

Innervation Zone of Orbicularis Oculi Muscle and Implications for Botulinum A Toxin Therapy

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Summary: Motor points (areas of maximal sensitivity to electrical stimulation) were found in constant locations over orbicularis oculi when measured in both eyes of six normal subjects. All subjects had a motor point at the lateral terminus of the upper lid crease and the medial extent of the lower lid crease. A study of the innervation zone [distribution of neuromuscular junctions (NMJ)] was conducted on strips of pretarsal and preseptal portions of the upper eyelid orbicularis that had been removed routinely during involutional ptosis surgery. There was no significant difference in NMJ concentration between the medial and lateral sections, as determined by cholinesterase staining. Therefore, we concluded that the innervation zone is diffuse for the orbicularis muscle within this portion of the upper eyelid. Single-point injections of botulinum toxin were then compared to the conventional multiple injection sites on separate eyes in 10 patients with benign essential blepharospasm. Eight of the 10 patients reported greater relief on the side given injections into multiple points; the other two patients experienced no difference between the two methods. Both histologic data and clinical observation of response to botulinum toxin injection suggest the innervation zone for the upper orbicularis is diffuse. Thus, we conclude that multiple injections are superior to the injection of a single motor point.

Key Words: Botulinum toxin—Blepharospasm—Facial nerve—Orbicularis oculi—Innervation zone.

Since the introduction of botulinum toxin for strabismus therapy in 1978 by Scott (1), it has been used for other movement disorders, including blepharospasm (2), spasmodic torticollis (3), and spasmodic dysphonia (4). Botulinum toxin injection into the orbicularis oculi muscle is also effective in the treatment of essential blepharospasm, Meige Syndrome, and hemifacial spasm (5, 6). The site for the intramuscular injection has heretofore been determined empirically, with the goal of achieving maximum response with minimal complications (AB Scott, personal communication). Therefore we set out to analyze the injection technique by studying the anatomy of the innervation of the orbicularis oculi muscle.

Botulinum toxin produces a variable state of neuromuscular paralysis by blocking release of acetylcholine from presynaptic vesicles at myoneural junctions (7). The toxin binds rapidly at the presynaptic membrane and undergoes internalization, followed by prolonged neuromuscular blockade (8), clinical weakness, and reduction of spasms with eventual atrophy of muscle fibers (1). When nonlethal amounts of botulinum toxin are injected into individual muscles, recovery of function requires regeneration or reconstitution of neuromuscular junctions. This process occurs over a 3- to 5-month period, as has been demonstrated in animal models (9).

Because the effectiveness of botulinum toxin therapy appears to depend on blocking of neuromuscular junctions, knowledge of their distribution within the orbicularis oculi muscle would be helpful in localizing the most sensitive regions into which injections of the toxin would be most effective. An understanding of the distribution of neuromuscular junctions (the innervation zone) also can be used to study the terminus of facial nerve projection into orbicularis oculi. In this investigation, we employed

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acetylcholinesterase staining to identify neuromuscular junctions on strips of orbicularis oculi. We also compared the neuromuscular junction concentration in the medial and lateral orbicularis muscle strips to survey the portion of the muscle that is most actively involved in the blink reflex. These findings will be discussed in relation to variations in the injection method used to treat Meige Syndrome and benign essential blepharospasm.

MATERIALS AND METHODS

Motor Point Determination of Orbicularis Oculi Muscle

A pencil-shaped stimulating electrode (diameter, 2 mm) delivering 100 μ s pulses of variable voltage (20–120 V) at 1-s intervals was used to determine the response threshold of the orbicularis oculi muscle. After being coated with conductive gel, this cathode was placed on the skin, and the voltage was increased until a small contraction of the orbicularis oculi was seen. The anode was a 5-mm disc electrode taped to the forehead several centimeters from the orbicularis oculi. Skin over the preorbital, preseptal, and pretarsal portions of the muscle was surveyed by moving the stimulating cathode around and noting the responses. When an electrically sensitive area of the muscle was found, the voltage was reduced until we were able to identify the region (motor point) at which the lowest voltage would produce a contraction (threshold voltage). Motor points were marked with a surgical marking pen and photographed to establish their location relative to surface anatomy.

