Surgical Intervention and Accommodative Responses, II: Forward Ciliary Body Accommodative Movement Is Facilitated by Zonular Attachments to the Lens Capsule

Rainer Wasilewski,[1] *Jared P. McDonald,*[2] *Gregg Heatley,*[2] *Elke Lütjen-Drecoll,*[1] *Paul L. Kaufman,*[2,3] *and Mary Ann Croft*[2]

Purpose. To determine the role of the lens and the lens capsule in the three-dimensional architecture of the ciliary muscle at rest and during accommodation, in live rhesus monkeys and in histologic sections, by removing the entire lens, or only the lens nucleus and cortex, while leaving the posterior capsule in place.

Methods. In 15 rhesus monkey eyes, aged 6 to 27 years, accommodation was induced by central stimulation of the Edinger-Westphal nucleus before and after intra- or extracapsular lens extraction (ICLE, ECLE). Forward ciliary body movement and ciliary body width were measured by ultrasound biomicroscopy (UBM, 50 MHz). The monkeys were then killed, the eyes were examined morphologically in 1-μm sections, and the shape of the ciliary muscle was compared with that obtained from UBM images.

Results. The shape of the ciliary muscle in eyes undergoing ECLE ($n = 5$) did not differ from that in control eyes. In contrast, after ICLE ($n = 10$), accommodative forward ciliary body movement ($P < 0.01$) and thickness were decreased ($P < 0.001$), length was increased ($P = 0.058$), and the inner apex was located more posteriorly than in control eyes ($P < 0.005$). Histologic and in vivo data were similar and showed that the ciliary muscle maintained its triangular shape only if the lens capsule (with or without the lens substance) was present.

Conclusions. The posterior lens capsule and anterior zonular attachments facilitate forward accommodative ciliary body movement. Lens substance extraction procedures that leave the posterior capsule intact, similar to those used clinically, do not affect the capsule/zonular/muscular system movements, an important finding for accommodating intraocular lens development. (*Invest Ophthalmol Vis Sci.* 2008;49:5495–5502) DOI:10.1167/iovs.08-1917

From the [1]Department of Anatomy, University of Erlangen-Nürnberg, Erlangen, Germany; the [2]Department of Ophthalmology and Visual Sciences and the [3]Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, Wisconsin.

Supported in part by National Eye Institute Grants R01 EY10213 (PLK), the Ocular Physiology Research and Education Foundation; DFG Grant DR 124/7 (ELD); Base Grant 5P51 RR 000167 to the Wisconsin National Primate Research Center, University of Wisconsin-Madison; and Core Grant for Vision Research P30 EY016665.

Submitted for publication February 21, 2008; revised April 25, 2008; accepted September 25, 2008.

Disclosure: **R. Wasilewski**, None; **J.P. McDonald**, None; **G. Heatley**, None; **E. Lütjen-Drecoll**, None; **P.L. Kaufman**, None; **M.A. Croft**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Mary Ann Croft, Department of Ophthalmology and Visual Sciences, University of Wisconsin Clinical Sciences Center, 600 Highland Avenue, Madison, WI 53792-3220; macroft@wisc.edu.

According to Helmholtz,[1] accommodation is induced by the ciliary muscle's moving forward and inward, releasing tension on the zonula and allowing the lens equator to move away from the sclera.[1] Rohen[2] showed that the zonula consists of two major systems. The posterior zonula covers the pars plana epithelium and runs straight forward from the region of the ora serrata toward the ciliary valleys, where it is attached and intermingles with the anterior zonula in the area of the zonular plexus. From there, the anterior zonula, comprising the posterior and anterior zonular tines, courses directly to the posterior and anterior aspects of the equatorial lens capsule.[2] Rohen theorized that during accommodative anterior–inward movement of the ciliary muscle, and thereby of the zonular plexus, the tension is mainly taken over by the posterior zonula, while the anterior zonula relaxes.[2] Because of the elasticity of the lens capsule, the lens can achieve a rounded shape. The lens equatorial diameter decreases, and the lens anterior–posterior thickness increases.[1,3–6]

To study human accommodation and presbyopia, the best animal model has proven to be the rhesus monkey. Its accommodative apparatus and mechanism are very similar to that of the human,[7–9] whereas subprimate species either do not accommodate or accommodate by mechanisms very different from that of humans. Also, rhesus accommodation declines on a relative time scale essentially identical to that of humans.[10] Surgical procedures that facilitate the study of accommodation and presbyopia (such as total iridectomy and lens extraction) can readily be performed on rhesus monkeys. Several high-resolution contact imaging techniques, superior in resolution and magnification to the magnetic resonance imaging (MRI) techniques reported elsewhere,[11,12] can be performed during accommodation in anesthetized monkeys but are not possible in humans.[4,13–16] High-resolution imaging of the dynamic accommodative movements in the normal primate eye and after surgical intervention to perturb the accommodative apparatus is needed to determine normal accommodative function and its change with age.

We have reported studies of centripetal ciliary process movement by using goniovideography methods,[17] but both centripetal and *forward* movements of the ciliary muscle are necessary for accommodation. Thus, we investigated the forward ciliary muscle/body movements in the same animals by using ultrasound biomicroscopic (UBM) imaging and determined the role of the lens capsule and lens substance in ciliary body configuration and forward movement during accommodation through surgical intervention in prepresbyopic and presbyopic rhesus monkeys. Since the resolution of UBM images have limitations, the morphology and shape of the muscle, encompassing its entire circumference, were also investigated histologically in the same monkey eyes.

