

AMERICAN UNIVERSITY OF BEIRUT

THE EFFECT OF SYMPATHETIC NEURAL SUPPLY ON THE
ERUPTION RATE OF RATS' MANDIBULAR INCISORS

By

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A thesis

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for the degree of Master of Science in Human Morphology
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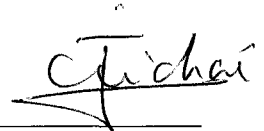
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ABSTRACT OF THESIS

Thesis title: The effect of sympathetic neural supply on the eruption rate of Rats' mandibular incisors.

By: Ammar Kassab

Major: Human Morphology

Background: Tooth eruption is defined as the movement of a tooth from its site of development within the alveolar process to its functional position in the oral cavity. The control of eruption mechanism is multifactorial, where the nervous system plays important role in the control of different contributors to this process.

Aims: (1) to investigate the effect of neurotomy on the eruption rate of rats' incisors, (2) and to evaluate the role of the sympathetic nervous system, in particular, in the eruption rate, (3) to explore the differences in eruption rate between intact and shortened incisors without altering the neural supply.

Material and methods: Forty nine (49) adult female Sprague-Dawley rats divided into seven groups; group 1: (n=8) had guanethidine treatment (30 mg/kg/day; for one week) to block the sympathetic transmission at effector level; group 2: (n=8) received hexamethonium treatment (10 mg/kg/day; for one week) to produce sympathetic block at ganglionic level; group 3: (n=7) subjected to chemical ablation of capsaicin sensitive primary afferents, and followed by daily treatment with guanethidine (30 mg/kg/day; for one week); group 4: (n=7) had the left inferior alveolar nerve (IAN) cut; group 5: (n=5) served as a sham for axotomy group; group 6: (n=7) had their left mandibular incisor cut out of occlusion by 2-3 mm; group 7: (n=7) served as a control group. Two landmarks were used to measure eruption rate: the first landmark was a groove placed on the distal aspect of the incisor, while the second landmark was a tattoo placed at the attached gingiva at the distal margin of the tooth. Measurements were registered every 48hrs for a period of 144hrs. Statistical analysis of results was performed (ANOVA) and the significance was tested by post hoc Bonferroni's multiple range test.

Results: The temporal evolution of the eruption of intact incisors elicited an initial fast ascension, followed by a phase of deceleration and decline at the end of the observation period. The eruption was significantly reduced in rats treated with guanethidine in 1st time segment (0.87 ± 0.06 mm vs. 1.18 ± 0.15 mm in control, $P < 0.05$) and at total observation period (2.57 ± 0.06 mm vs. 3.00 ± 0.16 mm; $P < 0.01$). The rate of eruption was attenuated in hexamethonium treated rats but measurements were not statistically significant. IAN section significantly attenuated eruption rate in 2nd (0.44 ± 0.13 mm, $p < 0.001$), 3rd (0.47 ± 0.11 mm, $p < 0.001$), and total time segments (1.8 ± 0.15 mm, $p < 0.001$). Guanethidine treatment in rats with ablated CSPA fibers reduced eruption rate during the first (0.79 ± 0.07 mm; $p < 0.05$), second (0.66 ± 0.7 mm; $P < 0.001$), and total (2.24 ± 0.08 mm; $p < 0.001$) time segments. The rate of eruption of shortened incisors significantly increased at the 1st (1.67 ± 0.2 mm; $p < 0.01$) and 2nd (1.66 ± 0.2 mm, $p < 0.05$) time segments then presented a compensatory deceleration until the tooth reached the incisal plane (0.37 ± 0.17 mm, $p < 0.01$).

Conclusion: the nervous system plays a key role in the control of the eruption process of rats' mandibular incisors. Sympathetic supply appears to constitute a major component in this control.

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LIST OF ABBREVIATIONS

A δ :	A-delta fibers
ANOVA:	Analysis of variance
CGRP:	Calcitonin gene-related peptide
CSPA:	Capsaicin sensitive primary afferents
IAN:	Inferior alveolar nerve
i.p:	Intraperitoneal
M1:	First molar
M2:	Second molar
M3:	Third molar
mAChR:	Muscarinic acetylcholine receptors
nACh:	Nicotinic cholinergic receptors
NSE+:	Non-specific esterase
SEM:	Standard error of the mean
SP:	Substance P
TRAP+:	Tartrate-resistant acid phosphatase-positive

Introduction

1. Preamble

About five hundred million years ago, Ordovician species developed teeth with the basic microscopic anatomy similar to that of recent vertebrates. Some jawless fish developed superficial, dermal structures known as odontodes (Smith, 1995; Butler et. al, 1998), which are small tooth-like structures that are located outside the mouth and serve various functions. The developmental process led to the encroachment of those odontodes into the oropharyngeal cavity creating the buccal teeth. Dietary habits and ecological adaptations drove the teeth of vertebrates to acquire numerous anatomical forms and shapes, as represented by incisors, canines, premolars and molars. Dentine, the main body of a tooth, is secreted by odontoblasts, which are cells of cranial neural crest origin (Lumsden; 1988). It is composed of collagen, dentine sialophosphoprotein, dentine matrix protein and hydroxyapatite. Dentine surrounds the pulp, which is rich in fibroblast-like cells, blood vessels and nerves. Enamel, the hardest tissue in the body, covers the coronal part of the dentine. It is secreted by ameloblasts, and is collagen-free. Teeth form through highly orchestrated mutual inductive interactions between two major cell types; (1) stomodeal ectodermal cells; and (2) cranial, neural crest-derived ectomesenchymal cells. Morphological differences between individual teeth of a dentition arise mainly from differences in expression of odontogenic genes. These genes encode transcription factors that regulate signaling factors (Thesleff et.al; 1995) that mediate inductive interactions between the odontogenic layers and affect cell multiplication, apoptosis and cell

differentiation (Matalova et.al; 2004). Those genes and signaling factors, which are involved in teeth morphogenesis, are also involved in the development of many other organs in various animals (Thesleff et.al; 1995, Koussoulakos; 2004). As roots of teeth form, teeth tend to start the eruption process, where teeth move in an axial direction from their location within the alveolar crypt of the jaw into their final functional position within the oral cavity (Cahill et. al, 1982).

2. Tooth eruption

Tooth eruption is defined as the movement of a tooth from its developmental site in the alveolar bone to its final functional position in the oral cavity (Masler, 1941). Eruption of teeth can be limited like in human dentition, or can be continuous like in rodents' incisors. The latter have provided valuable opportunities to study the eruptive process (Cahill et. al, 1988).

