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ScienceDirect
Experimental Eye Research 84 (2007) 302–313
EXPERIMENTAL EYE RESEARCH
www.elsevier.com/locate/yexer

Edinger-Westphal and pharmacologically stimulated accommodative refractive changes and lens and ciliary process movements in rhesus monkeys

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Received 2 June 2006; accepted in revised form 2 October 2006
Available online 28 November 2006

Abstract

During accommodation, the refractive changes occur when the ciliary muscle contracts, releasing resting zonular tension and allowing the lens capsule to mold the lens into an accommodated form. This results in centripetal movement of the ciliary processes and lens edge. The goal of this study was to understand the relationship between accommodative refractive changes, ciliary process movements and lens edge movements during Edinger-Westphal (EW) and pharmacologically stimulated accommodation in adolescent rhesus monkeys. Experiments were performed on one eye each of three rhesus monkeys with permanent indwelling electrodes in the EW nucleus of the midbrain. EW stimulated accommodative refractive changes were measured with infrared photorefraction, and ciliary process and lens edge movements were measured with slit-lamp goniovideography on the temporal aspect of the eye. Images were recorded on the nasal aspect for one eye during EW stimulation. Image analysis was performed off-line at 30 Hz to determine refractive changes and ciliary body and lens edge movements during EW stimulated accommodation and after carbachol iontophoresis to determine drug induced accommodative movements. Maximum EW stimulated accommodation was 7.36 ± 0.49 D and pharmacologically stimulated accommodation was 14.44 ± 1.21 D. During EW stimulated accommodation, the ciliary processes and lens edge moved centripetally linearly by 0.030 ± 0.001 mm/D and 0.027 ± 0.001 mm/D, with a total movement of 0.219 ± 0.034 mm and 0.189 ± 0.023 mm, respectively. There was no significant nasal/temporal difference in ciliary process or lens edge movements. 30–40 min after pharmacologically stimulated accommodation, the ciliary processes moved centripetally a total of 0.411 ± 0.048 mm, or 0.030 ± 0.005 mm/D, and the lens edge moved centripetally 0.258 ± 0.014 mm, or 0.019 ± 0.003 mm/D. The peaks and valleys of the ciliary processes moved by similar amounts during both supramaximal EW and pharmacologically stimulated accommodation. In conclusion, this study shows, for the first time, that the ciliary processes and lens edge move centripetally, linearly with refraction during EW stimulated accommodation. During pharmacological stimulation, the ciliary processes move to a greater extent than the lens edge, confirming that in adolescent monkeys, lens movement limits the accommodative optical change in the eye.

Keywords: accommodation; crystalline lens; ciliary body; Edinger-Westphal nucleus; image analysis; pharmacological stimulation; central stimulation

1. Introduction

Accommodation is a dioptric change in power of the eye to focus on objects at different distances. In the unaccommodated eye, zonular fibers extending from the lens equatorial region to the walls of the ciliary processes apply an outward directed force to hold the lens in a relatively flattened state (Glasser and Kaufman, 1999; Helmholtz von, 1855, 1909). During accommodation, the ciliary muscle contracts, releasing zonular tension and allowing the capsule to mold the lens into an accommodated form. Lens axial thickness increases, equatorial diameter decreases and anterior and posterior radii of curvature decrease to increase the optical power of the lens and bring about accommodative refractive change.

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doi:10.1016/j.exer.2006.10.003

Aspects of the mechanistic relationship between the ciliary processes and lens equator have been determined from prior studies using goniovideography in anesthetized, rhesus monkeys during Edinger-Westphal (EW) stimulated accommodation (Croft et al., 2006a,b; Glasser and Kaufman, 1999; Neider et al., 1990). A Swan-Jacob gonioscopy lens placed on the iridectomized eye allows direct visualization of the ciliary processes, zonular fibers and adjacent lens equator. Such studies have provided important information on the accommodative mechanism and age changes that contribute to the progression of presbyopia, but have not determined the relationship between the physical movements and the accommodative refractive change.

Prior studies in rhesus monkeys have related lens axial thickness and diameter changes to the accommodative refractive changes (Glasser et al., 2006; Ostrin and Glasser, 2005; Vilupuru and Glasser, 2005), showing highly systematic physical and optical accommodative changes that occur to bring about refractive change. Because the ciliary body movements indirectly cause the refractive changes, it is equally important to understand how the ciliary body accommodative movements relate to the refractive change.

Information on the accommodative relationships between ciliary process and lens edge movements are not available in humans due to the presence of the iris. Potentially, this could be done in an aniridic subject, but it would not be possible to achieve the same rigorous contact gonioscopy imaging conditions and control over the accommodative response as can be achieved with EW stimulated accommodation in anesthetized monkeys. The accommodative anatomy and mechanism in rhesus monkey eyes are similar to that of humans (Glasser and Kaufman, 1999; Koretz et al., 1987a,b; Lütjen-Drecoll et al., 1988a,b; Neider et al., 1990; Tamm et al., 1992). Rhesus monkeys also develop presbyopia with the same relative age course (Bito et al., 1982, 1987; Kaufman et al., 1982) and etiology as humans (Croft et al., 2006a; Strenk et al., 1999). Rhesus monkeys are therefore an ideal model for studies of accommodation and presbyopia.

Magnetic resonance imaging (MRI), ultrasound biomicroscopy (UBM) and goniovideography are the only techniques by which the ciliary processes and lens equator can be imaged in the living eye. MRI is of low spatial and temporal resolution, and can only provide static measurements. UBM is also relatively low spatial resolution but does provide dynamic measurements at about 7–12 Hz. Goniovideography is an optical imaging technique of much higher spatial and temporal resolution. It is recorded at high magnification at video frame rates (30 Hz), allowing high resolution measurements of accommodative movements of the ciliary processes and lens equator. Prior such studies have not related the accommodative movements of the ciliary processes and lens equator to the accommodative refractive changes (Croft et al., 2006a,b). This information is important and necessary to understand how physical movements produce accommodative optical changes and how these relationships change with age.

In anesthetized monkeys, accommodation can be induced with EW and pharmacological stimulation (Ostrin and Glasser, 2005; Vilupuru and Glasser, 2002). Recent studies have shown important differences between neuronally controlled and drug stimulated accommodation. Drug stimulation produces a stronger accommodative response and a forward translation of the lens that does not occur with EW stimulation in monkeys or with voluntary accommodation in humans (Koeppl et al., 2005; Ostrin and Glasser, 2005). It is thought that drug stimulation produces complete saturation of receptors at the neuromuscular junction and a supramaximal contraction of the ciliary muscle. It is of interest to understand the relationship between the ciliary body and lens accommodative movements during both EW and drug stimulated accommodation. These relationships are important for providing a more comprehensive description of the accommodative mechanism, identifying factors that limit the accommodative response, and understanding how and why presbyopia occurs.

2. Methods

Experiments were performed on three rhesus monkeys, ages 6 (#111) and 8 (#54 and #58) years. The monkeys had previously been bilaterally, surgically iridectomized (Kaufman and Lütjen-Drecoll, 1975) and each had a stimulating electrode implanted in the EW nucleus of the midbrain (Crawford et al., 1989; Vilupuru and Glasser, 2002). All experiments conformed to the ARVO Statement for Use of Animals in Ophthalmic and Vision Research, and were conducted under an institutionally approved animal protocol.

Reference marks were permanently tattooed on the nasal and temporal corneal limbus of the right eye in each monkey for goniovideography image analysis. For this, monkeys were anesthetized with 10 mg/kg intramuscular ketamine and 0.5 mg/kg intramuscular acepromazine (Phoenix Pharmaceutical, St. Joseph, MO), and supplemented with 6.25 mg/kg ketamine approximately every 30 min as required. Monkeys were placed supine, facing up beneath a surgical microscope. The eyelids were held open with a speculum. Corneal tattoos were made by inserting the tip of a 27 gauge syringe needle dipped in sterile black India ink into the stroma of the clear cornea, approximately 1 mm from the limbus along the 180 degree (horizontal) meridian. This resulted in a permanent black corneal tattoo about 250–500 μm in diameter.

