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Differential Response of Pig Masseter to Botulinum Neurotoxin Serotypes A and B

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Abstract

Introduction—Pigs respond to direct administration of botulinum neurotoxins (BoNTs), although they are resistant to botulism. The human masseter is frequently targeted for BoNT therapy. We aimed to understand how BoNT affects chewing by injecting porcine masseters.

Methods—One masseter of minipigs was injected with BoNT serotype A or B at doses comparable to those used in humans. Masticatory function was evaluated electromyographically. Muscle force was measured during tetany. Four weeks after injection, strain gauges affixed to the mandible assessed bone strain during chewing. Masseter mass and fiber diameter were measured after euthanasia.

Results—BoNT-A had no measurable effect. In contrast, BoNT-B reduced electrical activity and muscle force, producing substantial asymmetry between injected and uninjected muscles.

Discussion—The pig masseter is highly resistant to direct injection of BoNT-A, but it is affected by BoNT-B.

Keywords

botulinum neurotoxin; pig; muscle atrophy; mastication; masseter

Introduction

The neurotoxins produced by *Clostridium botulinum* (BoNT-A through G) produce flaccid paralysis by attacking proteins involved in vesicular release of neurotransmitters.¹ Pigs are resistant to orally administered BoNTs. Of the few documented botulism incidents, only 1 identified toxin in the gut, specifically BoNT-C.² As BoNT-C³ and BoNT-B⁴ can be found in the viscera of healthy pigs, botulism remains unproven. Smith et al.⁵ found that even intravenous delivery required huge doses to produce pelvic weakness. The only serotype to which pigs were moderately susceptible was BoNT-B, for which the lethal dose (LD) was

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180 mouse LD/kg. In contrast, the pig LD for BoNT-A was 6,000 mouse LD/kg. These results suggest that resistance is systemic.

Yet pigs are often used in studies which deliver BoNT-A by muscle injection. After direct injection, smooth muscle of the gastrointestinal tract relaxes,^{6,7} as do myocutaneous flaps.⁸ In addition, the masseter, an important chewing muscle,⁹ showed altered ion content and fiber type after injection of 100 units (mouse LD50/kg) of BoNT-A.¹⁰ These studies imply that, despite systemic resistance to oral and intravenous BoNT, porcine muscles respond to direct injection.

Pigs are an excellent model of the human masticatory apparatus¹¹ and were chosen to address the translational value of a previous rabbit study on the masseter.¹² Pigs may provide valuable information on how BoNT use in the masseter affects mastication in humans. Our original goals were to assess chewing behavior, mandibular loading, and muscle atrophy of animals injected unilaterally with BoNT-A. Although clinical use of BoNT in the masseter is usually bilateral rather than unilateral, the one-sided treatment allows the uninjected side to be used as an internal control and is the protocol used in other preclinical animal studies.^{10, 12} When, surprisingly, BoNT-A produced neither paralysis nor atrophy, we performed an additional study using BoNT-B.

Materials and methods

Animals and experimental treatment

To avoid increasing sample size, a single sex was used. Females were chosen by coin toss. A total of 12 minipigs (Sinclair Bio-resources, Columbia MO), all 4 months old, were used (Supplementary Material, Table). The animals were of 3 different minipig strains (Hanford, Sinclair, and Yucatan), depending on availability. Weight varied from 11-24 kg (unrelated to strain) and averaged 17.9 ± 4.2 kg. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Washington under protocol #2331-20, approved Aug. 2, 2011. Animals were housed under standard conditions (12 hour light cycle, pelleted minipig chow) in the University's main facility.

After acclimation to the laboratory environment and pre-injection measurements (see below), unsedated animals were anesthetized with isoflurane delivered by mask (5% induction, 3% maintenance). One masseter muscle per animal was injected, with treatment side determined by coin toss. The skin over the muscle was shaved and cleaned with betadine. Animals received either BoNT-A (onabotulinumtoxinA, Botox® Cosmetic, Allergan, Irvine CA, n=7), BoNT-B (rimabotulinumtoxinB, Myobloc, Solstice Neurosciences, San Francisco CA, n=3), or 0.09% saline (n=2) of similar volume to the BoNT injection (Supplementary Material, Table). The toxins were handled and mixed according to each manufacturer's instructions, and a new vial was used for each animal. Methods for BoNT-A preparation were the same as in our previous rabbit study,¹² in which the toxin was highly effective. Dosage of BoNT-A was originally planned to be about 75 units/muscle, because the minipig masseter is larger than the human masseter, for which the maximum dose is usually 50 units/muscle.¹³ When this was ineffective, dosage was increased to 100 and then to 200 units (Supplementary Material, Table, available online). As