Neuromuscular Junction Analysis Using Acetylcholinesterase Stain

Fourteen strips of orbicularis oculi muscle normally removed during involutional ptosis surgery to gain access to the levator aponeurosis were divided into medial and lateral portions and stained for cholinesterase in order to outline the positions of the neuromuscular junctions. These muscle strips usually measure 2–3 mm in diameter and 20–25 mm in length. They are removed in order to debulk the upper lid, thereby preventing an excess prominence in the upper lid fold, enhancing symmetry between lid folds, and creating a prominent lid crease (10, 11).

After remaining in Baker's solution (10% formol calcium) for 24 h, the muscle specimens were placed in 0.88M gum sucrose for 2–3 h. The tissue was sectioned at 10 μ in a cryostat at 20°C, placed

on a gelatin-coated slide, and allowed to dry. Acetylcholinesterase staining was carried out using Karnovsky's method of acetylcholine iodide in a 13:2:1:2 solution of 0.1 M sodium hydroxide malate buffer, 0.03 M cupric sulfate, 0.1 M sodium citrate, and 0.5 M potassium ferricyanide. The slides were incubated in this solution for 90 min at 37°C, then mounted with a cover slip.

The total surface area of all muscles assayed by this technique was 57 mm² for the medial strips and 50 mm² for the lateral strips. Myoneural junctions stained as discrete black dots on muscle fibers, as shown in Fig. 1 (see arrows).

Botulinum Toxin Injections: Conventional (Multiple-Point) Method Compared to Motor-Point Injection

Ten patients with benign essential blepharospasm or Meige Syndrome were treated by injecting the upper lid motor point alone on one side (Fig. 2) while multiple-point injections were made in the usual locations around the second eye (Fig. 3). Equivalent doses were administered to each side. Each patient selected for this part of the study had complained of discomfort after receiving multiple-point injections during previous sessions. Because this study group had been previously treated, the dose of botulinum toxin had been individualized to achieve the most beneficial effects. For this reason, the botulinum toxin dose was different for each patient.

Patients were reevaluated within 2–4 weeks from the time of the injection so that the examining physician (G.E.B.) could assess the relative effectiveness of the two injection strategies. The exact dosage given to each patient is shown in Table 1. It should be noted that patients receiving higher doses of toxin (e.g., patient 4) had not achieved beneficial responses at lower doses, but they did achieve benefit at the higher dose on a previous injection. Each of the patients was asked if there was any difference in effectiveness between the motor point-treated eye and the eye receiving injections into the conventional four to six points. One of us (G.E.B.) judged the differences in orbicularis weakness by direct observation. Weakness was assessed by asking the patient to make a maximal effort at eyelid closure while the examiner attempted to open the palpebral fissure. With normal orbicularis function, it is difficult to open the palpebral fissure but it is usually easy several weeks after botulinum toxin injection. Relative differences between the sides were compared 3–6 weeks after infection.