Specific factual observations were deemed necessary to exist in order to explain the eruptive mechanism; (1) teeth are moved in three-dimensional space, not just along their axis; (2) teeth erupt with varying speed; (3) teeth arrive at a functional position that is inheritable (Sandy et. al, 1996).

Considering the previous requirements for eruption, several hypotheses of tooth eruption like pulpal pressure, pulpal growth, periodontal fibroblasts traction, vascular pressure, and blood-vessel thrust are considered inconclusive in the explanation of the eruptive process (Herzberg et. al 1941; Bryer, 1957; Sutton et. al, 1985). In contrast, root elongation (Masler, 1941; Witkop, 1975; Shields et. al, 1973; Pietrokovski et. al, 1966; Perl et. al, 1977; Gowgiel, 1961; Carl et. al, 1980; Cahill et. al, 1980) hydrostatic pressure (Van

Hassel et.al, 1972; Moxham et. al, 1974), alveolar bone remodeling (Brash, 1928; Landsberger, 1924; Marks, 1989; Jensen et. al, 1990), and possibly, periodontal ligament formation (Berkovitz et. al, 1969; Berkovitz 1971) hypotheses are more elaborate in the explanation of tooth eruption in man and other mammalian species (Sandy et. al, 1996).

3. Neural supplies of the mandible and teeth

3.1 Anatomy of mandibular teeth innervation:

Rats have relatively simple dentition; three molars and one continuously erupting incisor are present in each quadrant. The Inferior alveolar nerve (IAN) supplies the mandibular teeth, and the 2nd and 3rd molars have alternative innervation as well (Berger et. al, 1983a/b). The IAN courses the mandibular canal as a single large trunk accompanied by one to three smaller nerves that proceed forward above the main IAN trunk to innervate the first molar and part of the second molar (anterior molars nerve). The main trunk of the inferior alveolar nerve is divided into two large branches (dorsal and ventral) at the level of the roots apices of incisors. The dorsal branch is larger and is designated as the mental nerve. It proceeds forward through the length of the mandibular canal and exits through the mental foramen which is located anterior to the first molar. The ventral branch, the incisor nerve, goes forward beneath and medial to the mental nerve. The incisor nerve divides into two branches (medial and lateral) at a level corresponding to the distal root of the second molar. Both branches further divide as they proceed forward along their path. Most of the fascicles of the larger, medial bundle and a small component of the lateral bundle descend through a thin layer of bone to enter the adjacent socket of the incisor. These fascicles

divide into numerous branches of forward and backward orientations, and form a conspicuous array of longitudinally oriented nerve fiber bundles within the periodontium of the incisor. The lateral incisor bundle continues forward within the mandibular canal. At the level of the posterior edge of the first molar, its branches exit from the mandibular canal and courses obliquely forward for about 0.5 mm in its own bony canal then enters the incisor socket, contributing to the formation of the periodontal plexus. Within the incisor socket, small branches from the periodontal plexus enter the apical foramen of the incisor root in accompany with blood vessels heading towards the core of the pulp (Naftel et. al, 1999). Figure one summarizes the distribution of the inferior alveolar nerve and its branches.

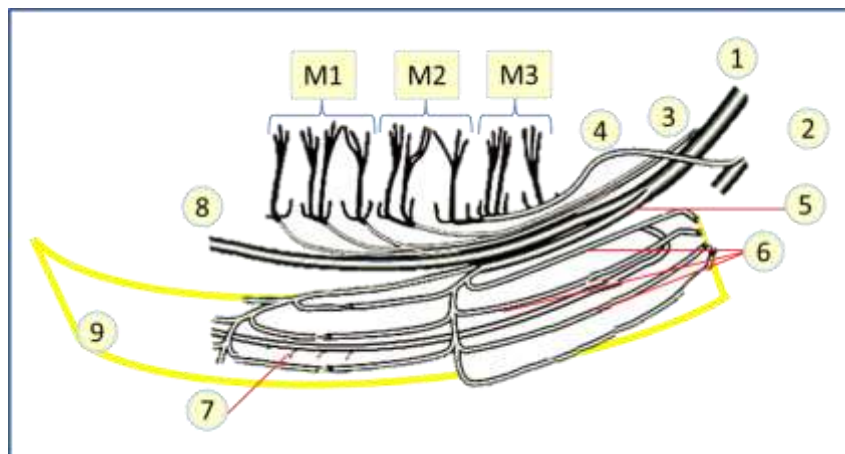


Figure 1: IAN nerve course and branches in the adult rat

(1) IAN trunk; (2) Lingual nerve; (3) Nerves to M1/M2; (4) Nerve to M2/M3; (5) Incisor branch; (6) Incisor periodontal plexus; (7) Incisor pulpal fibers; (8): Mental nerve; (9): Mandibular incisor; (M1): Nerve terminals to first molar; (M2): Nerve terminals to second molar; (M3): Nerve terminals to third molar (Naftel et. al, 1999).

The innervation to the teeth and their surrounding alveoli is made of the sympathetic efferents and sensory innervation through the myelinated A-delta ($A\delta$) that becomes unmyelinated as it enters the tooth pulp, and the unmyelinated C-fibers. Substance P (SP)-containing sensory fibers dissociate from the blood vessels after entering the marrow space and terminate as free-nerve endings (Shinji et. al, 2002).

The sympathetic innervation to teeth comes from unmyelinated axons originating in the ipsilateral superior cervical ganglion. The sympathetic nerve endings contain norepinephrine. Other neuropeptides are also present like neuropeptide Y and vasoactive intestinal peptide (Ladizesky et. al, 2001).

3.2 Mechanisms of sympathetic control of tooth eruption:

The nervous supply to bone and periosteum is made of autonomic and sensory nerve fibers (Miller et. al, 1963; Milgram et. al, 1965). Autonomic nerve fibers are found in the periosteum, endosteum, and cortical bone. The free-running fibers are usually associated with blood vessels that enter the bone through Volkmann's canals (Fristad et al., 1994).

Bone modeling and remodeling are important to form the shape and maintain balance in the bony skeleton. Bone turnover and remodeling are mediated through osteogenesis and osteoclastogenesis. Development and differentiation of osteoblasts and osteoclasts are locally controlled by growth factors and cytokines produced in the bone marrow microenvironment as well as by adhesion molecules that mediate cell-cell and cell-matrix interactions. Signals derived from the endocrine and the nervous systems also exert potent effects on osteoclast and osteoblast development and differentiation (Cardinali et al., 2003).

Intact neural supply is essential in bone metabolism, bone resorption, and in tooth movement. Osteoclasts are needed to resorb the alveolar bone as the tooth erupts. The formation of eruption pathways is considered a distinctive feature in bone. Even if the tooth is stopped from eruption, the pathway still forms (Cahill, 1969a; Wise et. al, 2008).