Materials and Methods

The in vivo data were collected during UBM (50 MHz) imaging, using the same equipment, animals, animal handling procedures, stimulus settings, and experimental time window reported in Croft et al.[17]

Table 1. Division of Study Animal Eyes According to Age and Surgical Procedure

	ICLE	(ME*)	ECLE	(ME*)
Young eyes (n = 7; 6–13 y)	5†	(1)	2	(1)
Older eyes (n = 8; 17–27 y)	5	(3)	3	(1)
Total (n = 15)	10		5	

* ME (morphologic examination), number of monkey eyes, within each surgical procedure group, undergoing measurements of ciliary muscle width and apical position by morphologic examination.

† The presence (n = 2) or absence (n = 3) of Wieger's ligament[17] did not affect forward ciliary body movement or ciliary body width; thus, these data were not separated according to whether Wieger's ligament was intact[17] and remained grouped together.

Monkeys

Fourteen rhesus monkeys (*Macaca mulatta*) of either sex, ages 6 to 27 years and weighing 5.7 to 14.8 kg, with normal eyes were studied. The monkeys were bilaterally iridectomized, and a bipolar electrode was placed in the Edinger-Westphal nucleus to stimulate accommodation.[13,18] Baseline measurements of the normal accommodative response in both eyes of each animal were taken before the lens surgical procedures were performed, thus providing ipsilateral and contralateral control eye data for each eye.

Based on the time course of presbyopia in rhesus monkeys and humans, the animals were grouped according to age as follows: young, 6 to 13 years, and older, 17 to 26 years (respectively equivalent to humans of ages ~15–30 years and 45–65 years). Additional subgroups were identified according to the surgical procedures used (Table 1).

Surgical Procedures

Intracapsular lens extraction (ICLE: removal of the lens nucleus, cortex, and capsule) and extracapsular lens extraction (ECLE: ~4 mm anterior capsulorrhexis, removal of the lens nucleus and cortex) surgery procedures were performed as described in the companion article.[17] The surgery procedures were assigned to the 15 eyes of the 14 study monkeys as follows

Seven monkeys (three young; four older) each provided one eye for surgical intervention (ICLE, n = 5; ECLE, n = 2); the opposite eye was iridectomized but otherwise was surgically untouched and served as a contralateral control eye for morphologic examination. Two of these monkeys (older ICLE, n = 1; older ECLE, n = 1) did not undergo UBM imaging, but the eyes did undergo measurements of the ciliary muscle during morphologic examination.

One additional young monkey also received unilateral ICLE (Wieger's ligament remained intact) but was not euthanatized and was retained for another study.

Another older monkey contributed one eye to the ICLE group and the opposite eye to the ECLE group. Surgery in this monkey's second eye was allowed by the veterinary staff of our institution and the Institutional Animal Care and Use Committee, since the surgery was performed at a separate time point from the procedure in the first eye, and cognitive behavior was observed by laboratory personnel and veterinary staff, to ensure that the animal was functioning normally before and after surgery. If signs of visual or other distress had been observed, the animal would have been euthanatized. However, no overt signs of distress were noted in any animal.

In the five other monkeys (three young; two older), surgical procedures were performed in one eye (ICLE, n = 3; ECLE, n = 2). However, the postsurgical clinical examination of these five eyes uncovered surgical or technical complications: ciliary body degeneration (n = 2, ICLE); severing of the posterior zonular attachments (n = 1, ICLE); and perforation of the posterior capsule; capsular fibrosis, and lens cell regrowth with pronounced presence of pearls and Soemmering's ring (n = 2, ECLE). These complications probably would have affected the accommodative apparatus in ways not intended by the surgery, which was designed to disrupt a specific part of the accommodative apparatus. Therefore, the decision was made, before postsurgical imaging, not to include postsurgical imaging data for these five eyes in the study. Subsequently, in each of these five monkeys the contralateral eye also underwent surgery (ICLE, n = 3; ECLE, n = 2). These eyes were free of postsurgical complications, based on clinical examination, and the postsurgical imaging was completed according to protocol. Again, cognitive behavior was observed for overt signs of distress after surgery in the second eye. However, the animals' function in their cage environment appeared normal. For the older animals, the loss in accommodative ability (through either ECLE or ICLE) is not really a change since most, if not all, of their ability to accommodate has already been lost. For the younger animals, this meant an adjustment to the presbyopic condition at an earlier age. In either situation (young or older animals), the visual space is restricted when looking around the room. The strategy of using the second eye in these monkeys under the careful constraints indicated was used to avoid major intracranial surgery in additional monkeys. All the eyes underwent morphologic examination with the exception of the monkey that was retained for further study and not euthanized.

In total, 15 eyes of 14 monkeys were included: for ICLE, 10 monkey eyes; 5 young, 5 older; and for ECLE, 5 monkey eyes; 2 young, 3 older.

Accommodation Stimulation and Response Measurements

Refractometry. Accommodation was induced by midbrain electrical stimulation and measured by Hartinger coincidence refractometry as described elsewhere.[13] Stimulus settings were chosen that induced maximum accommodative responses (i.e., maximum centripetal ciliary process and lens/capsule movement, maximum forward ciliary body movement) and maximum accommodation, allowing comparisons to be made between pre- and postsurgical accommodative responses.

Ultrasound Biomicroscopy (UBM). Dynamic UBM images were obtained during stimulation of accommodation and then recorded to videotape.[13] Pre- and postsurgical UBM imaging was performed in the same region of the eye temporally, encompassing ~25% of the entire circumference. The temporal region was selected, because this region provides the best and most distinct ultrasound imagery, with clear and distinct edges of the ciliary body and cornea. The postsurgery imaging sessions were performed 2.5 weeks to 3 months after surgery. Twelve of the animals were imaged within 1 month of surgery. Six were imaged within 2.5 weeks, as the eyes were completely healed and the cornea and anterior chamber were clear. The remaining eyes required additional time for postsurgical effects to resolve, such as corneal edema, anterior chamber cells, and flare. Care was taken to be sure that the eyes were in a completely recovered and healthy state, to ensure that accommodative responses during stimulation, image clarity, and image contrast would be optimal.