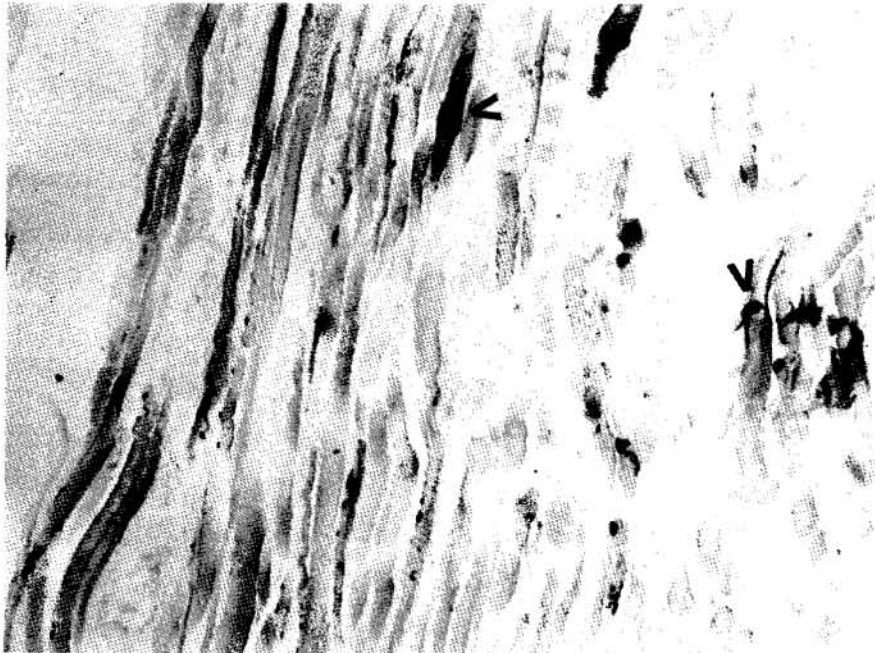


FIG. 1. Representative section showing neuromuscular junctions as small black dots on surfaces of orbicularis muscle fibers (arrows). Multiple junctions are seen in this field. (original magnification, $\times 25$)

RESULTS

Motor Point of Orbicularis Oculi

Motor points of orbicularis oculi were determined in both eyes for six normal subjects. In all cases, two major points were found in similar locations over the orbicularis muscle. One was found in the superior lateral orbicularis at the lateral terminus of the upper eyelid crease. A second was found on the inferior and medial portion of the muscle in close proximity to the anterior lacrimal crest (Fig. 4). Stimulation over the upper or lower motor point produced a twitch or contraction of the orbicularis muscle with threshold voltages of 36–60 V. Over

other areas of muscle, stimuli of 80–120 V were needed to produce a twitch. All stimuli were non-painful.

Effects of Botulinum Toxin on Motor Point Determination

Two patients with Meige Syndrome who had received multiple injections into orbicularis oculi for involuntary blepharospasm were evaluated to locate the motor points. The first patient had received 30–80 IU of botulinum type A toxin at seven treatment sessions over a period of 2.5 years; the second patient received 30–100 IU at 10 treatment

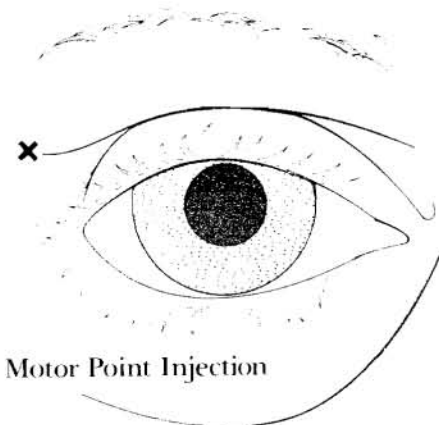


FIG. 2. The upper motor point was injected as shown here. Only a one-point injection was administered in this test group.

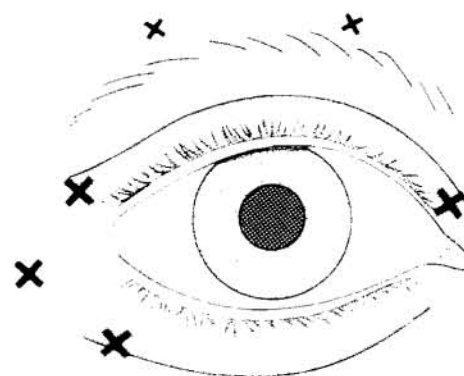


FIG. 3. Multiple-point injection strategy. This diagram shows the usual injection points for botulinum toxin in the treatment of blepharospasm. There are four points where the toxin is injected; in addition, injections may be given in the brow region.

TABLE 1. Clinical results: single (motor-point) injection vs. multiple-point injections^a

Patient	Multiple-point injection	Single-point injection	Result	Complications
1. A.Z.	15 IU ^b	15 IU	Less symptoms on multiple injection side; right side weaker	None
2. M.S.	15 IU	15 IU	No difference between eyes; no difference in strength	None
3. J.M.	30 IU	30 IU	Less symptoms on multiple injected side; no difference in strength	None
4. H.O.	75 IU	75 IU	Less symptoms on multiple injected side; right side weaker	None
5. C.G.	20 IU	20 IU	Less symptoms on the multiple injected side; right orbicularis weaker	Ptosis 1.5 mm OS
6. B.R.	35 IU	30 IU	Less symptoms on the single injection side; no difference in strength	None
7. E.P.	50 IU	35 IU	Less symptoms on multiple injected side; right orbicularis weaker	Ptosis 2.5 mm OS
8. R.K.	40 IU	40 IU	Less symptoms on the multiple injection side; right orbicularis weaker	None
9. E.B.	10 IU	10 IU	No difference; no difference in strength	None
10. M.P.	20 IU	20 IU	Less symptoms on multiple injected side; right orbicularis weaker	None

^a All multiple-point injections were given in the right eye; all single-point injections were given in the left eye.

