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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,

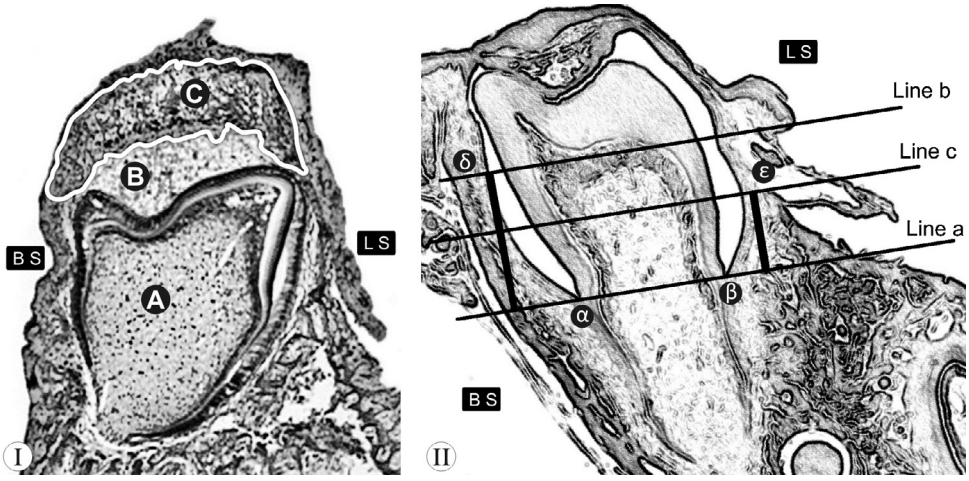

Fig. 1 – (I) Representative illustration of a buccolingual section of 3-day-old control pup mandible. A: first mandibular molar; B: stellate reticulum; C: Bone overlying the developing tooth germ. All histomorphometric parameters were measured in the bone overlying the developing tooth germ of the first mandibular molar of 3-day-old pups (contoured area). Total bone volume was calculated by adding cancellous bone tissue plus mesenchymal tissue found in the studied area. (II) The degree of tooth eruption of the first mandibular molar was measured on microphotographs of buccolingual sections stained with H&E. Line "a" was drawn from the lingual (α) to the buccal (β) cement enamel junction. Line "b" was drawn parallel to line "a" and through the upper point of the buccal alveolar bone crest (δ). The distance between lines "a" and "b" was considered a measure of the degree of buccal tooth eruption (DBTE). Line "c" was drawn parallel to lines "a" and "b" and through the upper point of the lingual alveolar bone crest (ε). The distance between lines "a" and "c" was considered a measure of the degree of lingual tooth eruption (DLTE). All tooth eruption measurements were expressed as negative values. Thus, the greater the distance, the lesser the degree of tooth eruption (BS: buccal side; LS: lingual side).

were obtained and stained with haematoxylin and eosin (H&E) or for histochemical detection of tartrate resistant acid phosphatase enzyme (TRAP).[28] The latter is a protein expressed by osteoclasts, preosteoclasts, and dendritic cells. Sections were photographed at 2.5× and 4×, digitalized, and histomorphometrically evaluated[29] using an Image Pro Plus 6.1 computer software (Media Cybernetics Inc; EE.UU., 2006). The best three sections, in terms of quality and buccolingual orientation, obtained from each animal were selected, and measurements were performed twice on each section. The obtained values were averaged to calculate the mean for each animal. A total of twelve pups were evaluated in each group (LL and C).

The following histomorphometric determinations were performed on microphotographs of H&E stained sections of the bone overlying the developing tooth germ of the first mandibular molar of 3-day-old pups (Fig. 1.I).

1. BV/TV (%): bone volume was expressed as cancellous bone to total volume ratio. Total volume was calculated by adding cancellous bone tissue plus mesenchymal tissue.
2. N.Oc/mm^2: number of osteoclasts with 2 or more nuclei per mm^2.
3. N.Nc/Oc: number of nuclei in each osteoclast located in the studied area.

