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Impairment of rat tooth eruption in pups born to mothers exposed to chronic stress during pregnancy

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ABSTRACT

Objective: Tooth eruption is a multifactorial process in which bone tissue plays a prevailing role. In this study we evaluated the bone overlying the developing tooth germ and the degree of tooth eruption of the first mandibular molar in pups born to mothers subjected to constant light during pregnancy.

Design: Pregnant rats were divided into two groups: mothers chronically exposed to a 12:12 light/light cycle (LL) from day 10 to 20 of pregnancy and controls (C) maintained on a 12:12 h light/dark cycle. Pups from each group were euthanized at the age 3 or 15 days.

Buccolingually oriented sections of mandibles were stained with haematoxylin–eosin or for histochemical detection of tartrate resistant acid phosphatase (TRAP). The histomorphometric parameters evaluated were bone volume, number of osteoclasts, TRAP+ bone surface, number of TRAP+ and TRAP− osteoclasts per mm^2 and degree of tooth eruption (mm).

Results: It was found an increase in bone volume (LL: 58.14 ± 4.24 vs. C: 32.31 ± 2.16; $p < 0.01$) and a decrease in the number of osteoclasts (LL: 3.5 ± 0.65 vs. C: 8.03 ± 1.31; $p < 0.01$) and TRAP+ cells (LL: 0.84 ± 0.53 vs. C: 8.59 ± 1.26; $p < 0.01$) in 3-day-old pups born to LL-exposed mothers. These observations are consistent with the decrease in the degree of tooth eruption observed in 15-day-old experimental pups (LL: -0.605 ± 0.05 vs. C: -0.342 ± 0.02; $p < 0.0001$).

Conclusion: Our results suggest that chronic constant light applied as a pre-natal stressor impairs the resorptive capacity of osteoclasts involved in the formation of the eruption pathway and consequently the degree of tooth eruption.

1. Introduction

Prenatal development in mammals appears to proceed largely under instruction and direction from the individual's genes. However, it has been extensively demonstrated that during the course of prenatal development, it is also affected by influences external to the mother and associated to different susceptibility of the foetus to "prenatal programming" as a result of its genetic makeup.[1] A large body of human epidemiological data as well as experimental studies suggest that external factors operating in prenatal life strongly affect

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developmental processes throughout life.[2–4] These environmental factors can influence the ontogenesis of organisms and could be crucial for establishing postnatal plasticity.[5,6] In line with this, it is known that mammals are synchronized with the environmental photoperiod, which entrains the main circadian clock located in the hypothalamic suprachiasmatic nuclei.[7] The latter nuclei integrate environmental signals, generating circadian rhythms synchronized to daily variations.[8] This complex process integrates and interrelates three physiological systems (nervous, immune, and endocrine), translating environmental information by means of neurotransmitters, cytokines and hormones.[9–11] This environmental information is transmitted by the mother to her foetus during early development, generating optimal maternal programming of offspring plasticity to postnatal life.[12]

Prenatal environment may become adverse under certain circumstances, such as poor placental function, maternal infection, or when mothers are subjected to stressing conditions. Thus, during foetal life, maternal exposure to chronic stress would seemingly act as a disruptive factor for optimal offspring development, and generate short- and long-term alterations in the temporal organization of neuroendocrine, biochemical, physiological, and behavioural processes.[9–11] In addition, prenatal stress is associated with the secretion of high concentrations of maternal glucocorticoids (GCs) by stimulation of the hypothalamic–pituitary–adrenal (HPA) axis.[9,13] The increase in circulating maternal GCs is known to strongly influence maternal programming.[11,14]

Continuous constant light (LL) is used as a chronic stressor in a number of animal models.[15–17] This LL stressing stimulus desynchronizes the individual neurons of the principal circadian clock in adult rodents.[18] Besides, in rat pups, prenatal exposure to LL suppresses lactate and malate dehydrogenase enzymatic daily variations in testis and epididymis,[19,20] suppresses maternal plasma melatonin rhythm,[21] increases anxiety-like behaviour and decreases copulatory behaviour.[10,22]