^b 1 IU = LD 50 for a white mouse.

sessions over a period of 3.5 years. Each patient had received an injection of botulinum toxin within 3 months of this evaluation. Discrete motor points could not be determined in either patient. When the stimulus voltage was increased far above that used in control subjects (100–140 V), there was very little contraction of orbicularis oculi along with more substantial contraction of the frontalis muscle underlying the anode (Fig. 5), a result not seen in normal subjects.

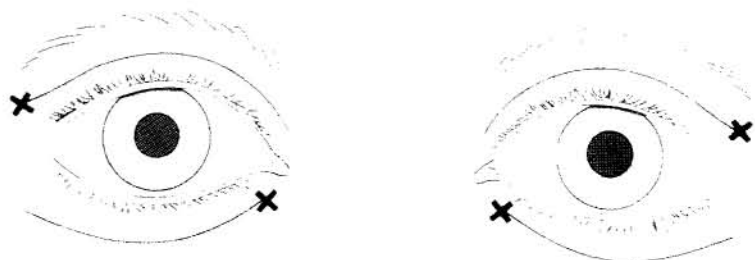
Neuromuscular Junction Topography within Orbicularis Oculi Muscle: Two-Point Determination

Absolute counts of neuromuscular junctions were made on each specimen cut in serial section. The neuromuscular junctions stained as black dots on the surface of muscle fibers (Fig. 1). Although

clusters were commonly seen within a microscopic field, the numbers of neuromuscular junction per serial section appeared consistent (range 20–65). The absolute number of sections was divided by the surface area surveyed to derive an expression of relative concentration between medial and lateral positions of the upper lid.

No significant difference in concentration was found between the medial and lateral specimens. The medial specimen concentration was 5.42 NMJ/mm²; the lateral concentration was 4.39 NMJ/mm² (Table 2). The lateral specimen included an area of orbicularis oculi just under the lateral extreme of the lid fold. This was the same area where the motor point of the upper portion of the orbicularis muscle was located with surface electrode studies.

FIG. 4. This diagram illustrates the motor points of the orbicularis oculi as determined using the surface-stimulating electrode. All 12 eyes studied had an area with the lowest electrical threshold in the superlateral and inferomedial portion of the muscle. These areas are believed to correspond to the major branches of the facial nerve penetrating the muscle.



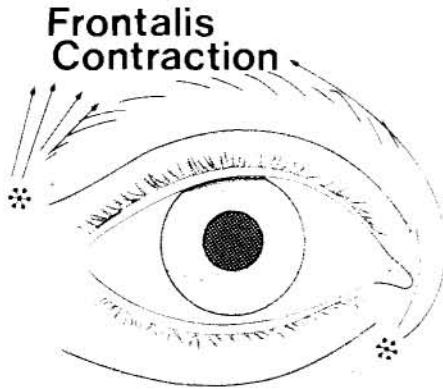


FIG. 5. This figure shows the effect of botulinum toxin on motor point determination using a surface stimulating electrode. When we tested patients who had received multiple toxin injections, the motor points of the upper and lower lid could not be found, because of uncoupling nerve impulses from muscular contraction. When these areas were stimulated with larger currents, contraction of surrounding muscles was observed.

DISCUSSION

Since the initial report of the efficacy of intramuscular injections of botulinum A toxin for the treatment of essential blepharospasm by Scott et al. (2), many patients have been managed with repeated injections given at multiple points around the eye (Fig. 3). The sites of injection were initially chosen based on empirical observation as to where injections produced the best clinical response with the lowest incidence of adverse effects (ptosis, diplopia, epiphora, lid malpositions). The injection sites shown in Fig. 3 result in a lower incidence of ptosis, diplopia, and epiphora.