Osteoclasts responsible for creating this pathway arise from the dental follicle at specific time prior to eruption. Bone resorption is essential in tooth eruption, and therefore, the interference with the resorptive process leads to the inhibition or the slowing down of eruption (Wise et. al, 1989).

The sympathetic system appears to play a significant role in bone resorption by acting on the differentiation of the recruited non-specific esterase (NSE)+ osteoclast precursors into tartrate-resistant acid phosphatase-positive (TRAP+) preosteoclasts, and ultimately on the size of the osteoclast population. It plays a role in the control of the access of the recruited osteoclasts to the bone surface (Cherruau et.al, 1998). It also controls bone metabolism through the inhibition of preosteoclast differentiation and osteoclast activation (Cherruau et. al, 1999, 2003); thus the depletion of sympathetic mediators may disturb osteogenic cell-mediated osteoclast differentiation.

Fat-derived hormone leptin controls bone formation through a hypothalamic relay. Leptin binding to its hypothalamic receptor is sufficient to induce bone loss by decreasing osteoblastic function (Ducy et. al, 2000). The central effects of leptin on bone are mediated via the sympathetic nervous system (Takeda et. al, 2002).

The effect of the sympathetic system on bone metabolism can also be indirect through the blood flow. It may also be possible that neuromediators released by nerve fibers affect osteoclasts and osteoblasts through the regulation of cytokine expression by immune cells

(Chenu, 2004). It was shown that chemical and surgical denervation of sympathetic and/or sensory nerves modulate the number of bone-resorbing osteoclasts, and thus affect bone resorption (Hill et. al, 1991; Sherman et. al, 2000; Chenu, 2004).

4. Chemical block of sympathetic efferents:

Different surgical and chemical techniques are used in order to block the sympathetic nervous system. The chemical approach can target the sympathetic block at the effector (peripheral) level (e.g. guanethidine), or at the ganglionic level (e.g. hexamethonium). Surgical sympathectomy can also be done, but with less selectivity and more secondary complications, compared to the aforementioned approaches. Surgical sympathectomy by superior cervical gangliectomy may interfere with the sympathetic supply to all organs in the head and neck area with the resulting denervation supersensitivity and other complications known to result from lesions to the peripheral nerves.

4.1 Sympathetic block at the target level:

4.1.1 Guanethidine:

Guanethidine is considered as an antiadrenergic agent acting exclusively at the level of peripheral sympathetic synapses (i.e. contacts with the effectors) without a direct effect at the level of central synapses. It inhibits the function of postganglionic adrenergic neurons at the level of their synaptic contacts with the effector organs. It can reach the synaptic vesicles through the norepinephrine (N.E.) re-uptake transporter. It replaces norepinephrine in the vesicles and is released instead of the normal transmitter. As being an inactive

transmitter, the replacement of N.E. by guanethidine is responsible of its antihypertensive effects (Freis, 1960, 1965; Kadzielawa, 1962).

Sympathetic blockade by guanethidine produces venodilatation, reduction in cardiac output due to inhibition of cardiac sympathetic innervations; it blocks sympathetic arteriolar reflex response to the reduction in cardiac output and produces symptomatic hypotension (due to sympathetic reflex blockade), sexual dysfunction (delayed ejaculation), and diarrhea (Freis, 1965). The neuropeptides (like neuropeptide Y, and vasoactive intestinal peptide) that are usually released from the sympathetic terminals are blocked by the action of guanethidine. It was also found that the administration of guanethidine at birth (in rats) increases bone resorption (Hill et. al, 1991), while treatment with guanethidine in adult rats decreased bone resorption (Cherruau et. al, 1999, 2003).

Guanethidine was also used in the treatment of sympathetic disorders such as complex regional pain syndrome, but other sympathetic block techniques proved to be superior to the guanethidine administration in the treatment of such disorder (Ejls et. al, 2010).

4.2 Sympathetic block at the ganglionic level:

4.2.1 Hexamethonium

Hexamethonium is a ganglionic blocker acting at the level of nicotinic cholinergic receptors (nACh). It does not have any effect on the muscarinic acetylcholine receptors (mAChR) located on target organs of the parasympathetic nervous system (Sonoyama et. al, 2000).

Hexamethonium acts on receptors at ganglionic sites in both the sympathetic and parasympathetic nervous systems, which are both regulated by nicotinic ligand-gated ionotropic acetylcholine receptors. Although postganglionic sympathetic systems are usually regulated by norepinephrine, some post-ganglionic sympathetic neurons, such as those stimulating sweat glands, release acetylcholine. The parasympathetic system is acetylcholine-based, and relies on muscarinic receptors.

Systemic effects of hexamethonium include combined sympatholytic effects like orthostatic hypotension and sexual dysfunction, and parasympatholytic effects like constipation, urinary retention, glaucoma, blurry vision, decreased lacrimal secretion, and xerostomia.

Different reports on the effect of hexamethonium on teeth eruption are present in the literature (Moxham et. al, 1979 and 1988). The administration of hexamethonium reduced the rate of eruption in erupting ferrets' canines. However, it had no effect on fully erupted ferrets' canines (Moxham et. al, 1988). These effects on teeth with limited eruption are not similar to the drug's effect on teeth with continuous eruption; i.e. the effect on rabbits' incisors where hexamethonium administration led to an increase in the extrusive movement (Moxham et. al, 1979). These differences might be due to differences in animal species studied, as well as different types of eruption.

5. Aims of study

It has been shown that alterations of the function of the sympathetic efferents may interfere with tooth eruption through modulating bone resorption and blood flow (Cherruou et. al, 1999). The present evidence shows variation in the effect of sympathetic block on bone resorption and tooth eruption. Leist showed an increase in eruption rate of rodents incisors, after having sympathetic block (Leist, 1927). Other evidence showed the contrary (Taylor and Butcher, 1951; Miller, 1957).

The aim of the study is to evaluate the role of the sympathetic nervous system in the eruption rate of intact rats' mandibular incisors. We also aim to investigate the effect of neurotomy on the eruption rate of rats' incisors.

In the present research, nerve block and/or selective lesion will follow a sequence of interventions aiming at progressive and reversible block of the sympathetic fibers supplying the mandibular incisors.

Also, this study aims to explore the differences in eruption rate between intact and shortened incisors without altering the neural supply.