2.1. EW stimulated accommodation

Monkeys were anesthetized with 10 mg/kg intramuscular ketamine and 0.5 mg/kg intramuscular acepromazine. Surgical depth anesthesia was induced with an initial bolus of 1.5 mg/kg followed by constant perfusion at 0.5 mg/kg/min of intravenous propofol (Propoflo, Abbott Laboratories, North Chicago, IL). The monkey was placed prone in a head holder with the head upright and facing forward. Pulse rate and SpO_2 were monitored and the monkey was wrapped in a 37 °C water heated pad to maintain body temperature. The eyelid was held open with a speculum, a PMMA contact lens (MetroOptics, Dallas, TX) was placed on the cornea to maintain clarity and corneal hydration, and sutures were tied beneath the

lateral and medial rectus muscles to reduce accommodative and wandering eye movements.

A static accommodative stimulus response function was measured with a Hartinger coincidence refractometer (HCR) (Zeiss, Jena, Germany) (Fincham, 1937a). Increasing current amplitudes were delivered to the EW nucleus to achieve the full range of accommodative responses available to each eye. In addition, for two of the monkeys, one supramaximal stimulus current amplitude was delivered, i.e., a current amplitude greater than that required to produce the maximum accommodative response. Accommodation was stimulated at each amplitude with five, four-second long stimulus trains (0.6 ms pulse durations at 72 Hz). Refraction of the eye was measured with the HCR during the last second of each stimulus train. The last three of the five refraction measurements were recorded and averaged. These measurements were later used to generate a calibration function for dynamic accommodation measurements.

Infrared photorefraction was used to measure the dynamic accommodative refractive changes for increasing current amplitudes (Vilupuru and Glasser, 2002). Photorefraction was performed at a working distance of 0.3 meters and recorded to digital video cassette recorder (VCR) using an infrared sensitive black and white charge-coupled display (CCD) camera (Cohu, San Diego, CA) with a bank of infrared light emitting diodes. Accommodation was stimulated five times at each of the same stimulus current amplitudes used in the static stimulus response function. Optimas 6.5 (MediaCybernetics, Silver Springs, MD) image analysis software was used to analyze the last three of five responses. The slope of the vertical luminance profile for the central 40% of the iridectomized pupil diameter was determined for each stimulus current amplitude and converted to refraction in diopters using the static stimulus response function for the same stimulus current amplitudes (Vilupuru and Glasser, 2002). Once a unique calibration curve was generated, images were analyzed at 30 Hz to determine dynamic changes in refraction. Each accommodative response was fitted with an exponential function to determine velocity of the response. The relationship between the peak velocity and the amplitude of the accommodative responses (a main sequence relationship) was calculated (Vilupuru and Glasser, 2002).

Following infrared photorefraction, the contact lens was removed from the eye and a Swan-Jacob gonioscopy lens was placed on the temporal aspect of the cornea to view the corneal reference tattoo, ciliary processes and crystalline lens edge through a slit-lamp microscope at high magnification. The gonioscopy lens was clamped and held in place by a micromanipulator, and gonioscopic prism solution (hypromellose ophthalmic demulcent solution 2.5%, Wilson Ophthalmic Corp., OK) was placed between the gonioscopy lens and cornea. The gonioscopy lens and the slit-lamp were visually aligned as close to the optical axis of the eye as possible to attain a constant angle between the measurement plane and axis of observation for all experiments. The same CCD camera as used for photorefraction measurements, with a resolution of 768×494 pixels, was attached to the slit-lamp to record the gonioscopy images to digital VCR. Accommodation was again stimulated with the same stimulus current amplitudes as used during photorefraction measurements. Gonioscopy images were analyzed off-line at 30 Hz with sub-pixel resolution, to determine ciliary process and lens vertical edge movements for the last three of five stimulations for each increasing stimulus current amplitude. The horizontal distance from five clearly distinguishable, adjacent ciliary process peaks and the vertical lens edge (at the 180 degree meridian) to the corneal reference tattoo was determined (Fig. 1). Image analysis was also performed on five clearly distinguishable, adjacent ciliary process valleys for the supramaximal current stimulus amplitude. Initially, an attempt was made to fit an arc to the lens edge to find the lens center and use the lens center for a vector for the measurements. However, since such a small arc of the lens is visible, the diameter and center of the circle fit to this lens edge varied unacceptably from one image to the next and this approach was abandoned. Main sequence relationships were determined for lens edge and ciliary process movement. The experiment was repeated in monkey #58 (experiments a and b). For monkey #111, the gonioscopy lens was moved to the nasal aspect of the cornea and the experiment was repeated.

Image magnification at the ciliary processes and lens equator as viewed through the Swan-Jacob gonioscopy lens was determined by inserting a 27 gauge needle into the anterior chamber of one monkey eye near the plane of the ciliary processes. The needle was imaged in the anterior chamber with the slit-lamp through the gonioscopy lens and calibrated according to the international standard for stainless steel tubing for the manufacture of medical devices, ISO 9626, which identifies that the outside diameter of a 27 gauge needle is 0.41 mm. This was confirmed by measuring the needle in air against 1 mm ruled graph paper at high magnification with image analysis. This calibration was used to convert all measurements through the gonioscopy lens into millimeters.

2.2. Pharmacologically stimulated accommodation

Pharmacological stimulation was done during separate experimental sessions from the EW stimulation experiments and at least one week later. Monkeys were anesthetized with 10 mg/kg intramuscular ketamine and 0.5 mg/kg intramuscular acepromazine, and supplemented with 6.25 mg/kg ketamine approximately every 30 min as required. The eyelid was held open with a lid speculum. Sutures were placed beneath the lateral and medial rectus muscles to reduce spontaneous eye movements. A contact lens was placed on the cornea and three baseline refraction measurements were recorded with the HCR and averaged. The contact lens was removed and the gonioscopy lens was placed on the temporal aspect of the cornea as described above. After baseline ciliary process and lens equator images were recorded for approximately 15–30 s in the unaccommodated eye, 40% carbachol in agar was applied iontophoretically (Vilupuru and Glasser, 2002) to the exposed nasal aspect of the cornea for ten seconds, without removing the gonioscopy lens. Goniovideography images