yet there is no standard dose for BoNT-B in the human masseter, but the dose of 5000 units used is the middle of the 3 sizes available (2500, 5000, and 10,000 units). The injection volume was divided into 5 equally spaced locations parallel to the lower border of the mandibular ramus and approximately between the inferior one-third and superior two-thirds of the muscle where the motor endplates are rich¹⁴ (Supplementary Material, Fig. A, available online). In 2 pilot pigs these locations plus 3-5 others in the thickest part of the muscle were injected (#408 and #409, Supplementary Material, Table). The procedure required less than 30 minutes, whereupon pigs were permitted to awaken and feed.

In vivo recordings

Animals were acclimated to the laboratory and experimental environment for 5-7 days. Except in the 2 pilot pigs, assessments of muscle activity and muscle force were recorded pre-injection. Muscle force was measured as the bite force produced by masseter tetanus. Animals were mask-anesthetized. Sterile fine-wire stimulation electrodes were inserted anteriorly and posteriorly into each masseter and connected to a stimulator (Grass S48 with SIU5, Astro-Med, Warwick RI). Each masseter was tetanized separately (550 ms trains, 55 pps, 5 ms/pulse); voltage was advanced gradually until the force produced at the teeth no longer increased, typically 25-30V. The bite force was measured using a custom transducer¹⁵ situated at the mandibular first molar of the tetanized side. Output voltage was measured with a DC amplifier (BioPac, Goleta, CA) and converted to Newtons (N) using a calibration equation determined by placing known weights on the transducer. Following the force recording, the pig was allowed to awaken and was offered its regular diet of pig chow pellets. Muscle activity was assessed during chewing via electromyography (EMG). While the pig was eating, a pair of snap surface electrodes (Vermed, Bellows Falls VT) was affixed to the shaved skin overlying each masseter muscle (Supplementary Material, Fig. A), and a ground electrode was placed on the shaved forehead. The lead wires of the electrodes were connected to differential amplifiers (MEC100, Biopac), and EMG signals were acquired at 1KHz using an MP150 data acquisition interface and AcqKnowledge software (BioPac). A webcam synchronized videos of the jaws with the EMG recording, and both were used to establish the side of each chewing stroke. The pig was permitted to eat to satiation and was then returned to its pen. Masticatory EMG was recorded again 2 days after BoNT or saline injection and then weekly for 3 weeks. Masseter force (bite force produced by the tetanized masseters) was recorded again 3 weeks after injection.

Final day recordings and muscle harvest

Four weeks after the injection, additional sensors were installed in a surgical procedure. Animals were anesthetized (isoflurane) for placement of 3-channel rosette strain gauges (SK-06-030WR-120, Vishay Micro-Measurements, Raleigh NC) bilaterally on the lateral surfaces of the condylar neck and the mandibular alveolar process at the molar region (Supplementary Material, Fig. A). This was accomplished by creating skin flaps to expose the bone, cleaning and drying the bone surface, and fixation of a waterproofed, pre-wired gauge to each site with cyanoacrylate glue. After measuring the orientation of the gauges relative to the occlusal plane, the lead wires were exited from the incisions, which were then sutured closed and infiltrated with 2% lidocaine. The lead wires were connected to conditioner/amplifiers (2100 system, Vishay) which in turn were connected to the Biopac

MP150 interface. After the strain gauges were installed, fine-wire EMG electrodes were inserted into the masseter muscles and led to the Biopac amplifiers. Analgesics (ketorolac 1mg/Kg and buprenorphine 0.1mg/Kg, both IM) were delivered, and anesthesia was removed. Pigs awakened within 30 minutes and were hungry, indicating that dosages of analgesics were appropriate. Regular pig chow was offered, and bone strains were recorded during mastication. Synchronous EMG and video served to identify the side of chewing.

