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Prenatal stress alters the developmental pattern of behavioral indices of sexual maturation and copulation in male rats

Enrique Hernández-Arteaga [a], Marisela Hernández-González [a,*], Mayra Liliana Ramírez- Rentería [a], Mayra Linné Almanza-Sepúlveda [a], Miguel Angel Guevara [a], Marcela Arteaga Silva [b], Herlinda Bonilla Jaime [b]

[a] Instituto de Neurociencias, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico
[b] Departamento de Biología de la Reproducción, Universidad Autónoma Metropolitana, Iztapalapa, P.O. Box 55 535, México 09340, D.F., Mexico

HIGHLIGHTS

- Prenatal stress alters the development of the behavioral indices of sexual maturation.
- Prenatal stress decreases the frequency of genital grooming during puberty in rats.
- Prenatal stress decreases the frequency of penile erections during puberty in rats.
- Prenatal stress delays the occurrence of preputial separation in rats.
- Prenatal stress alters the sexual behavior of adult male rats.

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ABSTRACT

Gestation and pre-puberty are critical periods during which several environmental factors can drastically affect the adequate development of subjects. Considering that stress is one of the most common factors to which subjects may be exposed during gestation, the present study evaluated the effects of prenatal stress on the behavioral indices of sexual maturation in male rats, including genital grooming (GG), preputial separation (PS), and spontaneous penile erections (SPE) during puberty, and on copulatory parameters during adulthood. Stress was exerted by immobilizing the female rats once per day for 2 h from days 14–21 of pregnancy. The young rats born to the dams in the stressed group (SG) later presented a delayed occurrence of PS with a delayed onset and lower frequency and duration of GG compared to a control group (CG). Less than half of the subjects in SG presented SPE, and those that did showed delayed onset and lower frequency and duration. In adulthood, fewer subjects in SG showed sexual behavior responses (intromission and ejaculation), and their mount and intromission latencies on the first day they ejaculated were longer than those of the CG rats. Findings from this study provide additional evidence that stress caused by immobilization during the third period of pregnancy exerts a negative effect in the short-term (*i.e.*, around puberty) by altering the typical development of GG and SPE and the occurrence of PS, while also demonstrating that this effect persists in the long-term, when it affects the performance of copulatory behavior in mature male rats.

1. Introduction

The biological mechanism through which the body attempts to re-establish homeostasis when affected by internal and/or external adverse forces (stressors) is known as stress [1]. Stress can exert significant effects on the expression of several physiological and behavioral indices, depending on the nature and temporality of the stressors involved [2,3,4], as well as on the period of the life-cycle in which it is experienced.

Gestation refers to the period in which a fetus develops, beginning with fertilization and ending at birth, while puberty is defined as the transitional period between the juvenile and adult states, characterized by the onset of sexual maturation in the hypothalamic-pituitary-gonadal (HPG) system. This, in turn, leads to the development of secondary sex characteristics and fertility. Both periods are characterized by rapid interactive neural, endocrine, and morphological changes and hence, are highly-sensitive to stress.

Around puberty, male rats present morphological and behavioral changes that are considered indices of sexual maturation. These include

* Corresponding author at: Instituto de Neurociencias, Universidad de Guadalajara, Francisco de Quevedo 180, Col. Arcos-Vallarta, CP 44130 Guadalajara, Jalisco, Mexico.
E-mail address: mariselh@cencar.udg.mx (M. Hernández-González).

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testicular descent, preputial separation (PS), genital self-grooming (GG), and spontaneous penile erections (SPE). Expression of these behaviors has been associated with improved sexual performance in male rats [5,6,7].

Preputial separation is a prerequisite for attaining complete ejaculation [8,9]. In Long-Evans and Sprague-Dawley rats this normally occurs at an average of 45 days of age [5]. This morphological parameter has been considered a reliable non-invasive indicator of the progression of puberty and of the androgen status of pubertal rats [10].

Genital grooming is a complex motor act that appears relatively late in ontogeny. It shows a characteristic developmental pattern around puberty, increases markedly between weaning and early post-puberty [11] and reaches its highest manifestation around 45–48 days post-birth [7,12]. GG is also associated with pubertal growth of the prostate gland and seminal vesicles in juvenile rats. Thus, the level of anogenital stimulation received during their developmental period may play an important role in the copulatory behavior of adult male rats [7,11,13,14].

Penile erections that occur in rats with no female present are called spontaneous penile erections (SPE). They are usually characterized by the extension of the engorged glans beyond the sheath, and frequently coincide with GG, or are followed quickly by it [15,16]. A significant relationship between the patterns of occurrence of GG and SPE in male rats around puberty has been reported [7], and a high correlation between greater frequency and longer duration of GG and SPE and early onset and better performance of sexual behavior in adult male rats has also been described.

