Bupivacaine Injection Remodels Extraocular Muscles And Corrects Comitant Strabismus

Joel M Miller, PhD [1, 2], Alan B Scott, MD [2], Kenneth K Danh, BS [1,2], Dirk Strasser, BS [2], Mona Sane, MD [2]

[1] Eidactics • San Francisco CA • USA

[2] Smith-Kettlewell Eye Research Institute • San Francisco CA • USA

Correspondence

Joel M Miller, PhD
Eidactics
Medical Arts Building • Suite 210
2000 Van Ness Avenue
San Francisco, CA 94109-3021
jmm@eidactics.com

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Disclosures

Orbit™ 1.8 (Miller 1999; Miller et al 1999) is a product of Eidactics.

Scott AB (2009). Medical Treatment of Muscles by Exposure to Anesthetic Drugs. Patent US 7,632,848 B1, filed 2007.10.04, issued 2009.12.15.

Scott AB (2012). Method of changing muscle lengths with anesthetic drugs. Patent US 8,193,220 B1, filed 2009.08.20, issued 2012.06.05.

Précis

Bupivacaine injection is shown to alter extraocular muscle size and shape, leading to enduring alignment corrections in non-paralytic horizontal strabismus.

Keywords

bupivacaine, EOM, hypertrophy, myotoxicity, strabismus

Abstract

Purpose: Evaluate clinical effectiveness and anatomic changes resulting from bupivacaine injection into extraocular muscles to treat comitant horizontal strabismus.

Design: Prospective observational clinical series.

Participants: Thirty-one comitant horizontal strabismus patients.

Methods: Nineteen patients with esotropia received bupivacaine injections in the lateral rectus muscle, and 12 with exotropia, in the medial rectus. Sixteen of these, with large strabismus angles, also received botulinum type A toxin injections in the antagonist muscle at the same treatment session. A second treatment was given to 13 patients who had residual strabismus after the first.

Outcome Measures: Clinical alignment measures and muscle volume, maximum crossectional area, and shape derived from magnetic resonance imaging, with followups from 6 mo to 3 yrs.

Results: At average 15.3 mo after the final treatment, original misalignment was reduced by 10.5Δ (6.0°) with residual deviations ≤10Δ in 53% of patients. A single treatment with bupivacaine alone reduced misalignment at 11.3 mo by 4.7Δ (2.7°) with residual deviations ≤10Δ in 50% of patients. Alignment corrections were remarkably stable over followups as long as 3 yrs. Six months after bupivacaine injection, muscle volume had increased by 6.6%, and maximum crossectional area by 8.5%, gradually relaxing towards pre-treatment values thereafter. Computer modeling with Orbit™ 1.8 (Eidactics, San Francisco, CA) suggested that changes in agonist and antagonist muscle lengths were responsible for the enduring changes in eye alignment.

Conclusions: Bupivacaine injection alone or together with botulium toxin injection in the antagonist muscle improves eye alignment in comitant horizontal strabismus by inducing changes in rectus muscle structure and length.

Introduction

Extraocular muscle resection, a common surgical treatment for strabismus, sacrifices insertion-end tissue to stretch a muscle, increasing its elastic force. Most other surgeries rely on compensatory muscle impairment. Recession, for example, reduces the force of a muscle to balance a weak antagonist or contralateral yoke muscle, transposition sacrifices one direction of muscle action for another, and posterior fixation moves a muscle's effective insertion to a mechanically disadvantageous position. Pharmacologic agents, in contrast, offer the possibility of increasing contractile strength and elastic stiffness, as well as changing muscle length, without resecting tissue or compromising orbital mechanics. Injection can be accurately targeted with electromyographic (EMG) guidance under local anesthesia in an outpatient setting or under light general anesthesia.

The amino amide anesthetic bupivacaine (BPX) injected into a skeletal muscle has the remarkable effect of destroying the myofibrillar system while preserving intact satellite cells, basal lamina, capillaries, and peripheral nerves (Nonaka et al 1983). BPX induces Ca^{2+} release from the sarcoplasmic reticulum, inhibits reuptake, and sensitizes the contractile apparatus to Ca^{2+} (Zink et al 2002), so that within a few minutes myofibrils hypercontract and damage to plasma membranes is evident (Bradley 1979). Within a few hours, calcium-activated neutral protease (CANP) localized in Z-lines (Ishiura et al 1980) cleaves the sarcomeres, which are then digested by other proteases and lysosomal enzymes. (Duncan 1978; Imahori 1982; Murachi 1983). Within a few weeks the muscle rebuilds (Hall-Craggs 1974; Bradley 1979), generally with increased size.

When skeletal muscles are mechanically overloaded, particularly during eccentric contraction (elongation under activation), they also sustain damage to myofibrils (Brooks & Faulkner 2001) and plasma membranes (Petrof et al 1993). Subsequent to both pharmacologically and mechanically induced damage, satellite cells proliferate and fuse into new muscle fibers. The role of various growth factors in mediating muscle enlargement is unsettled (Yang et al 1996; Yang & Goldspink 2002; Hill & Goldspink 2003; Matheny et al 2010).

Inadvertent injection of local anesthetics (lidocaine, BPX, or both) into human extraocular muscle can result in strabismus. In 1982, Scott examined several of Rainin's strabismus patients, and suggested incorrectly that BPX myotoxicity might be simply causal (ABS, personal communication). Rainin and Carlson (1985) published findings on these patients, concluding that enduring myotoxic damage was the principal etiology. Observation of the clinical time course was then made by Goldchmit and Scott (1994), who documented an initially *paretic* muscle pattern (limitation of gaze in the field of action of the affected muscle) that gradually transformed into *overaction* (deviation towards the affected muscle, increasing as the eye moves further into that muscle's field of action) or *contracture* (limitation of gaze in the opposite field). Many studies of inadvertent anesthetic injection followed. Han, et al (2004), for example, reviewed 14 cases of diplopia secondary to surgical or anesthetic trauma and found overaction in 12. In several cases they were able to document an initially paretic alignment pattern progressing to overaction and, using forced duction testing under general anesthesia, normal or slightly elevated stiffness, confirming Goldchmit and Scott (1994).

