Applicability of Infrared Photorefraction for Measurement of Accommodation in Awake-Behaving Normal and Strabismic Monkeys

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Purpose. This study was designed to use infrared photorefraction to measure accommodation in awake-behaving normal and strabismic monkeys and describe properties of photorefraction calibrations in these monkeys.

Methods. Ophthalmic trial lenses were used to calibrate the slope of pupil vertical pixel intensity profile measurements that were made with a custom-built infrared photorefractor. Day to day variability in photorefraction calibration curves, variability in calibration coefficients due to misalignment of the photorefractor Purkinje image and the center of the pupil, and variability in refractive error due to off-axis measurements were evaluated.

Results. The linear range of calibration of the photorefractor was found for ophthalmic lenses ranging from -1 D to $+4$ D. Calibration coefficients were different across monkeys tested (two strabismic, one normal) but were similar for each monkey over different experimental days. In both normal and strabismic monkeys, small misalignment of the photorefractor Purkinje image with the center of pupil resulted in only small changes in calibration coefficients, that were not statistically significant ($P > 0.05$). Off-axis measurement of refractive error was also small in the normal and strabismic monkeys ($\sim$1 D to 2 D) as long as the magnitude of misalignment was $<10°$.

Conclusions. Remote infrared photorefraction is suitable for measuring accommodation in awake, behaving normal, and strabismic monkeys. Specific challenges posed by the strabismic monkeys, such as possible misalignment of the photorefractor Purkinje image and the center of the pupil during either calibration or measurement of accommodation, that may arise due to unsteady fixation or small eye movements including nystagmus, results in small changes in measured refractive error. (*Invest Ophthalmol Vis Sci.* 2009;50:966–973) DOI: 10.1167/iovs.08-2686

Ocular accommodation is the process by which the eye changes its optical power to focus on objects at different viewing distances. Remote infrared (IR) photorefraction, first described by Schaeffel, Howland, and others,[1–3] is now a well validated technique to measure accommodation. The basic principle of IR photorefraction is that when the eye is illuminated by multiple eccentric point-like light sources mounted on a knife edge aperture in front of a video camera lens, it produces an IR light gradient in the pupil that depends on the optical defocus of the eye. The actual refractive error is proportional to the slope of the pixel intensity profile measured across the pupil. The use of photorefraction in different species, including humans, has been described elsewhere.[4–11] An advantage of infrared photorefraction is that it is a remote measurement technique suitable for uncooperative subjects and the subject can be unaware that the measurements are being made. Therefore, this technique is attractive for use in the awake-behaving monkey.

A fundamental issue with infrared photorefraction lies in calibrating the equipment so that reliable measures of accommodation are obtained from the pixel intensity profiles from within the pupil. Studies in animals and humans have generally used ophthalmic trial lens induced defocus to calibrate the photorefraction slopes.[7,12–14] Reports of accommodation measurement in anesthetized animals have sometimes used an independent measure (such as a static Hartinger coincidence refractometer) to calibrate the IR photorefraction measurements.[11]

Our long-term research goal is to measure accommodation dynamically in conjunction with eye movements in awake-behaving normal monkeys and monkeys with strabismus. Previous studies measuring accommodation in awake monkeys have used Scheiner principle optometers[15–18] or dynamic infrared optometers based on streak retinoscopy[19–21] but the working distance of these custom built instruments is typically short, and on-axis alignment of the eye with these instruments is critical for accurate measurements, thus making naturalistic behavioral viewing difficult. This study was therefore designed to examine applicability of infrared photorefraction, a technique developed for remote and unobstructed measurement of the eye, for measuring accommodation in the normal and strabismic monkeys. We specifically examined applicability of the calibration technique commonly used in humans for the awake strabismic monkey. As part of our evaluation of IR photorefraction calibration, we also examined the day-to-day repeatability of photorefractor calibration curves and possible calibration and refraction measurement errors due to misalignment of the center of the pupil and the photorefractor first Purkinje image.