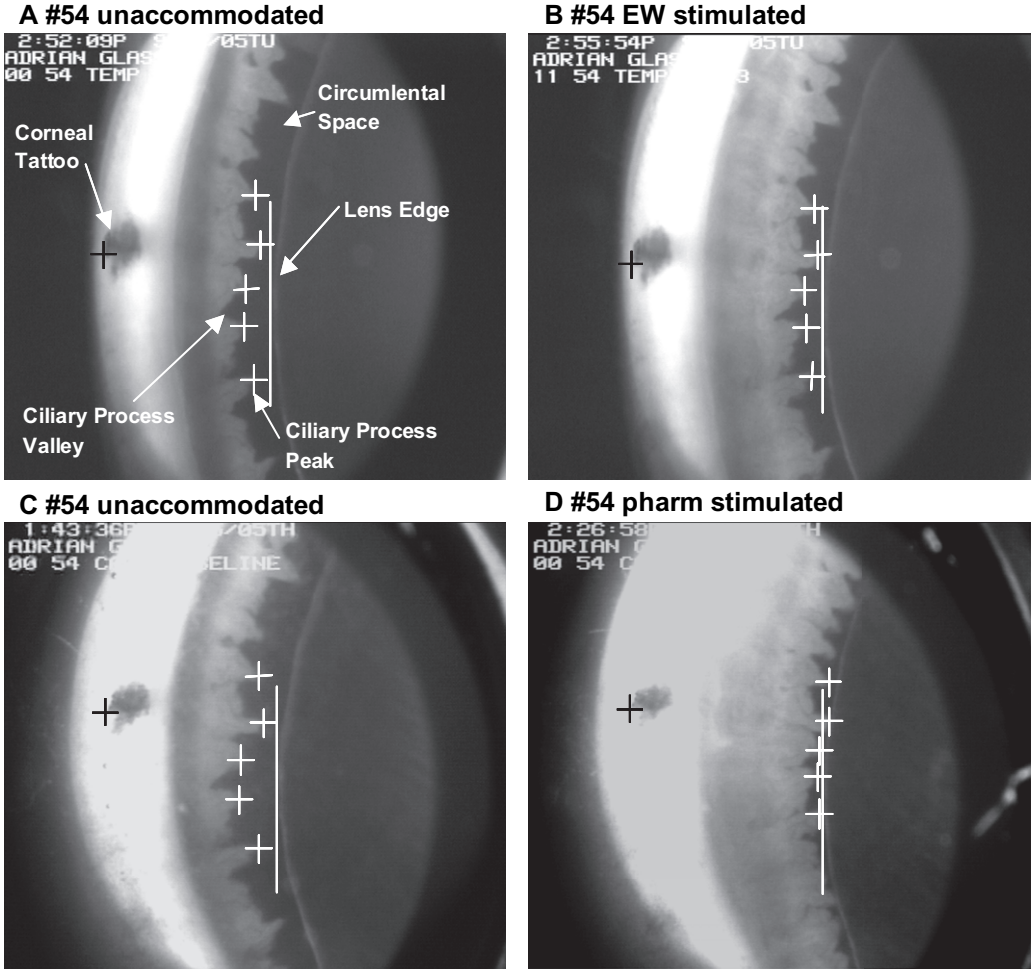

Fig. 1. Slit-lamp goniovideography images of (A) an unaccommodated and (B) maximally EW stimulated accommodated eye, and (C) an unaccommodated and (D) maximally pharmacologically stimulated accommodated eye. The black crosses mark the edge of the corneal tattoo, the white crosses mark five corresponding ciliary process peaks, and the vertical white line marks the 9 o'clock equatorial edge of the lens.

were then recorded continuously for 30 min to video tape. After 30 min, in three of the four eyes, carbachol was reapplied to the nasal cornea for 5 s and images were recorded for 10 more minutes to ensure a maximal accommodative response was achieved. One image per 30 s was analyzed off-line as described above to determine movements of the ciliary process tips, ciliary process valleys and lens edge from the corneal reference mark. At the end of the experiment, the gonioscopy lens was removed from the eye, the contact lens was replaced, and three final HCR accommodated refraction measurements were made and averaged. The difference between the baseline and final refraction measurements was recorded as the accommodative response amplitude. The experiment was repeated in one monkey (#111, experiments a and b).

3. Results

The precision of goniovideography image analysis was determined by calculating the standard deviation of five baseline (unaccommodated) video images, each immediately preceding 23 stimulations from three different monkey eyes. The mean standard deviation from all of these measurements was 0.006 mm with a standard deviation of 0.002 mm. Because the precision is better than 10 μm, goniovideography measurements are rounded and reported to three decimal places, although this is not intended to imply single micron precision.

Maximum EW stimulated accommodative amplitude was 7.36 ± 0.49 D (mean $\pm$ standard error of the mean (SEM), Table 1) (average values include the repeat experiments in the same eyes). The ciliary processes and the lens edge showed increasing centripetal movement for increasing current amplitudes (Fig. 2). Each trace graphed is an average response from the first 2 s of the last three of five stimuli delivered for each stimulus amplitude, with standard deviations (for clarity, only every second data point is shown). For one monkey in which measurements were made at both the nasal and temporal aspects of the eye, Bland-Altman analysis showed a mean difference for nasal vs temporal ciliary process movement of 0.020 ± 0.001 mm (mean $\pm$ SEM) and for lens edge movement of 0.000 (zero) $\pm$ 0.001 mm. Over the full range of EW stimulated accommodation available to

Table 1
Accommodative amplitude, lens edge movement and ciliary process movement during maximal EW stimulated accommodation

	Accommodative Amplitude (D)	Maximum Lens Edge Mvmt (mm)	Lens Edge Movement (mm/D)	Maximum Ciliary Process Mvmt (mm)	Ciliary Process Movement (mm/D)
#111 Nasal	7.39 ± 0.03	0.255 ± 0.002	0.031 ± 0.0005	0.271 ± 0.002	0.034 ± 0.0004
#111 Temporal	7.39 ± 0.03	0.241 ± 0.001	0.032 ± 0.0007	0.271 ± 0.002	0.032 ± 0.0006
#54 Temporal	8.41 ± 0.04	0.216 ± 0.002	0.027 ± 0.0005	0.286 ± 0.001	0.031 ± 0.0005
#58 Temporal	7.60 ± 0.04	0.167 ± 0.001	0.024 ± 0.0003	0.219 ± 0.001	0.027 ± 0.0006
#58 Temporal	6.04 ± 0.03	0.147 ± 0.002	0.028 ± 0.0006	0.152 ± 0.001	0.027 ± 0.0005
Average Temporal	7.36 ± 0.49	0.189 ± 0.023	0.028 ± 0.0017	0.219 ± 0.034	0.029 ± 0.0013

The averages are calculated from all values including the repeat experiments in the same eye. Values are mean ± SEM. SEMs for mm/D changes are calculated from the variance of the linear regression lines.

each eye, the ciliary processes and lens edge moved linearly with refraction (Fig. 3).

On average, centripetal temporal ciliary process movement resulted in an accommodative change of 31.66 D/mm (or ciliary process movement of 0.030 ± 0.001 mm/D), and centripetal temporal lens edge movement resulted in an accommodative change of 31.86 D/mm (or lens edge movement of 0.027 ± 0.001 mm/D). Because the standard deviations of the ciliary process and lens edge movements were small (0.007–0.008 mm), linear regression analysis

