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ORIGINAL ARTICLE

Contribution of capsaicin-sensitive innervation to the continuous eruption of the rat mandibular incisors

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Abstract

A major component of tooth innervation is made of capsaicin-sensitive primary afferents (CSPA). These fibers play a key role in tooth pain and inflammation; little is known, however, about the role of CSPA in tooth eruption. The aim of this study was to examine the role of the capsaicin-sensitive afferents in the process of eruption of intact rat incisors. CSPA fibers in several rat groups, were subjected to one of the following experimental procedures: systemic chemical ablation, systemic ablation followed by chemical sympathectomy and localized activation. The observed effects on incisor eruption were compared to those made on controls. The total amount of eruption in control/naïve rats, measured over a total period of 144 h, was 3.18 ± 0.07 mm and decreased to 2.43 ± 0.08 mm ($n = 7$; $p < 0.001$) following systemic ablation of CSPA. Further decrease to 2.24 ± 0.08 mm ($n = 7$; $p < 0.001$) was noticed when chemical sympathectomy was added to CSPA ablation. The average rate of eruption was 1.7 ± 0.25 mm following CSPA activation, compared to an average of 0.8 ± 0.07 mm for controls ($n = 7$; $p < 0.001$). Capsaicin sensitive fibers play an important role in tooth homeostasis, and intact neural supply is required for tooth growth under normal conditions.

Keywords Rat incisor · Capsaicin · Guanethidine · Sympathectomy · Tooth eruption · Tooth innervation

Abbreviations

CSPA	Capsaicin-sensitive primary afferents
IAN	Inferior alveolar nerve
SP	Substance P
CGRP	Calcitonin gene-related peptide

Introduction

Tooth eruption is the process by which a tooth moves from the pre-eruptive position in the alveolar bone through the mucosa into the oral cavity. It is believed that the dental follicle (sac containing the developing tooth and its odontogenic organ), rather than the tooth itself, plays the essential role in this process (Markman 2009). Recent studies show that innervation is essential for tooth development, tooth eruption and tooth homeostasis (Pagella et al. 2014). In this context, both sympathetic efferents and afferent nerve fibers are candidates to play a role in the eruption and homeostasis of teeth in the rat. Surgical sympathectomy exerts variable effects on the eruption rate of the intact and the injured tooth (Ladizesky et al. 2001). Our group has recently shown that chemical sympathectomy results in a consistent and significant reduction of the rate of eruption of intact rat incisors (Chidiac et al. 2018). On the other hand, cutting the inferior alveolar nerve (IAN) delays the eruption rate of rat incisors (Chidiac et al. 2018). Therefore, both efferent and afferent fibers seem to have an important role in the homeostasis of rat teeth.

Sensory nerves are known to release substance P (SP), calcitonin gene-related peptide (CGRP), and other neuropeptides in response to orthodontic forces and concomitant biochemical events (Miller et al. 2007). There seems, also, to be a close relationship between the nerve endings surrounding the odontoblasts and the primary cilia of these odontoblasts. This interaction being responsible for the pain sensation induced by thermal or chemical changes around the tooth pulp (Magloire et al. 2010) and may also influence tooth eruption.

Tooth pulp innervation consists primarily of sensory unmyelinated fibers that belong to the group of capsaicin

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sensory primary afferents (CSPA). Immuno-histochemical studies showed that (SP) and (CGRP) immunoreactivity co-exist in capsaicin-sensitive primary sensory neurons (Franco-Cereceda et al. 1987). In rat molars, extensive innervation of dentin by CGRP-immunoreactive (IR) nerve fibers is seen during sensory maturation with the appearance of the mature avascular odontoblast layer next to cell-free and cell-rich zones in the pulp horns (Veerayutthwilai et al. 2006). It seems that CGRP and substance P are also seen in teething (eruption of teeth in babies) as this eruption process involves inflammation and discomfort (Markman 2009). This is also happening even after eruption during orthodontic treatment movements (Fleming et al. 2016). It has been reported, however, that IAN denervation or neonatal capsaicin desensitization in the rat did not affect the rate of orthodontic tooth movement (Miller et al. 2007; Davila et al. 2011). These apparently controversial observations leave the floor open for further investigation into the potential role of CSPA in tooth growth.

The aim of the present study was to investigate further the role of teeth afferents in tooth growth. Rat incisors, known to have continuous growth, constitute a good material for this investigation. For this purpose the effects of either stimulation or selective ablation of these afferents were observed in intact incisors.