Methods

Subjects and Rearing Paradigms

Behavioral data were collected from one normal monkey (N1) and two strabismic (S1 and S2) juvenile rhesus monkeys (*Macaca mulatta*) weighing 8 to 11 kg. Monkeys with strabismus were reared at the Yerkes National Primate Research Center using an alternate monocular occlusion (AMO) paradigm for the first 4 months of life. The AMO method has been described in detail in our other publications[22,23] and is based on disrupting binocular vision during the first few months of

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life, the critical period during which the monkeys normally develop proper eye alignment, stereovision, and binocular sensitivity in the brain.[24–26]

Surgical Procedures and Eye Movement Measurements

After special rearing, the monkeys were allowed to grow normally until they were approximately 3 to 4 years old before starting behavioral experiments. Monkeys were approximately 6 years old when the current experiments were performed. Sterile surgical procedures carried out under aseptic conditions using isoflurane anesthesia (1.25%–2.5%) were used to stereotaxically implant a head stabilization post. In the same surgery a scleral search coil was also implanted in one eye using the technique of Judge and colleagues.[27] Later in a second surgery, a second scleral search coil was implanted in the other eye for the strabismic monkeys. All procedures were performed in strict compliance with NIH and ARVO guidelines and the protocols were reviewed and approved by the Institutional Animal Care and Use Committee at Emory University.

The monkeys used in this study were well-trained in oculomotor tasks and had been a part of other studies before collecting data for this study. Binocular eye position was measured using the magnetic search coil method (Primelec Industries, Regensdorf, Switzerland).[28,29] Calibration of the eye coil signal was achieved by rewarding the monkey with a small amount of juice or other reward when the monkey looked within a small region (± 2° window) surrounding a 0.25° target spot that was rear projected on a tangent screen 60 cm away from the monkey. All stimuli were under computer control. Calibration of each eye was performed independently during monocular viewing. During the accommodation experiments described here, eye position was continuously monitored to verify that the monkeys were maintaining fixation on the target.

Infrared Photorefraction to Measure Accommodation

The photorefractor consisted of 20 IR LEDs encased in a plastic mount shaped as a semi-circle with a knife-edge aperture. The photorefractor was mounted on a 12.5 mm lens (Pentax C21228TH; Image Labs International, Bozeman, MT) of a camera (DMK21F04; The Imaging Source, Bremen, Germany). The photorefractor-camera setup was visually aligned with the monkey's non-fixating eye at the beginning of each experiment as the monkey monocularly fixated a straight-ahead target projected on a screen in front of the monkey. Because the non-fixating eye was eccentric in the strabismic monkeys, the photorefraction camera, directly aligned with the non-fixating eye, was positioned to the side of the screen. A cartoon of the setup used is shown in Figure 1. For the normal, non-strabismic monkey, a cold mirror angled at 45° was placed in front of the monkey's eye and the camera was placed at 90° with respect to the monkey so that the monkey could continue to fixate the straight-ahead target during image acquisition. The camera to monkey distance was 40 cm for the strabismic monkeys and 45 cm for the normal monkey. The camera was directly connected to a computer (via an IEEE1394 firewire connection) to enable acquisition of the video images. The digital video signal was acquired at 30 Hz using commercial software (IC Capture 2.0; The Imaging Source). Video sequences were saved as avi files and used for subsequent off-line image processing.

Experimental Paradigms, Data Acquisition, and Analysis

For the dynamic accommodation calibration experiments, monkeys viewed a 2° × 2° Maltese cross that was back-projected onto a tangent screen at a distance of 60 cm. During the experiments, one of the eyes was occluded with a visible-block infrared-pass filter. The fixating eye viewed the target through corrective trial lenses to correct for distance refractive error only. For each of the monkeys, baseline distance refractive

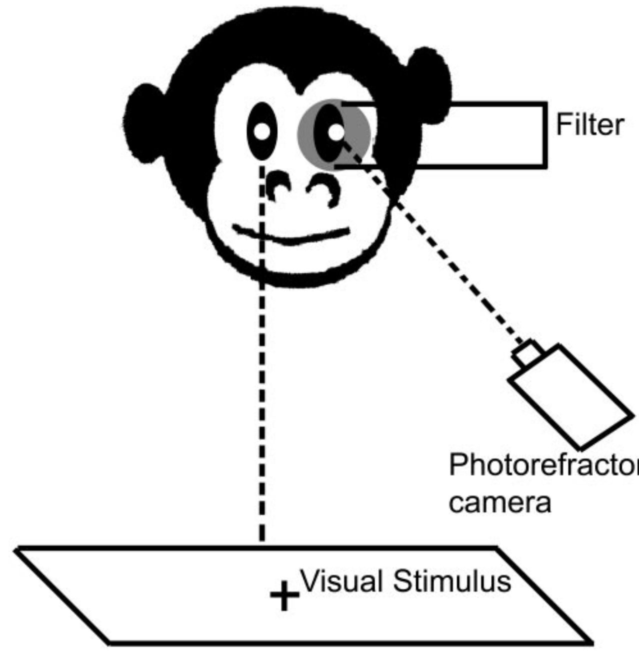

Figure 1. Cartoon illustration showing experimental setup for dynamic measurement of accommodation in a strabismic monkey. The monkey is fixating a target located straight ahead. The eye under cover is eccentric in the orbit. The placement of the photorefractor is such that its Purkinje image is aligned to the center of the pupil of the covered (strabismic) eye.

