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Research Article

Orthodontic pain: *c-Fos* expression in rat brain nuclei after rapid maxillary expansion

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ABSTRACT

Background: The aim of this in vivo study was to quantitatively evaluate pain after rapid maxillary expansion (RME) in young rats by analyzing the activation of nociception-related structures, that is, the caudalis, interpolaris, and oralis subnuclei, according to the *Fos* expression.
Methods: A total of 65 Wistar rats were assigned to three groups: control group (n = 15) with no treatment, positive control group (n = 25), and experimental group (n = 25) with RME. The experimental animals were euthanized at 6, 12, 24, 48, and 72 hours after RME, and the brain was later carefully collected. Coronal sections through the spinal trigeminal caudalis, spinal trigeminal interpolaris, and spinal trigeminal oralis were cut (thickness of 40 µm) on a cryostat and processed for *Fos* immunohistochemistry. Images from the sections were captured under light microscopy, and ImageJ software was used to count *Fos*-like immunoreactive neurons. The Analysis of variance (ANOVA) and Tukey test were used for statistical analysis, and the significance level was set at 5%.
Results: RME induced incisor distalization and opening of the midpalatal suture, as well as neuronal activation of the spinal trigeminal nucleus. The experimental group demonstrated significantly more *Fos*-positive neurons in subnuclei caudalis and subnuclei interpolaris 6 hours after the maxillary expansion. The *Fos* immunoreactivity significantly decreased at 12 hours and increased again at 24 and 48 hours ($P < 0.001$).
Conclusions: The RME increases the neural activation of brain regions involved in the nociception region, as determined by the Fos expression. The most intense *Fos*-like immunoreactive expression was detected in the brain 6 hours after the start of the palatal expansion.

1. Introduction

Malocclusion caused by a narrow maxillary dental arch width is often treated with rapid maxillary expansion (RME). This procedure induces pain during and after the opening of the midpalatal suture [1].

Pain is a complex experience and often accompanies orthodontic appointments [2]. The fear of pain is a key factor that may discourage a patient from seeking orthodontic treatment [3–5]. Usually, orthodontic patients are informed that there may be a degree of discomfort during the periodic adjustment of orthodontic appliances. The intensity and duration of discomfort may not always be discussed. Studies have shown that the response of patients

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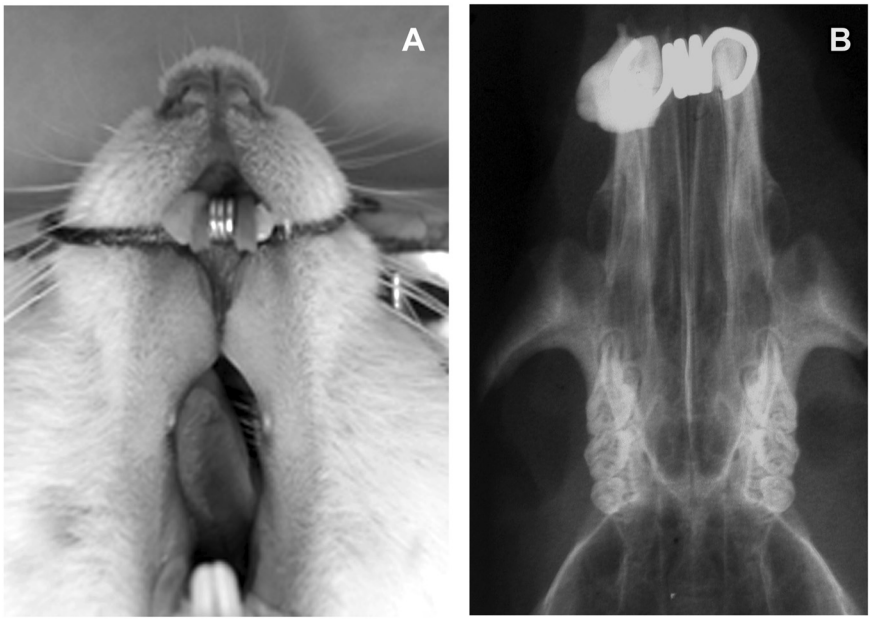

Fig. 1. Insertion of the orthodontic appliance (A). Radiographs of the maxillary region of an animal that received an activated orthodontic appliance (B).

to the initial movement of teeth varies [4], and it is difficult for the orthodontist to respond accurately to prospective patient inquiries regarding the extent and duration of discomfort that might be endured. The cause of the pain resulting from orthodontic tooth movement is not entirely clear [6,7].