BPX injections in rabbit extraocular muscle at concentrations above 0.75% produce extensive myofiber destruction, followed by regeneration with localized scarring, whereas lower concentrations have little effect (Zhang et al 2010). Studies by Rosenblatt's group (1992; 1992) in rat skeletal muscle (EDL) showed that after approximately 3 weeks, BPX-injected muscle recovers normal morphology and function, except for having slightly more Type I (slow) fibers. Subsequently, BPX-injected muscle undergoes hypertrophy, finally resulting in a muscle with increased total contractile force, but lower contractile force per unit crossectional area, suggesting that non-contractile tissue also is added to the muscle during the hypertrophy phase. Both contractility and stiffness of such a muscle should be higher than normal. These findings, supported by biomechanical simulations (Miller 1999; Miller et al 1999) showing that overaction and increased range of gaze could result only from increased contractility, convinced us that hypertrophy of the injected muscle was the principal cause of BPX-related strabismus. Scott then injected strabismus patients with BPX, with good clinical results (Scott et al 2007; Scott et al 2009b).

The picture provided by Bradley (1979) of gross sarcomere dissociation after BPX injection suggested that length of the regenerated muscle would be determined by the length at which it was held in the weeks following injection, during regeneration. Scott et al (2009a) then reported a series of patients in which BPX injection was combined with botulinum type A toxin (BTXA) injection in the antagonist muscle, to prevent the antagonist from stretching the BPX-injected muscle during the later's regeneration. Improvement in eye alignment in these cases was roughly twice that with BPX alone. BPX-injected muscle size, estimated by magnetic resonance imaging (MRI), increased, but no more than that in the earlier BPX-only cases. The increased effect of combined treatment could not be attributed to enduring effects of BTXA itself, because the BTXA dosage in most cases was below that seen to cause lasting alignment changes (Scott et al 2009a).

Herein we report refined analyses of muscle size, muscle shape, and eye alignment in 31 patients, with followups as long as 3 yrs, confirming and extending our earlier findings.

Methods

Patients

We offered BPX injection as an alternative experimental treatment to all adult patients requesting correction of their comitant horizontal strabismus. Those who understood the experimental nature of the treatment and wished to participate, gave written consent. All experimental procedures were approved by the Smith-Kettlewell Eye Research Institutional Review Board and adhered to the Declaration of Helsinki. Patient data was handled in accord with the Health Insurance Portability and Accountability Act (HIPAA) of 1996. Treatments and associated measurements were provided without cost to patients. The present report concerns 31 patients – 19 with esodeviations, average 18.9Δ (prism diopters; 10.7°), and 12 with exodeviations, average 31.9Δ (17.7°) – who received BPX injections in one horizontal muscle, and in some cases, concurrent BTXA injections in the antagonist muscle. Five additional enrolled patients were lost to followup and are not included in this report.

Decisions concerning BPX dose and concurrent injection of BTXA in the antagonist muscle were made clinically on the basis of deviation size, using larger doses for larger misalignments ($r = 0.61$, $p < 0.001$). Where alignment improved, but not as much as desired, an additional treatment was given, generally with a larger dose, sometimes by adding BTXA in the antagonist where the original treatment was with BPX only. Patients returned for alignment measurements and scans as they were able, and "6 mo", "1 yr", "2 yr", and "3 yr" followup data were collected as close as possible to those time points (see *Tables 1* and *2*).

Alignment Measurement

Eye alignment was measured using Hess screen and prism-cover tests with a viewing distance of 3 m. Alignment was estimated by prism and corneal reflex for patients without steady central fixation. Alignment was measured before injection to determine baseline, and at intervals thereafter to assess corrections and their stability. To validate our measurement technique, two independent ophthalmologists repeated a random subset of our alignment measurements, showing good agreement ($r = 0.997$, $p < 0.001$, $n = 31$).

MRI

We imaged muscles using 1.5 T magnetic resonance imaging (MRI) with standard head coils, 2 averaged series, and a T1-FSE pulse sequence (TR ~416 ms, TE ~13 ms). The field of view included both orbits, yielding in-slice-plane resolution of 0.5 x 0.5 mm. Apart from a few early cases, we collected 2 mm thick contiguous slices, extending from the orbital apex to the equator of the globe, and a few axial slices through the horizontal recti to check horizontal gaze in the scanner. Scanning was performed just prior to injections to establish baselines, within a few minutes after injections to visualize the muscle filled with injected fluid, at about 6 mo, and at roughly 1 yr intervals thereafter.

We used two measures of muscle size: volume (VOL), and maximum crossectional area (Max CSA; see *Figure 1*). *VOL* is the total volume, from the origin to the most anterior point in the orbit at which the muscle could be segmented in every MRI scan session for a given patient, typically approaching the point of tangency, where the muscle becomes too flat to distinguish from the globe. VOL therefore included roughly the posterior 2/3, almost all of the muscle with substantial thickness. *Max CSA* reflects only one region of the muscle, but a particularly important one, and has the methodologic virtue of being independent

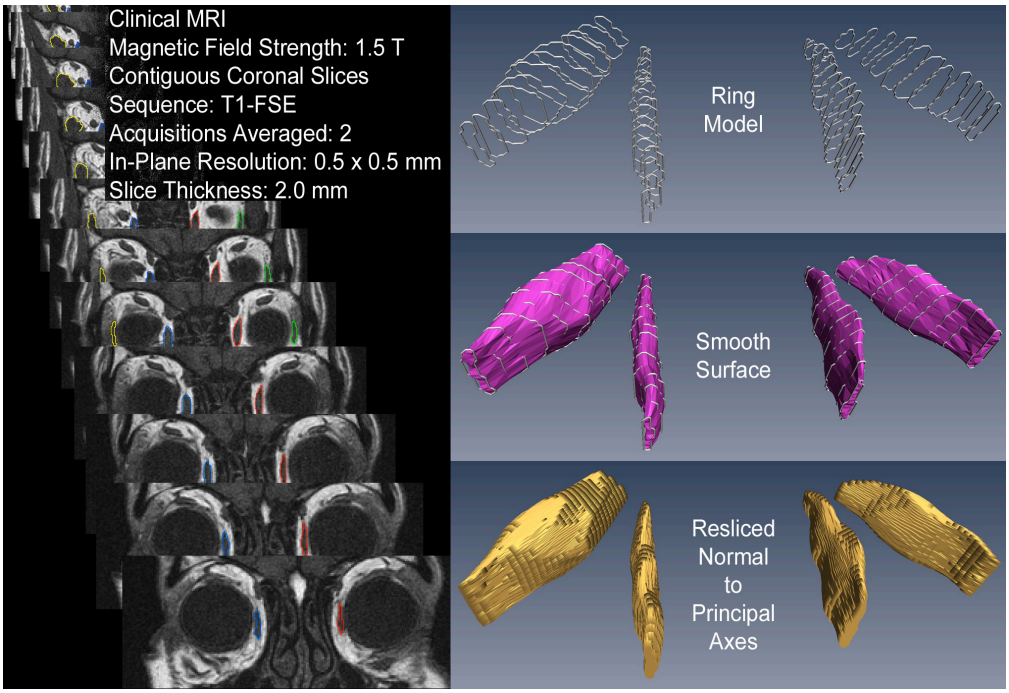

Figure 1: MRI Analysis. Coronal scans, collected as described in the text, were segmented manually with computer assistance, and the muscle contours used to build ring models of individual muscles. A smooth surface was then fitted using an automated, spline-based method, verified to approximate the ring model closely, which then was resliced normal to the principle axis of each muscle to yield accurate volume estimates, and true crossections that can be meaningfully associated with longitudinal positions at better than 2 mm resolution.