Sexual activity in male rats is characterized by the performance of a series of behavioral patterns that appear after puberty and depend on circulating hormone levels in the prenatal and peripubertal periods, as well as on interactions with the environment [16,17]. The rat's sexual behavior is sensitive to stress; for example, there are reports that mount frequency and intromission rates are altered after acute and chronic immersion in cold water, while electrical shocks applied to the feet affect copulatory parameters only when exposure is chronic [18]. Similarly, it has been reported that sexual performance in adult male rats is highly-sensitive to prenatal stress which can affect the functionality of various brain structures involved in modulating sexual behavior [19–24]. Ward [19], for example, showed that stress induced by immobilization and exposure to constant light three times a day during the final third of gestation, reduced the number of male rats that copulated, and those that did showed an increased ejaculation latency.

Although several works have focused on the impact of stress on sexual behavior, most have used adult male rats. These studies has clearly demonstrated the deleterious effect of several types of stressors on the copulatory parameters [19,21,24]; however, to the best of our knowledge, one issue that has not yet been addressed is the effect of prenatal stress on the developmental pattern of the indices of sexual maturation and how these may, in turn, alter sexual performance in adulthood. Considering that the restraint is one of the stressors most often used to induce stress in pregnant rats because it does not cause pain and does no serious damage to fetuses, but does impair sexual behavior by facilitating the occurrence of lordosis in the male progeny [24], the aim of this study was to determine whether prenatal stress provoked by immobilization (a form of restraint) affects indices related to sexual maturation, such as preputial separation, genital grooming, and spontaneous penile erections, during the peripubertal period and sexual behavior in adulthood.

2. Methods

2.1. Animals

Twelve pregnant Wistar rats were obtained from a colony bred at the Institute of Neurosciences at the University of Guadalajara. These dams were maintained under a 12/12-h light/dark cycle (lights on at 08:00 h) at 22 ± 23 °C, with food and water *ad libitum* during gestation and lactation. On days 14–21 of pregnancy, 6 of the dams were randomly assigned to a stress group (SG), and the other 6 to a control group (CG). On the experimental days, the dams from both groups were kept for 2 h in a soundproofed room, but only the SG females were placed in Plexiglas animal holders (17 × 7.5 × 5.8 cm) once per day for 2 h (11:00 h–13:00 h), following the protocol for immobilization-induced stress modified by Velazquez-Moctezuma, et al., [24]. The control dams remained in their home cages in the soundproofed room also for 2 h.

All of the prepubescent rats (n = 26 males; 13 per group) used in this study were the progeny of these 12 females. At 22 days of age (day of birth = 1), the pups were weaned and sexed. Both groups were nourished with food and water *ad libitum* throughout the experiment. Animal care and all procedures involving the rats were approved by our Institutional Animal Care and Use Committee, in accordance with NIH specifications.

2.2. Procedure

The young male rats from each group (SG, CG) were housed four or five animals per cage, and maintained throughout the experiment in a testing room located far from the colony room. This measure was designed to ensure that all behavioral testing carried out around the time of puberty was performed in the absence of any olfactory, visual or auditory stimuli emitted by females and to minimize any disturbance that might affect the males during the observation period. In this way, we ensured that the male pups were exposed to females only during the copulatory tests conducted from 42–105 days of age.

2.3. Behavioral testing

Subjects were observed in their home cages, which measured 32 × 47 × 20 cm. The cages were made of polycarbonate with wire tops and equipped with shavings as bedding. The occurrence of GG and SPE was observed every second day during days of age 25–47, from 12:00–14:00 h. Behavioral data were recorded in real time for 2 h daily by at least two experimenters who remained seated 0.5 m from the test cage inside the testing room. An episode of GG was recorded every time one of the males licked its testicles or penis. An SPE was identified when light pelvic thrusts (less vigorous than those characteristic of mounts and intromission responses) were followed by an upright seated posture and the emergence of the engorged glans penis from the distal penile shaft that coincided with, or was quickly followed by, penile grooming. The frequency and duration of each episode were recorded for each behavior. To ensure correct identification during simultaneous observation, the four male subjects in each cage were color-coded.

Preputial separation was monitored daily from 30 days of age between 11:00 and 13:00 h of the dark phase. Each male rat was placed on its back, and light manual pressure was applied at the posterior base of the penis, which is normally retracted into the sheath. Total preputial separation was confirmed when the penile sheath could be retracted to fully expose the dorsal surface and approximately half of the ventral surface of the glans penis.

Sexual behavior tests were performed between 17:00 and 21:00 h every second day from days of age 42–105. The male rats were placed in the test cage (43 × 53 × 20 cm) individually and allowed 5 min to adapt before a sexually-experienced, receptive female was introduced into the cage. The stimulus females of the same strain were rendered sexually-receptive by subcutaneous injection of 5 μg of estradiol benzoate 36 h before testing and then 500 μg of progesterone 3 h before the test session. The following behavioral variables were measured: (1) number of mounts (NM), mount with pelvic thrusting but no vaginal penetration; (2) mount latency (ML), the time from the entrance of the receptive female into the observation cage to the first mount;