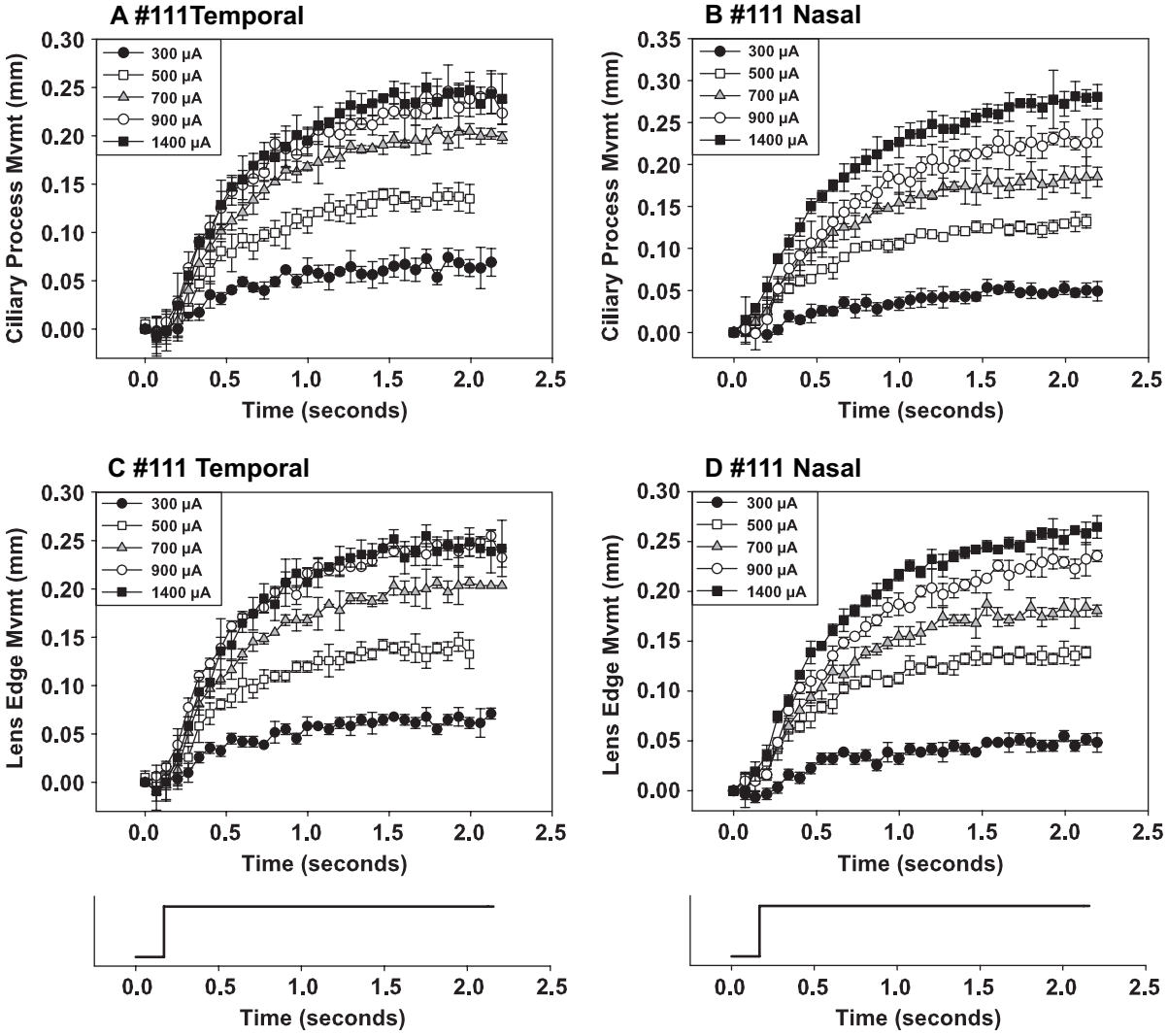

Fig. 2. (A, B) Ciliary process and (C, D) lens edge accommodative movement for increasing stimulus current amplitudes for the (A, C) temporal and (B, D) nasal aspects of one eye (monkey #111) spanning the full accommodative range available. For clarity, only every second data point is shown. The bottom traces show the onset of the stimulus.

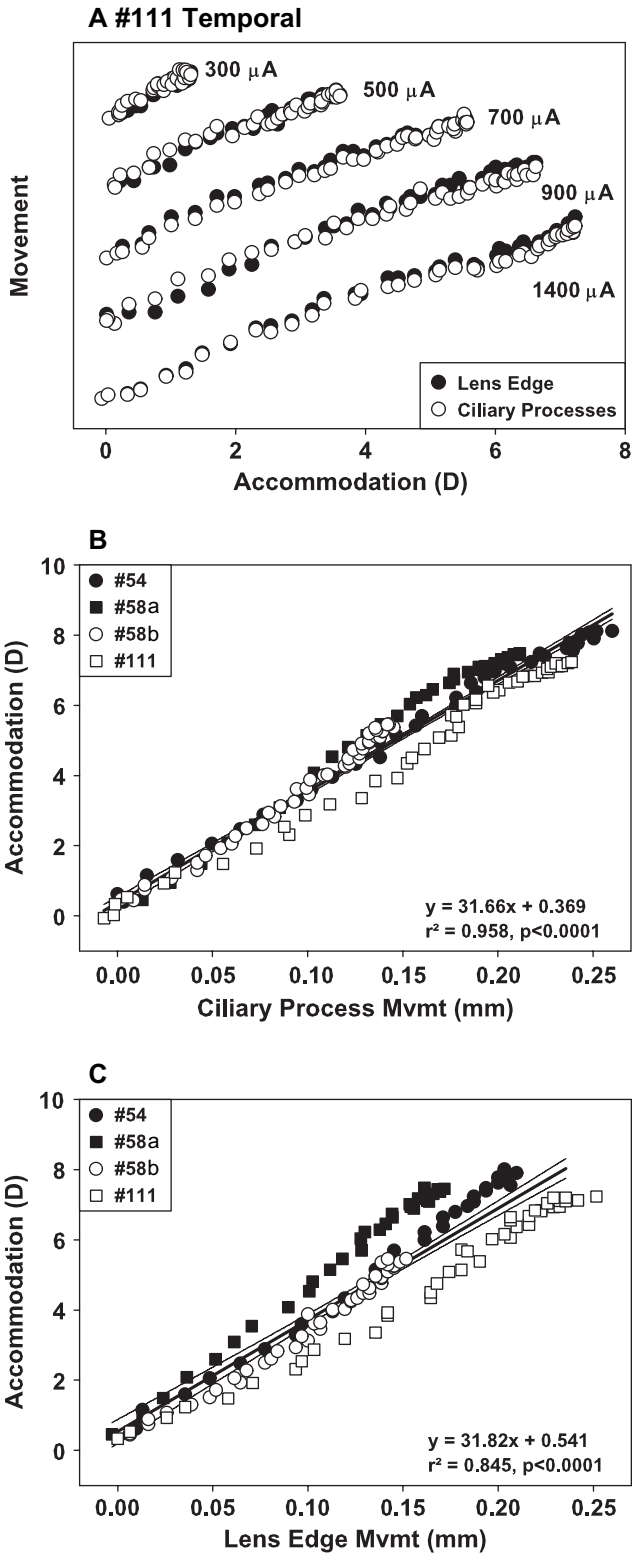

Fig. 3. (A) Ciliary process and lens edge movements were linearly related to accommodation for all amplitudes spanning the full accommodative range, shown for the temporal aspect of monkey #111, vertically offset for visibility. Accommodative stimuli ranged from 300 to 1400 μA. For the maximum amplitude for all monkeys, (B) ciliary process movement occurs at a rate of 0.030 mm/D, and (C) lens edge movement occurs at a rate of 0.026 mm/D. Linear regression line shown with 95% confidence intervals.

was used rather than orthogonal regression to compare the relationships. Comparing ciliary process movement vs accommodation and lens edge movement vs. accommodation, the slopes and intercepts were not significantly different ($p = 0.87$ and 0.37, respectively). However, when examined individually, two monkeys (#54 & #58b) showed greater ciliary process movement than lens edge movement at the highest current stimulus amplitudes (Fig. 4A and B). For those two monkeys, there was a significant decrease in circumlental space (distance from the tips of the processes to the lens edge) during maximal EW stimulated accommodation (-0.035 ± 0.001 mm/D, slope significantly different from zero, $p < 0.0001$). Monkey #111 and monkey #58b showed no change in circumlental space (Fig. 5A).

The main sequence relationships for ciliary process movement, lens edge movement and refraction were linear over the full range of accommodation available to each eye (Fig. 6). The main sequence for lens edge movement was not statistically different from the main sequence for ciliary process movement (variance: $F_{(2,\ 36)} = 0.716$, $p = 0.496$; slope: $p = 0.50$; y-intercept: $p = 0.33$).

For monkeys in which a supramaximal current amplitude was delivered, the ciliary process movements were slightly greater for the supramaximal stimulus amplitudes than for the maximal stimulus amplitudes, but the lens movements were similar (Fig. 7). For maximal EW stimulated accommodation, ciliary process movement was 0.029 ± 0.002 mm/D and lens edge movement was 0.026 ± 0.002 mm/D. For supramaximal EW stimulated accommodation, ciliary process movement was 0.033 ± 0.005 mm/D and lens edge movement was 0.024 ± 0.004 mm/D.