Materials and methods

Animals

Experiments were performed on male adult Sprague–Dawley rats (250–300 g) with completely developed mandibular incisors. Rats were kept in groups of three to five in plastic cages under standard colony conditions (12 h dark/light cycle, temperature 22 ± 2 °C) with free access to food and water. All procedures were carried out with strict adherence to the guidelines for experimentation animals (Swiss Academy of Medical Sciences 1997) and approved by the Institutional Animal Care Committee.

Experimental groups

This study is based on observations made on 28 rats, distributed into four equal groups. Group I was the control. In the second group rats were subjected to a systemic ablation of CSPA fibers. For group III, rats were subjected to a systemic (CSPA) ablation and a chemical sympathectomy. Finally, group IV rats had an exposed dentin in an intact incisor and topical application of capsaicin inside it to irritate (CSPA) fibers.

Experimental procedures

All experiments were performed under deep general anesthesia achieved by injection of atropine (Atropine sulfate, Laboratoire Aguettant; dilution 1:10 in saline, 0.05 mg/kg) and chlorpromazine (Largactil®, 8 mg/kg) intra-peritoneally, followed 10 min later by intraperitoneal (i.p.) injection of ketamine (Ketalar®, 50 mg/kg).

Systemic block of CSPA

CSPA fibers in adult rats were ablated by injecting capsaicin subcutaneously (dissolved in 10% Tween 80, 10% Ethanol, and PBS), on 2 consecutive days (McCafferty et al. 1997). Under light anesthesia, capsaicin (8-methyl-*N*-vanillyl-non-anamide; cat. no. M1022, Sigma, St. Louis, MO) was injected subcutaneously at a concentration of 25 mg/kg. The second injection was carried out after 8 h at a concentration of 50 mg/kg, and a third injection (50 mg/kg) was administered 32 h after the first injection. To assess if ablation was successful, the eye wiping test was performed, by applying a drop of capsaicin solution (10 mg/kg) in one eye and evaluating the behavioral response of the rat (Hammond and Ruda 1991).

Chemical sympathectomy (guanethidine)

A post-ganglionic block was applied chemically using guanethidine. Drug concentrations and optimal dosage were determined based on previous work from our group on the optimal dosage with minor side effects (Saadé et al. 2008; Safieh-Garabedian et al. 2002). Guanethidine: 0.2 ml guanethidine (1-[2-guanethinoethl] octahydroazocine) monosulfate (1:1), cat. no. G-8520, Sigma) (30 mg/kg) was administered daily subcutaneously for a period of 1 week (Coderre et al. 1984).

Topical application of capsaicin in dentinal tubules

A small cavity (about 1.5 mm) was prepared on the lingual sides of both incisors using a round dental carbide bur (Meisinger, Centennial, CO) of 1 mm in diameter mounted on a straight hand piece (G.Dental shop, Washington DC). Care was taken to expose the dentinal tubules and avoid reaching the dental pulp. Teeth were thoroughly washed with sterile saline during drilling, then air-dried at the end of the procedure. An aluminum foil was inserted between the two incisors to prevent the leakage of substances from one tooth to the other. On the experimental side, a small piece of gelfoam soaked with a solution of 10% of capsaicin dissolved in 10% of Tween 80 and 90% of olive oil, was

applied inside the hole. Capsaicin solution was mixed with Congo red dye (Sigma-Aldrich, St. Louis, MO), to track the infiltration of capsaicin through the dentinal tubules. On the second incisor (control side) the gelfoam was soaked with sterile saline. After a 30-min period of exposure, the holes were thoroughly washed with sterile saline, then treated with an etch and bond resin (GC America, Alsip, IL) and closed by a transparent flow composite resin (Ultradent Products, South Jordan, UT) using a light cure system (SDI Radii plus LED, SDI, Itasca, IL).

At the end of the experiment, the animals were euthanized, teeth were extracted and kept in formalin (Sigma-Aldrich) and later examined under an endodontic microscope at 12.5 × 2.5 magnification (Kaps SOM 22, endodontic microscope, Kaps, Asslar, Germany) to trace the infiltration of capsaicin in the pulp chamber (Fig. 1).

Measurement of tooth eruption

Measuring tooth eruption consisted of measuring the distance in millimeters between two reference landmarks. The first one is placed, as a tattoo, on the distal margin of the fixed attached gingiva. The moving landmark is made, as a narrow groove, on the tooth itself, at the junction of the buccal and distal surface of the tooth (clear differentiation in enamel color).