Material and Methods

1. Animals:

Forty nine (49) adult female Sprague-Dawley rats of (200-250) g were used in the experiments with strict adherence to ethical guidelines (Zimmermann, 1983) and following approval by the Institutional Animal Care and Use Committee (IACUC). Rats were placed in plastic cages, and were housed on a 12 hours light/dark cycles (7am-7pm) at a temperature of 20-22°C with standard rodent chow and water provided *ad libitum*. The experimental sessions were conducted during day hours between 08:00 and 18:00. Rats were divided into seven (7) groups according to following experimental protocol: Group (1) consisted of 8 rats and received guanethidine treatment; Group (2) consisted of 8 rats and received hexamethonium treatment; Group (3) consisted of 7 rats who had their capsaicin sensitive primary afferents blocked by capsaicin and treated with guanethidine; Group (4) consisted of 7 rats that had the left inferior alveolar nerve exposed and cut; Group (5) consisted of 5 rats and served as a sham group to the previous group by exposing the left inferior alveolar nerve and keeping it intact; Group (6) consisted of 7 rats that had no chemical or surgical intervention, and had their left mandibular incisor cut out of occlusion; Group (7) consisted of 7 rats and served as a control group. All animals had their mandibular incisors eruption rate measured every 48 hours for a period of 144 hours.

2. Drugs used for animals anesthesia:

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

3. Exposure of the inferior alveolar nerve:

The exposure of the inferior alveolar dental nerve was performed as described by (Dreyer and Rerief, 1969) with some modifications.

Three imaginary lines are identified on the rat's face (Fig. 2); (1) line A-B is extended from the outer canthus of the eye to the base of the ear; (2) E-F line extends from the corner of the mouth and runs parallel to A-B line; (3) C-D line runs in the middle between the A-B and E-F , this line represents the incision line on the skin; (4) G-H line bisects the C-D line and represents the direction of dissecting the masseters muscle fibers.

Once the masseter muscle is exposed, the tip of the scissors is introduced at the masseteric fascia, until contact with bone and muscle fibers are displaced by opening the scissors without cutting. The muscle displaced will be held in position using metallic hooks.

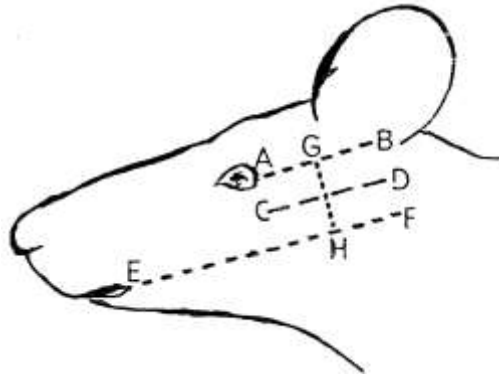


Figure 2: diagrammatic representation of the surgical access guide on the skin of the rats (left side); AB: outer canthus of the eye to the base of the ear; EF: elongation of the lip line; CD: incision line (equals to AB); and GH: vertical releasing incision (bisector of AB).

The bony prominence overlying the incisors germ was identified and a bony ridge extending from the prominence to the condyle is identified. The outer part of the cortex just distal to the bony prominence is removed by using a round carbide bur, 1 mm in diameter; this region was removed exposing 2-3 mm of the inferior alveolar nerve (Fig. 3).

At the end of the surgical intervention, the skin was sutured with non-resorbable 3-0 suture material (MERSILK, ETHICON, Auneau-France). Antibiotic ointment (bacitracin zinc and neomycin sulphate 250 IU 5000 IU) was applied topically to the wound after closure.

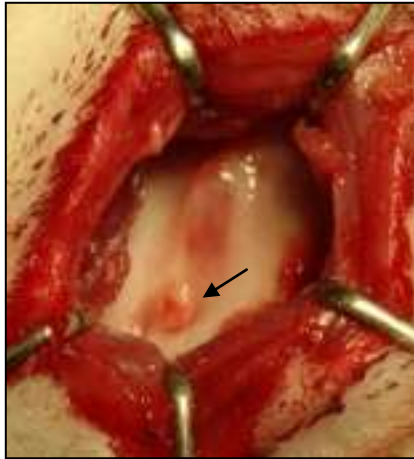


Figure 3: Exposure of the left inferior alveolar nerve (arrow).

4. Denervation:

After exposing the left inferior alveolar nerve, the nerve was removed from its bony canal using glass probes; the nerve bundle was cut leaving the two ends of the nerve separated at a distance of about 2 mm to prevent sprouting and re-communication of the two terminals. Care was taken to keep the vascular bundle intact.

5. Chemical block of the sympathetic efferents:

a. Guanethidine:

0.2 ml of guanethidine ((1-[2-Guanethinoethyl]octahydroazocine) monosulfate (1:1), cat. no. G-8520, Sigma, St. Louis, MO, USA) (30 mg / kg) was daily administered subcutaneously for a period of one week. This treatment regimen was used in both groups (1) and (3) (Coderne et. al, 1984).

b. Hexamethonium:

0.2 ml of hexamethonium Chloride ((Hexane-1,6-bis[trimethylammonium chloride]), cat. no. H-2138, Sigma, St. Louis, MO, USA) (10 mg / kg) was administered subcutaneously for rats in group (2) daily for a period of one week. The rate of eruption of mandibular incisors in this group was measured before and after the administration of hexamethonium (Hong and Henry, 1992).

6. Capsaicin sensitive primary afferents block:

Ablation of the capsaicin sensitive primary afferents in group (3) was carried out using three subcutaneous injections of capsaicin (8-methyl-N-vanillyl-non-anamide; cat. no. M1022, Sigma, St. Louis, MO, USA). The first injection was (25 mg/kg), the second injection was (50 mg/kg) and administered 8 hours later, and the third dose (50 mg/kg) was administered 32 hours after the first injection (Saadé et.al, 2002).

Treated animals were left to recover for 2 weeks, after which they were subjected to the eye-wiping capsaicin test (10mg/kg) to assess the successful ablation of CSPA (Hammond & Ruda, 1991).

7. Incisors cutting:

The left mandibular incisor was cut out of occlusion by 2-3 mm (Fig. 4), and the rate of eruption was measured for both incisors.



Figure 4: Left mandibular incisor height reduction.

8. Landmarks and measurements:

Two landmarks were used for measuring the rate of eruption of each mandibular incisor. The first landmark is a groove made at the distolabial margin of the tooth (Fig 5A&B). The groove was done by using a metallic disk (HI-FLEX, EDENTA AG, Hauptstrasse 7, CH-9434 AU/SG Switzerland) mounted on a hand piece. The second landmark is a tattoo (Drawing Ink, Rotring, Sanford GmbH D-22510 Hamburg, Germany) placed at the distal marginal gingiva (Fig. 5A&B). The first landmark will fade with eruption while the tattoos will remain permanent.