Injecting the toxin into the pretarsal portion of the orbicularis oculi close to the lash line and at the extreme medial and lateral extent of the upper eyelid allows for maximum separation of the injected orbicularis muscle from the levator muscle.

TABLE 2. Relative concentrations of neuromuscular junctions in human orbicularis oculi muscle by two-point determination (superior pretarsal-preseptal portion of the muscle)

Medial	Lateral
Total specimens = 8	6
Surface area = 57 mm ²	50 mm ²
Mean number of NMJ in each longitudinal section = 31	36.1
Concentration NMJ/mm ² = 5.42 ^a	4.34 ^a

^a No statistical difference was noted between the lateral and medial portions of the muscle.

NMJ = neuromuscular junctions.

Injections into more superior portions of the upper eyelid (above the lid fold) are more likely to produce ptosis because of the proximity of the muscular portion of the levator and minimal anatomic barriers to diffusion of the toxin into deeper orbital tissues. Patients with an attenuated preaponeurotic fat pad and orbital septum are more likely to develop ptosis because that structure represents the major anatomic barrier separating the orbicularis oculi from the levator palpebralis superioris muscle. This anatomic configuration of the upper eyelid has allowed for a selective weakening of the orbicularis muscle without impairing function of the lid retractors. Muscular fibers of the levator palpebralis superioris are located primarily within the superior orbit, with its aponeurosis extending under the fat pad, orbital septum, and orbicularis muscle before inserting on the tarsal plate. Because the aponeurosis is not affected by the toxin, lid protraction can be weakened selectively by injecting botulinum toxin into the pretarsal portions of the muscle.

Although the gross anatomic configuration of the eyelid has been helpful in interpreting the clinical responses achieved with botulinum toxin, a better understanding of the anatomic details of the organization of nerve fibers within the orbicularis oculi might serve to improve the choice of injection sites. Theoretically, because botulinum toxin acts at the neuromuscular junction, the areas within the muscle with the highest concentration of neuromuscular junctions would be most sensitive to the toxin. The region in which neuromuscular junctions are distributed within a muscle is known as "the innervation zone." If neuromuscular junctions were distributed in a homogeneous fashion throughout the muscle, the innervation zone would include the entire muscle and be *diffuse*. However, if the neuromuscular junctions are usually clustered in well-defined areas, innervation zones are described as *focal*. Theoretically, when innervation zones are focal in any given muscle, a precise injection of botulinum toxin into those zones should produce an optimum effect on muscular spasms with a minimum quantity of the toxin.

In this study, both electrostimulation and histochemical analysis were used to define more clearly the innervation of the orbicularis oculi muscle. Most textbooks of anatomy depict the facial nerve entering the orbicularis laterally from the temporal and zygomatic branches. More detailed anatomic representations described by Fugita (12) show the facial nerve projecting into the orbicularis muscle superolateral from multiple temporalis branches as well as inferior medial from zygomatic and buccal

branches. Other small branches penetrate the muscle throughout its circumference. Although these gross anatomic findings are helpful in understanding orbicularis innervation, simple dissection is insufficient to define the innervation zone for a muscle. Branches of the facial nerve may either project to neuromuscular junctions in one portion of the muscle (focal innervation zone) or ramify diffusely, projecting to neuromuscular junctions in many parts of the muscle (diffuse innervation zones).

Electrical stimulation of striated muscle by means of brief (less than 1 ms) current pulses occurs because the stimulus depolarizes alpha motor axons innervating the muscle; muscle fibers cannot be stimulated directly by the brief (100 μ s) current pulses used in this protocol. Impulses arise near the cathode along the course of a nerve as determined by the path of current flow and the anatomy of the nerve as it courses through the muscle.

A motor point is defined as a small area on the skin near a particular muscle where the threshold is lowest for electrical excitability of that muscle. With transcutaneous electric stimuli, the muscle is most excitable at its motor point, because it is there that the current most easily excites its motor nerve fibers. In some situations, the motor point overlies the entrance of the nerve into the muscle through its deep surface; in other words, it is where a motor nerve and its terminal branches happen to be nearest to the skin rather than where the axons directly penetrate the muscle.