of the extent of the measurable region. We also report the distance of Max CSA from the origin as an index of muscle shape. As illustrated in *Figure 1*, by fitting a 3-dimensional surface to MRI slice data (a kind of interpolation and smoothing) and then reslicing it perpendicular to the muscle's long axis, we were able to compare true crossections regardless of the muscle's orientation relative to the scan plane, and resolve longitudinal positions with resolution better than the slice separation in the raw data.

We achieved good gaze stability during scans by stabilizing the head with pads, and instructing patients to lie still with eyes closed gently. Gaze instability caused obvious motion artifacts in MR images, and affected scans were reacquired. The various stages of muscle paresis, destruction, and regeneration caused eye position in the scanner to vary across sessions. This should minimally affect VOL, which is essentially the entire muscle volume, by rotating a few mm of the flat insertional end of the muscle into or out of the region of interest. Analysis of uninjected antagonists showed that VOL was insensitive to these variations in eye position. Although it is well-known that Max CSA increases and moves posteriorly with extraocular muscle contraction, (Miller 1989) gaze was relaxed in all scans, so changes in Max CSA in our data were not related to contraction. Finally, our MRI indices gradually returned to pre-treatment values in later followups, when eye position continued to improve (eg, *Figure 2*) or was stable (*Figure 3*), showing that factors other than eye position drove our muscle size measures. MRI data were received as DICOM files, and logged into an OsiriX® (Pixmeo, Geneva, Switzerland) Picture Archiving and Communication System (PACS; osirix-viewer.com), running on a Mac Pro® computer (Apple, Inc, Cupertino, CA).

Segmentation of muscles from surrounding tissues involved judgments of muscle margins, and the locations of muscle origins. Scan readers were taught orbital anatomy, and trained to criterion. Scans were segmented and quantified using Amira® software (Visualization Sciences Group, Burlington MA) on large-screen iMac® computers (Apple, Inc). Readers drew muscle contours, reconstructed 3-dimensional muscle surfaces, and calculated muscle CSAs corrected for orientation, and muscle volumes (*Figure 1*). Scan images were processed in random order to minimize systematic errors related to knowledge of the patient, injected muscle, or stage of treatment. At least two readers independently made all MRI measurements, and data reported are averages. Discrepancies among readers were resolved by repeating all readings. In practice, most discrepancies were frank errors, which were easily identified and corrected (see *Appendix* for more on MRI analysis).

Our predictions about correction of alignment, VOL, and Max CSA were directional (positive corrections, and size increases in BPX-injected muscles), and were therefore evaluated with 1-tail statistical tests. Change in position of Max CSA was unpredicted, and so was evaluated with 2-tail tests.

EMG-Guided Injection

We injected extraocular muscles, guided by electrical activity in the target muscle, as awake patients made voluntary gaze shifts. With the needle tip inside the belly of the target muscle, we injected BPX (Leiter's Rx Compounding, San Jose, CA) in concentrations 0.75-3.0 g/dL and volumes of 1.0-4.5 mL. The volume range was narrowed to 1.5-3.0 mL after MRI showed that 3.0 mL filled muscles. There is evidence that BPX does not diffuse freely throughout an extraocular muscle (Park et al 2004), so we sought to broaden exposure by injecting most of the BPX in the posterior third and the remainder in the middle of the muscle, withdrawing the needle slowly to allow anterior spread along the needle track. We injected the antagonist muscle with BTXA in some cases, usually in low doses of 1.0-3.0 u, which caused mild paresis lasting under a month (*Table 2*).

Results

General Response Pattern

The mean response pattern of a horizontal rectus muscle, exemplified by Patient KD8 (*Figure 2*), had the following characteristics: [1] Before injection the muscle's Max CSA was near its center (blue graphics and text). [2] Immediately after injection, VOL was increased markedly, with a similar shape (green). [3] Several weeks after injection, VOL and Max CSA were increased moderately, and Max CSA was displaced posteriorly, compared with before injection (yellow). [4] Later, VOL and Max CSA stabilized at lower levels, approaching pre-injection baselines, with the altered shape partly retained (red).

Eye alignment generally improved as a result of BPX treatment, although the temporal relationship to muscle size was not simple. In the example shown (*Figure 2*), during the period from 53 to 253 days after injection, both measures of muscle size decreased, and yet alignment continued to improve (compare yellow to red in left panel).

Patient Characteristics, Treatments and Outcomes

At the latest examination, average 15.3 mo after the final treatment, original misalignments averaging 24.0Δ (13.5°), were reduced by 10.5Δ (6.0°) with residual deviations ≤10Δ in 53% of patients (*Table 1*). There were no significant differences in corrections of esotropia and exotropia.

Alignment corrections were notably stable over time: 12.3Δ (7.0°) with residual deviations ≤10Δ in 57% of patients at 6 mo, 9.3Δ (5.3°) with residual deviations ≤10Δ in 57% at 1 yr, 9.8Δ (5.6°) with residual deviations ≤10Δ in 90% at 2 yrs, and 14.0Δ (8.0°) with residual deviation ≤10Δ in all 5