The distance between the tattoo on the gingiva and the groove on the tooth was labeled (*X*), while the distance between the tattoo and the incisal edge of the tooth was labeled (*Y*) (Fig. 2). A digital caliper (Electronic Digital Caliper, accuracy 0.01 mm) was used. As incisor growth is maintained throughout life at an average rate of 0.5 mm/day (Chiba et al. 1973), the measurements were obtained every 48 h over a period of 144 h as, following this time period, both teeth (experimental and control) usually reached full eruption and the landmarks were no longer visible. Each session involved three repeated measurements performed on each rat, and the average was used as final value for the indicated time interval. A detailed description can be found elsewhere (Chidiac et al. 2018).

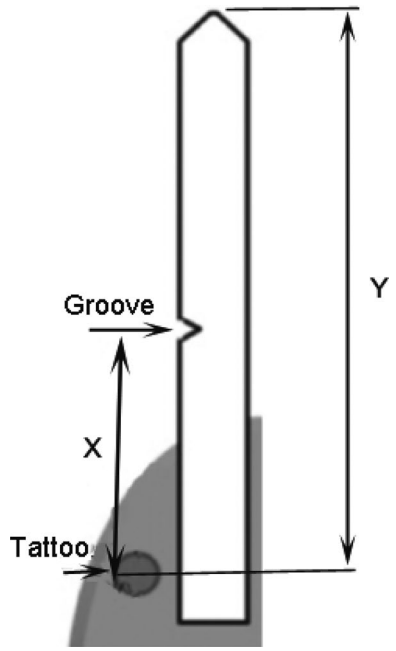

Fig. 2 Representation of the landmarks placed on the incisor and attached gingiva: distance between the groove made on the tooth (first reference point) and the tattoo on the attached gingiva (*X*). Distance between the tattoo on the attached gingiva and the incisal edge (*Y*)

Animal euthanasia

At the end of each experiment, each animal received a lethal injection of ketamine (Ketalar®, 100 mg/kg; i.p.) followed by a cervical dislocation. Incisors were extracted and preserved in jars containing 4% formalin (Sigma-Aldrich).

Fig. 1 Close up magnification of sectioned incisors to show the infiltration of capsaicin into the pulp chamber. **a** Composite resin still present, **b** composite resin removed

Statistical analysis

The rate of eruption is represented by the differences between measures *Y* and *X* in Fig. 2, registered at four time intervals (0, 48, 96 and 144 h) for each experimental group. At each time point, measurements were made three times then averaged for each rat. The results obtained for each time point were presented as the mean and standard error of the mean (SEM) of measurements made on all rats in the same experimental group. Significance of variations was determined by the student *t* test using GraphPad Instat 3 and GraphPad Prism 6 (GraphPad Software Inc. San Diego, CA).

Results

Effect of systemic ablation of CSPA on the eruption rate of rats' mandibular incisors

Systemic ablation of CSPA fibers produced a significant attenuation of the eruption rate of the mandibular incisors. The mean rate of eruption in the treated animals ($n=7$), at the initial time segment (0–48 h), was 0.84 ± 0.05 mm. That of the control group was 1.10 ± 0.05 mm ($p<0.01$, $n=7$). At the second time segment (48–96 h), the mean measured value for treated animals was 0.8 ± 06 mm; for controls it was 1.05 ± 0.1 mm ($p<0.01$). At the third time segments (96–144 h), values were 0.85 ± 0.1 mm for the treated animals and 1.03 ± 0.11 mm for controls ($p<0.05$; Fig. 3a). The means of the total amounts of eruption were 2.43 ± 0.08 mm for treated animals and 3.18 ± 0.07 mm for controls ($p<0.001$; Fig. 3b).