Maximum pharmacologically stimulated accommodation was 14.44 ± 1.21 D (Table 2). The temporal ciliary processes moved centripetally a total of 0.411 ± 0.048 mm, or 0.030 ± 0.005 mm/D, and the temporal lens edge moved centripetally a total of 0.258 ± 0.014 mm, or 0.019 ± 0.003 mm/D (Fig. 8). The ciliary processes moved a total of 0.150 ± 0.05 mm, or 0.010 ± 0.004 mm/D, more than the lens edge (paired t-test: $p = 0.05$, df $= 3$).

The circumlental space decreased a total of 0.153 ± 0.050 mm, or 0.011 ± 0.004 mm/D during carbachol stimulated accommodation. In two monkeys (#54 and #58), the circumlental space decreased to nearly zero, with several processes touching the edge of the lens at maximal accommodation (Fig. 5B).

For both supramaximal EW and pharmacologically stimulated accommodation, the ciliary process valleys moved slightly more than the ciliary process peaks (differences $= 0.019 \pm 0.001$ mm and 0.017 ± 0.002 mm, respectively). For supramaximal EW stimulation, the ciliary process peak movement (0.262 ± 0.040 mm) and valley movement (0.289 ± 0.040 mm) was slightly greater than lens edge movement (0.190 ± 0.040 mm) and for pharmacological stimulation ciliary process movements were much greater (ciliary process peaks: 0.411 ± 0.048 mm; ciliary process valleys: 0.420 ± 0.060) than lens edge movements (0.258 ± 0.014 mm) (Fig. 7).

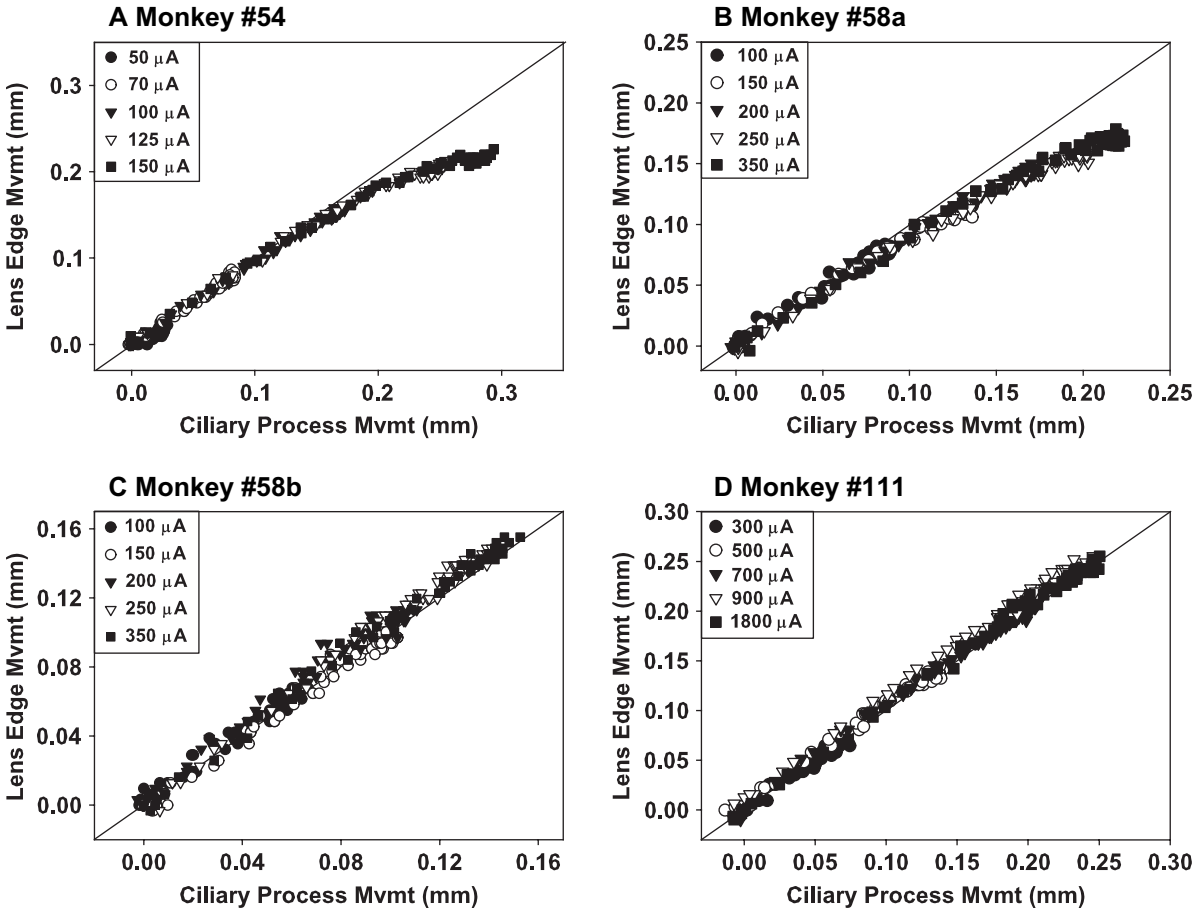

Fig. 4. Ciliary process movement and lens edge movement are similar for low accommodative amplitudes in all monkeys. Ciliary process movement was greater than lens edge movement at high amplitudes in (A) monkey #54 and (B) monkey #58a, and similar at all amplitudes in (C) monkey #58b and (D) monkey #111.

4. Discussion

In this study, movements of the ciliary processes and lens edge were correlated with dynamic changes in refraction to relate accommodative physical and optical changes during EW stimulated accommodation in three rhesus monkeys. The methodology presented here allows these dynamic measures to be correlated for the first time. While previous studies have described centripetal ciliary body and lens edge movements during EW stimulated accommodation in rhesus monkeys, movements have either not been quantified (Neider et al., 1990), or have only been compared to maximum refractive change (Croft et al., 2006a). While the number of monkeys used in the current study is small, the measurements were repeated for many responses and responses of different amplitudes including repeat experiments in the same monkeys. The results are consistent between monkeys and, in agreement with prior such studies in which relatively few monkeys were used, show striking correlations between the accommodative physical changes and the optical refractive changes (Glasser et al., 2006; Vilupuru and Glasser, 2005). The study was intended to determine the optical and physical relationships during the act of accommodation, and this can be achieved with relatively few monkeys. Although EW stimulation is different from the natural input to the EW nucleus during behaviorally elicited accommodation, the EW nucleus provides the parasympathetic innervation to the ciliary muscles, and EW stimulation produces a ciliary muscle contraction and an accommodative response via the same natural neuronal pathways. Understanding how movements of the accommodative structures relate to the dynamic refractive changes is important for modeling the accommodative mechanism (Burd et al., 1999; Schachar et al., 1993), understanding ciliary body and lens displacement in finite element modeling (Judge and Burd, 2002) and relating optical and physical changes during in vitro mechanical stretching experiments (Glasser and Campbell, 1998; Koopmans et al., 2003). Results from this study show that the lens edge and ciliary processes move centripetally with accommodation, and that these changes are linearly related to refractive changes during EW stimulated accommodation. These systematic changes in ciliary process and lens edge movement, along with systematic changes in anterior segment axial biometry (Ostrin and Glasser, 2005; Vilupuru and Glasser, 2003, 2005), and lens diameter (Glasser et al., 2006) show that the anterior segment physical changes are highly correlated with optical changes during accommodation.