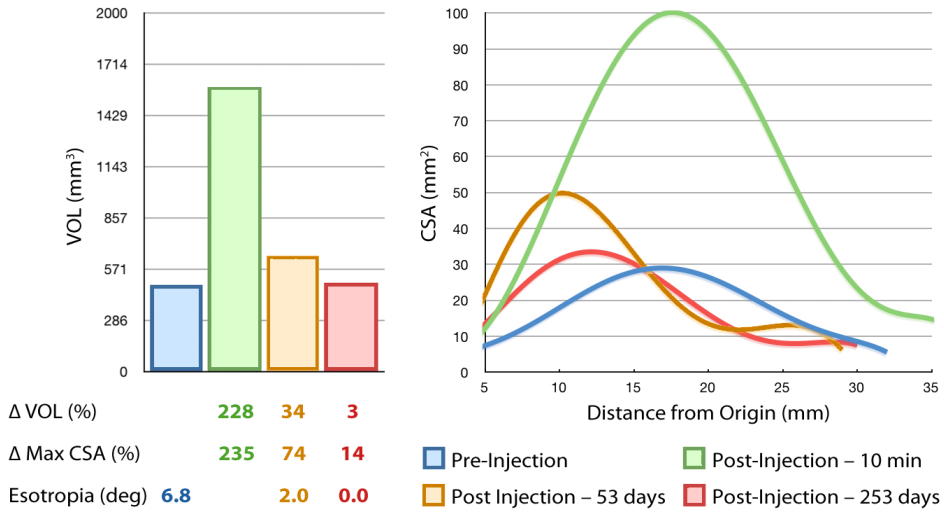

Figure 2: Muscle Size, Muscle Shape, and Eye Alignment. Patient KD8, a 58 yo woman in good health, presenting with comitant esotropia, exhibited all the features that otherwise emerged only in means (see Table 2). This patient received a single 3 mL injection of 2.0% BPX in the lateral rectus muscle as treatment for esotropia of 12.0Δ (6.8°). It can be seen from MRI data 10 min after injection that the muscle was well-filled (compare green to blue). Almost 2 months later, when muscle rebuilding was likely complete, the injected muscle was substantially larger than before injection (orange – blue). As shown in the plot on the right, the post-injection muscle developed an altered shape, with the point of maximum crossection moved posteriorly (compare red and orange with blue). Eye alignment (esotropia) improved throughout the followup period, finally achieving orthophoria, while muscle size relaxed towards normal (red – blue). ΔVOL and ΔMaxCSA are changes relative to pre-injection (blue).

depressed by inclusion of several patients whose injections did not fill the muscle, as determined by MRI, and who showed little effect of treatment. These patients had second injections, usually with BTXA in the antagonist, removing them from the BPX-only subgroup, and causing an apparent increase in mean correction with time. From the BPX-only data, we can make a rough estimate of the dose-response relationship as 0.1Δ correction/mg BPX (0.06°/mg; r = 0.4, n = 15).

Two patients presenting with small deviations were over-corrected, complained of diplopia. Six months after injection the injected muscle was tenotomized to achieve exact alignment. We have since amended our injection protocol to reduce BPX dosage for small deviations.

patients followed 3 yrs after their final injection (*Table 1*). The apparent improvements with time, here and below, were caused by patients returning for 2 and 3 year followups having better than average outcomes. Considered individually these patient's corrections were roughly constant over the entire followup period. Otherwise, patients who returned after the 6 mo followup had outcomes similar to those who did not: patients returning for at least one followup after 6 mo had corrections of 12.6Δ (7.2°, n = 23) at 6 mo, whereas those not returning had corrections of 9.1Δ (6.2°, n = 7; p = 0.8).

Patients with smaller initial deviations, mean 14.0Δ (8.0°), who received BPX only had corrections of 5.2Δ (3.0°) with residual deviations ≤10Δ in 60% at the latest examination, an average 16.7 mo after their final treatment. Corrections over time were: 6.3Δ (3.6°) with residual deviations ≤10Δ in 67% at 6 mo, 7.7Δ (4.4°) with residual deviations ≤10Δ in 62% at 1 yr, 8.6Δ (4.9°) with residual deviations ≤10Δ in all 6 at 2 yrs, and 12.5Δ (7.1°) with residual deviations ≤10Δ in all 3 at 3 yrs (*Table 1*). Mean corrections in this subgroup are

Treatment:	All					BPX-Only				
Examination:	Latest	"6 mo"	"1 yr"	"2 yr"	"3 yr"	Latest	"6 mo"	"1 yr"	"2 yr"	"3 yr"
Initial Misalignment	24.0Δ 13.5°	23.3Δ 13.1°	21.1Δ 11.9°	15.5Δ 8.8°	16.9Δ 9.6°	14.0Δ 8.0°	7.7Δ 4.4°	13.9Δ 7.9°	13.5Δ 7.7°	16.2Δ 9.2°
Time After Final Treatment	460 d	169 d	364 d	690 d	1041 d	502 d	147 d	361 d	711 d	1056 d
Absolute Correction	10.5Δ 6.0°	12.3Δ 7.0°	9.3Δ 5.3°	9.8Δ 5.6°	14.0Δ 8.0°	5.2Δ 3.0°	6.3Δ 3.6°	7.7Δ 4.4°	8.6Δ 4.9°	12.5Δ 7.1°
Relative Correction	50%	58%	56%	64%	85%	40%	48%	62%	65%	81%
T-Test (p)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.003	< 0.001	0.001	0.001	0.03
Residual Deviation ≤ 10Δ	53%	57%	57%	90%	100%	60%	67%	62%	100%	100%
Number of Patients	30*	30*	14	10	5	15	15	8	6	3

Table 1: Alignment After Final Treatment. Patients received 1 or 2 treatments. BPX-Only patients received no BTXA; others received BTXA injections in the antagonist muscle. Means of the latest examination data for each patient are shown, along with nominal 6 mo, 1 yr, 2 yr, and 3 yr followups. Initial misalignment is the presenting primary position deviation, measured as described in the text. Time after final treatment is the actual mean time in days of each followup exam. Absolute correction is the change in primary position alignment from initial misalignment in prism diopters (Δ) and degrees (°). Relative correction is the fraction of full correction of the initial misalignment. T-test (p) refers to a 1-tail test. Residual deviation ≤ 10Δ is the fraction of patients having successful outcomes by this conventional criterion. Number of patients is the number in the specified subgroup for whom followup data was available.

** One patient, over-corrected with a second treatment, is excluded to avoid inflating means; this patient subsequently chose to have surgery.*

Single Treatment and Two Treatments

A single treatment reduced binocular misalignment at the final examination by 7.9Δ (4.5°, $p < 0.001$) with residual deviation ≤10Δ in 30% of patients. Two treatments reduced binocular misalignment by 10.5Δ (6.0°, $p < 0.001$) with residual deviation ≤10Δ in 73% of patients. Correction with 2 treatments tended to be greater than with 1, but the difference was not statistically significant ($p = 0.1$). Alignment trends over time are shown in *Figure 3*.