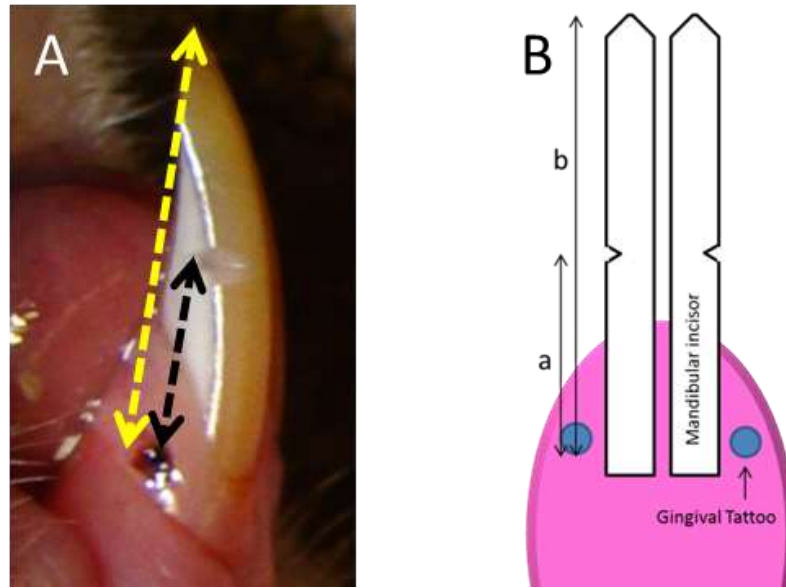


Figure 5: (A): Landmarks placed on the incisor and attached gingiva; black arrow: distance (a), yellow arrow: distance (b). (B): diagrammatic representation of the landmarks and distances used.

The distance between the tattoo on the gingiva and the groove on the tooth is called (a), while the distance between the tattoo and the incisal edge of the tooth is called (b) (Fig. 2.4 A&B). A digital caliper (Electronic Digital Caliper, accuracy 0.01mm) was used for measurements. The measurements were obtained every (48hrs) and for 144 hours. Each measurement was registered three times, after that they were averaged.

9. Animals sacrifice:

At the end of each experiments at the final time point, each animal received a lethal injection of ketamine (Ketalar®, 100mg/kg) (i.p.) followed by a cervical dislocation. Mandibles were sampled and kept under cold temperature (deep freeze, -80° C).

10. Statistical analysis and data presentation:

At each time point, every measurement was performed three times then averaged; the results obtained for each time point was presented as mean and standard error of the mean (SEM). Four time points were present in each experimental group; at time zero (0); at 48 hours; at 96 hours; and at 144 hours, leaving three readings of mean and standard error of the mean for 48 hour time difference between the time points, and one ultimate reading. The amount of eruption is represented by the differences between the mean of readings of the distance (a) on the incisors at each time point.

Statistics were made using GraphPad InStat 3 (GraphPad Software Inc. San Diego, CA, USA) and the significance of variations were calculated using one-way analysis of variance (ANOVA) and the significance tested by post hoc Bonferroni's multiple range test. The data were represented in graphs using the GraphPad Prizm 3 (GraphPad Software Inc. San Diego, CA, USA).

RESULTS

1. Rate of eruption of mandibular incisors in rats with intact innervation versus incisors with cut inferior alveolar nerve:

The eruption trend of the rats' incisors shows a tendency of attenuation throughout the observation period of 144h (Fig. 6A). The amount of eruption was (18 ± 0.15 mm) at the beginning and became 0.79 ± 0.2 mm at the end of the observation. Mandibular incisors with axotomized inferior alveolar nerve displayed a significant decrease in their rate of eruption starting 48 h after neurectomy, and the reduction in rate was maintained till the end of the observation period (Fig. 6B). The mean amount of eruption in the neurectomised incisors ($n = 7$) was 0.90 ± 0.19 mm during the first time period (0-48 h) and did not differ from that elicited by the sham group ($n = 5$; 0.79 ± 0.15 mm). This rate was significantly reduced during the second (0.44 ± 0.13 mm) and the third (0.47 ± 0.11 mm) time periods. The values measured in sham rats were: 0.79 ± 0.20 mm and 1.02 ± 0.14 mm, for the second and third time periods, respectively. The overall eruption period of 144 h, was 1.8 ± 0.15 mm for axotomized rats as compared to 2.6 ± 0.30 mm in sham rats, $P < 0.01$ (Fig. 6 B). There were no significant differences between the intact side (right side) and the sham group in each time segment and in the total amount of eruption ($p > 0.05$) (Fig. 6B).

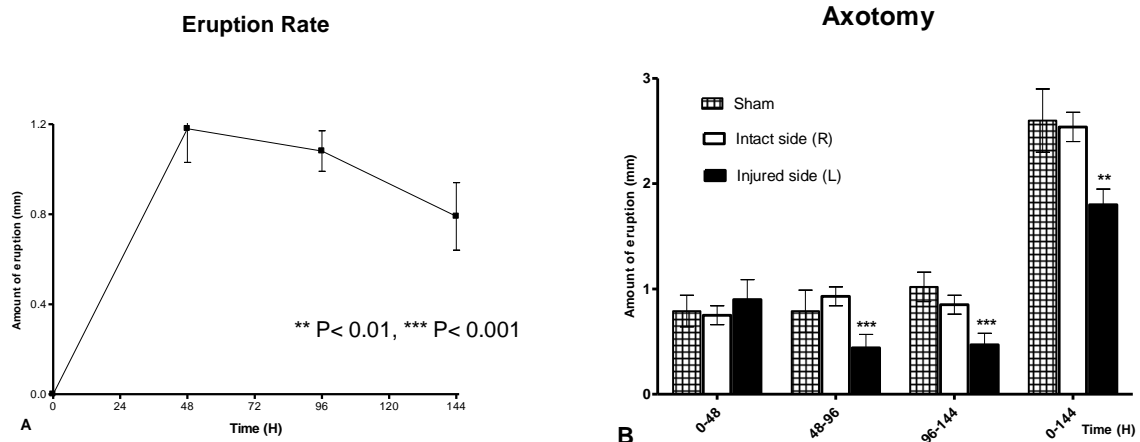


Figure 6: Time courses of eruption in rat incisors with intact (A) or lesioned (B) inferior alveolar nerves. Eruption of mandibular incisors shows an initial fast followed by a slower rate in control (n=7) experiments (A). Lesion of the left inferior alveolar nerve (B) produced a delayed and highly significant attenuation of the eruption rate (n=7), as compared to the incisor with intact innervations (contralateral side) or to sham group (n=5). All rats had the measurements taken under deep general anesthesia.