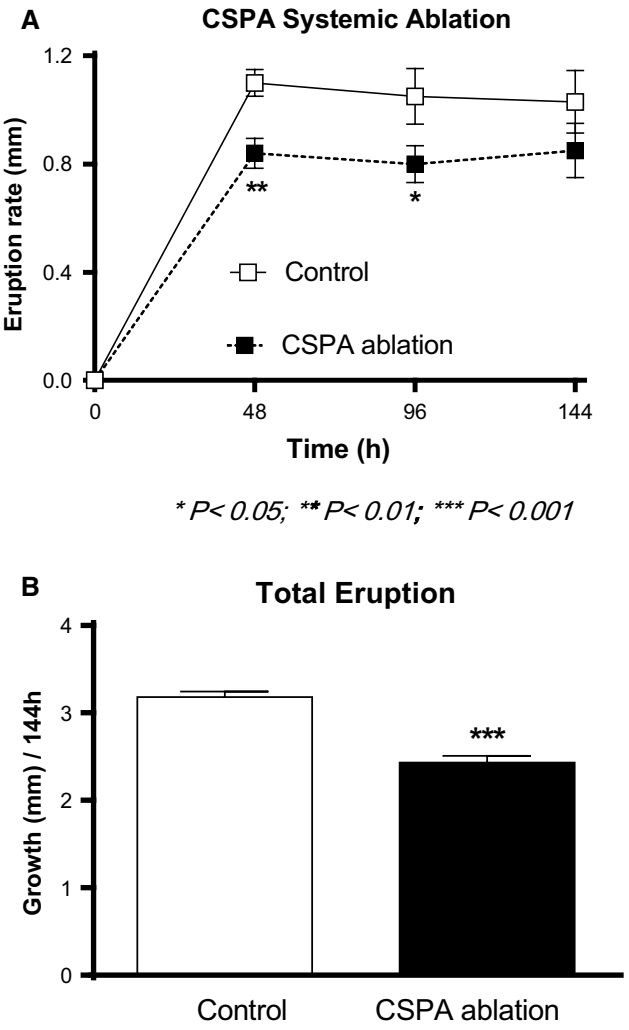

Fig. 3 Systemic ablation of capsaicin-sensitive primary afferents (CSPA) fibers produced significant attenuation of the growth rate of rat incisors. Capsaicin ablation procedure was performed 2 weeks before starting growth rate measurements ($n=7$). **a** Values of measurements at different time intervals. **b** The total amount of growth in control and capsaicin treated rats

Effect of combined ablation of CSPA fibers and sympathetic block on the eruption rate

Treating rats subjected to prior systemic ablation of their (CSPA) fibers with guanethidine produced a significant further attenuation of the eruption rate of the mandibular incisors. The mean rate of eruption in the treated animals, at the initial time segment, was 0.79 ± 0.07 mm, as compared to the control group 1.18 ± 0.15 mm ($n=7$ rats; $p<0.01$). The mean eruption at the second time segment (48–96 h) was 0.66 ± 0.7 mm, for treated animals and 1.08 ± 0.09 mm for controls ($p<0.001$). For the third time segments (96–144 h), values were 0.80 ± 0.06 mm for the treated animals and 0.79 ± 0.15 mm for controls. The means of total eruption were 2.24 ± 0.08 mm for treated animals subjected to combined treatments, as compared to control (3.00 ± 0.16 mm) or to rats subjected to CSPA ablation only (2.43 ± 0.08 mm) (Fig. 4).

Effect of acute application of capsaicin in the drilled hole in the incisor

Application of capsaicin in the drilled hole on the tip of incisor ($n=7$ rats) produced a consistent and significant acceleration of the eruption rate of the mandibular incisors, as compared to the rate of increase of the opposite incisor treated with sterile saline. At the day of surgery, the mean distance between tattoos and grooves in the capsaicin treated incisors was 4.2 ± 0.15 mm, as compared to the saline treated (control) incisor ,which was $3.8 \pm 0.0.19$ mm ($p>0.05$; $n=7$). The mean of measured distances at day 2 post treatments, was 4.5 ± 0.1 mm for capsaicin treated incisors, as compared to 4.1 ± 0.19 mm for saline-treated

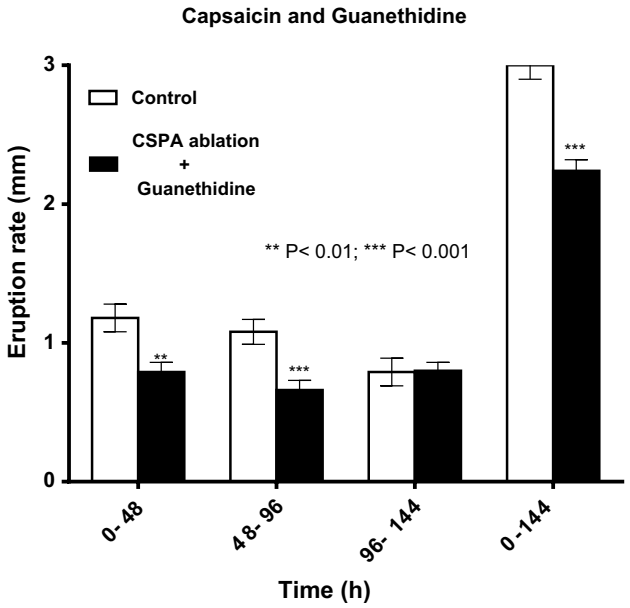

Fig. 4 Combined CSPA ablation and sympathetic block induced further decrease of the rate of tooth eruption. Each bar represents the average ± SEM of the value measured in each group at the corresponding time interval ($n = 7$ rats each)