To directly see the anterior segment accommodative structures, including the ciliary processes, zonular fibers and lens edge, the iridectomy is necessary. The iridectomy is

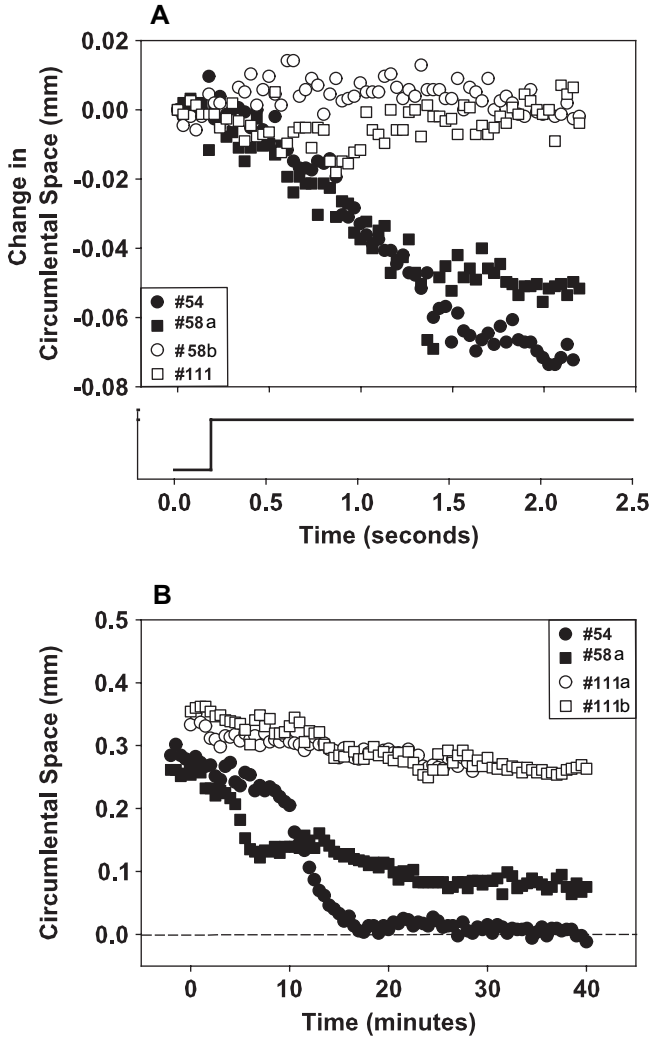

Fig. 5. (A) During maximal EW stimulated accommodation, the circumlental space remained constant in monkeys #58b and #111, and decreased in monkeys #54 and #58a. (B) Circumlental space decreased with time after carbachol iontophoresis. For monkeys #54 and #58, circumlental space decreased to nearly zero.

accomplished by removing the iris at its root without damage to the ciliary muscle or ciliary body (Kaufman and Lütjen-Drecoll, 1975). Previous studies have shown that the removal of the iris does not affect centrally stimulated accommodation, but may reduce accommodation to supramaximal pharmacological stimulation, including carbachol iontophoresis (Crawford et al., 1990). Pharmacological stimulation with the iris present causes a strong iris constriction which may further pull the ciliary body and lens forward, increasing the accommodative refractive change. In iridectomized rhesus monkeys, a forward movement of the entire lens has been demonstrated to occur with pharmacological stimulation that does not occur with EW stimulation, in addition to a higher accommodative response being measured, indicating that a supramaximal pharmacologically induced accommodative response still ensues in the absence of the iris (Ostrin and Glasser, 2005). It is unlikely that iridectomy has affected the results reported here.

Croft et al., report a nasal/temporal asymmetry in the ciliary process and lens edge movement during maximal EW stimulated accommodation in young monkeys, with movements being greater in the nasal quadrant (Croft et al., 2006a). For the one monkey reported here in which

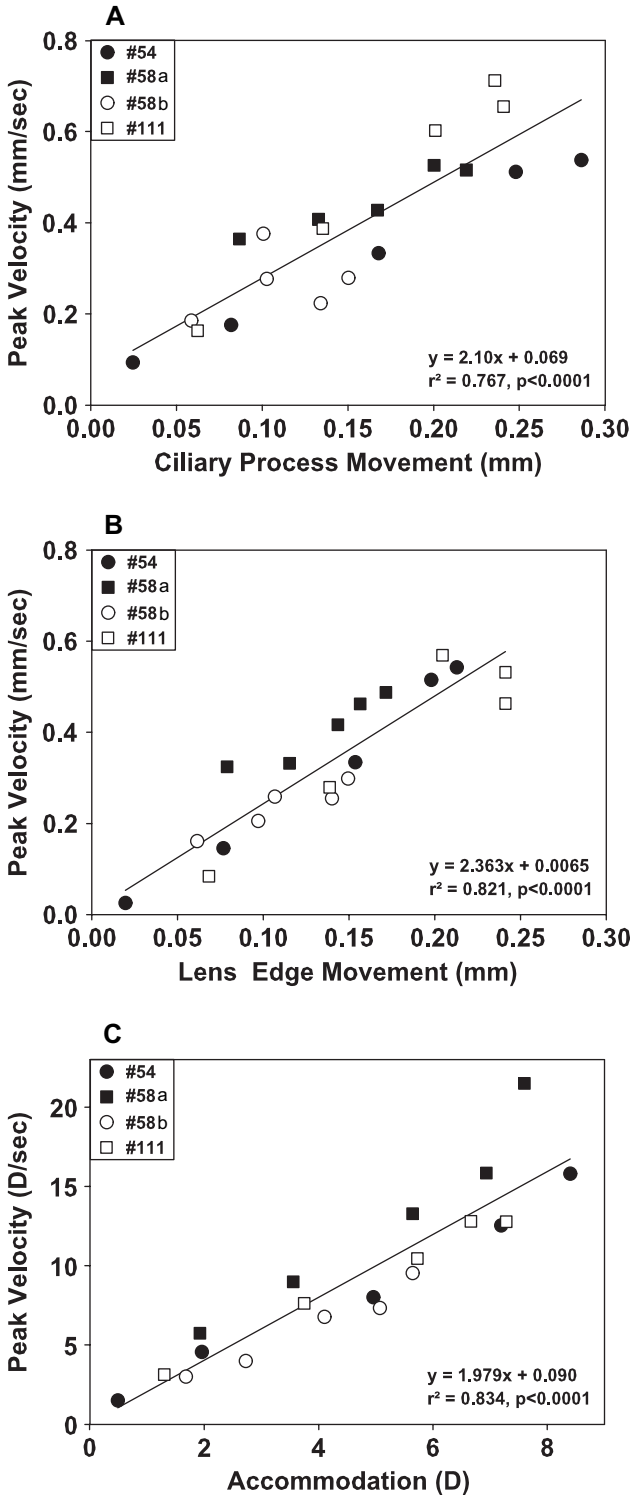

Fig. 6. The slope of the main sequence relationship for (A) ciliary process movement and (B) lens edge movement were not statistically different. (C) Main sequence relationship for accommodation.