2. Effects of peripheral sympathetic block by guanethidine treatment on the eruption rate of mandibular incisors

Blockage of the peripheral sympathetic synapses, at the level of the effectors, by guanethidine treatment (30mg/kg) (n=8), attenuated the eruption rate over the period of monitoring the eruption of the incisors (0-144 h, Fig. 7). Compared to the control group (n=7), a significant reduction in the eruption rate was noted during the initial time period as illustrated in the comparative measurements of the mean amount of eruption (0-48h; 0.87 ± 0.06 mm versus 1.18 ± 0.15 mm in control, $P < 0.05$). The trend toward reduction was maintained during the second and third time period, but without reaching significance. As

shown in figure 7, the mean of the total amount of eruption in the treated group (2.57 ± 0.06 mm; $p < 0.01$) was significantly lower than that of eruption in the control group (3.00 ± 0.16 mm; $P < 0.01$).

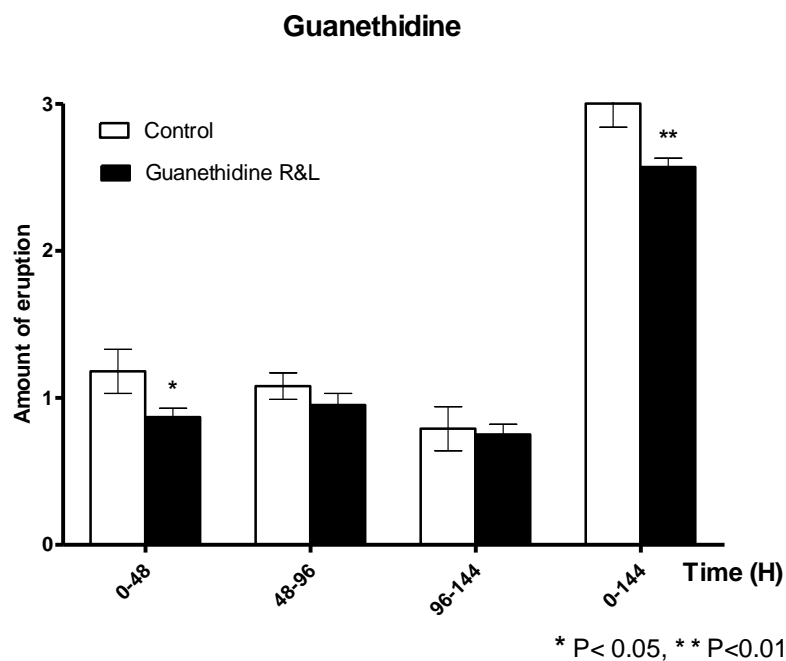


Figure 7: Blocking peripheral sympathetic synapses with guanethidine attenuated the eruption rate in rats' mandibular incisors.

Each bar represents the mean of the amount of eruption in (mm) + SEM measured in the treated ($n=8$) or control ($n=7$) at the indicated time interval. The degree of significance of the differences in the measured values was calculated with reference to control group. Guanethidine injection (30mg/kg) for seven days led to a consistent decline in the eruption rate of the rats' mandibular incisors. The reported values for the treated group are the based on the mean measurements made on both right (R) and left (L) paws, since systemic Guanethidine injections are supposed to affect both sides.

3. Effects of ganglionic sympathetic block by hexamethonium treatment on the eruption rate of mandibular incisors:

Ganglionic blockade of the sympathetic supply by hexamethonium treatment (10 mg/kg), elicited a general trend of attenuation in the eruption rate throughout the

observational period (Fig. 8). As illustration, during the initial time segment, the mean amount of eruption in the treated animals was reduced to (0.93 ± 0.06 mm; $p > 0.05$) as compared to the control group (mean amount of eruption \pm SEM: 1.18 ± 0.15 mm). Although consistent, this attenuation did not reach significance level at any time interval or in the total amount of eruption (2.8 ± 0.10 mm in treated versus 3.00 ± 0.16 mm in control group; Fig. 8).

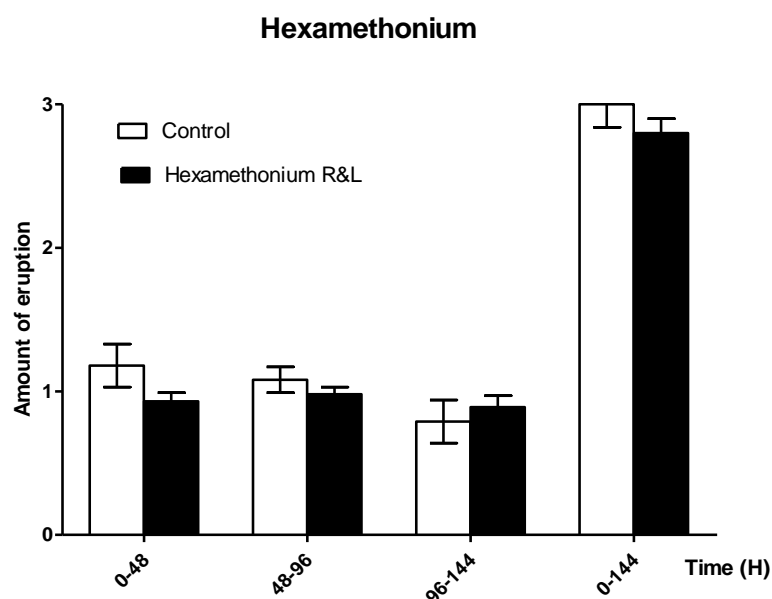


Figure 8: Ganglionic sympathetic block by hexamethonium treatment did not significantly attenuate the rete of eruption in rats' mandibular incisors.

Each set of bars indicates the mean + SEM amount of eruption of the mandibular incisors in a group of rats (control or treated, $n=7$ each) at the indicated time interval (0-48h, 48-96, 96-144 and total 0-144). The degree of significance of the differences in the measured values was calculated with reference to control group.

4. Effects of combined sympathetic block and CSPA ablation on the eruption rate of mandibular incisors:

Peripheral blockade of the sympathetic supply, by guanethidine treatment (30mg/kg), after ablation of the CSPA fibers in a group of rats (n = 7), significantly attenuated the eruption rate over the period of monitoring the eruption of the incisors (0-144 h, $p < 0.001$). Compared to the control group (n = 7), a general trend of reduction in the eruption rate was noted (Fig. 9). At the initial time segment, the mean amount of eruption of the treated animals was significantly reduced (0.79 ± 0.07 mm; $p < 0.05$), this reduction reached a peak during the second time period (0.66 ± 0.7 mm; $P < 0.001$) and returned to the control level at the end of the observation period. The mean of the total amount of eruption in the treated group (2.24 ± 0.08 mm) was significantly lower than that observed in the control group (mean amount of eruption (mm) \pm SEM: 3.00 ± 0.16 , $p < 0.001$).