error had been previously measured by an experienced retinoscopist using streak retinoscopy while the monkeys were anesthetized and cyclopleged. During the calibration runs, trial lenses of powers ranging from −2 D to +5.5 D were placed in front of the eye that was occluded with the IR-pass filter (Optical Cast IR Longpass Filter; Edmund Optics, Barrington, NJ). Therefore, the defocus from the trial lens provides no stimulus to the monkey to alter the accommodative state, but a change in the slope of pupil pixel intensity profile was induced. In experiments to determine whether calibration or measurement of refraction was affected by misalignment between the photorefractor induced first Purkinje image and actual center of the pupil, the fixation target was placed at one of nine horizontal or vertical locations (Left 20°, Left 10°, 0°, Right 10°, Right 20°, Up 10°, Up 20°, Down 10°, and Down 15°) to cause systematic misalignments (with respect to the photorefractor) of the eye being used for the calibration.

Processing of video images was performed offline using custom software developed in Matlab (Mathworks Inc., Natick, MA) that incorporated routines available via the image processing toolbox. Image analysis involved identifying the first Purkinje image in each video frame and drawing two vertical lines within the pupil boundary equidistant on either side of the Purkinje image. A linear regression was performed on the pixel intensity values across the vertical lines and the mean slope of the pixel intensity profile was calculated. The slope of the pixel intensity profile is the measure that has previously been shown to be proportional to the refractive state of the eye. Video clips for each fixation condition were approximately 5 to 10 seconds (150 to 300 video frames) long. These were analyzed with the Matlab code to obtain averages and standard deviations of pixel intensity slope values for each fixation condition. Even though the image analysis was automated, the investigator was able to view each stage of the analysis procedure and thereby visually verify that the automated analysis was appropriate. Finally, a linear regression of the mean pixel intensity slope and the dioptric power of the ophthalmic lens yielded the calibration coefficients that convert photorefraction pixel intensity slope measurements to dioptric values. The calibration coefficients developed could then be used in other experiments where the accom-