In addition, Meek et al.[23] reported by means of macroscopic observation a delay in tooth eruption of 9-day-old mice pups born to stressed mothers. Tooth eruption is a multifactorial tightly regulated process which starts prenatally and is a programmed and localized event. It involves the movement of a tooth from its position inside the osseous crypt into the oral cavity, where it appears in a specific position, at an appointed time.[24] During the intraosseous phase of tooth eruption, at the ultrastructural level, bone resorption is carried out by mature osteoclasts in the bone overlying the developing tooth germ where the eruption pathway forms. At the same time, there is an increase in the number of osteoclasts on the alveolar bone surface, due to the fusion of mononuclear preosteoclast cells. In rats, the great influx of these cells into the dental follicle occurs on post-natal day 3, resulting in a peak of osteoclasts.[25] An osteoclast is a multinucleated cell that dissolves bone mineral and enzymatically degrades extracellular matrix proteins. Therefore, the maturation of osteoclasts and the regulation of resorption of the bone overlying the developing tooth are critical events that involve molecular signals synthesized by dental follicle cells.[26] Tooth eruption is a valuable model to study the physiology of bone remodelling given that the emergence of a tooth into the oral cavity involves bone turnover events such as bone formation and bone resorption.

Despite the fact that prenatal adverse events have complex influences on development of rat pups, the effects of prenatal LL stress on bone modelling and remodelling are poorly known. The present study is the first to report data on the influence of constant light applied as a chronic prenatal stressor during the second half of gestation on the osteoclasts involved in the formation of the tooth eruption pathway of the first mandibular molar in 3-day-old rats, and on the degree of tooth eruption in 15-day-old rats.

2. Materials and methods

2.1. Animals

Three months old female Wistar rats weighing 250–300 g were used. The Animal protocol was approved by the local Bioethics Committee of School of Dentistry, Universidad Nacional de Córdoba, Argentina, and is in keeping with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe Ǹ 123, Strasbourg 1985). We made all possible efforts to minimize both the suffering and the number of animals used.

The animals were maintained on a 12:12 light/dark cycle (lights on at 07:00 a.m.) under controlled temperature (23 ± 1 °C) and were fed laboratory chow and water ad libitum. Estrous cycle was determined daily by examination of vaginal smears, and females were mated with males on the night of ovulation. The onset of pregnancy was confirmed by the presence of spermatozoa in vaginal smears on the following morning, considered day 0 of gestation.

On day 10 of gestation, 16 mothers were assigned to the following groups: Group LL, which included 8 mothers chronically exposed to a 12:12 light/light cycle (LL) of cool white light at 100 lux during 11 consecutive days (between days 10 and 20 of gestation); and Group C including 8 mothers maintained on a 12:12 light/dark cycle, which served as control (C). This constant light model was validated in previous studies performed at our laboratory demonstrating that constant light initiated on day 10 of gestation interrupts maternal foetal synchronization, which begins between days 11 and 12 of gestation.[27] Food ingestion of pregnant rats was measured daily during prenatal stress. After delivery, the litter was adjusted to eight pups per mother. Two or three male pups of each mother were selected so that each group (LL and C) included 12 pups. All the pups were weighed on post-natal days 3, 5, and 15 and euthanized on post-natal days 3 or 15 in order to perform histomorphometric analysis.

2.2. Histological and histomorphometrical analyses

After euthanasia, pup mandibles were resected, fixed in 10% phosphate-buffered saline-formaldehyde for 24 h, decalcified in EDTA (pH 7.2) for 30 days, dehydrated in acetone and xylol solutions, and embedded in paraffin at 58–60 °C for 4 h. Serial buccolingually oriented sections, approximately 7–8 μm thick,