A 50-MHz UBM instrument (model 840; Carl Zeiss Meditec, Inc., Dublin, CA) was used to image the lens edge, zonula, and ciliary body of the eye at rest and during accommodation,[14,16] allowing measurement of the resting ciliary body apex width, and accommodative forward movement.[13] The eye was stabilized with extraocular muscle sutures during UBM imaging, so that during accommodation there was minimal convergence eye movement, if any. The stabilizing arm of the ultrasound instrument held the transducer in place. Thus, there was very little if any change in angle of the transducer to the eye during accommodation.

Using the UBM images, we took measurements of the angle between the anterior aspect of the ciliary body and the inner aspect of the cornea (CB-cornea angle) in the unaccommodated (resting) eye and during supramaximum stimulation, with the change in angle (narrowing) serving as a surrogate indicator of forward ciliary body movement (since the sides of the angle are better defined than the anterior insertion, and it is more difficult to outline muscle tissue by UBM).[13] The width of the ciliary body apex was also measured from UBM

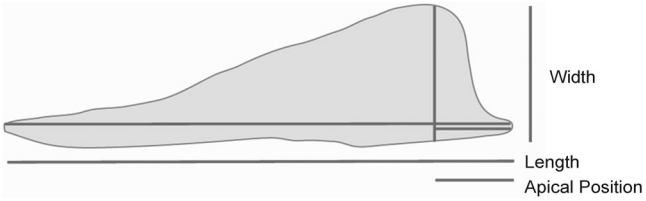

FIGURE 1. Schematic description of how measurements of muscle width, length, and apical position with respect to the scleral spur were taken in histologic sections. The eyes were not exposed to any pharmacologic agents to induce accommodation or relaxation.

images, analogous to those taken from histologic sections by Tamm et al.[19] For each group, the mean ± SEM width of the resting ciliary body apex, CB-cornea angle, and forward ciliary body movement were calculated. We did not measure ciliary muscle length by UBM, since we did not view the posterior extent of the ciliary muscle in the image and did not wish to extrapolate.

Histology

The monkeys were killed, and the eyes were fixed for morphologic examination: immediately ($n = 1$) and 1 ($n = 2$), 3 ($n = 3$), 5 ($n = 1$), 6 ($n = 1$), 8 ($n = 1$), and 16 ($n = 1$) months and 3 ($n = 2$), 5 ($n = 1$), and 6 ($n = 1$) years after postsurgical imaging.

The animals were perfusion fixed through the heart with 4% paraformaldehyde, after perfusion with 1 L of 0.1 M PBS (phosphate-buffered saline). After enucleation, slits were cut in the posterior sclera and a window was cut in the anterior cornea, to enhance fixative penetration and preserve the architecture of the ciliary muscle and its posterior attachment to Bruch's membrane. The eyes were then immersed in Ito's fixative[20] until they were sent to Erlangen for morphologic investigation. None of the eyes were exposed to pharmacologic stimulation before or during fixation. Small sectors of the anterior globe, including the entire ciliary body and adjacent cornea and sclera, were embedded in Epon, and 1-μm semithin sections were cut and stained with Richardson's stain.[21] In semithin sections from the different quadrants encompassing the entire circumference of the eye, the width, length, and apical position of the ciliary muscle (Fig. 1) were determined in six control eyes and six surgical eyes ($n = 4$, ICLE; $n = 2$, ECLE) of six adult monkeys selected randomly. The opposite eye of each monkey served as the control. The apical position is defined as the distance between the scleral spur and the midregion of the ciliary muscle apex. Quantitative evaluation of the width and apical position relative to the scleral spur was performed (Q Win program adapted to a microscope; Leica, Wetzlar, Germany).[19]

A two-tailed paired *t*-test was used to detect significant differences. $P \leq 0.05$ was considered significant; $0.05 \leq P \leq 0.10$ was considered to indicate a trend, given the small number of monkeys.

All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were in accordance with institutionally approved animal protocols.

RESULTS

Baseline Data and Technical Comparison

The three-dimensional structure of the ciliary muscle and the morphology of the muscle fibers were not affected by the surgical procedures. The same was true of the trabecular meshwork, indicating that valid results and conclusions can be obtained from this material. Even after ICLE, the posterior zonula appeared normal; only the anterior zonula was clumped, as expected.[17] A comparison of histology with UBM images showed similar absolute biometrics of the various ciliary muscle parameters and little variation around the circumference within individual eyes (histology) or between eyes (UBM; Fig. 2; Tables 2, 3).

UBM (Table 2) confirmed previous UBM findings[13] and earlier histologic findings that forward ciliary body movement diminishes significantly with age but is never completely lost, even in the oldest animals (e.g., 27 years in this series, comparable to approximately 65 to 70 years in humans).[19] Resting ciliary muscle width, corresponding to the histologic specimens, showed no age-related differences, analogous to earlier histologic findings in atropinized rhesus eyes.[22]

Surgical Results

There was no difference in ciliary muscle width (in millimeters), forward ciliary body movement, or apical position between post-ECLE versus the ipsilateral control baseline (Fig. 2; Tables 2, 3) or contralateral control (iridectomized-only control group; Table 2). There were no differences in any parameters by either technique between ECLE and control eyes, as defined earlier.