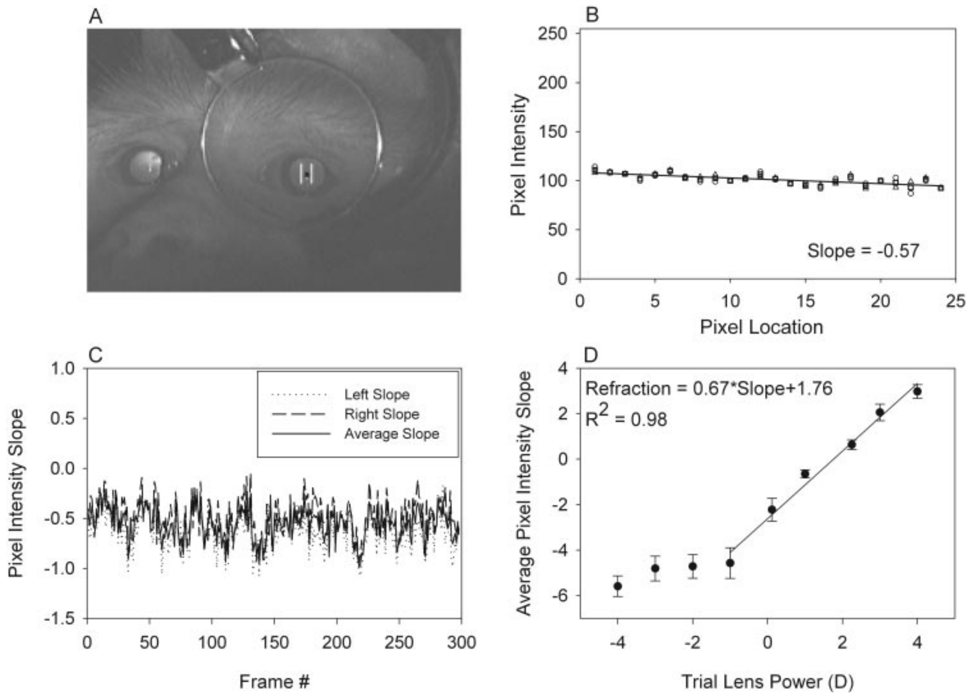

FIGURE 2. Calibration of IR photorefractor in the awake-behaving monkey. (**A**) Single frame of video obtained from a calibration experiment in S1. The monkey is viewing with his right eye and the left eye is covered with an IR-pass filter. A +1 D lens is placed in front of the covered eye. Purkinje image due to the photorefractor is identified by the *black asterisk*. Pixel intensity profiles are calculated on either side of the Purkinje image along the pixels identified by the *vertical lines*. (**B**) Data points indicate pixel intensity values along the vertical lines drawn on either side of the Purkinje image shown in (**A**). Units for pixel intensity are grayscale values that would range from 0 (*black*) to 255 (*white*). A linear regression of the pixel intensity values yields a pixel intensity slope measure that is proportional to accommodation. (**C**) Pixel intensity slopes calculated from each image frame over a 10-s fixation period. Left slope, right slope and average slope denote the pixel intensity slopes calculated from the regression of pixel intensity values along the vertical line on the left of the Purkinje image, right of the Purkinje image, and the average of the two. (**D**) Data points are the mean and SD of the pixel intensity slopes for different lenses placed in front of the covered eye. The linear regression yields calibration coefficients that are used to convert photorefractor pixel intensity slope measurements to dioptric accommodation values.

modative state was manipulated to obtain an accommodative response curve. Statistical analysis of data included using *t*-tests and ANOVA to compare calibration coefficients obtained on different days and compare calibration coefficients obtained during straight-ahead viewing and eccentric viewing. A significance value of 0.05 was used for all comparisons.

RESULTS

Static Ocular Alignment and Visual Capability

The strabismic monkeys in the study were both exotropic. S1 showed an exotropia of approximately 15° during right eye or left eye viewing of a straight ahead target. S2 showed an exotropia of approximately 13° during right eye viewing of a straight ahead target and an exotropia of approximately 18° during left eye viewing of the same target. The normal monkey showed no ocular misalignment. A cyclopegic refractive error was measured under anesthesia for each of these monkeys. The normal monkey had hyperopia of +4 D in both his right and left eyes, S1 was plano (i.e., no refractive error) in each eye and S2 had hyperopia of +4 D in both his right and left eyes. Cylindrical refractive error was small (<1 D) in all the monkeys. Results reported further, unless otherwise specified, were obtained when the monkeys viewed the target through spherical corrective lenses. All experiments were performed at a viewing distance of 60 cm and the evaluations are described relative to the assumed accommodation of 1.66 D due to this viewing distance.

Calibration of Infrared Photorefractor

The calibration method used was similar to that described in previous studies of accommodation in humans.[14] Figure 2 illustrates the calibration procedure in the awake monkey. Panel 2A shows a single video frame obtained in an experiment with the strabismic monkey S1. The monkey is viewing with the right eye and the eccentric left eye is covered with the infrared pass filter. The photorefractor is aligned with the covered left eye. The two pixel intensity profiles along the vertical lines drawn on either side of the Purkinje image visible in panel 2A are plotted in panel 2B along with the average pixel intensity profile. The slope of the mean pixel intensity profile is calculated from the linear regression and this slope is proportional to the accommodative state of the eye. Panel 2C shows the frame-by-frame slope measurements over a 10-second fixation period with a +1 D lens placed over the covered left eye. During calibration, the pixel intensity profile slope is proportional to the power of the trial lens placed in front of the occluded eye. Panel 2D plots the mean and SD of the photorefractor pixel intensity slopes during fixation for the different lenses used during such a calibration run. As can be observed from the plot, there is a linear change in photorefractor pixel intensity slopes for lenses ranging from −1 D to +4 D. Lenses with powers below −1 D did not fall within this linear range. A linear regression of the data from lens powers −1 D to +4 D in this plot yields the calibration coefficients that convert the photorefractor pixel intensity slopes to dioptric changes as would occur during accommodation.