After 10-15 minutes of feeding, the pig was re-anesthetized and euthanized by cardiac perfusion with 0.9% saline followed by 4% paraformaldehyde. Strain gauge attachments were verified. Masseter muscles were removed, blotted dry, weighed, and immersed in additional paraformaldehyde. Muscles were then cut in the horizontal plane to form blocks including the entire cross section. The blocks were processed for paraffin histology and sectioned in the horizontal plane at 7-10 μm . Sections estimated to be at the level of injection (Supplementary Material, Fig. A) were stained with hematoxylin and eosin (H & E) and imaged using a videocamera (Photometrics CoolSnapFX, Roper Scientific, Ottobrunn, Germany) mounted on an Eclipse E400 compound microscope (Nikon Instruments, Melville NY) and MetaVue software (Molecular Devices, Sunnyvale CA). Using a low-power photomontage of the complete section, the muscle was divided geometrically into anterior, middle, and posterior thirds, each split into a medial and a lateral half, totalling 6 anatomical parts. These parts were re-imaged under higher magnification (200x), and a 432 μm by 364 μm rectangular grid with 20 evenly spaced intersections was superimposed on the center of each part. An average of 100 fibers (range 60-275) was covered by each grid. The muscle fiber at or closest to each of the 20 intersections was measured for least (minimum) diameter (more accurate than area or maximum diameter, because some fibers were cut obliquely or longitudinally). Broken or indistinct fibers were not measured. In most cases, 120 muscle fibers were measured from each muscle section (20 from each of the 6 parts) and were used to calculate average least diameter for that muscle.

Signal Processing and Statistical Analysis

Masticatory signals were processed in AcqKnowledge. EMG recordings were filtered with a digital band stop of 60Hz and a bandpass of 60-500Hz to remove noise, and a lowpass filter of 30Hz was applied to bite force and bone strain recordings. Signals from 20-30 consecutive chewing cycles from each animal were analyzed for each feeding session and always included similar numbers of right and left cycles, identified using the timing of the left and right muscles.¹⁶ The mean rectified activity value per burst (bursts were defined as a 10% deviation from baseline) was computed by subtracting the corresponding baseline values. Strain signals from the rosette gauges were exported to Excel (Microsoft, Redmond WA), where a custom macro automatically calculated principal strains and shear strain (sum of absolute values of principal strains) using formulae provided by the manufacturer;¹⁷ shear strain baseline values were subtracted from peak values for each chewing cycle. To supplement the limited sample of saline controls and to compensate for failure of some gauge recordings in this group, strain data from control minipigs of the same age from a previous study¹⁸ were added.

For all aspects of data gathering, the observers were blinded to the treatment and side of treatment. Data were analyzed in Excel and SPSS (Ver. 14, SPSS Inc., Chicago IL). Statistics were carried out to compare BoNT-A and BoNT-B groups. Nonparametric statistics were used because of the small sample sizes, and for the same reason no tests were carried out for the saline animals (except when supplemented by controls), nor were comparisons between sides made except for the BoNT-A group. The question of interest was whether either type of BoNT treatment of the masseter decreased the muscle's mass, EMG activity, force production, or mechanical loading of the mandible.

Results

In all treatment groups, pigs continued to chew at typical rates and with typical left-right alternation.⁹ The amplitude of EMG from the masseter is typically greater on the side of chewing, so right and left side cycles were analyzed separately. Examples of EMG tracings are given in Supplementary Material, Fig. B, and Table 1 summarizes the mean amplitude of EMG bursts over time for same-side chewing (i.e., the injected muscle for cycles on the injected side and the uninjected muscle for cycles on the uninjected side). Variation was high, as is typical in EMG recording, which is subject to individual differences in function as well as anatomy. Pre-injection muscles, saline-injected muscles and uninjected muscles of all groups showed average values of 64-200 μ V throughout the experimental period. Surprisingly, BoNT-A injected muscles were also in this range throughout the experimental period; the injected side was always similar to the pre-injection level. In contrast, masseters that received BoNT-B showed notable reductions, especially at weeks 1 and 2 post-injection, with EMG amplitudes differing significantly from BoNT-A values ($P < 0.03$ at both time points).

Bite force elicited by unilateral masseter tetanus is listed in Table 2. Pre-injection values averaged about 330N and did not differ among groups. There was no evidence of a BoNT-A effect at week 3. In contrast, the BoNT-B-injected masseters showed decreased bite force ($P = 0.01$ compared to the pre-injection average). Muscle weight comparisons (Table 2) were consistent with EMG and bite force results in showing that, compared to the pigs injected with BoNT-A, those receiving BoNT-B had asymmetrical masseters, with the injected muscle smaller in all cases. Bone strain results are also summarized in Table 2. No differences were found, although values tended to be low on the injected side of BoNT-B pigs.