After ICLE, there were significant differences in the shape of the resting ciliary body between experimental and control eyes (Fig. 2). By UBM, the ciliary muscle at rest was thinner (length not measured; see the Methods section), and the apex appeared to be positioned more posteriorly compared with the ipsilateral control baseline or contralateral control eyes (Table 2, Fig. 3). Further, there was a 50% loss in accommodative forward ciliary body movement after ICLE compared with the ipsilateral (pretreated) or contralateral control (untreated) eyes (Fig. 3, Table 2, and Movie S1, http://www.iovs.org/cgi/content/full/49/12/5495/DC1), with no change in ciliary body width or accommodative forward movement in the contralateral untreated control eyes (Table 2). This loss of movement was seen in both the young and older eyes, even if the absolute width of the muscle and the forward movement differed (Table 2). By histology, after ICLE, the ciliary muscle was thinner and longer (i.e., extended further posteriorly), and the inner apex was located more posteriorly than in the control samples (Table 3). The inner edge of the ciliary muscle was absent, but the circularly running fibers formed a bandlike structure (Fig. 4) along the muscle portion facing the pars plicata of the ciliary processes stopping posteriorly at the transition zone between the pars plicata and the pars plana.

The pre- versus postsurgerical changes in accommodative responses (UBM) were not dependent on the time lapse between surgery and postsurgical imaging. Further, the morphologic differences between the control eyes and the surgically treated eyes were not dependent on the time lapse between surgery and morphologic examination.

DISCUSSION

Anterior capsulorrhexis and removal of the lens substance did not affect either the static geometry or the functional response of the ciliary muscle to central stimulation, so long as the posterior capsule remained. Removal of the entire lens and capsule caused changes in resting ciliary body geometry and a loss of forward ciliary body movement on central stimulation. The effect of leaving the posterior capsule in place but compromising its structural integrity remains unknown. Despite the large corneal incision and frequent vitreous loss with ICLE, the muscle retained a normal histopathologic appearance (i.e., healthy muscle fibers, no degeneration or connective tissue infiltration, normal anterior and posterior tendinous connections), as was true of the ECLE eyes. In both operations, the posterior zonula covering the pars plana appeared normal. After ICLE only, the anterior zonula, disconnected from the

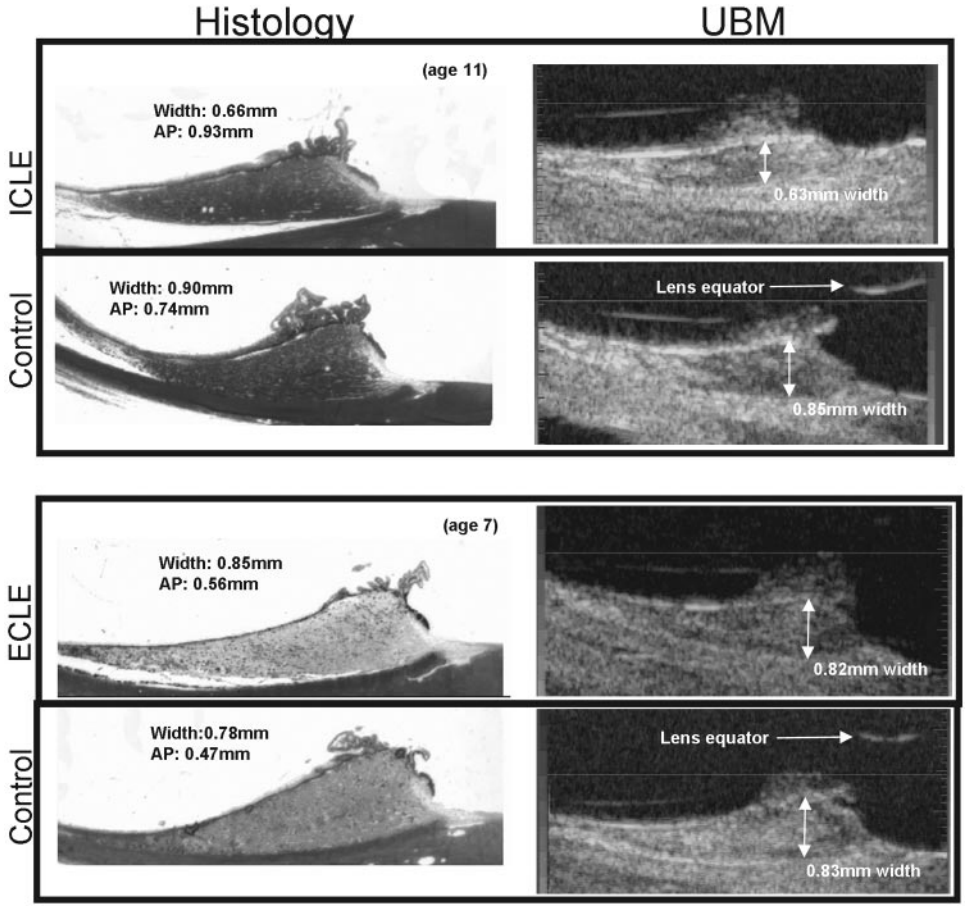

FIGURE 2. In vivo UBM images from both eyes of two rhesus monkeys (*right*), with the corresponding histologic sections of each of the imaged eyes (*left*) after euthanatization. Each monkey contributed one control (iridectomized phakic) eye and one surgically treated eye. One monkey underwent unilateral ICLE (*top*), and the other underwent unilateral ECLE (*bottom*). The numbers in the histologic sections represent the ciliary muscle width and apical position (AP) relative to the scleral spur[19] in the surgically altered and control eyes. In the ICLE histologic section, the detachment of the ciliary body from the sclera was an artifact. It was not included in the measurement of ciliary muscle width and did not affect the AP distance measurement from the scleral spur.

lens, appeared clumped together adjacent to the ciliary processes.[17] The loss of forward/inward "pull" from the anterior zonula may explain the thinner and more posteriorly positioned muscle after ICLE. Thus, in normal eyes, the presence of the lens and anterior zonula contribute to normal ciliary body configuration.

