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Botulinum neurotoxin type A in the masseter muscle: Effects on incisor eruption in rabbits

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Abstract

Introduction—Botulinum neurotoxins are responsible for the paralytic food poisoning, botulism. Commercial formulations such as botulinum neurotoxin type A are increasingly used for various conditions, including cosmetic recontouring of the lower face by injection of the large masseter muscles. The paralysis of a major muscle of mastication lowers occlusal force and thus might affect tooth eruption. The purpose of this study was to investigate the effects of unilateral masseter muscle injection of botulinum neurotoxin type A on the rate of eruption of incisors in a rabbit model. We hypothesized that the teeth would overerupt in an underloaded environment.

Methods—Forty rabbits were injected with either botulinum neurotoxin type A or saline solution in 1 masseter muscle. Mastication and muscle force production were monitored, and incisor eruption rate was assessed by caliper measurement of grooved teeth.

Results—The injection of saline solution had no effect. The masseter muscle injected with botulinum neurotoxin type A showed a dramatic loss of force 3 weeks after injection despite apparently normal mastication. Incisor eruption rate was significantly decreased for the botulinum neurotoxin type A group, an effect attributed to decreased attrition.

Conclusions—This study has implications for orthodontics. Although findings from ever-growing rabbit incisors cannot be extrapolated to human teeth, it is clear that botulinum neurotoxin type A caused a decrease in bite force that could influence dental eruption.

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Botulinum neurotoxins, produced by anaerobic Clostridium bacteria, are responsible for botulism, a paralytic food poison. These proteases interfere with the vesicle fusion apparatus, thus blocking the release of acetylcholine at neuromuscular junctions.[1] In 1989, botulinum neurotoxin type A serotype (BoNT/A) was approved by the FDA for treating blepharospasm. As a side effect of its use on muscles of the face, it was noted that it reduced facial wrinkles, leading to its popularity in cosmetic enhancement.[1] At therapeutic dosages, BoNT/A has a wide safety margin, leading many clinicians to use it off label.

One off-label use of BoNT/A is in jaw muscles. BoNT/A is currently used for the cosmetic reduction of large masseter muscles and in treating temporomandibular joint disorders, dystonias, and migraine headaches.[2,3] Even though published studies on the subject are generally "of low quality (noncomparative, nonrandomized trials)," the efficacy of the toxin for use in muscle spasms and cosmetic reduction is well established.[2] If an adequate dosage is used, muscle shrinkage occurs reliably, but not always evenly, as the denervated fibers undergo atrophy and dissolution.[3–5]

The clinical literature on BoNT/A in the masseter muscle indicates that maximal atrophy of the masseter follows loss of electromyography by about 2 months and is still sometimes observable at 1 to 2 years.[6,7] Occlusal force is related to muscle activity and size.[8,9] Yet these parameters do not covary after BoNT/A. In 1 report, voluntary bite force was subjectively normal in 8 days even though symptom relief persisted for 8 weeks.[10] In the best documented study to date, bite force began to recover in week 3 when muscle volume was still decreasing; bite force was fully recovered at 3 months, when the muscle was at minimal volume.[5] It is not clear how an atrophied muscle can produce a normal bite force, but a possible explanation is that the subjects had simply learned to produce normal voluntary force by using untreated muscles.

The presumed reduction of functional occlusal force after BoNT/A has consequences for the dentition, even if the loss is brief. The forces of occlusion are an important factor inhibiting the eruption of teeth.[11–14] The primary evidence for this inhibition comes from loss of occlusion. When an opposing tooth is removed experimentally[12,15–18] or clinically,[19] eruption speeds up, and overeruption occurs. Muscle weakness in humans[20–22] and muscle resection in rats[23] have the same effect. Because BoNT/A paralysis also causes muscle weakness, it can lead to supraeruption. At the same time, however, the loss of occlusal force would decrease the amount of attrition on the tooth crowns, and this effect might actually slow eruption, at least in rapidly worn, continuously erupting teeth such as rodent and rabbit incisors.[24] Eruption rate has not been studied systematically for BoNT/A treatment in either human or animal models. Control of the vertical dimension of occlusion is an important consideration for orthodontists in treating patients. Weak masticatory musculature, decreased occlusal force, and a tendency for extrusion have been associated with malocclusions characterized by increased anterior facial height and high mandibular plane angles.[21,25] Whereas the use of BoNT/A in these patients might exacerbate this extrusive tendency, it can prove useful in patients with a decreased lower facial height and large musculature, in which extrusion would be beneficial but difficult because of their strong biting forces.

Continuously growing teeth, such as rabbit and rat incisors, have a stable alveolar crest,[14] while the tooth-forming base remains in the same position in the socket.[12] The rapidity of eruption of rabbit incisors (10 times faster than human eruption) has made it a favorite study model.[12] For this reason, rabbits were chosen for this study, the purpose of which was to investigate the effects of unilateral masseter muscle injection of BoNT/A on the rate of tooth eruption. We expected that this loss of force would increase the rate of eruption of the incisors.

MATERIAL AND METHODS

We used rabbits from a previous study.[26] In brief, 40 female New Zealand white rabbits (Western Oregon Rabbit Company, Philomath, Ore) were obtained in groups of 8 at a time, except for 1 group of 9 because a rabbit expired prematurely in the previous group. This resulted in a sample of 21 animals treated with BoNT/A and 19 treated with saline solution, rather than 20 and 20 as originally planned. The sample size was based on a power analysis indicating that 20 animals per group would yield 95% power to detect a 1.7 effect-size difference in bite force with $\alpha = 0.05$. The rabbits were 5 months of age and weighed between 3.8 and 5.1 kg at the beginning of the study. They were fed only rabbit pellets (Rabbit 16%; Albers Animal Feed, Portland, Ore). The Institutional Animal Care and Use Committee at the University of Washington approved our protocol.