The following histomorphometric parameters were determined on TRAP stained sections of the studied area:

1. TRAP+/BS (%): TRAP+ bone surface/total bone surface.
2. N.Oc TRAP+/mm^2: number of TRAP+ osteoclasts/mm^2.
3. N.Oc TRAP−/mm^2: number of TRAP− osteoclasts/mm^2.

To allow for comparison, the sections were obtained from the same region of the bone overlying the developing tooth germ of the first mandibular molar. Using a stereoscopic microscope, the embedded specimens were sectioned upon visualization of the following anatomical structures: triangular shaped first molar, open apex and oval shaped continuously growing tooth.

2.3. *Degree of tooth eruption*

The degree of tooth eruption (DTE) of the first mandibular molar on the buccal and lingual sides was measured on microphotographs of buccolingually oriented sections stained with H&E. We employed a modified histomorphometric method described by Villarino et al.[36] Buccal and lingual cement enamel junctions were used as reference points for the lines drawn to determine tooth eruption as described below (Fig. 1.II):

Line "a" was drawn from the lingual (α) to the buccal (β) cement enamel junction.

Line "b" was drawn parallel to line "a" and through the upper point of the buccal alveolar bone crest (δ).

Line "c" was drawn parallel to lines "a" and "b" and through the upper point of the lingual alveolar bone crest (ε).

The distance between lines "a" and "b" was considered a measure of the degree of buccal tooth eruption (DBTE). The distance between lines "a" and "c" was considered a measure of the degree of lingual tooth eruption (DLTE). All tooth eruption measurements were expressed as negative values. Thus, the greater the distance, the lesser the degree of tooth eruption.

Table 1 – Effects of maternal exposure to constant light on pup body weight at the age of 3, 5 and 15 days. Results are given as mean ± SEM. Number of animals in parentheses.

Body weight (g)	Age 3 days	Age 5 days	Age 15 days
Control	8.92 ± 0.11 (16)	15.73 ± 0.46 (14)	30.66 ± 0.32 (12)
LL	7.55 ± 0.12 (16)*	12.71 ± 0.49 (14)*	25.53 ± 0.47 (12)*

* Significantly different from control: $p < 0.01$.

The coefficient of variation was calculated to determine intra-examiner error, and was found to be less than 5% in all measured parameters. Student's *t*-test for independent samples was used for statistical analysis. A *p*-value of less than 0.05 was considered statistically significant. All data are presented as mean ± standard error.

3. Results

Pregnant mothers exposed to constant light seemed to have more anxiety-like behaviour compared to control dams. No differences in the number of foetuses or in the signs of foetal resorption were observed between groups. Food intake increased in both groups between days 10 and 21; no significant differences in food intake were observed between groups at any time.

Body weight of pups born to control dams was 17% higher than that of 3, 5, and 15-day-old pups; the difference was statistically significant (Table 1).

3.1. *Histomorphometric analysis*

According to the results of the histomorphometric analysis of bone overlying the developing tooth germ of the first mandibular molar of 3-day-old pups, bone volume (BV/TV%) was 58.14 ± 4.24 in the LL group and 32.31 ± 2.16 in the control group ($p < 0.01$), indicating an approximate 80% increase in prenatally stressed pups (Fig. 2A–C). In agreement with the above finding, a significant decrease in the number of osteoclasts (N.Oc/mm^2) (LL: 3.5 ± 0.65 vs. C: 8.03 ± 1.31; $p < 0.01$) (Fig. 3A–C) and in the number of nuclei per osteoclast (N.Nc/Oc) (LL: 2.31 ± 0.19 vs. C: 3.42 ± 0.17; $p < 0.01$) was observed in pups born to LL dams (Fig. 3D).