Examples of muscle histology are shown in Fig. 1, and quantitative results are listed in Table 3. No fibrosis or fatty degeneration were seen. However, BoNT-B-injected muscles commonly had regions of smaller fibers adjacent to regions of larger fibers. BoNT-B pigs consistently had smaller average fiber diameters in the injected masseter relative to the uninjected masseter. Comparisons of the BoNT-A and BoNT-B groups were especially informative in showing that differences primarily involved the biggest fibers (maximum value of fiber least diameter, Table 3), which were enlarged on both sides in the BoNT-B group. Mean diameters differed only in the uninjected masseter, again due to larger values in the BoNT-B group, and the smallest fibers did not differ.

Although no conclusions can be drawn from a single case, it is worth noting that #437, which received twice the usual dose of BoNT-A, showed the same lack of EMG/muscle weight effect as the rest of the BoNT-A group.

Discussion

Study limitations

Sample sizes for saline and BoNT-B groups were very small, because the study was originally conceived as a comparison of masticatory function in minipigs injected with BoNT-A against untreated minipigs, for which we have a large historical sample.^{9,14,16,19,20} When no effect of BoNT-A was found, BoNT-B was tried on the basis of an old veterinary report.⁵ Because it was immediately obvious that BoNT-B did inhibit masseteric contraction, we thought it unethical to use more animals than was necessary for demonstration of this effect. It is also a limitation that several minipig strains were used, and that body weight varied. However, all treatments were used with at least 2 different strains with the same results each time, and body weight was not correlated with uninjected masseter weight ($r = 0.06$) or fiber size ($r = 0.53$, $P = 0.11$). Although it is possible that some vials of BoNT-A were inactive, this seems unlikely in view of our previous experience¹² and the fact that a different vial was used for each animal. Finally, because the tissue was formalin-fixed for other procedures, we could not perform standard muscle histochemistry for comparison with other studies.

Are pigs resistant to botulinum neurotoxin serotype A?

BoNT-A did not paralyze the minipig masseter or reduce its force. It is, of course possible that a response might have been seen with doses higher than 200 units/muscle, but this is already a very high dose. Thus these lab-bred swine with no exposure to field conditions have some resistance to BoNT-A. Unfortunately, we were unable to test for toxin antibodies in the serum because of difficulty in obtaining enough BoNT-A for Western blots.

Previous studies have shown that direct injection of BoNT-A into porcine smooth-muscle organs produces relaxation.^{6,7,21,22} In smooth muscle there are no specialized motor endplates; vesicle-bearing varicosities release neurotransmitters from distances as far as 1-2 μ m from the muscle bundle.²³ This open network may render the varicosities accessible to diffusing toxin.

It is more difficult to explain why Gedrange et al.^{10,24} showed mild but significant effects of BoNT-A in the masseter, even though the dosage was the same (100 units/muscle) and the animals about the same age as ours. Importantly, this group examined biochemical rather than functional parameters. Their findings of changes in ion content and myosin heavy chain expression indicate that muscle metabolism was changed, but metabolic alterations might not affect EMG activity or tetanic force as long as the poisoned neuromuscular junctions remained capable of producing action potentials. In contrast, our study examined *in vivo* muscle activity and strength and demonstrated clearly that neither paresis nor atrophy occurred with BoNT-A, in contrast to BoNT-B.

Functional effects of BoNT-B on the porcine masseter muscle

BoNT-B produced differential effects on our outcome measures. EMG activity fell to about 30% that of the uninjected masseter during weeks 1 and 2 post-injection (Table 1), the most striking result. Reduced EMG is a direct consequence of failed neurotransmission. Recovery of EMG from BoNT-B may have occurred at week 3, somewhat faster than the 6-week duration of the drug in humans,¹ but still providing prolonged inactivity. Atrophy (e.g., muscle weight and fiber diameter) follows inactivity and in turn causes loss of muscle contractile strength (e.g., bite force and bone strain). As expected, these effects were delayed relative to EMG, in both their appearance and their recovery.

The more surprising outcome of BoNT-B involved fiber diameter. Although fibers of the injected masseter were smaller than those on the opposite side, they were not small relative to other groups. Rather, hypertrophy was greatly accentuated on the uninjected side, accounting for the asymmetry observed. The muscles of mastication produce chewing through complex, centrally controlled coordination.^{25,26} Alteration of untreated jaw muscles is to be expected. Indeed, the fiber type changes observed by Gedrange et al. with BoNT-A were more dramatic in the uninjected than in the injected masseter.¹⁰

Is the masseter muscle unique?