The rabbits were acclimated to feeding in the laboratory environment; they were placed and fed in an acrylic plastic holding box for approximately 1 to 2 hours per day for 1 to 2 weeks. They received 8 oz of pellets per day from a food tray attached to the front of the holding box. For the rest of the time, they were housed in the animal facility without food except for the weekends. If the rabbits did not finish the 8 oz of food provided during the laboratory session, the rest was given in their cages.

After the initial acclimatization period, the animals were randomized to receive either BoNT/A or saline solution injections by coin toss. The investigators were blinded to treatment. BoNT/A (Botox Cosmetic; Allergan, Irvine, Calif) was reconstituted according to the package insert (100 units of toxin in 2.5 mL 0.9% of sterile saline solution). The animals were shaved to expose the skin over the masseter muscle, the forehead, and the submandibular area (Fig 1). Each animal received injections of either BoNT/A or saline solution in 1 masseter muscle, chosen by coin toss. The volume injected was 0.25 mL of BoNT/A (10 units) or saline solution, divided into thirds and injected into 3 target areas paralleling the lower border of the angle of the mandible into the middle thickness of the muscle, with injection continuing as the needle was withdrawn. These locations were chosen because they approximated the location of the motor end plates.[27] The injection sites were massaged for a few minutes to encourage dispersion of the fluid as per clinical usage.

This study was carried out during week 3 (days 15–22 after the injections), when incisor bite force was expected to be minimal. The investigators were blinded as to treatment group and side of treatment. The animals were weighed before treatment and several times per week after treatment with a digital scale (model 6745; Detecto, Webb City, Mo).

Electromyographic readings and simultaneous videos were recorded weekly while the animals were allowed to eat in their holding boxes. These data, previously published, indicated that injection of saline solution had no effect on muscle activity, nor was there any change in the activity levels of the noninjected masseter muscles of the BoNT/A animals. However, the effect of BoNT/A injection on masseter activity was profound. Electromyographic values were reduced by 40% to 70% compared with pretreatment values 1 to 2 weeks after the injection.[26]

As an index of masticatory efficiency during week 3, a random subset of 10 rabbits injected with saline solution and 10 injected with BoNT/A were individually recorded consuming a measured volume of food. The number of cycles required to masticate a specified amount of food was determined by putting a bolus of pellets (approximately 1 g) into the rabbit's mouth with a 1-mL insulin syringe with the needle portion removed (Becton Dickinson, Franklin Lakes, NJ). The bolus was weighed on a scientific scale (PC 400;Mettler, Columbus, Ohio) accurate to 0.01 g. An effort was made to vary the side of the mouth for delivery, but a previous study demonstrated that the side of food insertion is independent of the chewing side in rabbits.[28] Any pellet not consumed or that fell out of the mouth was collected and weighed. This was repeated 10 times for each rabbit, allowing sufficient time between to determine that all the food was consumed. Rabbits that did not voluntarily masticate the entire food bolus or held it in their cheeks were given another attempt later and excluded from this analysis if voluntary mastication was not observed. Approximately half of the rabbits did not completely chew the bolus, keeping the food in their cheeks, resulting in a final sample size of 6 saline solution and 5 BoNT/A rabbits. The delivery of the bolus and its subsequent mastication were recorded with a digital video camera. The number of chewing cycles for each bolus was counted on the video recording, and masticatory efficiency was calculated as the number of cycles per gram of food.

Incisor marking and assessment of tetanized muscle force were performed with the rabbits under isoflurane anesthesia (Narkovet 2; North American Drager, Telford, Pa) with a custom-built nasal mask. Rabbits have 2 maxillary incisors per quadrant: a large anterior tooth and a small posterior one behind the anterior incisor. Together, the maxillary incisors form a notch into which the mandibular incisor fits. The labial surfaces of the anterior maxillary incisors and the mandibular incisors were marked at the level of the free gingival margin in the midline of each tooth with a small-diameter bur (FG330; SS White, Lakewood Township, NJ) in an airpowered, high-speed dental hand piece (model 430K; Star Dental, Lancaster, Pa). This shallow reference groove did not penetrate the enamel layer (Fig 2). If any space remained between the apical margin of the reference groove and the most apical point of the free gingival margin, it was recorded. At weekly to biweekly intervals, the space between the most apical point of the free gingival margin and the most apical margin of the reference groove was measured with digital calipers. Incisor eruption measurements were initially taken while the animals were anesthetized; it was later possible, once the animals were acclimatized to the laboratory environment, to do it without anesthesia, simply by retracting their lips manually. As the marker reached the incisal edge, new reference grooves were placed. This resulted in most teeth having 2 grooves available for measurement on any given day. Both were measured, providing an assessment of repeatability. These paired measurements yielded essentially identical readings. The groove technique was adopted

after an attempt to mark the incisors with a bonded dental composite was abandoned because the rabbits wore off the composite. Attempts were also made to mark the molars, but access issues made accurate placement and measurement impossible. It was also impossible to track the posterior maxillary incisor, but it was assumed that its behavior mimicked that of the anterior maxillary incisor.

Bite force was measured at the incisors in anesthetized animals while each masseter muscle was separately tetanized. Wire electromyographic electrodes, situated anteriorly and posteriorly in the masseter, were stimulated (Grass models S48 and SIU5; Astro-Med, West Warwick, RI; or the stimulator function of model M150; Biopac, Goleta, Calif) with trains of 5 msec pulses delivered at 55 Hz for 550 msec at a voltage determined to be supramaximal by gradually ramping up the voltage, with intervals of 5 to 10 seconds between trains. This procedure was necessary to determine maximal bite force but might have resulted in muscle fatigue. However, all muscles received the same stimulation protocol, and all investigators were blinded to the treatment of each muscle. Thus, although muscle fatigue might underestimate the absolute value of bite force, differences measured between muscles are nonetheless valid. The bite force transducer was based on the design of Dechow and Carlson[29] and included a small groove for the maxillary incisors to ensure consistent placement. The transducer readings were converted to kg-force by using a previously determined calibration equation. The right and left masseter muscles were tetanized in random order. Repeat stimulations were not carried out to avoid fatiguing the muscles further.