A decrease in TRAP+ bone surface (%TRAP+/BS) was observed in sections corresponding to experimental 3-day-old pups as compared to their age-matched controls (LL: 8.89 ± 1.75 vs. C: 52.63 ± 3.76; $p < 0.01$) (Fig. 4A–C). Likewise, a significant decrease in the number of TRAP+ osteoclasts (N.Oc TRAP+/mm^2) was observed in experimental pups (LL: 0.84 ± 0.53 vs. C: 8.59 ± 1.26; $p < 0.01$) (Fig. 4A, B and D). In addition, pups born to stressed dams showed a significant increase in the number of TRAP− osteoclasts (N.Oc TRAP−/mm^2) compared to controls (LL: 2.8 ± 0.66 vs. C:0.76 ± 0.36; $p < 0.01$) (Fig. 4A, B and E).

Although a negative correlation in the number of TRAP+ and TRAP− osteoclasts was observed between the two groups of pups, the total number of osteoclasts, i.e. TRAP+ plus TRAP− osteoclasts, was still significantly lower in prenatally stressed pups. The latter result is consistent with the data obtained from H&E stained sections.

3.2. *Degree of tooth eruption*

H&E stained sections corresponding to 15-day-old pups born to stressed mothers showed significantly decreased DTE on the buccal (LL: −0.972 ± 0.04 vs. C: −0.726 ± 0.04; $p < 0.0001$) and lingual (LL: −0.605 ± 0.05 mm vs. C: −0.342 ± 0.02 mm; $p < 0.0001$) sides (Fig. 5A–C). Thus, both values showed a marked delay in tooth eruption in experimental pups.

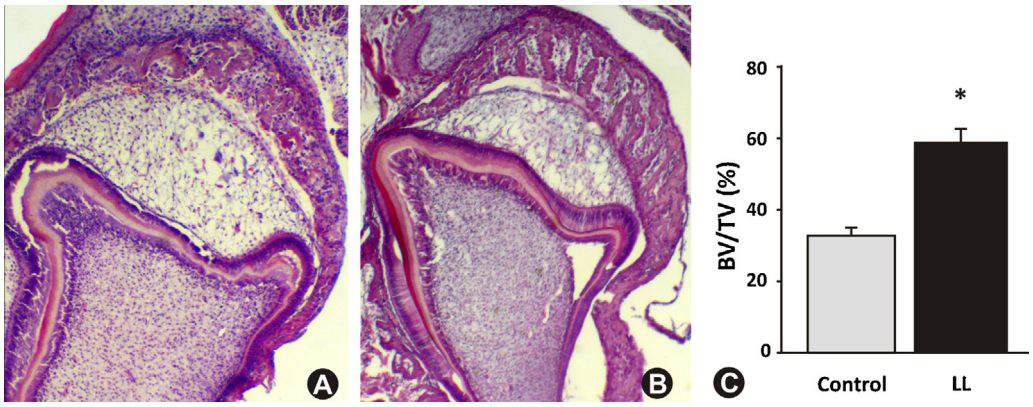

Fig. 2 – Microphotographs showing a marked increase in the volume of bone overlying the developing tooth germ of the first mandibular molar in 3-day-old prenatally stressed pup (B) compared to controls (A). Original magnification: 4×. The bar chart (C) shows bone volume; empty bars correspond to control pups and solid bars to pups born to LL mothers (*n* = 12 pups per group; **p* < 0.01).