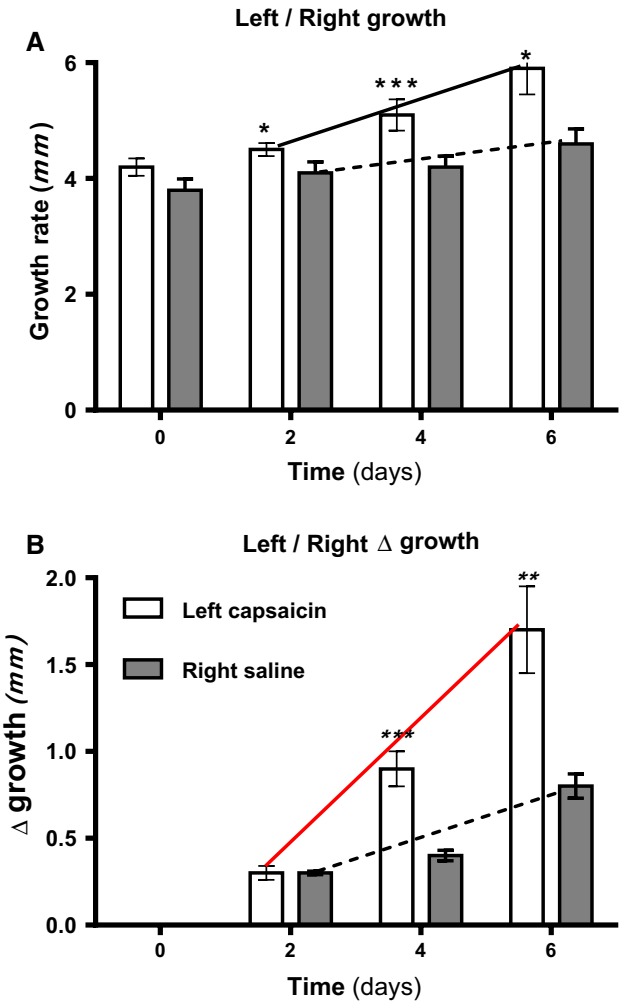

Fig. 5 Application of capsaicin in the open cavity in the incisor produced significant acceleration of the eruption rate. **a** The measured distances between the tattoo and grooves in capsaicin- and saline-treated incisors of the same rats at different time intervals. **b** Differences in growth of the treated incisors observed at the same time intervals

incisors/controls ($p < 0.05$). At day 4 post treatment, values were 5.1 ± 0.27 mm for capsaicin-treated animals and 4.2 ± 0.19 mm for saline controls ($p < 0.001$). At day 6 post treatment, the grooves reached the tip of the capsaicin treated incisors with an average total length of 5.9 ± 0.45 mm, as compared to 4.6 ± 0.26 mm for the saline-treated incisor ($p < 0.016$; Fig. 5a). The average differences in growth rates are illustrated in Fig. 5b. Measured eruption rates at 2, 4 and 6 days post treatments were in the capsaicin-treated incisors 0.3 ± 0.04, 0.9 ± 0.1 and 1.7 ± 0.25 mm, as compared to 0.3 ± 0.01, 0.4 ± 0.03 and 0.8 ± 0.07 mm in saline treated incisors, respectively.

Discussion

The main aim of the present study was to shed light on the role of capsaicin-sensitive fibers in tooth eruption, knowing that this group of afferents constitutes the major component of tooth innervation. For this purpose, the role of this group was assessed under two experimental conditions; the first addressed the effects of their ablation and the second the effect of their selective activation. The observed results revealed that selective ablation of CSPA fibers reduced the rate of tooth eruption; a reduction that was increased further by adding to ablation a chemical block of sympathetic efferents. On the other hand, selective activation of CSPA fibers, using an improvized procedure to insure the infiltration of capsaicin into the tooth pulp, elicited a significant acceleration of tooth eruption.

The fact that adult teeth in vertebrates do not show, under normal conditions, overt changes in their shape and size, led us to opt for the work on rat incisors, which are known for their continuous growth. This peculiarity, considered by some experts as reminiscent of an embryonic feature in adult animals (Ohshima et al. 2005), provided the opportunity to observe the effects of manipulating tooth innervation without interfering with the normal process of eruption.