Noxious stimulation in the trigeminal regions, which is caused by such phenomena as thermal stimulation of the dental pulp [8], noxious thermal and mechanical stimulation of the teeth [9], mechanical stimulation of oral mucous membranes [10], and pulp exposure or tooth extraction [11], causes a marked induction of *Fos*-like protein in the trigeminal subnucleus complex. Anatomical studies have indicated that the nociceptive information is further processed to the parabrachial nucleus [12–14]. The sites of processing for the nociceptive information originating in the midpalatal suture after RME have not been investigated to date in the trigeminal subnucleus complex and parabrachial area. In the present study, *Fos* expression was investigated in spinal trigeminal caudalis, spinal trigeminal interpolaris, and spinal trigeminal orali areas 72 hours after the commencement of orthopedic force application.

2. Methods and materials

2.1. Animals

A total of 65 male Wistar rats weighing 220 G were kept in Plexiglas cages in a room maintained at a controlled temperature (25°C) with a 12:12 hour light/dark cycle. The rats had free access to water and food. The experiments were reviewed and approved by the Ethics Committee on the Use of Experimental Animals of the University of São Paulo. The rats were divided by blind randomization into three groups: experimental group (25 rats) with midpalatal suture expansion for 6, 12, 24, 48, and 72 hours; control group (no treatment, n = 15); and positive control group (n = 25). The control group consisted of anesthetized animals without orthodontic appliances. In the positive control group (sham group), the orthodontic appliance, without activation, was fixed to the maxillary incisors of the rats (without midpalatal suture expansion), and it was immediately removed after insertion. We used five animals in the appliance groups for each time point.

2.2. Anesthetic and maxillary expansion procedure

All rats except the intact group (control group) were subjected to the RME of the midpalatal suture according to the method described by da Silva et al. (2012) [15]. For all procedures, the animals were intramuscularly anesthetized with a combination of ketamine (Agener, 40 mg/kg) and xylazine (Syntec, 20 mg/kg) at a 1:2 ratio (1 mL/kg body weight). We chose this anesthetic mixture because of its negligible effects on *c-Fos* expression in the brain stem [16]. A hole was drilled laterally in both incisors at the level of the gingival papilla with a nº one-fourth round burr. The immediate expansion of the midpalatal suture was performed by inserting a 1.5-mm-thick circular metal ring fabricated from a 0.5-mm-diameter stainless steel orthodontic wire (Dental Morelli Ltda, Sorocaba, São Paulo, Brazil) between the maxillary incisors. This appliance was kept in position with a light-cured adhesive (3M Unitek, Monrovia, CA) (Fig. 1A). To confirm the RME, radiographs of the maxillary region were taken from all control animals, and after the insertion of the appliance to verify the integrity of the midpalatal suture (Fig. 1B).

2.3. Fos immunohistochemistry

The animals were deeply anesthetized and transcardially perfused with 100 mL of phosphate-buffered saline (PBS 0.01 M, pH 7.4), followed by 400 mL of 4% paraformaldehyde in 0.1 M PBS. The brain was removed from the skull, post-fixed in the same fixative for 2 days, placed in PBS containing 30% sucrose, and stored at 4°C until cryostat sectioning.