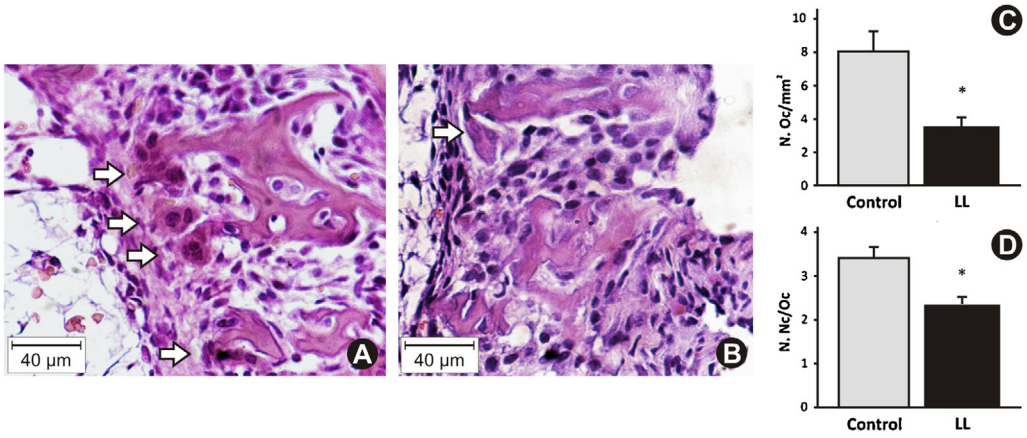

Fig. 3 – Microphotograph of a section corresponding to a control (A) and to a prenatally stressed pup (B). Original magnification: 40×. Note the significant decrease in the number of osteoclasts (C) and in the number of nuclei per osteoclast (D) in pups born to LL mothers compared to the controls (A); *$p < 0.01$ (empty bars correspond to Controls and solid bars to pups born to LL mothers; $n = 12$ per group).

4. Discussion

To the best of our knowledge, this is the first "*in vivo*" study aiming to explore the association between adverse prenatal conditions and certain aspects of bone modelling events involved in the tooth eruption process in rats. The results of the present study show that exposure of pregnant rats to constant light from day 10 to day 20 of gestation increased bone volume in the bone overlying the developing tooth germ of the first mandibular molar of their pups. At the same time, osteoclast recruitment and the number of nuclei per osteoclast were significantly lower in 3-day-old pups born to stressed mothers. The latter was associated with a significant decrease in the degree of tooth eruption in 15-day-old experimental pups. Our findings suggest a decrease in bone