Descriptive statistics were calculated for each variable. Incisor eruption rates were analyzed for each tooth and compared between the 2 groups. The primary hypothesis was that eruption would be faster in animals injected with BoNT/A than in those injected with saline solution. This was assessed as a 2-sample *t* test of the null hypothesis of no difference between the groups, performed for each tooth position. Because the incisors function as a unit, no difference was expected between eruption of the teeth on the injection side vs the noninjection side. This null hypothesis was tested within groups by using paired *t* test comparisons. The significance level was set at $P < 0.05$. The statistical analysis was accomplished by using SPSS software (version 17.0; SPSS, Chicago, Ill). Power calculations were carried out with G*Power.[30]

RESULTS

Overall, the rabbits tolerated the experiments well. Some data were missing, primarily because of equipment malfunction, and this is reflected in the varying sample sizes. There was no initial difference between the BoNT/A and saline solution groups in any parameter, including body weight, electromyographic activity levels, masticatory performance, or stimulated masseter incisor bite force.[26]

The Table compares these variables at week 3 postinjection for the 2 groups and shows that body weight and masticatory parameters still did not differ significantly. The BoNT/A animals were actually slightly heavier and chewed slightly more on the injected side than did the animals injected with saline solution. However, bite force produced by tetanus of the

muscles injected with BoNT/A had plummeted compared with that produced by tetanus of the muscles injected with saline solution (P <0.0001). The noninjected side was unaffected.

In contrast to body weight and masticatory parameters, the Table and Figure 3 also show that eruption rate was strongly influenced by BoNT/A injection. Unlike the change in tetanically produced bite force, the changes in eruption rate were manifested on the noninjected side as well as on the injected side. Maxillary and mandibular incisors in the BoNT/A animals all showed significant differences from their saline-solution counterparts ($P \leq 0.02$). Surprisingly, in view of our hypothesis, the teeth of the rabbits injected with BoNT/A erupted slower, not faster, than those of controls. In addition, the data suggest a possible difference between the mandibular incisors of the animals treated with BoNT/A, with the tooth of the injected side erupting even more slowly than that of the noninjected side ($P = 0.06$ in a paired t test; Table).

In both groups of rabbits, the maxillary incisors erupted more slowly than did the mandibular incisors (Fig 3; P <0.001). The relationship was unchanged by BoNT/A; in both groups, the ratio of maxillary rate to mandibular rate was 69% (Table).

DISCUSSION

The ability of BoNT/A to produce muscle paralysis by chemodenervation has been used to treat multiple conditions in a reliable and relatively safe manner. We examined the effects on incisor eruption of unilateral injection of BoNT/A into the masseter muscles of rabbits. As expected from the clinical literature[3,5,10] and as documented previously,[26] BoNT/A injection into the masseter muscle had little effect on chewing. Nevertheless, this treatment resulted in a significant decrease in the ability of the muscle to produce a biting force and striking changes in eruption rates.

An advantage of our experimental design is that bite force was measured at the same teeth (incisors) used to assess eruption, ensuring a relevant value. At week 3 postinjection, incisor bite force produced by tetanus of the masseters injected with BoNT/A was only about 20% of control values, verifying that this time point was appropriate for examining tooth eruption. This drop in bite force was greater than the estimate used to calculate sample size, and a post hoc calculation of actual power indicated that these data had 100% power to detect a statistically significant change in bite force. The magnitude of force loss was much greater in our rabbit study than in clinical reports, one of which claimed bite force to be “subjectively normal” after only 8 days.[10] More quantitative clinical studies have indicated that, at comparable time points, 60% to 80% of control force is still present.[31,32] Unlike our study, human bite force was measured with the subjects voluntarily clenching at a maximal force, a highly subjective procedure. This study, by isolating each masseter muscle, allowed a more reliable measure of muscular force production without the influence of other variables such as patient cooperation and of uninjected muscles of mastication such as the temporalis and medial pterygoid.

How can mastication remain normal with a largely nonfunctional masseter muscle? This phenomenon occurs in humans as well as rabbits. Patients report only brief periods of

problems with chewing and the return of normal function after a short time.[5,10] The rabbits showed no change in any masticatory parameter including feeding efficiency (Table). One possible explanation is that other muscles hypertrophy and compensate for the loss of masseter force. However, no clinical study has found evidence of compensation by other muscles,[5,6,10] and animal studies, including this one, have found minimal compensation at best[26,33] even after physical removal of the masseter.[34] Because compensation does not occur, maximal occlusal force must decrease. However, maximal occlusal force might not be important for ordinary mastication. Modern human diets are not challenging for the masticatory system, and rabbit pellets such as those we used are friable and offer only a slight resistance to a load.[35]

In contrast to mastication, our study offers clear evidence that the loss of masseter force is important for incisor function. Rabbits use their incisors to acquire food and probably also grind them against each other for sharpening, as do rodents.[36] These activities might require more occlusal force than mastication. Our evidence of a real drop in functional loading (as opposed to maximal bite or other activities that only occur under experimental conditions) is clinically significant, because 1 major rationale for BoNT/A usage on jaw muscles is to reduce functional loading for conditions such as temporomandibular joint pain and condylar fracture.[2,37,38]

As is known to occur in rats, the mandibular incisors of the rabbits, regardless of treatment, erupted faster than did the maxillary incisors (Fig 3).[24,39] This probably reflects the relative size of the teeth as well as usage patterns.