It is worth noting that the measurement technique described in the present study has been used and validated before by other researchers (Chiba et al. 1973). The results of our measurement of eruption rate in/control rats appear to be in line with the observations made by Law et al. 2003

and Ohshima et al. 2005. The measurements were made at four time intervals: 0 h, 48 h, 96 h and 144 h. These time periods were chosen to be comparable with data on normal growth based on measurement techniques used in previous studies (Law et al. 2003; Burn-Murdoch 1999; Main and Adams 1965).

A recent study by Hayano et al. (2018) reported comparable results, using a similar method of measurement. In addition, the authors observed the effect of IAN denervation on stem cells of the incisor. They reported a reduction in the eruption rate and they showed by immunohistochemistry that total IAN denervation produced changes in the formation of ameloblasts with disorganized, and a reduced number of, osteoblasts. Osteodentin-like formation cells were also present (Hayano et al. 2018). Previously published data by our group showed that selective ablation of CSPA fibers in the inferior alveolar nerve resulted in a significant attenuation of incisor eruption (Kassab et al. 2013). The results of the current study on the systemic ablation of CSPA are in line with those of local block; both results can be correlated with several reports published by others showing the role of CSPA fibers at the different embryonic steps of osteogenesis leading to tooth formation (Wang et al. 2009; Kjær 2012). Ablation of CSPA fibers in the inferior alveolar nerve has been shown to cause a consistent reduction in the numbers of CGRP and SP immunoreactive fibers in the jaws (Jacobsen and Heyeraas 1996). Other studies have shown a reduction in bone resorption in rats treated with capsaicin (Adam et al. 2000) and a reduction in the overall bone mass (Wang et al. 2009). Our results, established on continuously growing teeth in adult rat, appear to correlate well with the role of CSPA and their neurotransmitters during embryonic events leading to the development of teeth. Furthermore, the enhancement of the effects of CSPA ablation by peripheral sympathetic block is in line with the results of our previous study on the role of sympathetic efferents in tooth eruption (Chidiac et al. 2018). It appears that the results of these two complementary studies correlate well with previous reports on the key role of innervation in osteogenesis and early events of tooth formation (Pagella et al. 2014; Grässel 2014).

Activation of CSPA fibers leading to a significant acceleration of the eruption rate is an added significant result to the opposite finding observed following their ablation. The acceleration observed following application of capsaicin in the proximity of the exposed dentinal tubule cannot be attributed to the effects of tooth injury, since application of sterile saline in a hole drilled in the second incisor and in the same rat, did not produce significant alterations in its normal rate of eruption. Furthermore the infiltration of dye mixed to capsaicin solution (Fig. 1) confirms the infiltration of the solution through the dentinal tubules, which can lead to the irritation of the CSPA terminals in the tooth pulp. This infiltration has been shown previously to induce pain-related behavior in rats that was prevented by pretreatment with a composite paste leading to closure of dentinal tubule (Rifai et al. 2004). Results on the irritation of tooth pulp by the activation of capsaicin sensory afferents are in line with the reported pain and inflammation due to tooth orthodontic and experimental movements (Long et al. 2016; Zhou et al. 2016; Zhu et al. 2017).

Chemical sympathectomy was performed to block the sympathetic efferents reaching the tooth and to make sure that the remaining innervation consists essentially of the CSPA fiber group. This procedure, being chemical or surgical, has been shown, by us and others, to have no significant damage on the tooth itself; in all cases it induced a reduction in the eruption rate, meaning that the eruptive phenomena are still present (Ladizesky et al. 2001; Chidiac et al. 2018; Komorowski et al. 1996). The procedure used was based on the protocol described by Komorowski et al. (1996), who showed that chemical sympathectomy using guanetidine did not have an apparent effect on neurogenic inflammation (Komorowski et al. 1996). The possible effect of this chemical sympathectomy on the periodontal ligament was also not a major concern, following the work of Haug and Hereyaas who showed that sympathectomy induced an increase in the number of osteoclasts and the formation of resorptive lacunae lining the periphery of the periapical lesions (Haug and Heyeraas 2003).

Putting these results together reveals that afferent innervation with CSPA fibers can play a continuous role in the tooth defense and homeostatic mechanisms that start during embryogenesis and continue during postnatal life.