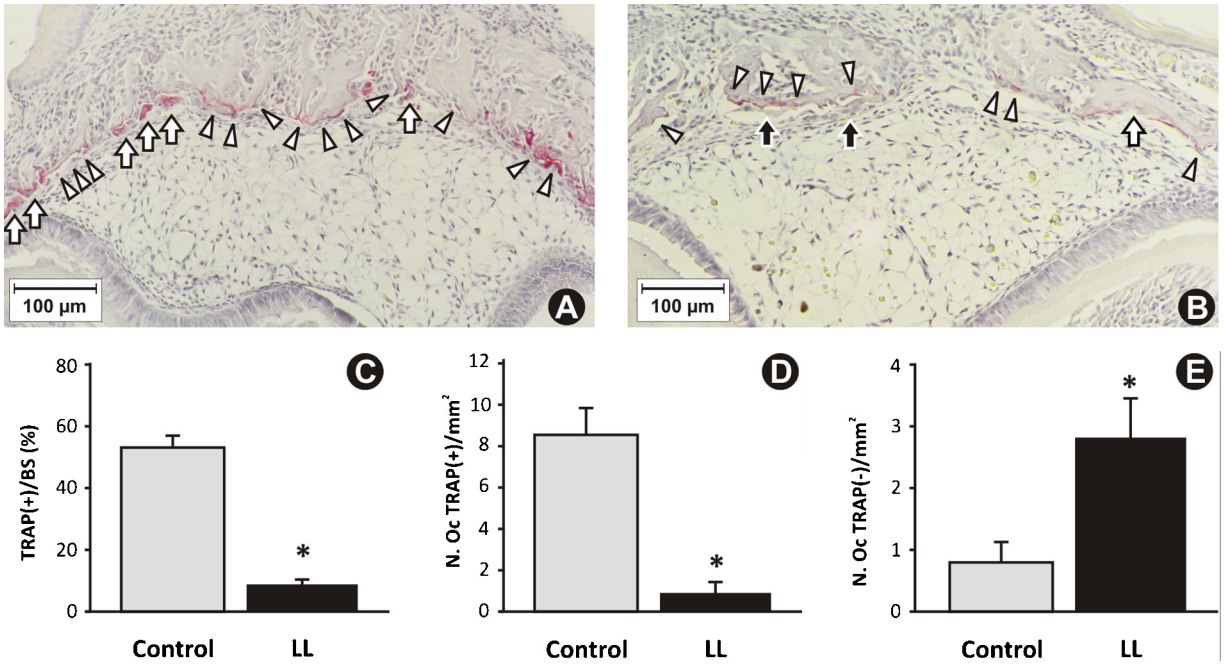

Fig. 4 – Representative microphotograph of a TRAP stained section of bone overlying the developing tooth germ of the first mandibular molar of a control (A) and a prenatally stressed (B) pup. Original magnification: 10×. Arrowheads: TRAP+ bone surface; white arrows: TRAP+ osteoclasts; black arrows: TRAP− osteoclasts. A substantial decrease in TRAP+ bone surface (C) and a significantly lower number of TRAP+ osteoclasts (D) was observed in pups born to LL mothers compared to controls, *$p < 0.01$. At the same time the number of TRAP− osteoclasts (E) was significantly higher in pups born to LL mothers; *$p < 0.01$ ($n = 12$ per group; empty bars: controls; solid bars: pups born to LL mothers).

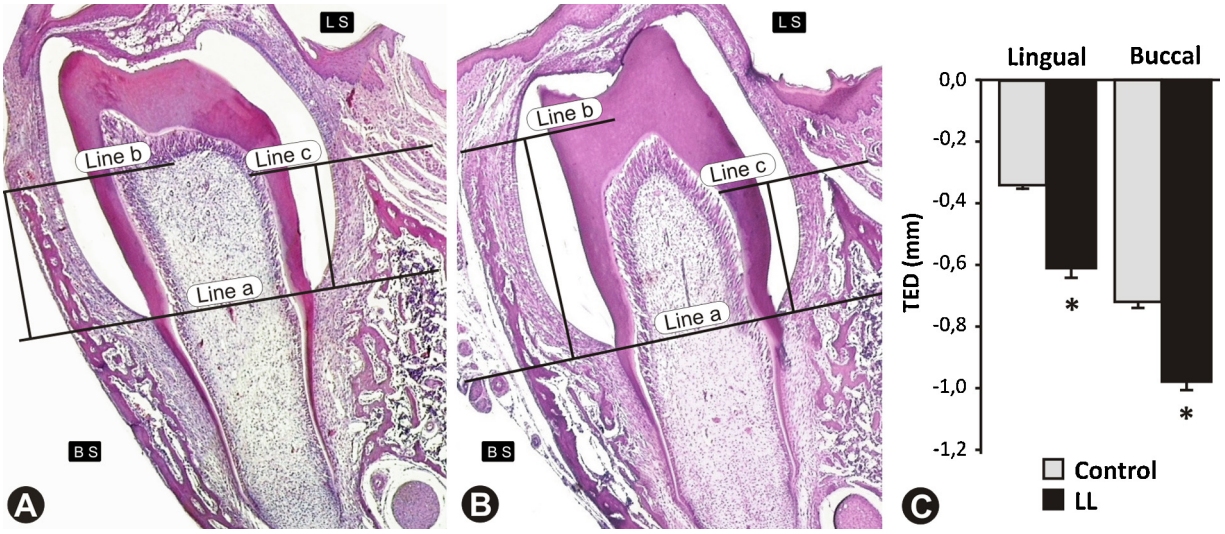

Fig. 5 – Representative microphotograph of an H&E stained section of a 15-day-old pup born to LL mother (B) and a control pup (A). Original magnification: 4×. The distance between lines "a" and "b" and between lines "a" and "c" was considered a measure of the degree of buccal and lingual tooth eruption respectively. The degree of tooth eruption on both the buccal and lingual sides (C) was significantly lower in pups born to LL mothers compared to controls; $^{*}p < 0.0001$ (empty bars: controls; solid bars: pups born to LL mothers; $n = 12$ per group; BS: buccal side, LS: lingual side).

resorption, which is a crucial event in the formation of the eruption pathway through the bone overlying the developing tooth germ of the first mandibular molar.