Coronal sections through the spinal trigeminal caudalis (SpVc–bregma: −15.96 and −14.28 mm), spinal trigeminal interpolaris (SpVi–bregma: −13.32 mm), and spinal trigeminal oralis (SpVo–bregma: −11.28 mm) were cut at a thickness of 40 µm on a cryostat (Fig. 2). Every third section was collected in PBS and processed for *Fos* immunohistochemistry. Free-floating sections were washed

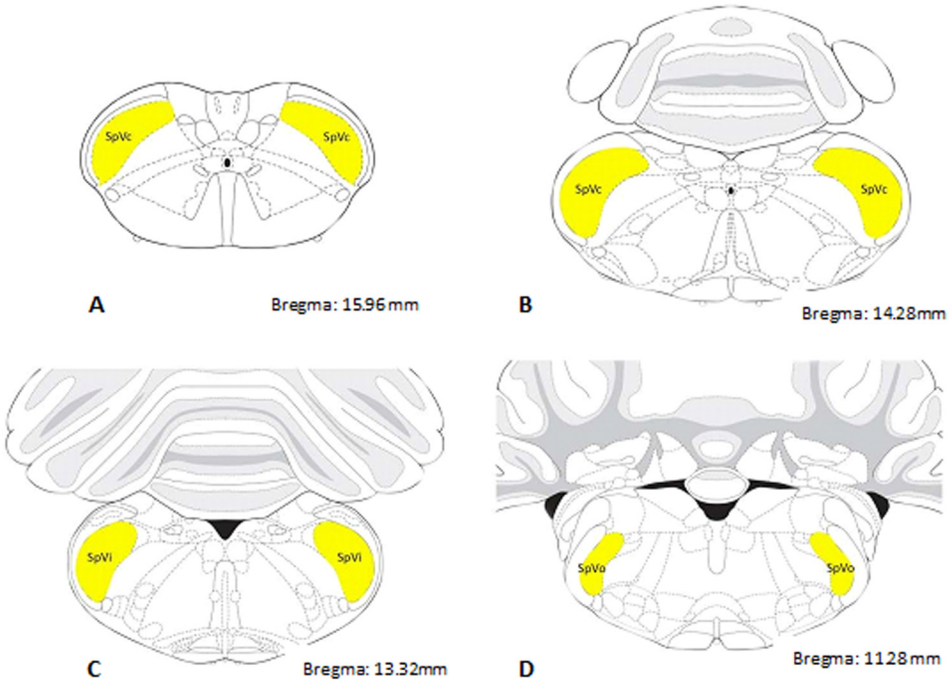

Fig. 2. Schemes of rat brain sections (Adapted from Paxinos and Watson[25]) through the caudal (A) and rostral (B) portions of the spinal trigeminal caudalis subnucleus (SpVc) and spinal trigeminal interpolaris (SpVi), and orali (SpVo) subnuclei, C and D, respectively.

three times (15 minutes per wash) in PBS (0.01 M, pH 7.4) and incubated in PBS containing 3% hydrogen peroxide for 10 minutes to inactivate the endogenous peroxidase activity. After several rinses in PBS for 30 minutes, the sections were placed in 5% normal goat serum (Vector, Burlingame, CA) for 45 minutes and subsequently incubated for 24 to 48 hours at 4°C with a polyclonal anti-*c-Fos* serum generated in rabbits (Santa Cruz Biotechnology, Santa Cruz, CA). The serum was diluted 1:2000 in PBS containing 1% normal goat serum, 1% bovine serum albumin, and 0.3% Triton X-100 (J.T. Baker, NJ). After being rinsed in PBS, the sections were incubated for 1.5 hours at room temperature with biotinylated goat-antirabbit IgG (1:200; Vector). Subsequently, the sections were washed in PBS and incubated for 30 minutes in an avidin-biotin-peroxidase complex (ABC kit, Vectastain, Vector, Burlingame, CA). Labeled neurons were visualized by 10 to 15 minutes of incubation with 0.05% 3.3′-diaminobenzidine tetrachloride and 0.001% hydrogen peroxide. The polyclonal anti-*c-Fos* antibody was omitted in negative controls. The sections were mounted onto gelatin-coated slides, dehydrated through an ascending series of ethanol, cleared with xylene, and coverslipped with Entellan. Because the antibody used in this study recognizes *c-Fos* and *Fos*-related proteins, the obtained immunoreactive neurons are described as *Fos*-like immunoreactive (Fos-IR) (Fig. 3).