The ciliary muscle structural findings were similar by two different techniques: one postmortem and one in vivo. The width of the ciliary body apex in the phakic eyes (0.79 ± 0.03 mm; Table 2) was nearly identical with that reported by Tamm et al.[19] measured histologically (i.e., 0.74 mm in atropinized sections, prepared in the same way, but without surgical intervention).[19] This result gives confidence that our biological conclusions are accurate, that the techniques are predictive of each other, and that the parameters under study do not change after death, enucleation, fixation, sectioning, and staining. This information is important for studying scarce nonhuman primates and for clinical studies in humans.

We used the accommodative ciliary body–corneal angle change as a surrogate marker for forward ciliary muscle movement[13] rather than the vector change in the ciliary body center of mass. Indeed, the angle is quite distinct, thus lessening variability of the data, and is no more subjective than determining the center of mass. In addition, since the zonula attach to the walls of the ciliary processes (internal periphery ciliary body), the change in angle may be a more important indicator of ciliary body forward movement, accommodative mechanism, and accommodative amplitude. Further, during UBM imaging, the stabilization arm held the transducer in place, and the anterior and posterior ends of the ciliary body were oriented in a horizontal direction within all images to ensure stability and reproducibility. The eye was also stabilized with extraocular muscle sutures during UBM imaging, so that during accommodation there was minimal convergence eye movement, thus eliminating changes in the angle of the transducer to the eye during accommodation. Nonetheless, choosing the center of mass and its vector may provide a separate and distinct piece of information and could be undertaken in a subsequent study, but this method was beyond the scope of the study.

In our previous study (Tamm et al.[19]) by histologic examination of the ciliary muscle apical position in pilocarpinized sections, in the older versus the young rhesus eyes, we reported an ~80% loss in accommodative forward positioning of the ciliary muscle. The present study showed ~50% loss in the older versus the young monkeys by UBM during central electrical stimulation. The 30% difference in the age-related loss of forward ciliary body movement between the eyes undergoing accommodation induced by cholinomimetic drugs versus the eyes undergoing central electrical stimulation most likely reflects that pharmacologic stimulation is a more potent inducer of accommodation.[18]

With both the UBM and the histologic method, forward ciliary body movement decreased with age, but some mobility was maintained even in the oldest eyes. Although removal of the lens substance increased centripetal ciliary body movement,[17] it neither increased nor decreased forward ciliary body movement. This finding suggests that the older lens substance does not play a role in the age-related loss of forward ciliary body movement. On the other hand, the fact that there was a loss in forward accommodative movement of the ciliary body after ICLE but not after ECLE suggests that the zonular/capsular attachments, and not the lens substance, facilitate forward accommodative movement of the ciliary body.

TABLE 2. Ciliary Body Width, CB-Comea Angle before and during Supramaximum Stimulation and Forward Ciliary Body Movement

	A. Resting Ciliary Body Apex Width (mm)			B. CB-Cornea Angle (degrees)				C. Forward Ciliary Body Movement (degrees)		
				Pre ICLE		Post ICLE				
	Pre-ICLE	Post-ICLE	Pre-Post	Resting	Smax	Resting	Smax	Pre-ICLE	Post-ICLE	Pre-Post
ICLE										
Young (n = 5)										
Mean	0.79	0.63	0.16	155.8	95.5	159.9	134.7	60.3	25.2	35.1
SEM	0.03	0.02	0.04	4.0	4.0	5.2	9.5	6.1	6.1	10.9
P			0.015							0.032
Older (n = 4)										
Mean	0.78	0.61	0.17	158.1	130.0	152.3	135.8	28.1	16.6	11.6
SEM	0.02	0.05	0.06	5.1	6.1	6.1	5.8	2.8	6.0	4.8
P			0.062					0.006		0.095
Young and older (n = 9)										
Mean	0.78	0.62	0.16	156.8	110.8	156.5	135.2	46.0	21.4	24.6
SEM	0.02	0.03	0.03	2.6	6.9	3.9	5.5	6.6	4.3	7.3
P			0.001							0.01

	A. Resting Ciliary Body Apex Width (mm)			B. CB-Cornea Angle (degrees)				C. Forward Ciliary Body Movement (degrees)		
				Session 1		Session 2				
	Session 1	Session 2	Session 1 Minus Session 2	Resting	Smax	Resting	Smax	Session 1	Session 2	Session 1 Minus Session 2
Contralateral Control Eyes (Surgically Unaltered)										
Young (n = 2) and older (n = 3)										
Mean	0.77	0.79	−0.02	152.1	123.1	152.6	122.8	29.1	29.9	−0.8
SEM	0.04	0.03	0.01	4.2	6.0	4.9	10.3	6.5	8.7	3.5
P			NS							NS

	A. Resting Ciliary Body Apex Width (mm)			B. CB-Cornea Angle (degrees)				C. Forward Ciliary Body Movement (degrees)		
				Pre-ECLE		Post ECLE		Pre-ECLE	Post-ECLE	
	Pre-ECLE	Post-ECLE	Pre-Post	Resting	Smax	Resting	Smax	Resting Smax	Resting Smax	Pre-Post
ECLE										
Young (n = 2)										
Mean	0.82	0.81	0.01	151.2	93.4	160.2	90.7	57.8	69.5	−11.7
SEM	0.01	0.01	0.00	4.8	18.2	2.6	19.6	22.9	17.0	8.3
Older (n = 2)										
Mean	0.81	0.76	0.05	145.9	110.8	156.5	120.4	35.1	36.0	−1.0
SEM	0.02	0.05	0.04	3.8	7.0	5.6	0.5	10.9	5.1	22.5
Young and older (n = 4)										
Mean	0.81	0.79	0.03	148.5	102.1	158.3	105.5	46.4	52.8	−6.3
SEM	0.01	0.03	0.02	2.9	9.4	2.7	11.7	12.3	12.1	7.6
P			NS							NS