The most surprising finding of our study on unilateral masseter BoNT/A injection was not that the eruption rate of the incisors was altered but the nature of the alteration. The incisors of the group treated with BoNT/A erupted more slowly compared with the control group, with statistical significance in every case. Post hoc analyses indicated statistical power of 64% to 78% for these findings. We had expected that, with a decreased force exerted on these teeth, an increased rate of eruption would be observed. This would be analogous to incisors that have been taken out of occlusion by shortening, a situation in which unloading increases the eruption rate.[40] The opposite response was observed here. In looking at possible explanations for this phenomenon, the results are similar to those obtained when the rate of attrition is modified. The length of rodent incisors is for the most part stable with balanced rates of eruption and attrition maintaining the occlusal plane.[41] Attrition is an ongoing process necessary to maintain an appropriate length of continually erupting teeth.[42] An inverse relationship between rat incisor length and attrition rate has been documented on several occasions.[40,43] Attrition in rabbits occurs in several ways, including ingestion and chewing of food, the sharpening movement in which the mandibular incisors grind on both pairs of maxillary incisors, and gnawing on objects such as the cage bars. It can be theorized that, because of the decreased incisor bite force, the BoNT/A-injected rabbit's ability to maintain a level of attrition comparable with the control group was affected, and this decreased rate of attrition was manifested as a decreased eruption rate. Unfortunately, the rate of attrition was not measured in our study nor was the total length of the incisors.

The slowing of eruption affected the incisors of the noninjected side as well as those of the side injected with BoNT/A. The mandibular incisors demonstrated a trend toward decreased eruption on the BoNT/A side ($P = 0.06$, Table; post hoc analysis indicated 42% power to detect a statistically significant difference), but, even if real, this marginal difference is small compared with the enormous difference in force production of the masseters of the injected and noninjected sides. Even though these paired incisors function in tandem and cannot be affected in isolation, the similarity of reaction of the 2 sides reflects the basic mechanics of the jaw.[40] The incisors are the farthest teeth from the masseter muscles and jaw joints, and are located next to each other in the midline. Functional (as opposed to stimulated) bite force at the incisors results from the sum of all muscle contractions on both sides. Thus, the incisors of the injected side and the noninjected side must receive similar reductions in occlusal loading after BoNT/A, explaining why their eruption was affected similarly.

This study verifies the loss of functional occlusal force after BoNT/A, and it is reasonable to extrapolate from this that patients also experience substantial loss of force. However, the message from our finding of slower eruption of the incisors of rabbits in the BoNT/A group cannot be similarly applied to the human situation. If our suggestion that decreased attrition was the immediate cause of decreased eruption is correct, then the rabbit incisor was a poor choice to model human eruption. Although attrition does contribute to human dental eruption, the effect is rarely clinically important in populations with a modern diet.[44] We speculate that the loss of occlusal force, rather than the loss of attrition, would be the predominant influence in the clinical situation, and thus would expect patients treated with BoNT/A to exhibit excessive eruption as in other types of muscle weakness rather than retarded eruption as exhibited by the rabbits.[20–22] This scenario is plausible but depends on 2 assumptions: (1) that slower attrition was the cause of slower eruption in the rabbits, and (2) that attrition would be a negligible factor for human eruption. If these assumptions are false, then BoNT/A could conceivably retard eruption in patients as well as rabbits.

The fact that incisor eruption (and probably attrition) was strongly affected while mastication was normal seems contradictory, but is probably explained by the fact that our functional observations did not include incision. The rabbits did not have to incise the pellets, which were small. Furthermore, the pellets were often put into their mouths with a modified syringe. This bypassed the incisors entirely. Presumably, however, the rabbits were using their incisors in nonfeeding activities, such as sharpening and gnawing on the toys in their cages when not under observation. The reduction of bite force used in these activities undoubtedly contributed to changes in tooth eruption.

In examining the published literature, it becomes evident that little scientific research has been conducted on BoNT/A, especially considering its increasing usage among the younger, still growing, population. The American Society of Plastic Surgeons has reported that Botox was used nearly 12,000 times in patients aged 13 through 19 in this country in 2009.[45] The increasing popularity of this procedure in immature persons is unsettling because there are few studies on the possible effects on craniofacial growth. As stated in a recent Cochrane review, “the authors in this review did not find any high quality studies evaluating the effectiveness and potential harms of botulinum toxin type A in the management of benign masseter hypertrophy.”[46] Because the teenage years are the most common age for

orthodontic treatment, it is important to be aware of the possible implications of the use of BoNT/A in our patients. Although the results obtained in this study with continuously erupting teeth cannot be extrapolated to the human dentition, they do raise several questions on how changing the forces of occlusion due to manipulating muscles might affect the dentition. This study showed effects on bite force and incisor eruption from only 1 unilateral injection. Bilateral and multiple injections of BoNT/A are more common clinical patterns and would be expected to have even greater effects.

CONCLUSIONS

This study, in which a unilateral masseter BoNT/A or saline solution injection was administered to a group of rabbits, showed that, although the treatment had no detectable effect on masticatory performance, bite force was strongly decreased, and this decrease was associated with statistically significant slowing of the eruption of the incisors.