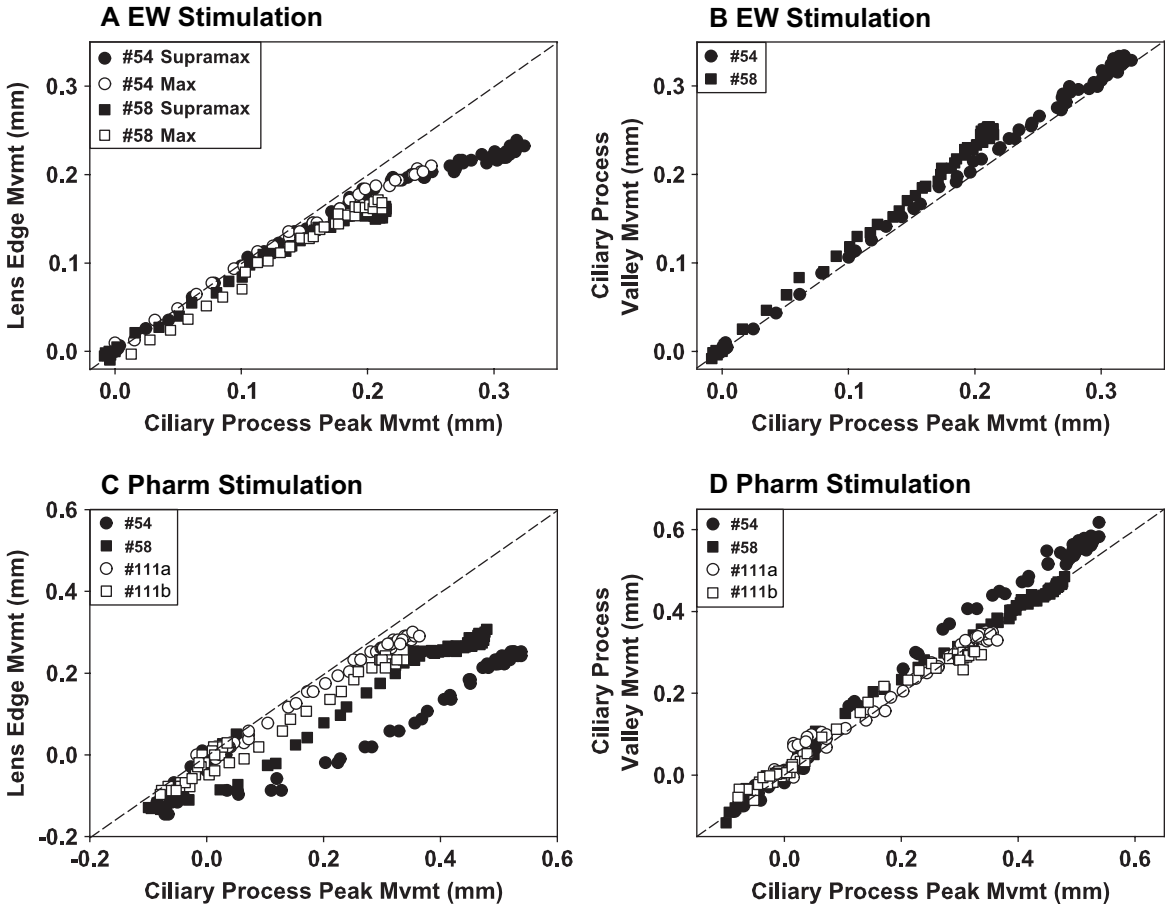

Fig. 7. Ciliary process movement was greater for supramaximal EW stimulation (A, closed symbols) than for maximal EW stimulation (A, open symbols). Ciliary process movement was greater than lens edge movement during (A) supramaximal EW stimulated accommodation and (C) pharmacologically stimulated accommodation. Ciliary process valley movement was slightly greater than ciliary process peak movement during (B) supramaximal EW stimulated accommodation and (D) pharmacologically stimulated accommodation.

measurements were made nasally and temporally, a small but significant asymmetry in the ciliary process, but not lens edge, movement was found. It is unclear whether the slightly larger movement in the nasal quadrant is due to the ciliary body configuration or alignment of the gonioscopy lens with respect to the ciliary process geometry.

It has been suggested that the accommodative movements of the ciliary processes may not track that of the ciliary muscle (Strenk et al., 2005), and the accommodative movements of the ciliary processes may be less than that of the muscle (Strenk et al., 2000). While ciliary muscle movement may exceed ciliary process movement, it is the ciliary processes that anchor the zonular fibers, allowing a release of zonular tension during accommodation. Results from the current study show that movements of the ciliary processes are linearly correlated with the accommodative optical changes resulting from ciliary muscle contraction. The movements of the ciliary process peaks and valleys are similar. The valleys may be more representative of ciliary muscle movement due to the proximity of the valleys to the muscle. There are no in vivo imaging techniques that can clearly distinguish the ciliary muscle from the ciliary body to discern if there are differences in their accommodative movements. Because the ciliary body surrounds the ciliary muscle and the zonular fibers are anchored in the ciliary body, it is not surprising that the ciliary body accommodative movements are well correlated with the accommodative refractive changes.

In this study, the ciliary processes of the two slightly older monkeys (#54 and #58) moved more than the lens edge during maximum EW stimulated accommodation. This difference was even greater when a supramaximal stimulus current was delivered. The greater ciliary process movement than lens edge movement has also been shown in previous studies of older monkeys (Croft et al., 2006a). When the experiment was repeated in one monkey (#58b), this non-linearity was not apparent (Fig. 4C). This is likely due to the lower accommodative amplitude achieved in the second experiment. Accommodative amplitude achieved with EW stimulation in one monkey can vary from one experimental session to the next due to various factors, including level of anesthesia, blood pressure and hydration. It is likely that in the second experiment the accommodative amplitude achieved was not limited by the ciliary muscle and lens movement, but rather due to conditions associated with EW stimulation.

Ciliary process centripetal movement was greater with pharmacological stimulation than EW stimulation, indicating

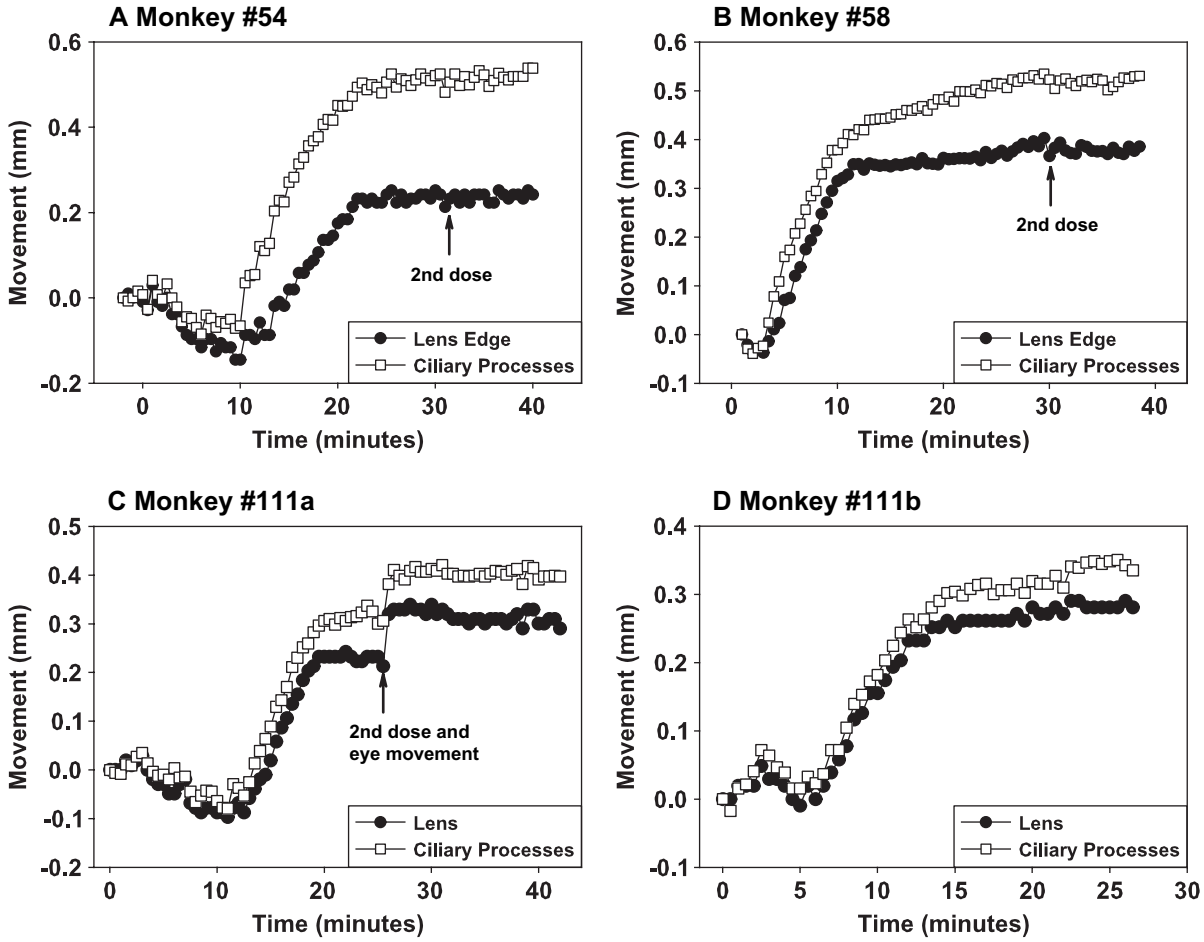

Fig. 8. Ciliary process and lens edge movement with time after carbachol iontophoresis for (A) monkey #54, (B) monkey #58, and (C, D) monkey #111. A second dose of carbachol was delivered (at arrows) after 30 min in monkeys #54, #58 and #111a (C) to ensure a maximal accommodative response had been induced.