Inter-day Variability of Calibration Coefficients

The next phase of the study was to examine if the calibration coefficients obtained on different experimental days were consistent. Figure 3A–C shows calibration curves for the normal and strabismic monkeys obtained on different experimental days. The slopes and intercepts of the different regression lines was compared using ANOVA and although variation was observed, no significant differences were observed ($P > 0.05$) suggesting that calibration curves generated on different days were similar. The average calibration equations (along with the standard deviations of measurements) for the normal monkeys and the strabismic monkeys are:

$$N1:lens = 1.16(0.21)*slope + 4.88(0.41)$$

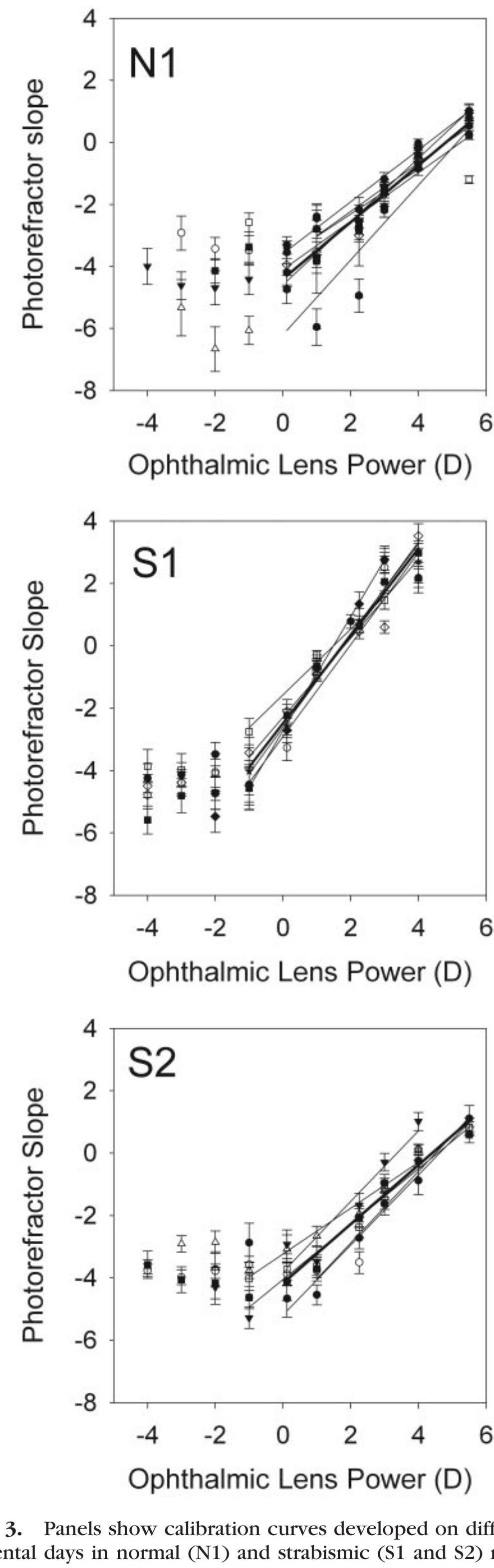

FIGURE 3. Panels show calibration curves developed on different experimental days in normal (N1) and strabismic (S1 and S2) monkeys. The average calibration curve is shown by the *darker line* in each of the panels.

$$\text{S1:lens} = 0.73(0.14)^{*}\text{slope} + 1.71(0.19)$$

$$\text{S2:lens} = 1.03(0.22)^{*}\text{slope} + 4.31(0.52).$$

The calibration coefficients between the different monkeys were significantly different even though the experimental parameters such as distance from camera to eye, camera aperture and visual stimuli were maintained the same.

Calibration Errors Due to Misalignment of Photorefractor Purkinje Image and Pupil Center

We also examined the variability of calibration coefficients that might arise due to misalignment of the photorefractor Purkinje image and the center of the pupil (caused by misalignments of the photorefractor with the eye). We were able simulate this source of error by acquiring data during fixation by the trained monkeys on targets located at known eccentricity. Figure 4 shows calibration curves developed from such eccentric viewing data in the strabismic monkey (S1) up to 20° off axis. Data from the normal and the two strabismic monkeys are summarized in Table 1. A qualitative examination of the data in Figure 4 and Table 1 suggests that there is no systematic variation of calibration coefficients with eccentric viewing.

To identify any statistical differences, we compared the calibration coefficients in the central 10° and the central 20° with the calibration coefficients obtained during straight ahead viewing. This data is summarized in Figure 5. Mean and SD of calibration coefficients during straight ahead viewing (0°) was obtained by combining data from experiments performed on different days. ANOVA comparison showed that neither the normal monkey nor the strabismic monkeys showed statistically significant differences for the central 10° of eccentric viewing or central 20° of eccentric viewing when compared to straight ahead viewing ($P > 0.05$). However, there appeared to be a trend for greater variability in the calibration coefficients for the eccentric viewing conditions (especially for 20° eccentricity) when compared to the straight ahead viewing conditions.