	A. Resting Ciliary Body Apex Width (mm)			B. CB-Cornea Angle (degrees)				C. Forward Ciliary Body Movement (degrees)		
				Session 1		Session 2				
	Session 1	Session 2	Session 1 Minus Session 2	Resting	Smax	Resting	Smax	Session 1	Session 2	Session 1 Minus Session 2
Contralateral Control Eyes (Surgically Unaltered)										
Young (n = 2) and older (n = 2)										
Mean	0.76	0.78	−0.02	142.7	116.6	151.8	123.1	26.1	28.8	−2.6
SEM	0.08	0.05	0.03	10.2	7.1	8.5	5.2	3.1	3.4	0.3
P			NS							NS

(A) Resting ciliary body apex width (obtained with UBM) in young and older monkey eyes before and after ICLE or ECLE, along with mean pre- minus post-ICLE or ECLE differences; P, mean difference by two-tailed paired t-test. (B) Ciliary body/cornea angle (CB-cornea angle; obtained with UBM)[13] in young and older monkey eyes before and after ICLE, or after ECLE, when the eye was at rest and during supramaximum (Smax) stimulation. (C) The difference between the CB-cornea angle at rest and during Smax stimulation was used as a surrogate indicator of forward ciliary body movement.[13] NS, not significant.

TABLE 3. Histological Measurements of Ciliary Muscle Width and Apical Position

	Ciliary Muscle Width (mm)				Apical Position (mm)				Length (mm)			
	Control	ICLE	Control − ICLE	*P*	Control	ICLE	Control − ICLE	*P*	Control	ICLE	Control − ICLE	*P*
ICLE (n = 4)												
Mean	0.84	0.66	0.19	0.047	0.79	0.99	−0.20	0.005	2.95	3.43	−0.52	0.058
SEM	0.01	0.06	0.06		0.03	0.05	0.03		0.10	0.15	0.13	
	Ciliary Muscle Width (mm)				**Apical Position (mm)**				**Length (mm)**			
	Control	**ECLE**	**Control − ECLE**		**Control**	**ECLE**	**Control − ECLE**		**Control**	**ECLE**	**Control − ECLE**	
ECLE (n = 2)												
Mean	0.81	0.90	−0.10		0.69	0.67	−0.02		3.01	3.06	−0.06	
SEM	0.03	0.04	0.06		0.09	0.03	0.06		0.08	0.15	0.06	

Data represent mean ± SEM width and length of the ciliary muscle, and the distance of the ciliary muscle apical position from the scleral spur,[19] obtained from histologic sections in control (iridectomized phakic) eyes and in eyes that had undergone ICLE and ECLE. *P,* probability of no significant difference between surgically treated eyes and the contralateral control eyes by the two-tailed paired *t*-test.

The design and properties of putatively accommodating intraocular lenses (IOLs) must take into account the decreased mobility of the driving system. However, recall findings in the companion study[17] showed that centripetal ciliary body movement increased after ECLE, even in old eyes and could contribute to accommodation in the presence of an appropriately designed IOL.

There was uniformity of the measurements within the circumference of and between eyes, which demonstrated both the precision of the techniques and the small biological variance of the parameters. If humans are similar to monkeys, this replication would be important for sample sizes and repetitive measurements in clinical trials. In addition, the uniformity of the data indicates that day-to-day variations in experimental technique, tissue processing, data analysis, and animal biological factors are small in terms of the parameters being measured (i.e., the signal-to-noise ratio may be high). Day-to-day variability in experimentally induced accommodative responses cannot account for all the findings: (1) In the resting eye, the ciliary body apex width was thinner in all monkeys