that carbachol acted as a supramaximal stimulus. All monkeys had greater ciliary process movement than lens edge movement during pharmacologically and supramaximal EW stimulation. These results suggest, in accordance with prior studies (Croft et al., 2006a), that while the ciliary body accommodative movements may decrease with age, lens movements limit the accommodative optical change in the adolescent monkey eye. The lens edge movement per diopter of accommodative change was significantly smaller with pharmacologically stimulation than with EW stimulation (0.019 mm/D and 0.024 mm/D, respectively), although the accommodative amplitude was greater with pharmacological stimulation. This suggests that pharmacologically induced accommodation is somehow more efficient at producing the optical change as gauged by lens edge movement. It is possible that pharmacologically induced accommodation causes the ciliary muscle to contract and move in a slightly different way than with EW stimulated accommodation. This may cause zonular tension around the lens equator to be released in slightly different ways to increase the efficiency of the accommodative response of the lens. Modeling studies show that changing the distribution of zonular fiber force around the lens equator can impact

Table 2
Accommodative amplitude, temporal lens edge and ciliary process movement 30–40 min after carbachol iontophoresis

	Accommodative Amplitude (D)	Maximum Lens Edge Mvmt (mm)	Lens Edge Mvmt (mm/D)	Maximum Ciliary Process Mvmt (mm)	Ciliary Process Mvmt (mm/D)	Maximum Change in Circumlental Space (mm)	Change in Circumlental Space (mm/D)
#111	15.08 ± 0.07	0.285 ± 0.003	0.019	0.347 ± 0.003	0.023	−0.0627 ± 0.002	−0.004
#111	16.42 ± 0.07	0.229 ± 0.003	0.014	0.314 ± 0.004	0.019	−0.0842 ± 0.005	−0.005
#54	15.33 ± 0.14	0.238 ± 0.003	0.016	0.519 ± 0.004	0.034	−0.2811 ± 0.002	−0.018
#58	10.92 ± 0.22	0.281 ± 0.002	0.026	0.465 ± 0.003	0.043	−0.1839 ± 0.003	−0.017
Average	14.44 ± 1.21	0.258 ± 0.014	0.019 ± 0.003	0.411 ± 0.048	0.030 ± 0.005	−0.1530 ± 0.050	−0.011 ± 0.004

The averages are calculated from all values including the repeat experiments in the same eye. Per diopter changes are calculated by dividing the total movement (mm) by accommodative change (D). Values are mean ± SEM.

the accommodative efficiency, although to a limited extent (Stachs et al., 2006). It is also possible that the greater pharmacologically stimulated accommodative optical change comes, in part, from the forward shift of the lens that occurs with carbachol stimulation (Ostrin and Glasser, 2005). In that study, there was also a slightly smaller increase in lens thickness with pharmacological stimulation compared to maximal EW stimulation, despite the higher accommodative amplitude achieved with pharmacological stimulation.

It has been suggested that centripetal movements of the crystalline lens edge during accommodation are an artifact due to extraocular eye movements (Schachar and Kamangar, 2006), and that in fact the lens edge moves towards the sclera during accommodation. In this study, lens edge movements were measured from a fixed reference mark on the cornea and clearly showed a centripetal movement away from the sclera during both pharmacologically and EW stimulated accommodation, in accordance with previous studies (Croft et al., 2006a,b; Fincham, 1937b; Glasser et al., 2006; Grossmann, 1903; Wilson, 1997). Here, measurements were made on the temporal and nasal sides of the same eye of one monkey during central stimulation, both of which showed an equivalent movement of the lens edge away from the sclera. Despite small eye movements, the accommodative movements demonstrated here, with both EW stimulated and pharmacologically stimulated accommodation, are in accordance with the Helmholtz accommodative mechanism.

The ciliary processes are composed of loose connective and vascular tissue (Tamm and Lütjen-Drecoll, 1996), surrounding the antero-inner apex of the ciliary muscle. The ciliary muscle is composed of three portions – longitudinal, circular and radial that do not extend into the ciliary processes. There are ultrastructural and histochemical differences between the longitudinal and circular portions of the ciliary muscle (Flugel et al., 1990) and some studies suggest that the circular portion is more important in accommodation (Poyer et al., 1994), while others suggest that all portions of the ciliary muscle contract together during accommodation (Lütjen-Drecoll, 2001; Poyer et al., 1993; Rohen, 1982). A contraction of the ciliary muscle serves to carry the surrounding ciliary processes and the zonular fiber anchor points antero-inward towards the lens equator. The ciliary muscle is completely contained within the ciliary body and is not visible with gonioscopy imaging, and so only ciliary process movement was evaluated. The main group of zonular fibers span from the inner walls of the ciliary processes to insert into the capsule around the lens equator (Stachs et al., 2006). From a biomechanical standpoint, it is of interest to understand to what extent the processes may be pulled centripetally by the zonular fibers, or pushed centripetally by ciliary muscle contraction. Accommodative movements of the ciliary process valleys were slightly greater than movements of the peaks during supramaximal EW and pharmacological stimulation. This suggests that with accommodation, the ciliary processes are being "driven" centripetally by the ciliary muscle. The extreme case of this is observed as the ciliary processes actually abut and push against the lens edge at maximal accommodation (Fig. 1D) (Croft et al., 2006a,b). The walls of the processes are essentially the anchor point of the zonular fibers going to the lens edge. Therefore any lens edge movement must be caused by and consequent to ciliary process movement. As the lens edge centripetal movement reaches its maximum, the fact that the processes move more means that they are being driven by the ciliary muscle and that it is this that allows zonular relaxation to allow the capsule to mold the lens into an accommodated form. The greater ciliary process movement than lens edge movement argues against the Coleman catenary theory (Coleman and Fish, 2001). If a pressure differential existed and was a primary force to induce accommodation, zonular tension would never be reduced because the lens would be pushed forward during accommodation by a pressure differential (Martin et al., 2005).

This study has shown that the accommodative ciliary body movement is well correlated with the optical refractive changes in adolescent monkeys. During maximal accommodation, the ciliary body movements are slightly greater than the lens edge movements. With supramaximal EW or drug stimulated accommodation, the ciliary body movements are even greater, but without an increased lens edge movement. The accommodative ciliary body movements release zonular tension around the lens equatorial region to allow the capsule to mold the lens into an accommodated form. Even in adolescent monkeys, there is a greater ciliary body movement than lens edge movement, indicating that ciliary body movement is not the factor limiting the accommodative optical change in the eye. Future studies in older monkeys directed at understanding age related changes in the relationship between accommodative ciliary body movement, lens edge movement and the refractive change will provide information on the age changes in the accommodative mechanism that lead to presbyopia.

Acknowledgements

Thanks to Dr. Ying-Sheng Hu for statistical support and Hope Queener for computer programming assistance. This study was funded in part by NEI grant RO1 EY014651 to AG, NEI grant P30 EY07751 to the University of Houston, College of Optometry, NEI grant #5 T32 EY07024 to the University of Texas Health Science Center at Houston (AG and LO), and Ezell Fellowship from the American Optometric Foundation to LO.

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