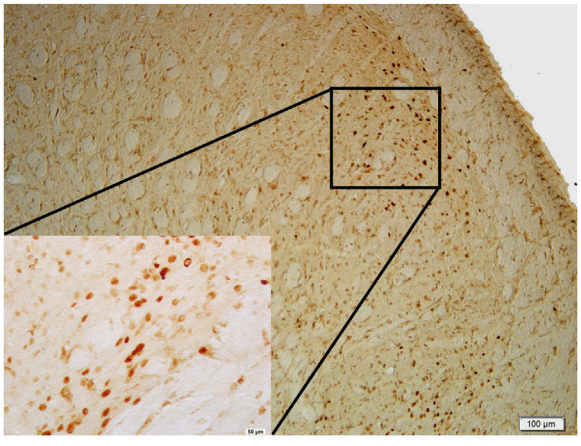

Fig. 3. Photomicrographs of the dorsal lateral portion of the SpVc showing the presence of *Fos*-IR neurons (black dots in nuclei) in animals that received RME. Scale bar: 100 µm.

2.4. Data analysis

ImageJ software 1.28 µ (National Institutes of Health, Bethesda, Maryland, USA) was used to quantify *Fos*-IR neurons in the sections under light microscopy (10 × objective), and labeled neurons were registered using an image analysis system (Zeiss KS 300). Neurons were considered to be *Fos*-IR-positive when their nuclei were of appropriate size (diameter ranging approximately 8–15 µm) and shape (oval or round) and distinct from the background at 10 × magnification. For quantification, brain sections of each subnucleus containing the maximum number of labeled neurons were selected for unilateral counts for each rat. The sections were selected from similar rostrocaudal positions based on the description of an anatomical atlas [17]. Each section was divided into three anteroposterior 2-mm^2 areas comprising at least 50% of the total surface area of the subnucleus. All results are expressed as the mean ± SD. Analysis of variance (ANOVA), followed by Tukey post hoc tests was used for the comparison of the groups. $P < 0.05$ was considered to represent a statistically significant difference.

3. Results

The radiographic analysis showed the separation of the midpalatal suture with the orthodontic appliance in the experimental group (Fig. 1b), whereas only a small tooth movement was observed with the orthodontic appliance inserted in the sham group