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REFERENCES

1. Davletov B, Bajohrs M, Binz T. Beyond BOTOX: advantages and limitations of individual botulinum neurotoxins. Trends Neurosci. 2005; 28:446–452. [PubMed: 15979165]
2. Ihde SKA, Konstantinovic VS. The therapeutic use of botulinum toxin in cervical and maxillofacial conditions: an evidence-based review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007; 104:e1–e11. [PubMed: 17560141]
3. Kim JH, Shin JH, Kim ST, Kim CY. Effects of two different units of botulinum toxin type A evaluated by computed tomography and electromyographic measurements of human masseter muscle. Plast Reconstr Surg. 2007; 119:711–717. [PubMed: 17230111]
4. Borodic GE, Ferrante R, Pearce LB, Smith K. Histologic assessment of dose-related diffusion and muscle fiber response after therapeutic botulinum A toxin injections. Mov Disord. 1994; 9:31–39. [PubMed: 8139603]
5. Yu CC, Chen PKT, Chen YR. Botulinum toxin A for lower facial contouring: a prospective study. Aesthetic Plast Surg. 2007; 31:445–451. [PubMed: 17674088]
6. Lee CJ, Kim SG, Kim YJ, Han JY, Choi SH, Lee SI. Electrophysiologic change and facial contour following botulinumtoxin A injection in square faces. Plast Reconstr Surg. 2007; 120:769–778. [PubMed: 17700130]
7. Kim NH, Chung JH, Park RH, Park JB. The use of botulinum toxin type A in aesthetic mandibular contouring. Plast Reconstr Surg. 2005; 115:919–930. [PubMed: 15731696]
8. Ferrario VF, Sforza C, Zanotti G, Tartaglia GM. Maximal bite forces in healthy young adults as predicted by surface electromyography. J Dent. 2004; 32:451–457. [PubMed: 15240063]
9. Sasaki K, Hannam AG, Wood WW. Relationships between the size, position, and angulation of human jaw muscles and unilateral first molar bite force. J Dent Res. 1989; 68:499–503. [PubMed: 2921394]
10. Freund B, Schwartz M, Symington JM. The use of botulinum toxin for the treatment of temporomandibular disorders: preliminary findings. J Oral Maxillofac Surg. 1999; 57:916–920. [PubMed: 10437718]
11. Weinmann JP. Bone changes related to eruption of the teeth. Angle Orthod. 1941; 11:83–99.

12. Ness AR. The response of the rabbit mandibular incisor to experimental shortening and to the prevention of its eruption. Proc R Soc Lond B Biol Sci. 1956; 146:129–154. [PubMed: 13379357]
13. Proffit WR. Equilibrium theory revisited: factors influencing position of the teeth. Angle Orthod. 1978; 48:175–186. [PubMed: 280125]
14. Steedle JR, Proffit WR. The pattern and control of eruptive tooth movements. Am J Orthod. 1985; 87:56–66. [PubMed: 3881035]
15. Ness AR. Eruption rates of impeded and unimpeded mandibular incisors of the adult laboratorymouse. Arch Oral Biol. 1965; 10:439–451. [PubMed: 5231526]
16. Anneroth G, Ericsson SG. An experimental histological study of monkey teeth without antagonist. Odontol Revy. 1967; 18:345–359. [PubMed: 4965146]
17. Michaeli Y, Weinreb MM. Role of attrition and occlusal contact in the physiology of the rat incisor. II. Diurnal rhythm in eruption and attrition. J Dent Res. 1968; 47:486–491. [PubMed: 5240802]
18. Moss JP, Picton DC. Mesial drift of teeth in adult monkeys (Macaca irus) when forces from the cheeks and tongue had been eliminated. Arch Oral Biol. 1970; 15:979–986. [PubMed: 4992597]
19. Darling AI, Levers BG. The pattern of eruption of some human teeth. Arch Oral Biol. 1975; 20:89–96. [PubMed: 1054585]
20. Proffit, WR. The facial musculature in its relation to the dental occlusion. In: Carlson, DS.; McNamara, JA., Jr, editors. Muscle adaptation in the craniofacial region. Ann Arbor, Mich: Center for Human Growth and Development, University of Michigan; 1978. p. 73-88.
21. Kiliaridis S, Mejersjo C, Thilander B. Muscle function and craniofacial morphology: a clinical study in patients with myotonic dystrophy. Eur J Orthod. 1989; 11:131–138. [PubMed: 2767145]
22. Suda N, Matsuda A, Yoda S, Ishizaki T, Higashibori N, Kim F, et al. Orthodontic treatment of a case of Becker muscular dystrophy. Orthod Craniofac Res. 2004; 7:55–62. [PubMed: 14989756]
23. Horowitz SL, Shapiro HH. Modification of skull and jaw architecture following removal of the masseter muscle in the rat. Am J Phys Anthropol. 1955; 13:301–308. [PubMed: 13258782]
24. Weinreb MM, Michaeli Y, Silberman G. Role of attrition and occlusal contact in the physiology of the rat incisor. IV. Prevention of attrition in the articulating incisor. J Dent Res. 1969; 48:120–130. [PubMed: 5252084]
25. Proffit WR, Fields HW. Occlusal forces in normal- and long-face children. J Dent Res. 1983; 62:571–574. [PubMed: 6573374]
26. Rafferty KL, Liu ZJ, Ye W, Navarrete AL, Nguyen TT, Salamati A, et al. Botulinum toxin in masticatory muscles: short- and longterm effects on muscle, bone, and craniofacial function in adult rabbits. Bone. 2012; 50:651–662. [PubMed: 22155510]
27. Weijs WA. Functional somatotopic organisation of motoneurons supplying the rabbit masseter muscle. J Comp Neurol. 1996; 364:279–289. [PubMed: 8788250]
28. Morimoto T, Inoue T, Nakamura T, Kawamura Y. Characteristics of rhythmic jaw movements of the rabbit. Arch Oral Biol. 1985; 30:673–677. [PubMed: 3865644]
29. Dechow PC, Carlson DS. A method of bite force measurements in primates. J Biomech. 1983; 16:797–802. [PubMed: 6643517]
30. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007; 39:175–191. [PubMed: 17695343]
31. Ahn KY, Kim ST. The change of maximum bite force after botulinum toxin type A injection for treating masseteric hypertrophy. Plast Reconstr Surg. 2007; 120:1662–1666. [PubMed: 18040203]
32. Kim KS, Byun YS, Kim YJ, Kim ST. Muscle weakness after repeated injection of botulinum toxin type A evaluated according to bite force measurement of human masseter muscle. Dermatol Surg. 2009; 35:1902–1907. [PubMed: 19737290]
33. Tsai CY, Chiu WC, Liao YH, Tsai CM. Effects on craniofacial growth and development of unilateral botulinum neurotoxin injection into the masseter muscles. Am J Orthod Dentofacial Orthop. 2009; 135:142.e1–142.e6. discussion 142–3. [PubMed: 19201315]
34. Guelinckx P, Dechow PC, Vanrusselt R, Carlson DS. Adaptations in the temporalis muscles of rabbits after masseter muscle removal. J Dent Res. 1986; 65:1294–1299. [PubMed: 2959691]