Muscles of mastication differ from postcranial muscles in many details of embryological derivation and gene expression^{27,28,29} and are spared under circumstances that produce marked deterioration of limb muscles.³⁰ In humans, however, BoNT treatments of the masseter have exceptionally long durations,^{31,32,33} so it is unlikely that jaw muscles are protected. The resistance of the pig masseter to BoNTs is therefore probably generalizable to other porcine striated muscles.

Clinical considerations and conclusions

This study was undertaken originally to develop a large animal model for the use of BoNT-A in the masseter muscle, a procedure frequently performed for pain relief and for cosmetic reasons. After studying the effect of a unilateral treatment on oral function, we intended to proceed to a more clinically relevant bilateral protocol. However, BoNT-A produced neither paresis nor atrophy in the minipig masseter. In contrast, treatment with BoNT-B greatly reduced muscle activity with associated decreases in muscle force and dimensions. BoNT-B is probably the better choice for future studies in porcine models.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

BoNT-A, BoNT-B, BoNT-C, BoNT-D	Botulinum neurotoxin serotypes A, B, C and D
EMG	Electromyography or electromyographic
H & E	Hematoxylin and eosin
LD	Lethal dose in mouse
LD50	Dose which is lethal to 50% of mice

Figure 1. Analysis of muscle fiber dimensions from histological sections

The upper panel is an unmagnified photograph of an entire muscle section (see Supplementary Material, Fig. A for plane of section). The black lines show the geometric division of the section into 6 parts. The small white rectangles in the center of each part show the areas photographed at high magnification and analyzed. The examples shown in the lower panels are from the anterior-lateral part (marked with an asterisk in the upper panel). Over each of the rectangular areas, a rectangular grid with 20 intersections was superimposed. The muscle fiber closest to each intersection (light colored fibers in lower panels) was measured for least fiber diameter for a total of 120 fibers measured per muscle. The lower panels compare the anterior-lateral part of a BoNT-A-injected masseter (#433) with that of a BoNT-B-injected masseter (#439). After BoNT-A, fibers were relatively uniform in size, as in uninjected muscles. After BoNT-B, however, both regions of smaller fibers, shrunk within their sarcolemmas, and regions of much larger fibers were seen, often in proximity. Scale bars in the lower panels are 100 μ m.

Table 1
Average amplitude of masseter EMG during chewing* (mean in $\mu V \pm S.D.$)

Treatment	n	Preinjection	48 hours	1 week	2 weeks	3 weeks
Saline Injected side	2	77 $\pm$ 25	177 $\pm$ 90	176 $\pm$ 90	106 $\pm$ 124	200 $\pm$ 46
BoNT-A Injected side	5	84 $\pm$ 30	85 $\pm$ 66	95 $\pm$ 73	95 $\pm$ 38	81 $\pm$ 57
BoNT-B Injected side	3	139 $\pm$ 84	47 $\pm$ 10	16 $\pm$ 3	14 $\pm$ 9	40 $\pm$ 28
<i>Mann-Whitney P (A vs B)</i>		<i>0.30</i>	<i>0.30</i>	<i>0.025</i>	<i>0.025</i>	<i>0.18</i>
Saline Uninjected side	2	64 $\pm$ 8	148 $\pm$ 3	119 $\pm$ 26	101 $\pm$ 57	69 $\pm$ 41
BoNT-A Uninjected side	5	78 $\pm$ 33	96 $\pm$ 45	107 $\pm$ 65	86 $\pm$ 55	73 $\pm$ 43
BoNT-B Uninjected side	3	141 $\pm$ 83	105 $\pm$ 40	46 $\pm$ 15	52 $\pm$ 17	66 $\pm$ 38
<i>Mann-Whitney p, A vs B</i>		<i>0.18</i>	<i>0.88</i>	<i>0.10</i>	<i>0.30</i>	<i>0.88</i>

* Chewing cycles in which the pig was chewing on the same side as the recorded masseter; 10-15 cycles were averaged for each animal at each time period. Bolded values indicate $P < 0.05$.