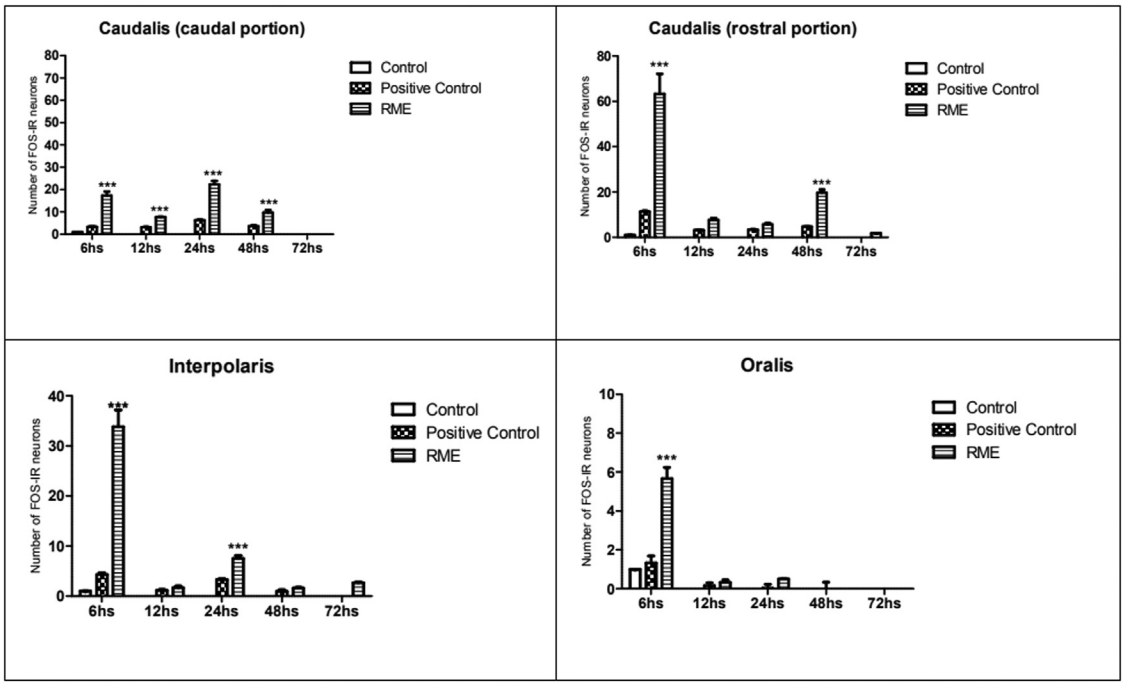

Fig. 4. Temporal changes in the number of *Fos*-IR neurons (means ± SEM) in the trigeminal subnuclei caudalis (SpVc), subnuclei interpolaris (SpVi), and subnuclei oralis (SpVo) in animals of the control group (no treatment), positive control (without RME), and experimental group with appliance activated (RME); $^{***}P < 0.001$ in RME versus positive control.
hs, hours.

with no expansion of the midpalatal suture. To examine the effects of RME, we evaluated *Fos* expression in the nuclei until 72 hours after RME. We observed *Fos*-IR expression mainly in the laminae I and II in the caudalis subnucleus, in the dorsal portion of the interpolaris subnucleus, and in the dorsal median portion of the oralis subnucleus.

No *Fos*-IR neurons appeared in the trigeminal spinal subnuclei (caudalis [SpVc], interpolaris [SpVi], and oralis) in the control group (0 hours). The brain sections of the group that received the activated appliance, which was removed shortly after insertion (positive control group) and received the same anesthesia and were subject to all manipulations associated with appliance adjustment, showed only a few *Fos*-IR neurons at each analyzed time point (Fig. 4). These low number of *Fos*-IR neurons in the positive control group indicate that a simple oral manipulation or a short insertion of an activated appliance did not induce a strong neural activation. The expression of *Fos*-IR in the positive control groups was greater than in the control groups ($P < 0.05$) and lower than in the experimental group ($P < 0.001$). These results may reflect some minor tooth movement that was simply caused by the appliance insertion in this group.

In the experimental groups, the orthodontic appliance promoted *Fos* expression in several nuclei. Because these nuclei exhibited almost identical *Fos*-IR labeling in both hemispheres, we chose the better-defined side for counting and photography. The experimental group showed a much higher number of *Fos*-IR neurons in all analyzed brain structures and at every time point when compared with the control and positive control group ($P < 0.001$).

In the experimental group, *Fos*-IR expression after 6 hours of expansion period was significantly stimulated ($P < 0.001$) compared with the positive control group (mean + SEM). Many *Fos*-IR neurons were observed in the rostral (63.33 ± 19.69 vs. 11.33 ± 0.8 positive control; $P < 0.0001$) and caudal (17.33 ± 6.11 vs. 3.33 positive control; $P < 0.0001$) portions of the SpVc at 6 hours after RME. Similar findings were obtained in the interpolaris (33.83 ± 8.34 vs. 4.33 positive control; $P < 0.0001$) and oralis (5.66 ± 1.34 vs. 1.33 positive control; $P < 0.0001$) (Fig. 4). Furthermore, *Fos*-IR expression during the later stage (48 hours) of the 72-hour expansion period was still significantly higher than that observed in the positive control group, mainly in the caudalis (caudal and rostral portion). In this group, the time course analysis revealed an increase in the number of *Fos*-IR neurons at 6, 24, and 48 hours ($P < 0.001$), followed by a generalized decrease in *Fos*-IR at 72 hours.

After RME (6 hours), numerous *Fos*-like immunoreactive neurons were clustered in the dorsomedial end of the subnucleus caudalis, predominantly near the obex, and in the transition zone to the trigeminal spinal subnuclei interpolaris (Fig. 3). The RME did not significantly increase the number *of Fos*-IR neurons in the trigeminal spinal subnuclei interpolaris or oralis at 12 hours, but we registered another increase in the number of *Fos*-IR neurons in the SpVc at 48 hours (Fig. 4).