35. Ravosa MJ, Kunwar R, Stock SR, Stack MS. Pushing the limit: masticatory stress and adaptive plasticity in mammalian craniomandibular joints. J Exp Biol. 2007; 210:628–641. [PubMed: 17267649]
36. Druzinsky RE. Incisal biting in themountain beaver (Aplodontia rufa) and woodchuck (Marmota monax). J Morphol. 1995; 226:79–101. [PubMed: 7473765]
37. Canter HI, Kayikcioglu A, Aksu M, Mavili ME. Botulinum toxin in closed treatment of mandibular condylar fracture. Ann Plast Surg. 2007; 58:474–478. [PubMed: 17452828]
38. Schwartz M, Freund B. Treatment of temporomandibular disorders with botulinum toxin. Clin J Pain. 2002; 18(Supp):S198–S203. [PubMed: 12569969]
39. Drevensek M, Volk J, Sprogar S, Drevensek G. Orthodontic force decreases the eruption rate of rat incisors. Eur J Orthod. 2009; 31:46–50. [PubMed: 19073954]
40. Risnes S, Septier D, Goldberg M. Accelerated eruption of rat lower incisor. Relationship between impeded and unimpeded eruption rates, rate of attrition, tooth length, and production of dentin and enamel. Connect Tissue Res. 1995; 32:183–189. [PubMed: 7554916]
41. Michaeli Y, Weinreb MM, Zajicek G. Role of attrition and occlusal contact in the physiology of the rat incisor: VIII. Tooth length and occlusal plane as regulating factors of eruption and attrition rates. J Dent Res. 1974; 53:1215–1218. [PubMed: 4528903]
42. Weinreb MM, Assif D, Michaeli Y. Role of attrition in the physiology of the rat incisor. I. The relative value of different components of attrition and their effect on eruption. J Dent Res. 1967; 46:527–531. [PubMed: 4961086]
43. Law KT, Lee CK, King NM, Rabie AB. The relationship between eruption and length of mandibular incisors in young rats. Med Sci Monit. 2003; 9:BR47–BR53. [PubMed: 12552237]
44. Kerr NW, Ringrose TJ. Factors affecting the lifespan of the human dentition in Britain prior to the seventeenth century. Br Dent J. 1998; 184:242–246. [PubMed: 9581041]
45. Louis CS. This teenage girl uses Botox. No, she's not alone. The New York Times. 2010 Aug 11. (Sect. E):1.
46. Al-Muharraqi MA, Fedorowicz Z, Bareeq A, Al Bareeq R, Nasser M. Botulinum toxin for masseter hypertrophy (review). Cochrane Database of Systematic Reviews. 2009 CD007510.