It is known that the molecular events that promote the resorption process are closely regulated and involve many systemic and local factors, including growth factors, hormones, transcription factors, enzymes and transporters.[30] Among the molecular signals synthesized by the dental follicle, expression of both chemoattractant molecules Monocyte Chemiotactic Protein-1 (MCP-1) and Colony Stimulating Factor-1 (CSF-1) peaks in normal rats at the age of 3 days. This peak is consistent with the burst of osteoclastogenesis observed in the dental follicle at that stage.[26] Osteoclastogenesis is also promoted by the receptor activator of nuclear factor-kappa B ligand (RANKL), that binds to the receptor activator of nuclear factor-kappa B (RANK) present in mononuclear pre-osteoclasts.[31] RANK-RANKL binding not only activates a variety of downstream signalling pathways required for osteoclast development, but also cross-talks with other pathways and fine-tunes bone homeostasis both in normal physiology and disease. These complex signals for osteoclast maturation are crucial to the formation of the eruption pathway.[24] In this work, the increase in bone volume found in 3-day-old pups born to stressed mothers suggests an inhibitory effect of exposure to constant light on pup osteoclastogenesis and, consequently, on the bone resorption process (Fig. 2).

The number of osteoclasts on sections stained for detection of TRAP was consistent with the number of osteoclasts found on H&E stained sections (Figs. 3 and 4). TRAP is synthesized as latent proenzyme and activated by proteolytic cleavage; it is released into Howship's lacunae during resorption and it is expressed by osteoclasts, macrophages, and dendritic cells.[32] According to the literature, the number of TRAP positive cells in the dental follicle of normal rats peaks on postnatal day 3 and then decreases steadily until post-natal day 16.[25] In the present study, the lower number of TRAP+ osteoclasts found in 3-day-old pups born to stressed mothers is directly associated with a significant decrease in TRAP+ bone surface in the bone overlying the developing tooth germ of the first mandibular molar. A significant increase in TRAP− osteoclasts was also observed (Fig. 4). It is not easy to explain why the number of TRAP− osteoclasts increased in the pups born to LL mothers. Even though TRAP enzyme is an indicator of osteoclast phenotype, it could be assumed that osteoclast activity may be decreased in those cells that are TRAP negative. Of particular interest is that TRAP activity is low in cultures of dendritic cells when these are immature, but it increases 5-fold on cell maturation.[33] In addition, studies in mice lacking TRAP as a result of targeted gene disruption showed decreased bone modelling, abundant TRAP− osteoclasts, and increased mineralization in long bones of older animals, reflecting mild osteopetrosis caused by reduced osteoclast activity.[32] In agreement with Hayman's findings, our results would seem to indicate that osteoclastogenesis is partially inhibited in pups born to LL exposed mothers. It could be posited that deregulation of osteoclastogenesis may lead to a high number of non-functioning osteoclasts, thus resulting in a decrease in bone resorption. The relationship between osteoclasts and tooth eruption was demonstrated in rat models of osteopetrosis.[34] In this genetic disease, osteoclast differentiation and maturation is affected by a dysplastic bone disorder characterized by a general increase in bone mass. Consequently, the lack of osteoclast formation generates a delay in, or complete failure of, the tooth eruption process.[24] Moreover, it has been shown that bisphosphonate treatment inhibits osteoclast activity and also significantly delays tooth eruption.[35] In addition, it is well documented that the process of tooth eruption is delayed or inhibited in other pathologies, such as diabetes,[36] or in intoxications. In previous studies performed at our laboratory, intoxication with chromium[37] or

uranium[38] was found to delay tooth eruption in rats. The results of the present study showed a 1.55-fold decrease in the DTE of the first lower molar in 15-day-old pups born to mothers exposed to constant light (LL) compared to control pups (Fig. 5).