There are, however, some limitations to this study because it was restricted to macroscopic observation of the global results of the manipulation (block or excitation) of tooth innervations. A step towards completion of our work would be the investigation of the cellular and molecular mechanisms underlying the observed effects. Since the work of Hayano et al. (2018) was based on an almost total ablation of innervation by cutting the IAN at its root entry in the mandible, it would be interesting to repeat their experiments but with selective ablation of either afferent (CSPA) or efferent (sympathetic) fiber systems.

In conclusion, the results of the present study provide clear evidence of the role of CSPA in the continuous eruption of rat incisors. These results are in line with those of several other studies, revealing an important trophic role of tooth innervation that is not restricted to pain signaling during embryogenesis and post-natal development.

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Compliance with ethical standards

Animal welfare All experiments have been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the American University of Beirut under a written protocol and in accordance with the Policy on Human Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health in the USA.

Ethics approval The animal experimental protocol in this study was approved by the Institutional Animal Care Committee, number IACUC # 16-03-466, approved on 31 March 2016.

Consent for publication All authors have approved the manuscript and given their consent for their data to be published in the report.

Conflict of interest The authors declare that they have no competing interests.

Availability of data The dataset supporting the conclusions of this article is included within the article.

References

Adam C, Llorens A, Baroukh B, Cherruau M, Saffar JL (2000) Effects of capsaicin induced sensory denervation on osteoclastic resorption in adult rats. Exp Physiol 85:62–66

Burn-Murdoch RA (1999) The length and eruption rates of incisor teeth in rats after one or more of them had been unimpeded. Eur J Orthod 21:49–56

Chiba M, Tsuruta M, Eto K (1973) A photographic method of measure eruption rates of rat mandibular incisors. Arch Oral Biol 18:1003–1010

Chidiac JJ, Kassab A, Rifai K, Saade NE, Al Chaer E (2018) Modulation of incisor eruption in rats by sympathetic efferents. Arch Oral Biol 89:31–36. https://doi.org/10.1016/j.archoralbio.2018.02.003

Coderre TJ, Abbott FV, Melzack R (1984) Effects of peripheral antisympathetic treatments in the tail-flick, formalin and autotomy tests. Pain 18:13–23

Davila JE, Miller JR, Hodges JS, Beyer JP, Larson BE (2011) Effect of neonatal capsaicin treatment on orthodontic tooth movement in male Sprague–Dawley rats. Am J Orthod Dentofac Orthop 139(4):e345–e352. https://doi.org/10.1016/j.ajodo.2009.07.019

Fleming PS, Strydom H, Katsaros C, Mac Donald L, Curatolo M, Fudalej P, Pandis N (2016) Non-pharmacological interventions for alleviating pain during orthodontic treatment. Cochrane Database Syst Rev 12:CDO 10263. https://doi.org/10.1002/14651858.cd010263.pub2

Franco-Cereceda A, Henke H, Lundberg JM, Petermann JB, Hökfelt T, Fischer JA (1987) Calcitonin gene-related peptide (CGRP) in capsaicin-sensitive substance P-immunoreactive sensory neurons in animals and man: distribution and release by capsaicin. Peptides 8(2):399–410

Grässel SG (2014) The role of peripheral nerve fibers and their neurotransmitters in cartilage and bone physiology and pathophysiology. Arthritis Res Ther 16(6):485

Hammond DL, Ruda MA (1991) Developmental alterations in nociceptive threshold, immunoreactive calcitonin gene-related peptide and substance P, and fluoride-resistant acid phosphatase in neonatally capsaicin-treated rats. J Comp Neurol 312(3):436–450

Haug SR, Heyeraas KJ (2003) Effects of sympathectomy on experimentally induced pulpal inflammation and periapical lesions in rats. Neuroscience 120(3):827–836

Hayano S, Fukui Y, Kawanabe N, Kono K, Nakamura M, Ishihara Y, Kamioka H (2018) Role of the inferior alveolar nerve in rodent lower incisor stem cells. J Dent Res 97(8):954–961. https://doi.org/10.1177/0022034518758244

Jacobsen EB, Heyeraas KJ (1996) Effect of capsaicin treatment or inferior alveolar nerve resection on dentine formation and calcitonin gene-related peptide- and substance p-immunoreactive nerve fibres in rat molar pulp. Arch Oral Biol 41:1121–1131

Kassab A, Hage M, Jabbur SJ, Chidiac JJ (2013) Modified technique for the exposure of the inferior alveolar nerve in rats. J Pharmacol Toxicol Methods 67(3):182–186. https://doi.org/10.1016/j.vascn.2013.01.006