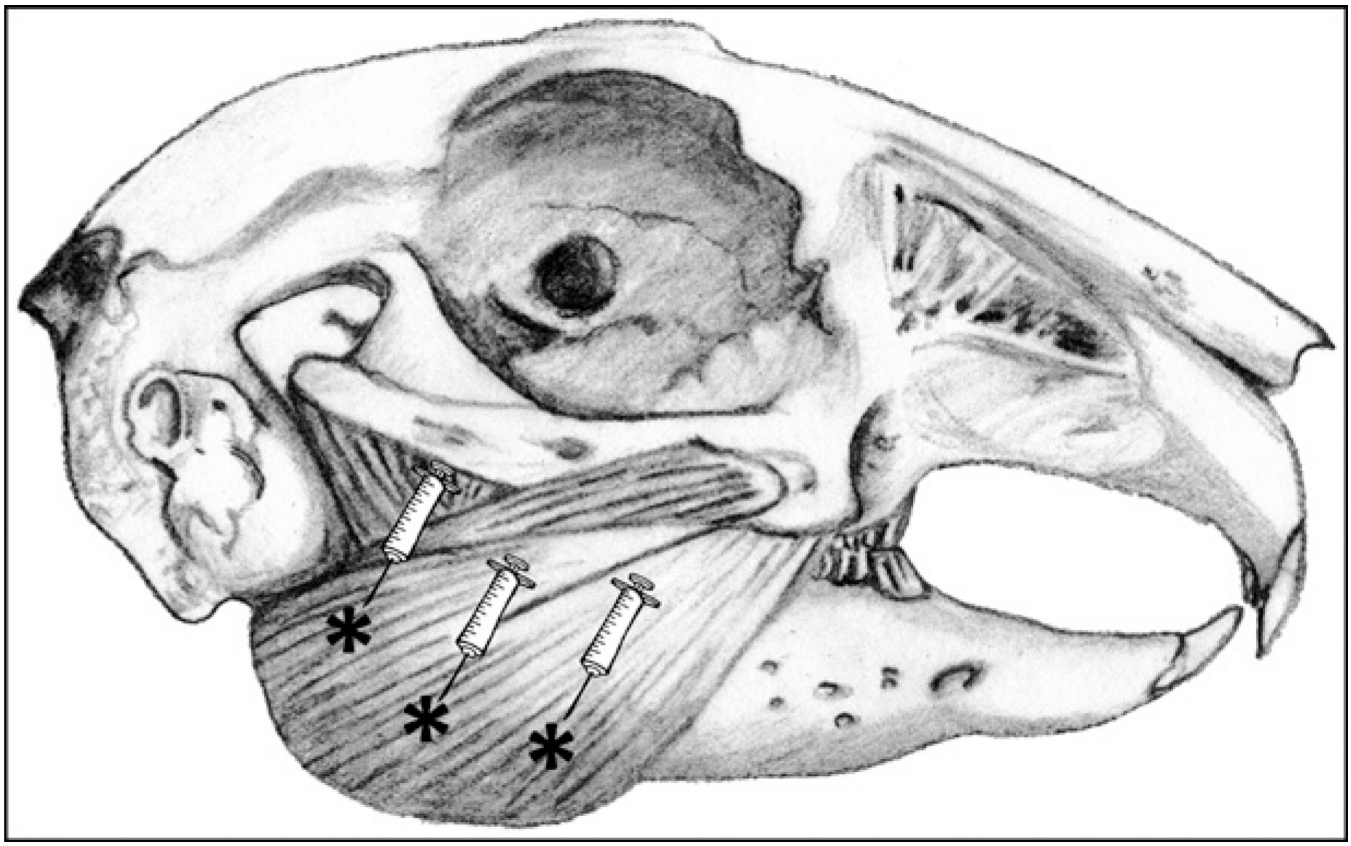

Fig 1.

The injection sites in the rabbit masseter muscle were parallel to the border of the mandibular angle in the superficial belly of the muscle. The *asterisks* with syringes show the 3 targeted locations where BoNT/A or saline solution was injected into 1 randomly chosen masseter muscle, in this case the right. One-third of the total dose was injected into each location.

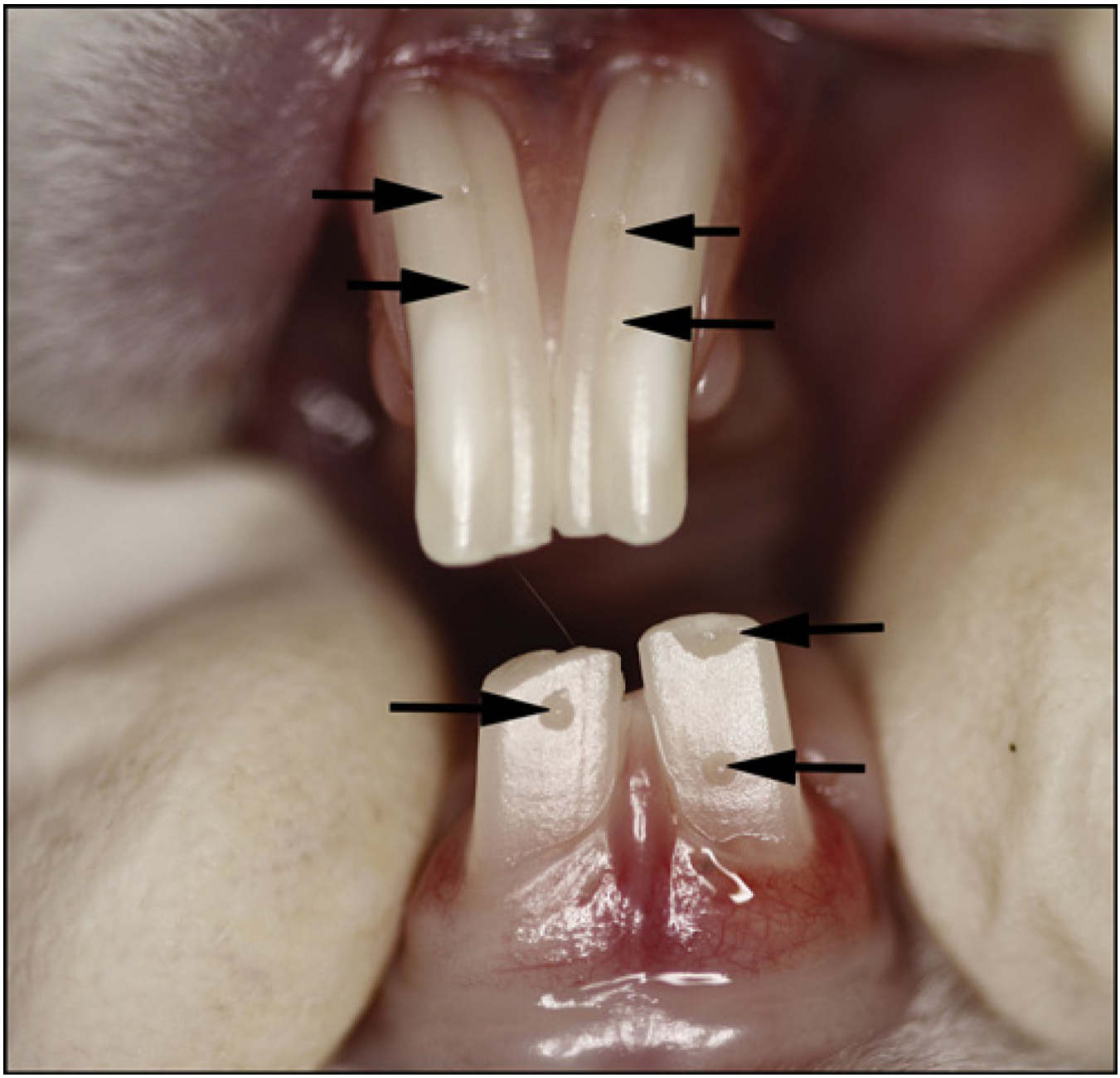

Fig 2.