Table 2
Measurements of bite force produced by masseter tetanus (pre-injection and week 3 post-injection), masseter weight (week 4), and mandibular shear strain during chewing (week 4)

		Pre-injection*		Week 3/4 Saline		Week 3/4 BoNT-A		Week 3/4 BoNT-B		Mann-Whitney P
Side	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	
Bite Force (N)										
Injected	10	322.1 ± 99.8	2	386.4 ± 146.8	5	274.9 ± 160.2	3	136.5 ± 74.9		preinj. vs A, 0.44
Uninjected	10	333.3 ± 95.0	2	350.8 ± 47.6	5	337.6 ± 169.7	3	218.5 ± 113.8		preinj. vs B, 0.01 [†]
Muscle Weight (g)										
Injected	N/A		2	42.2 ± 3.0	7	38.8 ± 10.2	3	36.9 ± 4.4		A vs B, 0.02 ^{†, ‡}
Uninjected	N/A		2	40.4 ± 0.1	7	38.5 ± 9.8	3	41.9 ± 4.8		
Mandibular Strain (µε)										
Body										
Injected	N/A		N/A		3	383 ± 157	3	275 ± 112		N/A
Uninjected	N/A		7§	454 ± 153	2	449 ± 135	3	372 ± 57		N/A
Condyle										
Injected	N/A		N/A		4	957 ± 552	2	834 ± 386		N/A
Uninjected	N/A		9§	862 ± 465	5	923 ± 322	2	931 ± 285		N/A

^{*} All groups averaged for pre-injection values of bite force.

[†] Bolded tests indicate $P < 0.05$.

[‡] Statistics were performed on asymmetry in muscle weight (uninjected minus injected weight, A vs B).

[§] Previous strain gauge data collected using the same techniques¹⁸ were added to improve sample size for the saline controls.

Table 3

Masseter muscle fiber diameters (least diameter of 60-120 muscle fibers per muscle: mean $\pm$ standard deviation, minimum and maximum; grand means $\pm$ standard deviation for treatment groups)

ID	Treatment	Injected Masseter (μm)			Uninjected Masseter (μm)		
		Mean $\pm$ SD	Minimum	Maximum	Mean $\pm$ SD	Minimum	Maximum
434	Saline	21.0 $\pm$ 4.1	13.7	31.7	20.6 $\pm$ 3.3	13.4	27.2
435	Saline	27.4 $\pm$ 4.4*	16.7	36.9	22.0 $\pm$ 3.3	13.2	30.1
<i>Saline grand means</i>							
431	BoNT-A	24.2 $\pm$ 4.5	15.2 $\pm$ 2.1	34.3 $\pm$ 3.7	21.3 $\pm$ 1.0	13.3 $\pm$ 0.1	28.6 $\pm$ 2.1
432	BoNT-A	25.4 $\pm$ 4.6	15.3	37.3	24.5 $\pm$ 4.3	15.0	36.9
433	BoNT-A	26.0 $\pm$ 5.1	14.7	38.6	28.9 $\pm$ 4.7*	17.2	41.4
436	BoNT-A	26.2 $\pm$ 6.4	12.0	40.9	25.9 $\pm$ 6.2	15.6	41.8
437	BoNT-A	23.2 $\pm$ 3.2	15.3	29.8	22.6 $\pm$ 3.2	14.0	29.9
<i>BoNT-A grand means</i>							
438	BoNT-B	28.8 $\pm$ 5.0*	17.0	40.6	26.5 $\pm$ 3.9	17.7	38.8
439	BoNT-B	25.9 $\pm$ 2.0	14.8 $\pm$ 1.8	37.4 $\pm$ 4.5	25.7 $\pm$ 2.4	15.9 $\pm$ 1.5	37.7 $\pm$ 4.8
440	BoNT-B	32.4 $\pm$ 8.8	14.3	54.9	35.1 $\pm$ 9.4*	14.9	60.5
<i>BoNT-B grand means</i>							
439	BoNT-B	25.9 $\pm$ 8.7	12.7	53.2	33.1 $\pm$ 6.6*	14.2	46.3
440	BoNT-B	30.4 $\pm$ 7.3	16.2	47.0	34.7 $\pm$ 7.6*	15.5	50.7
<i>BoNT-B grand means</i>							
439	BoNT-B	29.6 $\pm$ 3.3	14.4 $\pm$ 1.7	51.7 $\pm$ 4.2	34.3 $\pm$ 1.0	14.9 $\pm$ 0.7	52.5 $\pm$ 7.3
<i>Mann-Whitney p, A vs B†</i>							
		0.18	0.66	0.03	0.03	0.30	0.03

* Significantly larger than fibers of the opposite side in the same animal (*t*-tests). All differences $p < 0.0001$ except #438 ($P < 0.02$).

† Bolded entries in this row represent P -values < 0.05 in comparisons between BoNT-A and BoNT-B.