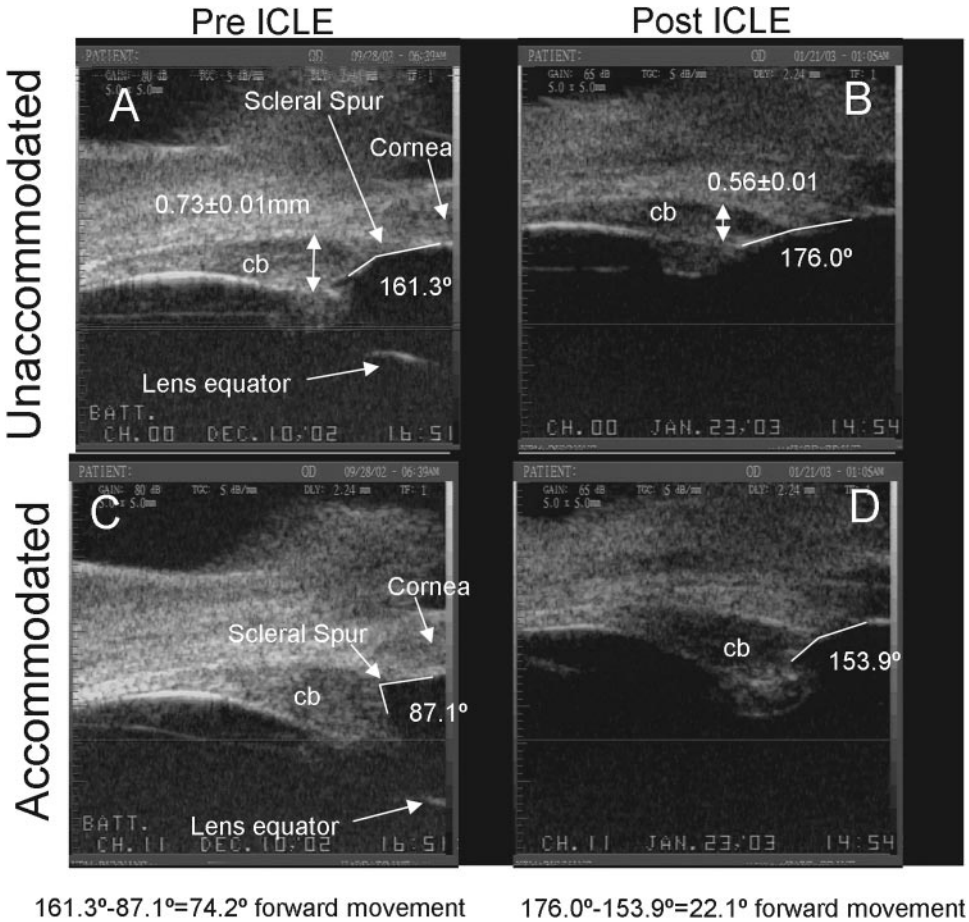

FIGURE 3. UBM in the temporal quadrant of a 7-year-old rhesus monkey eye before and after ICLE. Resting (**A**, **B**) and accommodated (**C**, **D**) ciliary body before (**A**, **C**) and after (**B**, **D**) ICLE. The width of the ciliary body apex was 0.73 ± 0.01 mm before ICLE (**A**) and decreased to 0.56 ± 0.01 mm after ICLE (**B**). The amount of accommodative forward movement diminished from 74.2° to 22.1° before versus after, respectively, as measured by ciliary body–cornea angle change.[13] During maximum accommodation, the anterior aspect of the ciliary body moved past the scleral spur before, but not after, ICLE. After ICLE, the ciliary body apex was flattened against the sclera. The numbers in degrees represent the angle between the inner aspect of the cornea and the anterior aspect of the ciliary body.

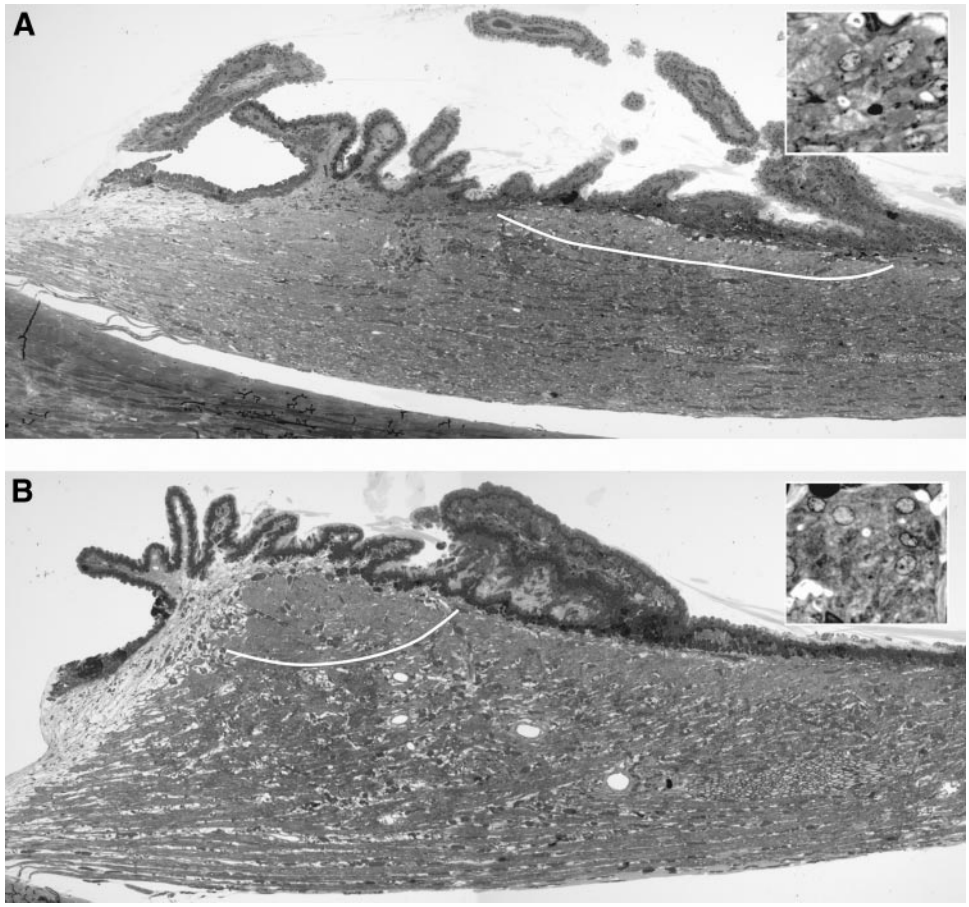

FIGURE 4. Images are of 1-μm-thick sagittal sections through the ciliary muscle of (**A**) a 16-year-old monkey after unilateral ICLE and (**B**) the contralateral control eye (Richardson's stain). Even in the extremely small muscle after ICLE there were circularly arranged muscle fibers. In contrast to the control eyes, in which the circular fibers formed an anterior inner edge (*white line*), in the ICLE eye they formed an elongated area at the inside of the muscle. *Insets*: cross sectioned muscle fibers.

after versus before ICLE, regardless of age; (2) accommodative forward movement was lost after ICLE in seven of eight monkeys. These postsurgical accommodative movement responses occurred consistently from monkey eye to monkey eye despite the complex nature of the surgical procedures and the varied intervals between pre- and postsurgical measurements.

Forward ciliary body movement was not reduced after ECLE, and there was increased centripetal ciliary body accommodative movement after ECLE and ICLE in which Wieger's ligament remained intact.[17] Collectively, these findings alleviate concern that these surgical procedures caused intraocular scarring that would likely diminish accommodative movements.