4. Discussion

The individual responses to pain caused by orthodontic appliances are variable. In addition, there are immediate and delayed pain responses after orthodontic force application. The reaction to orthodontic treatment is related to the initial compression of the periodontal ligament. The initial response to compression and later response that starts a few hours later can be called hyperalgesia of the periodontal ligament. All orthodontic procedures create tension and compression zones in the periodontal ligament space, resulting in a painful experience for the patients [2,4]. Based on the time of onset, Burstone [1962] [18] classified pain as follows: (1) immediate: associated with the sudden placement of heavy forces on the tooth, such as a hard figure eight tie between the central incisors to close a midline diastema. (2) delayed: produced by varying force

(light to heavy) and manifesting as hyperalgesia of the periodontal membrane. This type of pain response decreases with time.

Orthodontic tooth movement induces pain and discomfort because of inflammation within the periodontium. Some proteins, such as prostaglandins and substance P, are released, which could be related to the mechanism of pain sensation [4]. The *c-Fos* is an immediate early gene that encodes the nuclear protein *Fos*, and the expression of this gene can indicate the physiologic activity to identify specific neuronal pathways in the brain. *Fos* protein is the product of *c-Fos* expression and is detected by immunohistochemistry. Under normal physiologic conditions, the levels of *c-Fos* are low, but it may be upregulated rapidly and dramatically after neural activity associated with several stimuli, including pain [19]. Studies have shown that experimental tooth movement induces the expression of *c-Fos* in the trigeminal sensory complex [14,17,19]. Gene expression mostly appeared 2 and 4 hours after the onset of the experimental tooth movement, which indicates that such nociception could be related to the compression of the periodontal ligament immediately after the application of force to the teeth and unintentional stimulation of the oral cavity associated with the experimental procedure. In the clinical practice, the pain and discomfort usually appear 1 day after orthodontic force application [15,19]. The literature reported a similar study [20] that evaluates *Fos*-IR in the same brain regions that we investigate in this study; however, they evaluated the orthodontic force but not the RME that produces immediate suture opening as we get in orthodontic procedures. We know that the RME in clinical cases will generate an orthopedic force and, in some cases, causes microfracture of the suture.

In this study, significant induction of *Fos*-IR expression was observed 6 hours after RME in the trigeminal subnucleus caudalis. Electrophysiologic studies have demonstrated that the subnucleus caudalis contains nociceptive neurons [8]. The *Fos*-IR expression pattern in the present study was similar to that elicited by thermal stimulation of the dental pulp [8], mechanical stimulation of oral mucous membranes [10], pulp exposure, or tooth extraction [11]. Therefore, the present results indicate that RME elicited nociception in the palatal suture and that this peripheral nociceptive sensation is projected to the bilateral trigeminal subnucleus caudalis.

In the present study, the expression of *Fos*-IR was induced 6 hours after the commencement of RME. In a previous study, experimental tooth movement resulted in a similar *c-Fos* expression pattern in the trigeminal subnucleus caudalis after 1, 2, and 4 hours; however, expression was not detected after 24 hours. [19] This discrepancy may be related to the sensitivity of the technique employed to detect *Fos* protein or the magnitude of the force used. When a mechanical force is applied to teeth for a prolonged period, inflammation occurs within the periodontium [2]. In a recent study, the perceived pain was most pronounced in the first 3 days after multibanding [21]. The peak pain intensity was reached at 24 hours after a latency of 2 hours, which disagrees with our finding that the pain intensity peaked 6 hours after RME, with less pain at 24 hours.

All studied cerebral areas showed a statistically significant increase of *Fos*-IR expression after 6 hours of RME; however, there was a difference in the expression pattern between the areas after 12, 24, 48, and 72 hours of RME. This differential distribution of *c-Fos* immunoreactivity in the spinal trigeminal nucleus has already been shown previously after facial stimulus [22]. In this study, the *Fos*-IR expression in the caudal portion of spinal trigeminal caudalis was statistically significant in RME groups at 6, 12, 24, and 48 hours, whereas the rostral portion *Fos*-IR expression was observed only after 6 and 48 hours. This cerebral area has already been associated with persistent orofacial pain [23], which can explain the prolonged expression of *Fos*-IR. In the spinal trigeminal interpolaris area, the *Fos*-IR expression was statistically significant after 6 and 24 hours of RME, and in the spinal trigeminal oralis area, only after 6 hours. These results are different from the results of Fujiyoshi et al. (2000) [24], which showed an increase of *Fos*-IR expression in the spinal trigeminal oralis area after 24 hours of orthodontic tooth movement. The different orthodontic treatments applied may explain the differences between the results.