Effect of Misalignment of Photorefractor Purkinje Image and Pupil Center on Refractive Error Measurement

Another source of measurement error of the accommodative state of the monkey is if there were misalignment between the photorefractor and the center of the pupil, post-calibration (i.e., off-axis measurement of refraction). In strabismic monkeys, such misalignment might arise due to drift in the position of the non-fixating eye (due to nystagmus for example). We were able to estimate this error using a subset of the eccentric calibration data described previously. Only data collected as the monkey viewed the central and eccentrically located targets with a close to plano lens (+0.12 D) in front of the covered eye was used for this analysis (i.e., not all the lens conditions as required for calibration). The photorefractor pixel intensity slopes measured under this condition was converted to a dioptric refraction value using the calibration derived when the monkey was fixating the straight ahead target with the photorefractor camera aligned to the center of the pupil. For example, the pixel intensity slope value for fixation on a target that is 10° left of straight ahead in monkey S1 (from Fig. 4) is −1.01. This slope is converted to a refractive error measurement using the straight-ahead (0°) calibration coefficients (from Fig. 4: Slope, 0.55; Intercept, 1.49), resulting in a refractive error measurement of +0.93 D. Ideally, the refractive error measurement should have been +0.12D.

Figure 6 summarizes data from the normal and strabismic monkeys. For target eccentricities of 10° (i.e., misalignment of 10° between the photorefractor and the center of pupil), the errors in measurement of accommodation range from −1.77 D to +0.93 D across all the monkeys (means of absolute value of errors in measurement of refraction N1: 0.99 ± 0.36 D, S1: 0.45 ± 0.44 D, S2: 0.97 ± 0.63 D). For eccentricities of 20°, the errors in measurement of accommodation were larger (means