Frontal view of a rabbit with the lips retracted by a gloved hand, showing the shallow grooves in the enamel of the incisors (*arrows*) used for measurement of eruption rate. Two grooves are visible on every tooth except the mandibular right incisor, which has only 1. The more coronal groove on the mandibular left incisor's edge is almost worn away. New grooves were placed every several days as the old ones wore off.

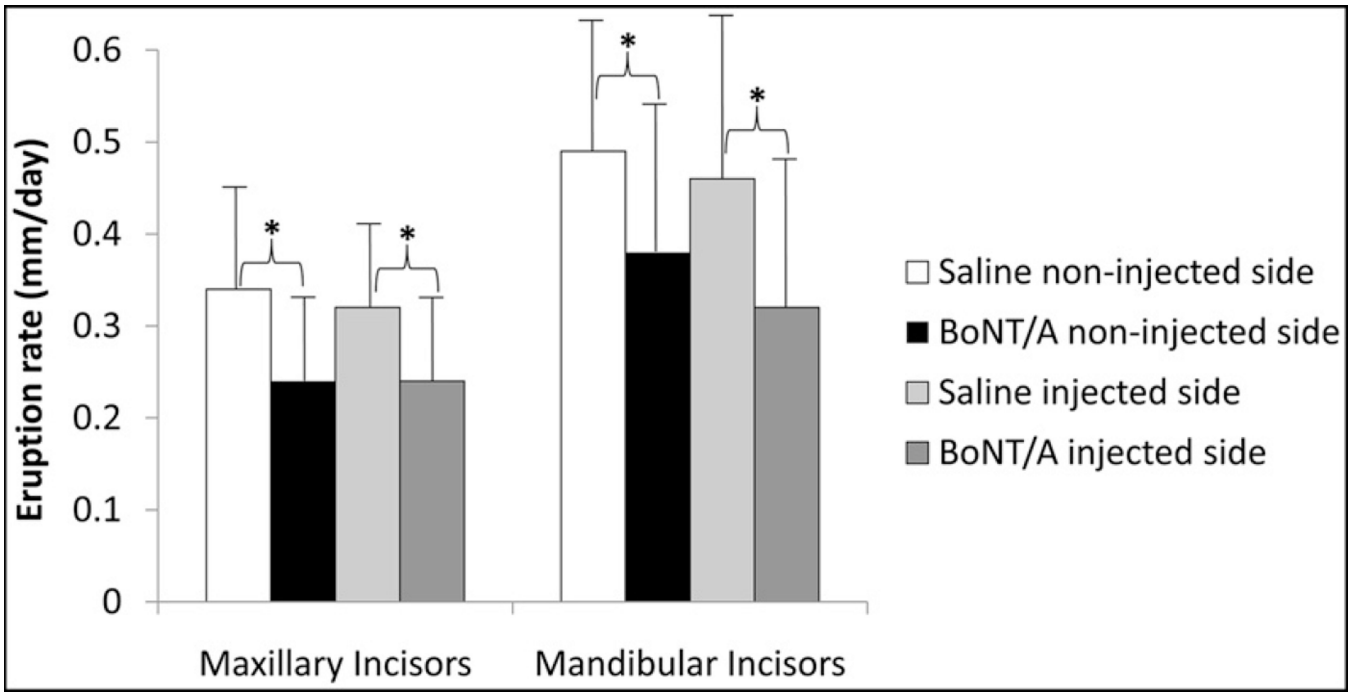

Fig 3.

Eruption rates of the incisors during the third week after injection of either BoNT/A or saline solution into 1 masseter muscle. The *error bars* represent 1 SD. Regardless of the group, the mandibular incisors erupted faster than did the maxillary incisors. All incisors of the rabbits injected with BoNT/A erupted significantly more slowly than the counterpart incisors of the rabbits injected with saline solution (*asterisks*; Table). There were no significant differences between the injected and the noninjected sides.

Table

Comparison of the rabbits 3 weeks after injection of either BoNT/A or saline solution into a masseter muscle (mean ± standard deviation, n)

	BoNT/A	Saline solution	*P* value
Body weight (kg)	4.04 ± 0.27, 21	3.95 ± 0.36, 19	0.40
Mastication			
Rate (Hz)	3.3 ± 0.33, 21	3.41 ± 0.29, 19	0.52
Side (% on injected side)	58 ± 24, 21	49 ± 29, 19	0.52
Efficiency (cycles/1-g bolus)	126 ± 39, 5	117 ± 20, 6	0.65
Bite force from tetanized masseter (kg)			
Noninjected side	0.98 ± 0.68, 18	1.04 ± 0.43, 18	0.40
Injected side	0.22 ± 0.21, 16	1.38 ± 0.90, 16	0.00*
P value between sides	0.00*	0.16	
Incisor eruption rate (mm/day)			
Maxillary noninjected side	0.24 ± 0.09, 16	0.34 ± 0.11, 16	0.01*
Maxillary injected side	0.24 ± 0.09, 16	0.32 ± 0.09, 16	0.02*
Paired *P* value between sides	0.81	0.45	
Mandibular noninjected side	0.38 ± 0.14, 16	0.49 ± 0.12, 16	0.02*
Mandibular injected side	0.32 ± 0.14, 16	0.46 ± 0.16, 16	0.01*
Paired *P* value between sides	0.06	0.45	

*Statistically significant difference.