A single treatment with BPX alone, at 11.3 mo, reduced original misalignments averaging 14.0Δ (8.0°) by 4.7Δ (2.7°) with residual deviation ≤10Δ in 50% of patients

Muscle Size

Six months after BPX injection, VOL of BPX-injected muscles increased by 6.6% ($p = 0.007$), and Max CSA increased by 8.5% ($p = 0.01$) and moved posteriorly by 2.0 mm ($p = 0.001$). Subsequently, VOL and Max CSA gradually returned to near pre-treatment values. These results are summarized in *Figure 3*.

Patient characteristics, treatments, and outcomes are detailed in *Table 2*.

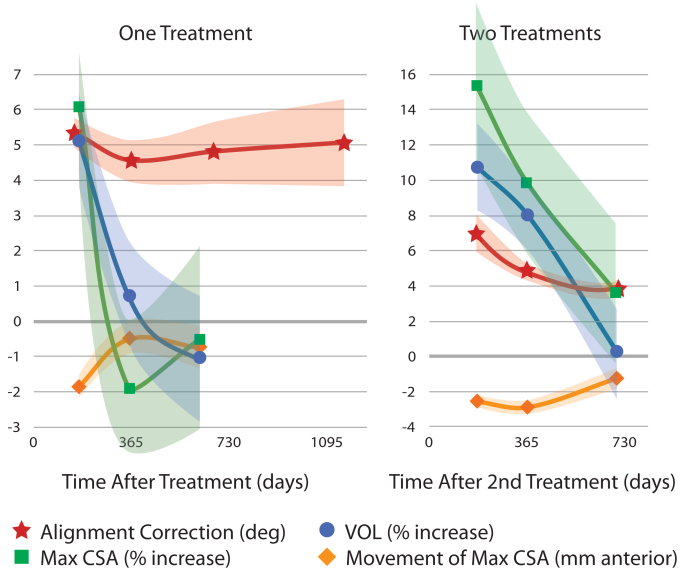

Figure 3: Alignment and MRI Trends. Alignment correction, VOL increase, Max CSA increase, and longitudinal movement of Max CSA are shown at times up to ~3 years after a single treatment (left panel), and after two treatments (right panel). The symbols connected by lines are means across all patients for whom data was available (see Methods). The vertical dimension of the light-colored bands is Standard Error of the Mean. For both 1 and 2 treatments, alignment correction was stable over time, while measures of muscle size and shape spiked initially and then relaxed back towards pre-treatment values.

Clinical Notes

Injection pain was minimized with topical proparacaine. With BPX doses above 60 mg, inflammation and edema related to myofiber destruction, comparable to to that following strabismus surgery, was significant for 1-2 days in a few patients, and then quickly resolved. Oral prednisone, 40 mg at time of treatment and 30 mg on each of the next 2 days, was effective in controlling it.

Paralysis caused by the anesthetic effect of BPX increased the initial strabismic deviation for a day. Myofibrillar destruction then resulted in a small increase in the initial misalignment, until regeneration began to straighten the eye around day 6-10. Thereafter, alignment progressively improved. Patients found this progression much less discomforting than the prolonged paralysis following injections of BTXA alone.

Twenty-four patients were contacted by mail to rate their experience and 14 responded. All were comfortable with the MRI scans, but 4 found the injections to be uncomfortable. Ten of 13 patients felt they had been helped by the procedure, and 13 of 14 were satisfied with their participation in the project overall. No vision loss or eye perforation occurred in any of the 58 patients we have treated with BPX.

Discussion

Strabismus Correction

Our results support previous findings that treatment with BPX alone results in clinically significant improvement in eye alignment (Scott et al 2007; Scott et al 2009b), and that larger deviations can be corrected by adding small doses of BTXA in the antagonist, allowing the BPX-injected muscle to rebuild at reduced length (Scott et al 2009a). Stability of alignment changes has now been demonstrated for up to 3 years.

Patients enjoying a satisfactory result (residual deviation ≤10Δ) with a single treatment had an initial misalignment of 14.8Δ (8.4°, n = 9), whereas those needing 2 treatments had initial misalignment of 17.1Δ (9.7°, n = 8). Thus, there was only a weak relationship between strabismus magnitude and number of treatments required ($p = 0.4$).

Reducing the amount of BTXA in the antagonist reduced the absolute (deg) correction, but did not significantly reduce the relative (percent) correction at any time point, because patients treated with BPX-BTXA had larger initial misalignments than those treated with BPX only. Although it is possible that larger BTXA doses may themselves have prolonged effects on alignment, our impression is that BTXA injection principally allows the BPX-injected muscle to rebuild shorter than it otherwise would, particularly where the antagonist is unusually short or strong.

It is reasonable to expect that after BPX-induced myofibril destruction, a muscle would rebuild at the length at which it was held, which would be longer than before treatment if the antagonist muscle were active, and that this effect could be harnessed to therapeutically lengthen muscles. However,