The results of this study provide new insights into accommodative ciliary body function. In summary: (1) After ICLE, UBM showed that accommodative ciliary body forward movement decreased and resting thickness decreased. (2) The lens capsule and its zonular connections to the ciliary body are necessary for the inner edge of the muscle to be well defined. (3) Ciliary body resting thickness and the magnitude of the accommodative forward ciliary body movement were not altered by ECLE.

This study is the first to show that opening the anterior chamber, creating a ~4 mm anterior capsulorrhexis, removing the lens nucleus and cortex, but leaving the posterior capsule intact, does not change the operation of the zonular/muscular system as a whole, even without replacement of the lens content. This finding is important for accommodating IOL development. In contrast, removing the entire lens does make a difference. Yet to be determined is the effect of opening the posterior capsule (i.e., to treat posterior capsule opacification).

Acknowledgments

The authors thank James Reed for lending his technical expertise with the image analysis systems; Joseph Sanchez, Mike Killips, and Charles Roth for expert editing of the video presentation; and Kate Fahl for editorial assistance.

References

1. von Helmholtz HH. Helmholtz's treatise on physiological optics. In: Southall JPC, ed. *Handbuch der Pysiologishen Optik.* Southall JPCT, trans. *Mechanism of Accommodation.* Vols. 1 and 2. New York: Dover Publications; 1909:143–172.
2. Rohen JW. Scanning electron microscopic studies of the zonular apparatus in human and monkey eyes. *Invest Ophthalmol Vis Sci*. 1979;18:133–144.
3. Koretz JF, Bertasso AM, Neider MW, Gabelt BT, Kaufman PL. Slit-lamp studies of the rhesus monkey eye: II. Changes in crystalline lens shape, thickness and position during accommodation and aging. *Exp Eye Res*. 1987;45:317–326.
4. Croft MA, Kaufman PL, Crawford KS, Neider MW, Glasser A, Bito LZ. Accommodation dynamics in aging rhesus monkeys. *Am J Physiol Regul Integr Comp Physiol*. 1998;44:R1885–R1897.
5. Glasser A, Wendt M, Ostrin L. Accommodative changes in lens diameter in rhesus monkeys. *Invest Ophthalmol Vis Sci*. 2006; 47(1):278–286.
6. Vilupuru AS, Glasser A. The relationship between refractive and biometric changes during Edinger-Westphal stimulated accommodation in rhesus monkeys. *Exp Eye Res*. 2005;80(3):349–360.
7. Neider MW, Crawford K, Kaufman PL, Bito LZ. In vivo videography of the rhesus monkey accommodative apparatus: age-related loss of ciliary muscle response to central stimulation. *Arch Ophthalmol*. 1990;108:69–74.

8. Lütjen-Drecoll E, Tamm E, Kaufman PL. Age changes in rhesus monkey ciliary muscle: light and electron microscopy. *Exp Eye Res*. 1988;47:885–899.
9. Bito LZ, DeRousseau CJ, Kaufman PL, Bito JW. Age-dependent loss of accommodative amplitude in rhesus monkeys: an animal model for presbyopia. *Invest Ophthalmol Vis Sci*. 1982;23:23–31.
10. Duane A. Studies in monocular and binocular accommodation with their clinical applications. *Am J Ophthalmol*. 1922;5:867–877.
11. Strenk SA, Stenk LM, Semmlow JL, DeMarco JK. Magnetic resonance imaging study of the effects of age and accommodation on the human lens. *Invest Ophthalmol Vis Sci*. 2004;45:539–545.
12. Strenk SA, Stenk LM, Guo S. Magnetic resonance imaging of aging, accommodating, phakic, and pseudophakic ciliary muscle diameters. *J Cataract Refract Surg*. 2006;32:1792–1798.
13. Croft MA, Glasser A, Heatley G, et al. Accommodative ciliary body and lens function in rhesus monkeys: I. Normal lens, zonule and ciliary process configuration in the iridectomized eye. *Invest Ophthalmol Vis Sci*. 2006;47(3):1076–1086.
14. Croft MA, Glasser A, Heatley G, et al. The zonula, lens, and circumlental space in the normal iridectomized rhesus monkey eye. *Invest Ophthalmol Vis Sci*. 2006;47(3):1087–1095.
15. Glasser A, Kaufman PL. The mechanism of accommodation in primates. *Ophthalmology*. 1999;106:863–872.
16. Glasser A, Croft MA, Brumback L, Kaufman PL. Ultrasound biomicroscopy of the aging rhesus monkey ciliary region. *Optom Vis Sci*. 2001;78:417–424.
17. Croft MA, McDonald JP, James RJ, et al. Surgical intervention and accommodative responses: I. Centripetal ciliary body, capsule and lens movement in rhesus monkeys of varying age. *Invest Ophthalmol Vis Sci*. 2008;49:5484–5494.
18. Crawford K, Terasawa E, Kaufman PL. Reproducible stimulation of ciliary muscle contraction in the cynomolgus monkey via a permanent indwelling midbrain electrode. *Brain Res*. 1989;503:265–272.
19. Tamm E, Croft MA, Jungkunz W, Lütjen-Drecoll E, Kaufman PL. Age-related loss of ciliary muscle mobility in the rhesus monkey: role of the choroid. *Arch Ophthalmol*. 1992;110:871–876.
20. Ito S, Karnovsky MJ. Formaldehyde-glutaraldehyde fixatives containing trinitro compounds. *J Cell Biol*. 1968;39:168.
21. Richardson KC, Jarrel L, Finke H. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain Technol*. 1960; 35:313–323.
22. Lütjen-Drecoll E, Tamm E, Kaufman PL. Age-related loss of morphologic responses to pilocarpine in rhesus monkey ciliary muscle. *Arch Ophthalmol*. 1988;106:1591–1598.