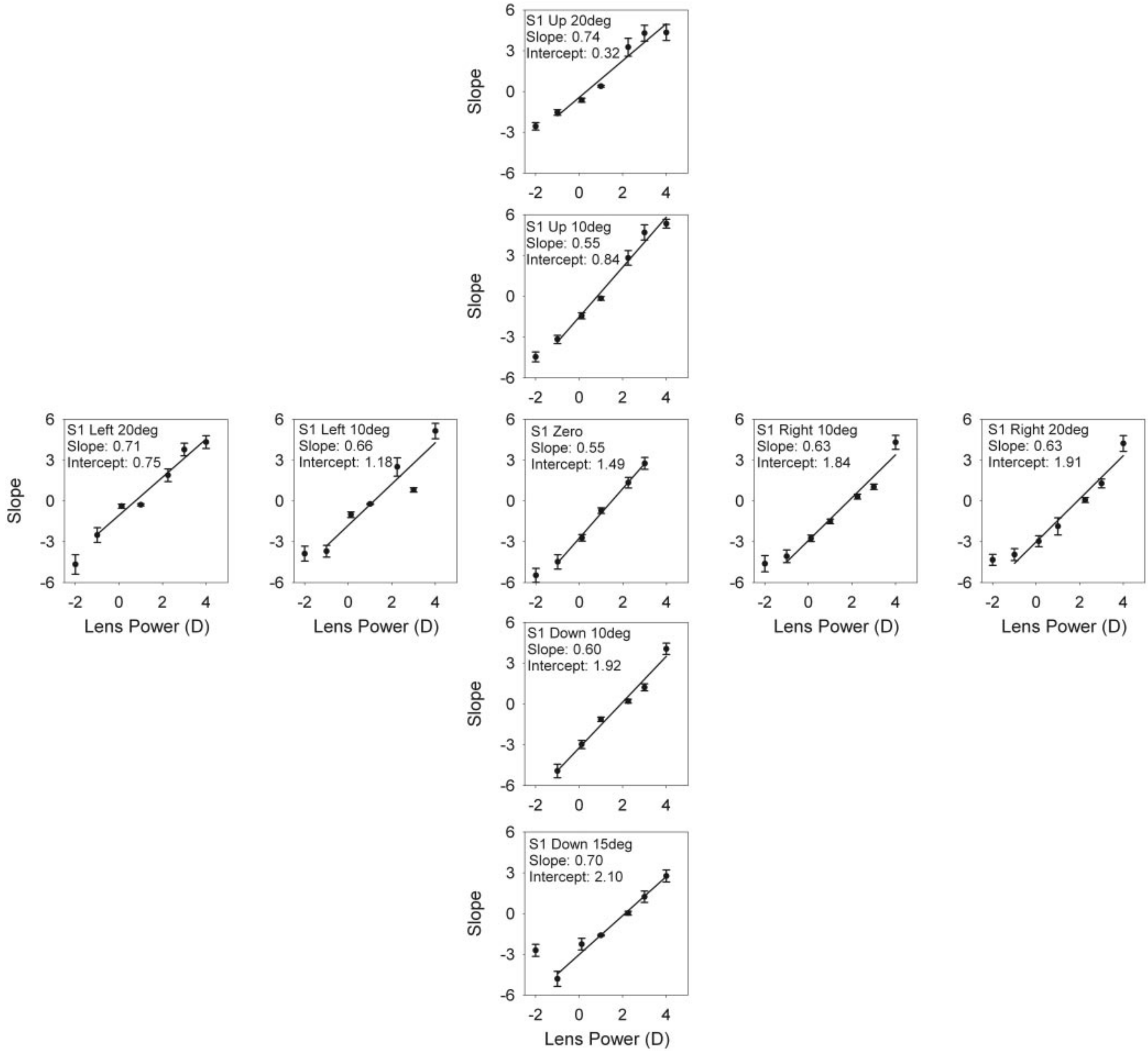

FIGURE 4. Individual panels show the calibration curves developed during experimental runs in which the strabismic monkey, S1, fixated central (0°) or eccentric targets (Left 20, Left 10, Right 10, Right 20, Up 10, Up 20, Down 10, Down 15°). The calibration coefficients (slope and intercept of the regression) are printed in each plot. Neither the normal monkey nor the strabismic monkeys showed significant differences in the linear range and in the calibration coefficients for 10° of misalignment between the Purkinje image and the center of the pupil.

of absolute value of measurement errors N1: 1.29 ± 0.75 D, S1: 0.70 ± 0.59 D, S2: 0.85 ± 0.21 D). We also examined the data to consider whether there was any systematic change in off-axis refractive error measurements as the eccentricity varied from left to right or from up to down. However, we did not observe any such relationship that was consistent across the monkeys (data not shown).

DISCUSSION

We have shown that remote infrared photorefraction can be successfully used to measure refraction and accommodation in awake-behaving monkeys. In this study we have focused on issues related to calibration of the IR photorefraction method for its use in awake monkeys. Below, we discuss our results pointing out particular challenges when studying control of accommodation in the awake strabismic monkey.

Ophthalmic Lens Method to Calibrate IR Photorefractor

We have been able to adopt an ophthalmic trial lens method used in human studies, to calibrate the IR photorefractor for measuring accommodation in awake-behaving monkeys. The results of the calibration procedure produced qualitatively similar data as those reported in the human work[14] and in anesthetized monkey studies.[11] Thus the calibration procedure yields a linear relationship between the slope of the pupil intensity profiles and refractive error within a certain range of refractive error. Previous studies in anesthetized monkeys by Vilupuru and Glasser[11] have shown that IR photorefraction

TABLE 1. Effect of Misalignment between the Photorefractor Purkinje Image and the Center of the Pupil on Calibration Coefficients

Target Eccentricity	N1		S1		S2	
	Slope	Intercept	Slope	Intercept	Slope	Intercept
Zero	1.27	4.86	0.55	1.49	0.85	3.35
Left 10	1.03	4.78	0.66	1.18	1.14	4.10
Left 20	1.28	5.15	0.71	0.75	0.88	3.91
Right 10	0.93	4.69	0.63	1.84	0.76	2.89
Right 20	1.82	4.95	0.63	1.91	0.53	2.82
Up 10	1.14	3.99	0.55	0.84	0.60	4.07
Up 20	1.15	3.23	0.74	0.32	0.70	3.60
Down 10	1.19	4.37	0.60	1.92	1.05	2.37
Down 15	1.32	4.11	0.70	2.10	0.75	1.65