Because the orbicularis muscle is flat and lies just beneath the skin surface, the motor point represents the area where the major motor nerve trunks penetrate muscle prior to arborizing into smaller branches. Axons are also most easily stimulated electrically before they branch into smaller-diameter, poorly myelinated terminals. The motor points of the orbicularis oculi were in similar locations in each of the 12 cases, a finding that reflects anatomic regularity of motor axon penetration into this muscle.

Neuromuscular junctions, as recognized by staining the motor end-plates, are usually situated near the center or equatorial region of each muscle fiber. Therefore, in any given muscle fascicle, they are generally found in a narrow band. Although the innervation zone for certain muscles may lie immediately beneath a motor point, this is not true for many muscles (13–16). For example, in flexor carpi radialis and palmaris longus, the fasciculi run parallel to the overlying fascia, and the motor nerve enters perpendicular to the muscle at a point near the center of the deep surface of the muscle so the

motor point and innervation zones coincide. In flexor carpi ulnaris and tibialis anterior (13–16), muscle fasciculi run deeply away from the surface toward the tendon so the innervation zone is buried. In these muscles, as in biceps brachii, precise correspondence between innervation zone and motor points is lacking. In peroneus longus and gastrocnemius, the motor nerve runs parallel to the long axis of muscle fibers; the innervation zone is aligned in that direction but does not underlie the motor point.

To learn more about the orbicularis oculi innervation zone from a microanatomic perspective, quantification of neuromuscular end-plates was attempted using cholinesterase staining of specimens of muscle routinely removed during ptosis surgery. Although these specimens only sampled the upper portion of orbicularis oculi, this portion of the muscle is clinically important to the blink reflex. This area of muscle is close to the recommended injection points for botulinum toxin to treat essential blepharospasm and Meige Syndrome. Data generated from these cholinesterase stains indicate a diffuse configuration of neuromuscular junction distribution. This suggests that the anatomic innervation zone is diffuse, or multifocal at least, for the upper portion of the orbicularis oculi muscle, with motor end-plates distributed evenly over the horizontal extent of the upper lid in those specimens studied. Although these specimens may be representative, they do not reflect an entire map of end-plates for this muscle. A complete distribution map will require a complete orbicularis specimen taken close to the time of death so that immediate placement into Baker's solution can be accomplished. These findings are not surprising, considering the extensive ramification of axons within the muscle (12).

These histological findings may explain the differences in clinical response to botulinum toxin when it was injected using the two different techniques described in this study. When only the motor point of the upper eyelid was injected, most patients found that the injection produced less favorable results than the multiple-point injections. The efficacy of the multiple-point method is probably due to the diffuse or multifocal nature of the innervation zones of orbicularis oculi. Because botulinum toxin binds to neuromuscular junctions almost immediately upon injection and because its specific effects are limited to those regions, a one-point injection would bind to a smaller fraction of the neuromuscular junctions than multiple injections over a greater portion of the innervation zone, even if

similar amounts of toxin were administered at the motor point. The clinical observation that multiple-point botulinum toxin injections appear to produce a superior effect is thus compatible with the concept of a diffuse configuration for the innervation zone of orbicularis oculi. This interpretation assumes that the regional toxin spread is confined to an area close to the injection site.

Inability to define a motor point over orbicularis muscle after repeated botulinum toxin injections is a result of neuromuscular blockade produced by the toxin. Because many or most of the orbicularis oculi neuromuscular junctions were blocked by the toxin, increasing electrostimulation caused contraction in the untreated frontalis muscle from current spread to surrounding axons innervating untreated facial muscle groups. The uncoupling of orbicularis contraction to surface electrostimulation is consistent with the mechanism by which botulinum toxin affects muscular contraction and neuromuscular transmission.

Our study suggests that motor point-directed injections of botulinum toxin provide no clinical advantage due to the nature of the innervation of the orbicularis oculi muscle. Furthermore, a diffuse innervation zone supplied by multiple branches of the facial nerve is consistent with orbicularis resilience against paralysis after major reconstructive eyelid procedures.

Further histologic evaluations of innervation zones may provide useful information in designing botulinum toxin injection techniques for other muscle groups.



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