Patient		BPX			BTXA (u)	Distance Deviation		"6 Month" Followup				"1 Year" Followup				"2 Year" Followup				"3 Year" Followup	
								PP Correction		BPX-Injected Muscle Size Increase (%)		PP Correction		BPX-Injected Muscle Size Increase (%)		PP Correction		BPX-Injected Muscle Size Increase (%)		PP Correction	
ID	Age (y)	Target	Conc (g/dL)	Vol (mL)		Type	Mag (deg)	Relative (%)	Abs (deg)	VOL	Max CSA	Relative (%)	Abs (deg)	VOL	Max CSA	Relative (%)	Abs (deg)	VOL	Max CSA	Relative (%)	Abs (deg)
RC1	72.3*	RLR	0.75	4.5	0	EsoT	8.5	66	5.7	-4.2	-5.0	73	6.2	-3.0	-3	73	6.2			63	5.4
DE2	52.7*	RLR	3	1	0	EsoT	5.1	84	4.3			100	5.1	-5.8	-3	101	5.2	5	10	105	5.4
CH3	41.8*	RLR	3	1	0	EsoT	14.0	39	5.5	22.9	26.9										
	42.2*		1.5	3	0			65	9.2			58	8.1			59	8.3	7	10	76	10.6
GW 4	70.9*	LLR	0.75	1.5	0	EsoT	9.1	6	0.5			-4	-0.4			-12	-1.1	-3	-2		
	72.6		0.75	4.5	1.5			34	3.1	-8.6	-8.3	20	1.8	0.2	-2	22	2				
OO5	38.7*	LLR	0.75	1	0	EsoT	5.7	40	2.3												
	39.1*		1.5	3	0			114	6.5	0.0	-5.6	110	6.3	-4.1	-8	50	2.8	-11	-12		
RH6	60.4	RLR	1.5	3	0	EsoT	8.0	28	2.3	3.3	0.8										
KR7	33.9*	RLR	1.5	3	0	EsoP	8.0	0	0.0												
	35.3		3	4	1.5			73	3.5	26.1	41.7	47	3.7	21.1	35			10	23		
SH1	52.7	LMR	3	3	3	eXoT	9.1	100	9.1	-4.4	-6.0			-9.4	-13	78	7.1	-13	-14	81	7.4
JJ2	74.4	LMR	0.75	4	2	eXoT	11.3	87	9.9			87	9.9	4.9	0	100	11.3	0	4	100	11.3
DM3	71.3	RMR	0.75	4	5	eXoT	21.8	53	11.8	-7.1	-2.8										
ED4	77.2	RLR	0.75	4.5	2.75	EsoT	16.7	0	0.0			1	0.1			-12	-2			-23	-3.9
	80.2		2	3	1.5			-16	-2.6												
JS5	48.1	RMR	0.75	4.5	0	eXoT	6.8	-25	-1.7	5.9	-0.4										
	48.7		3	2.75	0			62	4.3	5.9	-1.9	77	5.3	7.6	-1	50	3.4	-4	-7		
MA6	62.8	LLR	0.75	4	0	EsoP	5.7	60	3.4	11.8	8.1										
	63.2		0.75	3.5	0			60	3.4	10.5	-2.0	60	3.4	5.5	-0	55	3.1				
AC8	38.6	LMR	3	4	4	eXoT	26.6	27	7.3	22.0	29.3										
VB1	52.1	RLR	0.75	3	3	EsoT	14.0	35	4.9												
KL4	48.9	RMR	0.75	3	1.5	eXoT	14.0	35	4.9	-6.1	-7.0	1	0.2	2.5	3						
JP7	71.3	LLR	1.5	3	1.5	EsoT	21.8	64	14.0	15.0	27.4										
	72.0		3	3.25	4			115	25.1	18.8	42.4			18.3	34						
RH8	32.7	RLR	0.75	3	0	EsoT	8.0	33	2.6	7.0	15.7	18	1.4	2.5	7						
PM0	27.2	RMR	2.5	3	5	eXoT	21.8	0	0.0	3.0	2.3										
TM2	27.3	LMR	3	3	4.5	eXoA	40.4	21	8.5			18	7.3	20.2	16						
JP3	20.2	LLR	1.5	3	2	EsoT	12.7	72	9.1	5.1	4.1	75	9.5			55	7				
SS4	54.6	LLR	2.5	3	2	EsoT	16.7	58	9.6	25.3	17.7	37	6.2								
BC7	29.0**	RLR	1.5	3	0	EsoA	6.8														
	29.1		3	2.5	1.5			33	2.3												
KD8	58.2	RLR	2	3	0	EsoT	6.8	100	6.8	19.5	35.1										
BC0	50.9	LMR	1.5	3	5	eXoT	10.2	6	0.6	-9.6	-6.4										
CR1	58.6	RLR	2	3.1	0	EsoT	6.8	100	6.8	-11.0	-22.9			-14.0	-23						
KL3	74.2	LLR	1.5	4	1.25	EsoT	19.3	13	2.6	-1.2	10.0										
	74.5		2	3	2.5			57	11.0												
IN6	75.3	RMR	2	3	1.25	eXoT	21.8	45	9.9	6.0	-3.6			8.0	-1						
LK7	52.7	LMR	2	3	0	eXoT	11.3	25	2.8	-1.5	-2.1										
RR9	67.5	LMR	2.5	3	2.5	eXoT	16.7	105	17.6												
FA0	62.6	RLR	2	3	0	EsoT	8.5	-7	-0.6												
	62.8		2.5	3	4			100	8.5	23.0	41.5										

Table 2: Patient Characteristics, Treatments and Outcomes.

Patients are identified by codes (ID), and treatments by age at time of injection (Age); patients receiving 2 treatments, therefore, have 2 "age" lines. The muscle receiving bupivacaine (BPX) injection (Target), the concentration (Conc) and volume (Vol) injected, and the amount of botulinum type A toxin (BTXA) injected in the antagonist (0 = no injection) are shown for each treatment. Targeted muscles were left lateral rectus (LLR), left medial rectus (LMR), right lateral rectus (RLR), and right medial rectus (RMR). The presenting primary-position deviation with 3 m distant target (Distance Deviation) was an esotropia (EsoT), alternating esotropia (EsoA), esophoria (EsoP), exotropia (eXoT), or alternating exotropia (eXoA), of magnitude (Mag) shown. Primary position correction (PP Correction) is the reduction of pre-treatment strabismic deviation, while looking straight ahead with the good eye, shown as a fraction of full orthophoric correction (Relative), and as a number of degrees (Abs). Two measures are given for BPX-Injected Muscle Size Increase, total volume (VOL) and maximum crossectional area (Max CSA).

Mean "6 Month" Followup time was 169 days for alignment data and 172 days for magnetic resonance imaging (MRI) data; "1 Year" Followup was at 364 and 358 days, resp; "2 Year" Followup was at 690 and 656 days, resp; "3 Year" Followup was at 1041 days.

*Empty cells are unavailable data (see Methods). * = previously reported 24, 25. ** = first BPX injection was without effect and another treatment was given 3.0 months thereafter.*

the alignment corrections enjoyed by our BPX-only patients show that this does not generally occur. Furthermore, in 3 patients not included in this study we injected a compound of BPX and BTXA in the same muscle with the aim of inducing paresis during rebuilding so the muscle would rebuild at increased length, but were unsuccessful. Perhaps the elevated stiffness of the rebuilt muscle, related to its higher proportion of connective tissue (Rosenblatt & Woods 1992), resists elongation by the antagonist.

We recently amended our protocol to combine BPX with epinephrine 1/100,000 for patients with deviations over 20 Δ (11.3°) with the aim of reducing vascular flow and increasing the duration of exposure of muscle tissue to BPX. This appears to increase the effect on alignment by roughly 50%. We intend to investigate further if adding epinephrine can reduce the BPX dose needed to correct smaller deviations. Results of BPX-epinephrine treatment will be given in a subsequent report.

BPX injection is a useful treatment for non-paralytic strabismus where surgery is contraindicated. Ongoing refinements are expected to increase its effectiveness and broaden its applications.