Kjær I (2012) New diagnostics of the dentition on panoramic radiographs—focusing on the peripheral nervous system as an important aetiological factor behind dental anomalies. Orthod Waves 71(1):1–16

Komorowski RC, Torneck CD, Hu JW (1996) Neurogenic inflammation and tooth pulp innervation pattern in sympathectomized rats. J Endod 22(8):414–417

Ladizesky MG, Lama MA, Cutrera RA, Boggio V, Giglio MJ, Cardinali DP (2001) Effect of unilateral superior cervical gangionectomy on mandibular incisor eruption rate in rats. Auton Neurosci 93:65–70

Law KT, Lee CK, King NM, Rabie AB (2003) The relationship between eruption and length of mandibular incisors in young rats. Med Sci Monit 1:47–53

Long H, Wang Y, Jian F, Liao LN, Yang X, Lai WL (2016) Current advances in orthodontic pain. Int J Oral Sci 8(2):67–75

Magloire H, Maurin JC, Couble ML, Shibukawa Y, Tsumura M, Thivichon-Prince B, Bleicher F (2010) Topical review: dental pain and odontoblasts: facts and hypotheses. J Orofac Pain 24:335–349

Main JHP, Adams D (1965) Measurement of the rate of eruption of the rat incisor. Arch Oral Biol 10:999–1008

Markman L (2009) Teething: facts and fiction. Pediatr Rev 30(8):e59–e64. https://doi.org/10.1542/pir.30-8-e59

McCafferty DM, Wallace JL, Sharkey KA (1997) Effects of chemical sympathectomy and sensory nerve ablation on experimental colitis in the rat. Am J Physiol 272(2 Pt 1):G272–G280

Miller JR, Davila JE, Hodges JS, Tulkki MJ, Vaydae PM (2007) Effect of surgical denervation on orthodontic tooth movement in rats. Am J Orthod Dentofac Orthop 131:620–626

Ohshima H, Nakasone N, Hashimoto E, Sakai H, Nakakura-Ohshima K, Harada H (2005) The eternal tooth germ is formed at the apical end of continuously growing teeth. Arch Oral Biol 50(2):153–157

Pagella P, Jimenez-Rojo L, Mitsiadis TA (2014) Roles of innervation in developing and regenerating orofacial tissues. Cell Mol Life Sci 71(12):2241–2251

Rifai K, Chidiac JJ, Hawwa N, Baliki M, Jabbur SJ, Saadé NE (2004) Occlusion of dentinal tubules and selective block of pulp innervation prevent the nociceptive behaviour induced in rats by intradental application of irritants. Arch Oral Biol 49(6):457–468

Saadé NE, Farhat O, Rahal O, Safieh-Garabedian B, Le Bars D, Jabbur SJ (2008) Ultra violet-induced localized inflammatory hyperalgesia in awake rats and the role of sensory and sympathetic innervation of the skin. Brain Behav Immun 22(2):245–256

Safieh-Garabedian B, Poole S, Haddad JJ, Massaad CA, Jabbur SJ, Saadé NE (2002) The role of the sympathetic efferents in endotoxin-induced localized inflammatory hyperalgesia and cytokine upregulation. Neuropharmacology 42:864–872

Swiss Academy of Medical Sciences (1997) Swiss Academy of Sciences Ethical principles and guidelines for scientific experiments on animals. Altern Lab Anim 25(3):379–384

Veerayutthwilai O, Luis NA, Crumpton RM, MacDonald GH, Byers MR (2006) Peripherin- and CGRP-immunoreactive nerve fibers in rat molars have different locations and developmental timing. Arch Oral Biol 51(9):748–760

Wang L, Zhao R, Shi X, Wei T, Halloran BP, Clark DJ et al (2009) Substance P stimulates bone marrow stromal cell osteogenic activity, osteoclast differentiation, and resorption activity in vitro. Bone 45:309–320

Zhou Y, Long H, Ye N, Liao L, Yang X, Jian F, Wang Y (2016) The effect of capsaicin on expression patterns of CGRP in trigeminal ganglion and trigeminal nucleus caudalis following experimental tooth movement in rats. J Appl Oral Sci 24(6):597–606. https://doi.org/10.1590/1678-775720160150

Zhu Y, Wang S, Long H, Zhu J, Jian F, Ye N, Lai W (2017) Effect of static magnetic field on pain level and expression of P2X3 receptors in the trigeminal ganglion in mice following experimental tooth movement. Bioelectromagnetics 38(1):22–30