Capsaicin and Guanethidine

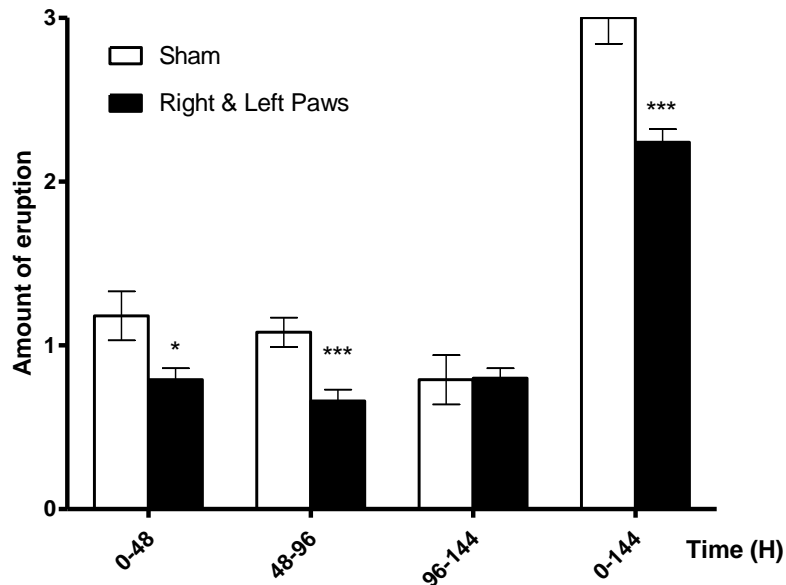


Figure 9: Guanethidine treatment in rats with ablated CSPA fibers further reduced the rate of eruption of mandibular incisors.

Each bar represents the mean amount of eruption in (mm) + SEM measured in control and in rats treated with guanethidine following CSPA ablation (n=7). The degree of significance of the differences in the measured values was calculated with reference to control group (n=7).

(* P < 0.05, ** P < 0.01, *** P < 0.001).

5. Eruption rate of shortened rats' mandibular incisors:

Shortening mandibular incisor unilaterally in rats increases the rate of eruption of this incisor, as the shortened tooth reaches the occlusal level, the rate of eruption gets attenuated again (Fig. 10). In a group of rats (n=7), the left mandibular incisors were cut out of occlusion (by 2-3 mm. Fig. 4). At the initial time segment the rate of eruption was significantly increased as illustrated by comparing the mean amount of eruption of the left mandibular incisors (1.67 ± 0.2 mm) to the contralateral incisor (0.92 ± 0.1 mm, $p < 0.01$) or to control group (n=7, 1.18 ± 0.15 mm, $p < 0.05$). The eruption rate of the shortened incisor

was also significantly increased at the second time segment (48-96 h) where the amount of eruption in the impeded incisors was (mean amount of eruption (mm) \pm SEM: 1.66 ± 0.2 mm), compared to the contralateral incisors (1.1 ± 0.19 mm, $p < 0.05$), and or to the control group (1.08 ± 0.09 mm, $p < 0.01$). As the shortened incisors reached the occlusal level at the third time segment (96-144 h.), the amount of eruption (0.37 ± 0.17 mm) was significantly lower than the right incisors (0.94 ± 0.12 mm, $p < 0.01$) or the control group (0.79 ± 0.15 mm, $p < 0.05$).

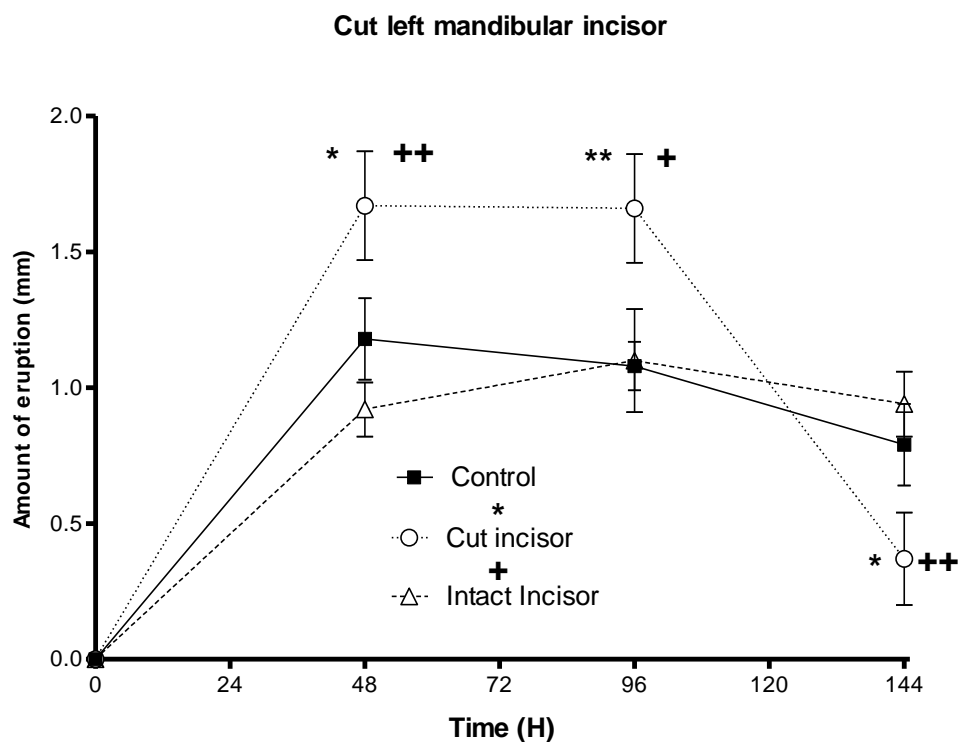


Figure 10: Shortening of the mandibular incisor induced significant acceleration of its eruption rate until reaching the occlusal level.

Each data point represents the mean \pm SEM of amount of eruption measured mandibular incisors at the indicated time interval in a group of rats with shortened left and intact right incisors ($n=7$), as compared to control ($n=7$). Determination of the significance of differences was made either with reference to control (*) or contralateral intact incisor (+).

Discussion

A characteristic feature of rodents is the presence of continuously growing incisors where new tissue production at the odontogenic base replaces what is lost during function (attrition), and continuous tooth eruption maintains a nearly constant tooth length in adult rats. In normal situations, the amount worn away from the incisal edges equates with the amount of tooth eruption (Bryer, 1957). This continuous growth depends on complex regenerative mechanisms that require an intricate regulation with important supply in nutrients and growth factors. The aim of our study was to investigate the role of the innervation on tooth eruption, in particular the role of the sympathetic efferents on the eruption rate of continuously erupting mandibular incisors in rats.

