molasses (3%), limestone (1%), monocalcium phosphate (1%), salt (0.3%), lysine (0.1%), methionine (0.1%), threonine (0.05%), choline (0.15%) and a vitamin & mineral premix (0.2%). Does had free access to water.

Rabbit kits were born after 30.7 ± 0.3 days of gestation. At the second day *post partum*, kits were cross-fostered within treatment groups in order to ensure equal litter sizes of ten pups per doe. After weaning (35th day of age), sixty-four (32 animals per sex) of a total of 317 rabbits were randomly selected and kept indoors in individual cages ($0.41 \times 0.33 \times 0.29$ m) with wire mesh floors, under the following environmental conditions (temperature: 22.5 ± 3.5 °C; relative humidity: $50 \pm 20\%$; lightning: 0:00–12:00 h/12:01–24:00 h light/dark cycle). Each cage was equipped with a metal feeder and an automatic nipple drinker. Feed was provided *ad libitum* and rabbits had free access to water. The diet consisted of a commercial concentrate mixture (in pellets) containing barley (17%), wheat bran (23%), soybean meal (7%), sunflower meal (11%), alfalfa meal (20%), sugar beet (15%), soybean oil (1%), molasses (3%), limestone (1%), monocalcium phosphate (1%), salt (0.4%), lysine (0.1%), methionine (0.1%), threonine (0.05%), choline (0.15%) and a vitamin & mineral premix (0.2%).

The methods used in the present experiment were approved by the Research Ethics Committee of the Department of Animal Science and Aquaculture of the Agricultural University of Athens under the guidelines of “Council Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes”.

2.2. Behavioral recordings of offspring in their cages

At the age of 50 and 65 days, behavior elements of thirty-two rabbits (four males and four females from each treatment group) were recorded on three consecutive days. The behaviors observed could be categorized in the five following groups: maintenance (eating and drinking), resting, comfort and locomotory–investigatory. The definitions of these behavioral patterns are [12,13]:

- Frequency of eating: Oral seizing of feed and chewing/munching of it or having the muzzle over the feeder for over 10 s
- Duration of eating (min): Cumulative duration of acts of eating in a feeder
- Frequency of drinking: Drinking water from nipple drinkers
- Duration of drinking (min): Cumulative duration of acts of drinking in a nipple drinker
- Resting: Sleeping, lying at any position (unsleeping, stretched) and sitting
- Comfort behaviors: Any behavior form connected with the own body of the animal (washing, licking, starching)
- Locomotory and investigatory behaviors: Any voluntary change of position (treading, running, hopping, prancing) and behavior forms connected to the cage or to its equipments (rubbing, licking, grawing, smelling, marking with the chin).

The behavior of the rabbits was recorded using four video cameras with infrared lighting (TX-14300A, Turbo-X). Each camera was placed in a fixed position in order to record behavior patterns in 8 cages of rabbits. The recorded data were stored in a digital video recorder equipped with a hard disk (TX168, Telexper Inc., USA). One person decoded all the recordings and was blind to the group for all the behavioral measurements.

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