5. Conclusions

Craniofacial orthopedics uses mechanical forces of a high magnitude that are absorbed and transmitted to the craniofacial complex. These forces produce a series of reactions characterized by tissue displacement, deformation, and the development of internal stress [2,25,26]. After sagittal expansion force was applied in rats, traumatic tears and exudates were observed, in addition to the death of fibroblasts, disruption of collagen fibers, and acute inflammation. As part of the inflammatory process, the patient perceives a painful sensation that is often expressed in the whole craniofacial region. Painful experiences have been reported in the literature after the application of expansive force with rapid palatal expanders [27,28]. Needleman et al. [2000] [28] concluded that the vast majority of children undergoing rapid palatal expansion experience pain that occurs during the initial phase and diminishes thereafter.

The correlation between neuronal activation and the sensation of pain and discomfort during orthodontic tooth movement is a matter of clinical interest. The expression and distribution of *c-Fos* in the neurons of the trigeminal sensory nuclear complex, parabranchial nucleus, and paraventricular nucleus of hypothalamus and the thalamus have been demonstrated [14,19]. It is clear from these studies that the trigeminal nucleus caudalis is an important relay nucleus for the processing of orofacial sensory information, and increased expression of *c-Fos* can be found in this nucleus after the application of orthodontic force. This rapidly advancing field within pain research uses orthodontic forces as a model to provide insight into the intricate mechanisms of neuronal activation. Although, further studies are required to elucidate the pathway of pain arising from mechanical procedures applied during orthopedic mechanotherapy. In conclusion, these findings indicate that RME evoked delayed and continuous nociception after the application of orthopedic force to the teeth and that the nociceptive information was conveyed to the trigeminal subnucleus.

CRediT authorship contribution statement

Caio Luiz Bitencourt Reis: Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft. **Elaine Machado Pingueiro-Okada:** Methodology, Writing – review & editing. **Kelly Galisteu Luiz:** Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Gabriela Leite Pedroso:** Methodology, Formal analysis, Investigation, Writing – review & editing. **Mirian Aiko Nakame Matsumoto:** Conceptualization, Resources, Writing – review & editing. **Luciane Macedo de Menezes:** Software, Resources, Writing – original draft, Writing – review & editing. **Erika Calvano Küchler:** Resources, Writing – review & editing. **Maria Bernadete Sasso Stuani:** Conceptualization, Methodology, Validation, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