Dental pulp is highly vascularized, and the pulpal pressure is among the highest of all body tissues. As the pulp is enclosed by walls of limited expansion capacity (dentine), it has a limited ability to expand or shrink. Another unique feature of pulp vessels is that they have very thin walls compared to systemic vessels (Dahl et. al, 1973; Harris et. al, 1971). The physiology of these vessels is rather unique in their response to vasodilatation in such a low compliance environment, where pulpal vasodilation in dog canine and rat incisor teeth has been shown to result in a sharp, transient increase of blood flow, followed by a marked, sustained decrease (Kim et. al, 1990). The enclosed environment is thus unique in its response to stimulations to the blood flow and to the fluctuation of neurotransmitters, and these responses might affect the behavior of the tooth, from its eruption to its own survival.

Eruption rate in intact rats' mandibular incisors demonstrated a pattern of initial acceleration at the first time segment, and then slowed down throughout the observation

period (Fig. 6A). This trend was more apparent in the shortened incisors group where the shortened incisors displayed an initial intense acceleration compared to the control group or to the contralateral intact incisor (Fig. 10). The increase in the rate of eruption was less as the tooth was approaching the incisal level of the contralateral incisor. Our findings are in line with the published literature, showing that cutting the incisors out from occlusion releases the full potential of tooth eruption as unimpeded eruption rates are between two and three times that of impeded rates (Bryer, 1957; Berkovitz et. al, 1969). The increased eruption rate and its deceleration as the tooth approaches the incisal level might be related to the neural activity in response to the injury and repair process. As the incisor was cut, the dentinal tubules were exposed; such event is well known to cause the release of neurotransmitters (SP and calcitonin gene-related peptide “CGRP”, Byers, 1994). These neuropeptides are known to modulate the blood supply to the pulp and to increase vascular permeability (neurogenic inflammation) (Heyeraas, 1992; Olgart et.al, 1994). This increase in blood flow promotes the accelerated eruption rate at the initial phase of cutting the incisors, and as the tertiary dentin is deposited and the tooth is erupting, the injury discharge comes to a halt and the release of neurotransmitters is reduced, leading to a deceleration in the rate of eruption in the injured incisors. From the preceding findings, one might speculate that stimulation of the nervous system might increase the rate of eruption in rats’ incisors.

Contradictory results have been reported in the literature regarding the effects of denervation on the eruption rate. Ladizesky showed an increase of eruption rate after superior cervical ganglionectomy, when the mandibular incisor is cut out of occlusion

(Ladizesky et. al, 2001). On the other hand, Jacobsen showed a decrease in SP and CGRP, and a reduction in dentin formation and eruption rate (Jacobsen et. al, 1996). Our results were in agreement with the reduction in the eruption rate, where a significant reduction was evident after the first time segment and throughout the total time segments. The observed lack of effect during the first time segment following denervation could be attributed to the time delay between denervation and degeneration of the nerve terminals at the effector level.

To rule out the added effect of surgery, and to further emphasize the role of complete denervation on the rate of eruption, a model of rats was used where the CSPA fibers were knocked out by systemic capsaicin injections, and later the sympathetic efferents were blocked by guanethidine administration (Fig. 9). The results show a marked reduction in the rate of eruption comparable to what was observed following denervation by IAN lesion. This reduction in eruption rate was more significant than the effect of CSPA ablation alone, and it was more significant than that observed with guanethidine treatment alone (Fig. 7).

The effects of blocking the sympathetic supply at target and at ganglionic levels were also investigated; blocking peripheral synapses elicited the most significant decline in tooth eruption (Fig. 11).

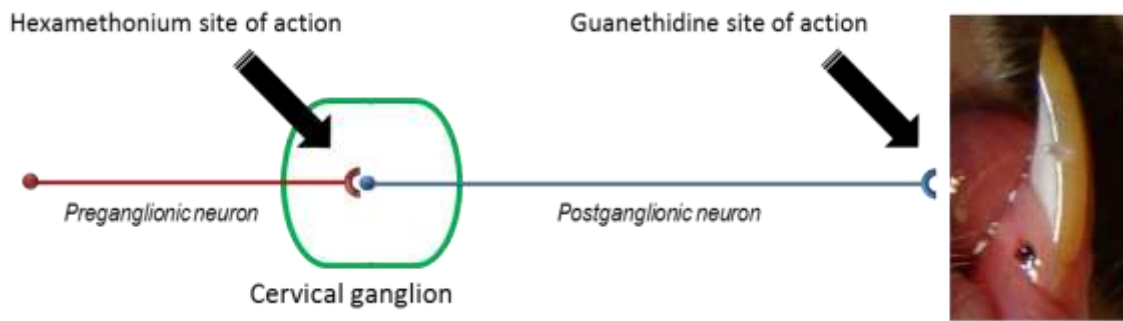


Figure 11: Site of action of different sympathetic blocking agents. Guanethidine site of action is at the terminal synapse (target tissue level), while hexamethonium site of action is at the ganglionic level.

Significant acceleration of eruption rate by sympathetic denervation was also observed in some studies (Leist, 1927; Bryer, 1957), but not in others (Taylor and Butcher, 1951; Miller, 1957). More recently, acceleration of movement of rabbit and cat incisors shortly after cervical ganglionectomy or adrenergic drug injection were observed (Moxham, 1981; Aars, 1982). These findings were observed in animals where the studied incisors were cut out of occlusion, and appear therefore, to be the end result of mixing accelerating factors, produced by cutting incisors and decelerating factors due to partial denervation by sympathectomy. An indirect confirmation of our results is provided by other studies showing that guanethidine treatment of adult rats strongly impairs osteoclastic resorption, which may result in a reduction in the rate of eruption (Cherruou et. al, 1999).

In our study, systemic administration of guanethidine reduced the rate of eruption in mandibular incisors which was significant during the first time segment and at the total duration (Fig. 7). The administration of hexamethonium reduced the rate of eruption in

erupting ferrets' canines (Moxham et. al, 1979 and 1988). However, it has no effect on fully erupted ferrets' canines (Moxham et. al, 1988). These effects on teeth with limited eruption are not similar to the drug's effect on teeth with continuous eruption, where hexamethonium administration led to an increase in the extrusive movement (Moxham et. al, 1979). These differences might be due to differences in animal species studied, as well as different types of eruption. Our finding in the sympathetic block by hexamethonium has shown that a general trend of eruption attenuation existed but was not significant (Fig. 8), this could thus be attributed to the site of action of hexamethonium in which the post ganglionic neurons were preserved and neurotransmitters are still released at the peripheral synapses.

In conclusion, the nervous system plays an essential in the control of the eruption process of rats' mandibular incisors. Sympathetic supply appears to constitute a major component in control of the tooth eruption. This contribution was confirmed by the reduction of the eruption rate following chemical sympathetic block.

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