One of the body's responses to stress is the secretion of high concentrations of GCs by stimulation of the HPA axis.[39] In stressed pregnant rodents, there is an elevated concentration of corticosterone, which crosses the placenta into the foetal circulation. In rats, the presence of placental enzyme 11β-hydroxysteroid dehydrogenase type 2 (11b-HSD2) acts as "barrier" to GCs within the maternal circulation. This enzyme catalyzes the conversion of corticosterone into inert 11-keto forms, regulating the serum GCs concentration that reaches the foetus and protecting it from relatively high levels of maternal corticosterone.[40] Under stressful conditions, over stimulation of the HPA axis could lead to deficient enzyme activity[41] and may underlie deleterious short- and long-term effects, including intrauterine growth retardation and reduced birth weight[9,14] which are strong predictors of chronic disease later in life.[42] The rat model of maternal stress used here indirectly proves the deleterious effect of increased corticosterone levels on foetal circulation. Our results showed significant differences in body weight between experimental and control pups. Determinations performed on postnatal days 3, 5, and 15 showed pups born to mothers exposed to constant light had significantly lower body weight as compared to control pups (Table 1).

Although it is known that GCs affect bone remodelling, their action on osteoclasts remains controversial. Whereas some authors have associated GCs to an increase in bone resorption,[43,44] others have found GCs to be associated with an inhibition of bone resorption, by means of a decrease in the number of osteoclasts and preosteoclasts,[45] the promotion of osteoclast apoptosis[46] or exerting an inhibitory effect on the colony stimulating factor, an essential signal during osteoclastogenesis.[47] The findings presented here are in line with the latter authors, who found GCs to inhibit bone resorption. Our results show a decrease in the number of mature osteoclasts and preosteoclasts, which would lead to an increase in bone volume in the bone overlying the developing tooth germ of the first mandibular molar of pups born to stressed mothers (Figs. 2 and 3).

On the other hand, it is known that chronic exposure of pregnant rats to constant light, a powerful maternal stressor, suppresses the maternal melatonin rhythm,[21] and disrupts maternal circadian clock programming.[22,48] In line with this, Otha et al.[18] demonstrated in rodents that chronic constant light acts as a stress stimulus, desynchronizing clock neurons but not compromising their ability to generate circadian rhythms. Cellular synchrony among clock neurons is disrupted. In addition, in a previous work we demonstrated that maternal circadian programming initiates between days 11 and 12 of gestation.[27] The results of the present study are in agreement with our previous findings regarding the effects of prenatal exposure to constant light initiated on day 10 of gestation on enzymatic activity, anxiety-like and sexual behaviour.[22] Furthermore, they support the idea that this adverse stimulus may desynchronize the maternal circadian clock during the embryonic stage and in turn, impairs the entrainment of the foetal clock. As a result, prenatal exposure to constant light could lead to a systemic foetal pattern of disruption and might subsequently affect lower molar eruption in rat pups. Further studies should be carried out in order to elucidate the possible effects of prenatal constant light on other cells involved in the process of tooth development and eruption, such as migratory cells passing through the dental follicle connective tissue, which are thought to contribute to tooth organogenesis.[49]

In conclusion, the present study suggests an association between prenatal exposure to constant light and a decrease in osteoclast-mediated bone resorption which is a crucial event in the formation of the eruption pathway through the bone overlying the developing tooth germ. The latter would explain the decrease in the degree of tooth eruption of the first mandibular molar observed in 15-day-old experimental pups. Our findings support the hypothesis that the synchronization of the pup circadian system function is crucial to the physiological processes of bone resorption and tooth eruption during early development. Further studies should be carried out in order to elucidate the molecular mechanisms involved in prenatal stress-associated delay in tooth eruption and the possible treatment to prevent it.

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Competing interests

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

Ethical approval

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.

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