References

[1] Sawada M, Shimizu N. Stimulation of bone formation in the expanding mid–palatal suture by transforming growth factor-beta 1 in the rat. Eur J Orthod 1996;18:169–79.
[2] Krishnan V. Orthodontic pain: from causes to management – a review. Eur J Orthod 2007;29:170–9.
[3] Oliver RG, Knapman YM. Attitudes to orthodontic treatment. Br J Orthod 1985;12:179–88.
[4] Ngan P, Kess B, Wilson S. Perception of discomfort by patients undergoing orthodontic treatment. Am J Orthod Dentofacial Orthop 1989;96:47–53.
[5] Keim RG. Managing orthodontic pain. J Clin Orthod 2004;38:641–2.
[6] Firestone AR, Scheurer PA, Bürgin WB. Patients' anticipation of pain and pain-related side effects, and their perception of pain as a result of orthodontic treatment with fixed appliances. Eur J Orthod 1999;21:387–96.
[7] Bergius M, Kiliaridis S, Berggren U. Pain in orthodontics. A review and discussion of the literature. J Orofac Orthop 2000;61:125–37.
[8] Hu JW, Sessle BJ. Comparison of responses of cutaneous nociceptive and non-nociceptive brain stem neurons in trigeminal subnucleus caudalis (medullary dorsal horn) and subnucleus oralis to natural and electrical stimulation of tooth pulp. J Neurophysiol 1984;52:39–53.
[9] Coimbra F, Coimbra A. Dental noxious input reaches the subnucleus caudalis of the trigeminal complex in the rat, as shown by c-fos expression upon thermal or mechanical stimulation. Neurosci Lett 1994;173:201–4.
[10] Sugimoto T, Hara T, Shirai H, Abe T, Ichikawa H, Sato T. c-fos induction in the subnucleus caudalis following noxious mechanical stimulation of the oral mucous membrane. Exp Neurol 1994;129:251–6.
[11] Wakisaka S, Sasaki Y, Ichikawa H, Matsuo S. Increase in c-fos-like immunoreactivity in the trigeminal nucleus complex after dental treatment. Proc Finn Dent Soc 1992;88(Suppl. 1):551–5.
[12] Ding YQ, Takada M, Shigemoto R, Mizumo N. Spinoparabrachial tract neurons showing substance P receptor-like immunoreactivity in the lumbar spinal cord of the rat. Brain Res 1995;674:336–40.
[13] Wang QP, Nakai Y. The dorsal raphe: an important nucleus in pain modulation. Brain Res Bull 1994;34:575–85.
[14] Yamashiro T, Satoh K, Nakagawa K, Moriyama H, Yagi T, Takada K. Expression of Fos in the rat forebrain following experimental tooth movement. J Dent Res 1998;77:1920–5.
[15] da Silva AP, Petri AD, Crippa GE, et al. Effect of low-level laser therapy after rapid maxillary expansion on proliferation and differentiation of osteoblastic cells. Lasers Med Sci 2012;27:777–83.
[16] Magdalena CM, Navarro VP, Park DM, Stuani MBS, Rocha MJ. c-Fos expression in rat brain nuclei following incisor tooth movement. J Dent Res 2004;83:50–4.
[17] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. Boston, MA: Elsevier Academic Press; 2005.
[18] Burstone CJ. The biomechanics of tooth movement. In: Kraus BS, Riedel RA, editors. Vistas in orthodontics. Philadelphia, PA: Lea & Febiger; 1962. p. 197–213.
[19] Kato J, Wakisaka S, Kurisu K. Immunohistochemical changes in the distribution of nerve fibers in the periodontal ligament during an experimental tooth movement in rat molar. Acta Anat (Basel) 1996;157:53–62.
[20] Joviliano P, Junqueira AA, Stabile AC, Leite-Panissi CR, Rocha MJ. Rapid maxillary expansion causes neural activation in brain structure of rats. Brain Res Bull 2008;76:396–401.
[21] Erdinç AM, Dinçer B. Perception of pain during orthodontic treatment with fixed appliances. Eur J Orthod 2004;26:79–85.
[22] Meng ID, Bereiter DA. Differential distribution of Fos-like immunoreactivity in the spinal trigeminal nucleus after noxious and innocuous thermal and chemical stimulation of rat cornea. Neuroscience 1996;72:243–54.
[23] Ren K, Dubner R. The role of trigeminal interpolaris-caudalis transition zone in persistent orofacial pain. Int Rev Neurobiol 2011;97:207–25.
[24] Fujiyoshi Y, Yamashiro T, Deguchi T, Sugimoto T, Takano-Yamamoto T. The difference in temporal distribution of c-Fos immunoreactive neurons between the medullary dorsal horn and the trigeminal subnucleus oralis in the rat following experimental tooth movement. Neurosci Lett 2000;283:205–8.
[25] Mao JJ, Wang X, Kopher RA. Biomechanics of craniofacial sutures: orthopedic implications. Angle Orthod 2003;73:128–35.
[26] Mao JJ. Mechanobiology of craniofacial sutures. J Dent Res 2002;81:810–16.
[27] Schuster G, Borel-Scherf I, Schopf PM. Frequency of and complications in the use of RPE appliances – results of a survey in the Federal State of Hesse, Germany. J Orofac Orthop 2005;66:148–61.
[28] Needleman HL, Hoang CD, Allred E, Hertzberg J, Berde C. Reports of pain by children undergoing rapid palatal expansion. Pediatr Dent 2000;22:221–6.