Biomechanical Mechanisms

Simulation using Orbit™ 1.8 (Miller 1999; Miller et al 1999; Eidactics, San Francisco, eidactics.com/Eidactics-branch/Products/Orbit1.8) shows that even our largest mean increase in muscle size (15.4% in Max CSA at 6 mo after 2 treatments), whether attributed to proportionally increased elastic and contractile muscle forces, or mostly to increased elastic tissue stiffness as Rosenblatt's (1992) findings suggest, cannot directly account for more than about 1.7Δ (1°) change in alignment, whereas our patients enjoyed much larger corrections. Other processes must therefore be involved. A striking aspect of our results is the stability of improved alignment in the period from 6 mo to 3 yrs after injection, while measures of muscle size (VOL and Max CSA) relaxed towards pre-injection values (see *Figure 3*). Similar results were seen in an earlier clinical series (Scott et al 2009a). We hypothesize that following BPX injection, extraocular muscles rebuild somewhat stronger and stiffer, causing a change in eye position that shortens the path of the injected muscle and lengthens the path of its antagonist. Muscles respond to chronic strain by removing or adding serial sarcomeres to optimize cross-bridge overlap (Tabary et al 1972; Williams & Goldspink 1973, 1978; Scott 1994), and accordingly, the BPX-treated muscle gradually shortens by removing serial sarcomeres, and its antagonist lengthens. Modest changes in agonist and antagonist lengths (unlike comparable relative changes in force) would result in significant changes in eye alignment; eg, length changes of just 1 mm in a muscle pair would cause ~9Δ (5.1°) change in eye position. Then, because muscle stretch regulates growth and repair (Zádor et al 1999; Christiansen & Mcloon 2006), once the antagonist of the BPX-injected muscle had lengthened to suit its new path, the load it presented would be reduced, and the agonist would down-regulate its hypertrophic growth, consistent with our data. That is, we propose that corrected eye position, initially achieved by a stronger and stiffer agonist muscle, is maintained by a shortened agonist and lengthened antagonist, which through stretch-regulated growth tends to restore normal muscle sizes, thereby mitigating initially high horizontal muscle forces and increasing mechanical stability of the orbit.

BPX injection seems to also increase the intrinsic stiffness of extraocular muscles, perhaps by adding fibrous tissue as might be expected to result from a cycle of myofiber damage and regeneration, and as suggested by Rosenblatt's (1992) findings. We directly measured increased stiffness in one of our patients (RH6) (Scott et al 2009a), and have the impression that greater stiffening is associated with larger BPX doses.

Extraocular muscle consists of several muscle fiber types that might be affected differently by BPX such that certain forces increase more than proportionally to overall size. The increase in Type I fibers found in regenerated muscle after BPX exposure (Rosenblatt 1992), for instance, raises the possibility that an oxidative fiber type found in extraocular muscle, perhaps the orbital singly-innervated fibers thought to determine primary position alignment (Porter et al 1995) or extraocular muscle pulley position (Demer et al 2000; Miller 2007), undergoes preferential hypertrophy. The change in muscle shape we documented as posterior movement of the point of Max CSA might be due to differential hypertrophy of orbital layer fibers, which are found in proximal and middle regions of extraocular muscles (Porter et al 1995; Oh et al 2001). If so, the fiber-specific effects of BPX at our higher concentrations are different from those Porter et al (1988) found at low (0.75%) concentrations.

Generality and Variability

The present pilot study was limited to patients with comitant, non-paralytic strabismus. Preliminary studies suggested that BPX injection does not work well in chronically paretic muscles, perhaps because atrophy decimates the population of satellite cells needed for rebuilding, or critically reduces the amount of muscle tissue available for BPX-induced destruction, and so, the signaling factors needed to activate satellite cells. Where innervation is at least partly intact, pharmacologic treatment for atrophic muscles would be particularly valuable, although merely increasing the stiffness of a denervated muscle would be clinically useful. Closer examination of BPX in atrophic muscles is clearly warranted. Otherwise, BPX-injection treatment seems generally effective.

The present study lacked a comparative base; a prospective randomized comparative trial of BPX vs strabismus surgery

is being developed for horizontal comitant strabismus of 10-30Δ.

It is apparent from *Table 2* that our findings concerning eye alignment, VOL, and Max CSA emerge against high inter-patient variability consequent to the diverse medical histories of enrolled patients and the evolving treatment parameters in this observational study. Some injections did not fill the target muscle (as assessed during treatment or from the immediately subsequent MRI scans), sometimes giving inferior results, but we included these cases with all others enrolled. Variability of our patients and their treatments certainly limits the conclusions we can draw (eg, we do not have sufficient data to say what subtypes of strabismus are most effectively treated), but there is an upside: greater variability means that where statistically significant differences are found they are associated with larger absolute effects and are generalizable to broader patient and treatment populations. That is, our statistically significant findings are more likely than those of a strictly-controlled, homogeneous-group study (one with high "statistical power") to have broad, real-world significance.

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Appendix – Extraocular Muscle Scan Analysis

Orbital image analysis techniques used in the present study are significantly improved over those we originally introduced (Miller 1989), versions of which remain in general use. The old techniques do not give accurate volumes or directly yield true muscle crossections, and do not adequately address errors and biases that can affect MRI quantification.

Determining size in tomographic images begins by segmenting the structure or object of interest, that is, delineating its outline. High contrast between object and surround and small pixel size aid accurate segmentation. For extraocular muscles, high contrast is achieved by viewing against contrasting orbital fat (eg, in quasi-coronal sections for the rectus muscles), rather than against poorly contrasting globe and optic nerve (eg, as in Lee et al 2007). Tightly framing the region of interest (eg, one or both eyes) in the MRI scanner's "field of view" (FOV) minimizes pixel size for given scanner settings. Volume averaging, a consequence of encoding as pixel density the average signal from an elemental volume of tissue (a voxel), can blur and displace object borders. Thinner slices have less volume averaging, but also weaker signals. With elongated structures, such as extraocular muscles, volume averaging artifacts can easily be minimized by orienting the scan plane perpendicular to the long axis. Nevertheless, one still sees extraocular muscle studies using longitudinal sections, under the erroneous assumption that they provide direct and complete pictures (eg, Lee et al 2007), though because of volume averaging apparent muscle sizes are strongly affected by slice thickness and position, and unless the slice precisely bisects the muscle along its entire path, muscle shape is also distorted.

Quasi-crossectional scans of extraocular muscles are therefore essential to view muscles against contrasting fat and minimize distortions due to volume averaging (Demer & Miller 1999). But individual coronal scans are still problematic. In comparisons of scan planes fixed relative to an orbital reference, a muscle may appear to have hypertrophied, eg, simply because a larger crossection moved into the chosen plane. One might instead compare maximum crossections in single coronal scans, but then orbital positions of the compared slices will be different, creating a confusing or contentious presentation. Finally, coronal scans approximate crossections for the medial rectus, but not for the lateral rectus, so projection corrections must be calculated. In summary, unless the effect one wishes to measure is large enough to survive volume averaging artifacts, projection distortions, and slice selection biases, the preferred method for quantifying elongated structures such as extraocular muscles is 3D reconstruction based on multiple quasi-crossectional slices.

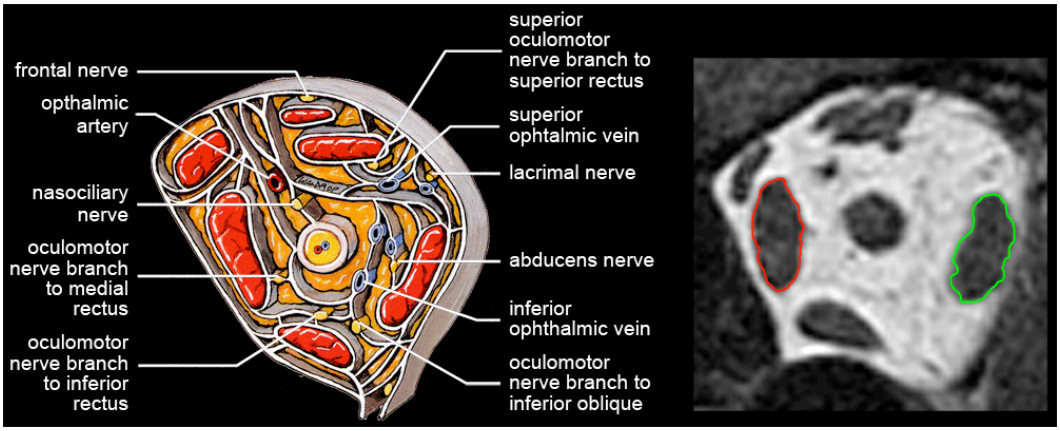

Figure A1: MRI Segmentation. Nerves, blood vessels, and associated fascia appear in MRIs similar to bits of muscle, but can be excluded based on known anatomy and known smooth shapes of muscle crossections. With this guidance, scan readers aimed to draw muscle contours so as to bisect luminance gradients. Scans were read without knowledge of which muscles had beee injected, or or when in the injection sequence the scan was collected. Results from multiple independent readers were averaged to reduce judgment bias. [Artist's drawing modified from Dutton et al (1994)].

Even with modern clinical scanners and quasi-crossectional slice orientation, muscle contours must be delineated manually, and are therefore subject to errors of judgment. It is essential to obtain multiple independent judgments of object boundaries. In the present study, multiple readers were taught orbital anatomy, and trained to criterion (*Figure A1*).

Nerves, blood vessels, and dense connective tissue bands can distort the apparent outline of a muscle, but can usually be excluded on the basis of known anatomy (*Figure A1*).

One of our measures of muscle size, VOL, was essentially the volume of the muscle, apart from the flat portion that approaches and wraps around the globe. Where it was not possible to accurately segment the muscle all the way to the point of tangency, we were careful to compare the same extent of muscle over time. This required reliable orbital reference points. When we introduced EOM path and size measurement (Miller 1989) we used the globe-optic nerve junction as a longitudinal position reference because it could be reliably visualized with the scanning technology of the time, although it did not yield an orbit-relative measurement. It is now possible, with generally available clinical scanners, to visualize the orbital apex sufficiently well to use muscle origins as head-fixed referents. For studies involving extraocular muscle contraction, which is known to be reflected mostly in posterior crossection increases (Miller 1989), it is also essential for measurements to extend to the origin, so this should become general practice.

In *Figure A2*, we show how we localized LR and MR muscle origins in order to align data across scan sessions.

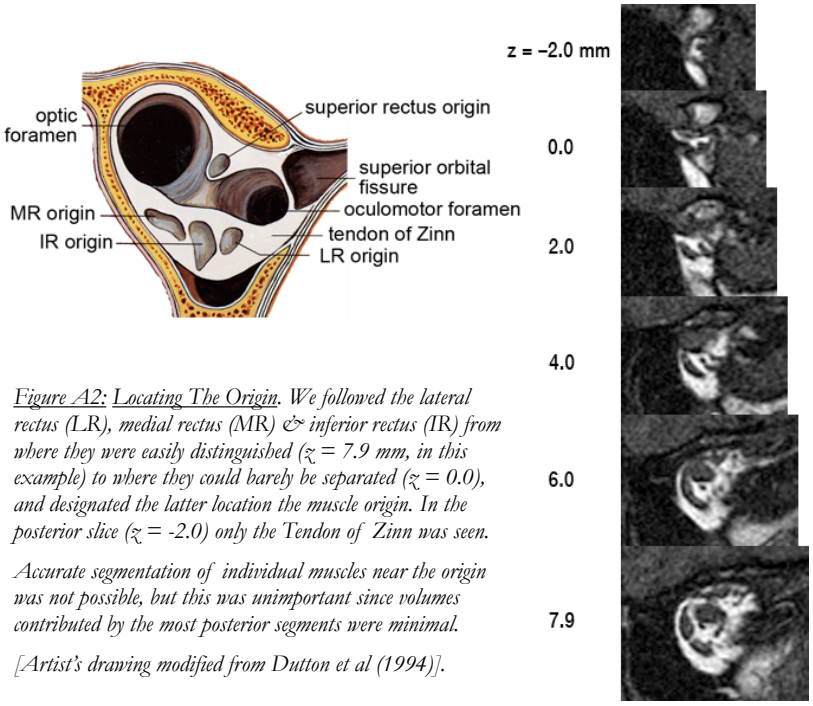

Figure A2: Locating The Origin. We followed the lateral rectus (LR), medial rectus (MR) & inferior rectus (IR) from where they were easily distinguished (z = 7.9 mm, in this example) to where they could barely be separated (z = 0.0), and designated the latter location the muscle origin. In the posterior slice (z = -2.0) only the Tendon of Zinn was seen.

Accurate segmentation of individual muscles near the origin was not possible, but this was unimportant since volumes contributed by the most posterior segments were minimal.

[Artist's drawing modified from Dutton et al (1994)].

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