calibration curves are nonlinear in some situations beyond a certain range and we found the same in our experiments with the awake monkey. The linear range from −1 D to +4 D ophthalmic trial lenses used for calibration represents a range from mild hyperopic toward increasingly myopic refractive errors. For the kinds of accommodation experiments to be done with strabismic monkeys, this is a sufficient and desirable range of refractive states; therefore, the non-linearities and saturation that occurs for higher powered minus lenses is not critical for this study or for future studies that will examine control of accommodation in the strabismic monkeys. An emmetropic eye accommodating accurately to a target at 60 cm would exhibit a refraction of −1.66 D with respect to infinity. A photorefractor at 40 cm from an emmetropic eye viewing a distant target would "see" 2.5 D of hyperopia. Therefore, an emmetropic eye accurately accommodating to a stimulus at 60 cm as viewed with a photorefractor at 40 cm from the eye should show +0.84 D of hyperopia. Trial lens powers from −1 to +5 D held in front of the eye would therefore represent the range of refractive errors of +1.84 D to −4.16 D. It is possible that measuring baseline refraction under cycloplegia might have resulted in a more hyperopic refractive error measurement than the actual refractive error during behavioral fixation. However, the effect of placing slightly higher power corrective lenses in front of the viewing eye is unlikely to have significant effects on calibration coefficients determined for the photorefractor aimed at the covered eye. Testing in the awake-behaving monkeys was not performed under cycloplegia because the intention was to simulate natural behavior. Furthermore, the monkeys were fixating on targets at a distance closer than optical infinity, so cycloplegia would be undesirable. Finally, cycloplegia would have caused dilation of pupils which once again would have been likely to disrupt the natural behavior of the monkeys.

One requirement for the calibration technique to work successfully is cooperation from the subject in fixating on the target with the uncovered eye. The monkeys that were a part of this study were all trained to perform oculomotor tasks, so reliable fixation performance was not a problem. We also monitored the eye movements continuously using implanted search coils and so were able to verify that the monkey was fixating the target during data collection. As far as methodology was concerned, calibration of the strabismic monkeys did not present any additional problems compared to the normal. For these experiments, we aligned the photorefractor with the covered eye. If it were necessary to measure accommodation in both eyes of such strabismic monkeys, it may be necessary to use two photorefractors (one aimed at each eye) with a shield between the eyes to block stray infrared light from the contralateral photorefractor. We believe this will be possible with the cooperation that is achieved with trained monkeys. Discussion of the degree of precision necessary for alignment is presented later.

Inter-day Variability in Measurement of Calibration Coefficients

As part of our evaluation of the technique, we repeated the experiment on several different days and compared calibration curves. Our basic finding was that the calibration curves generated on different days were not significantly different. We were careful to maintain critical factors such as camera distance, camera aperture, power to the photorefractor LEDs, room illumination, and position and size of visual stimulus the

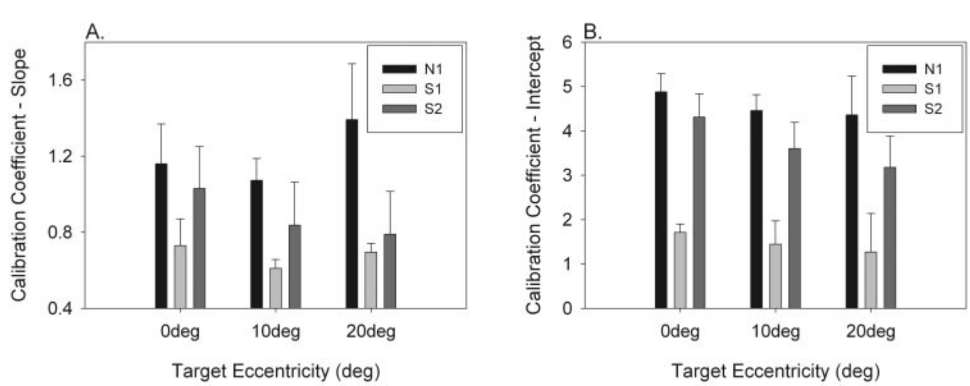

FIGURE 5. Summary data from the normal and strabismic monkeys showing variation in calibration coefficients (**A**) slope, (**B**) intercept, due to misalignment between the photorefractor Purkinje image and the center of the pupil. (0deg) Data obtained when the animal is fixating a straight ahead target and the Purkinje image and the center of the pupil are aligned. (10deg) Data obtained when the monkey is fixating a target that is eccentric by 10°, therefore simulating a 10°-misalignment between the Purkinje image and the center of the pupil. All target eccentricities of 10° (Right 10, Left 10, Up 10 and Down 10) or 20° (Right 20, Left 20, Up 20, Down 15) were averaged to enable statistical comparisons. Statistical analysis using ANOVA showed that there were no significant differences ($P > 0.05$) for 10° or 20° of misalignment though there was a trend for larger differences between the 0° and 20° calibrations compared to the 0° and 10° calibrations.

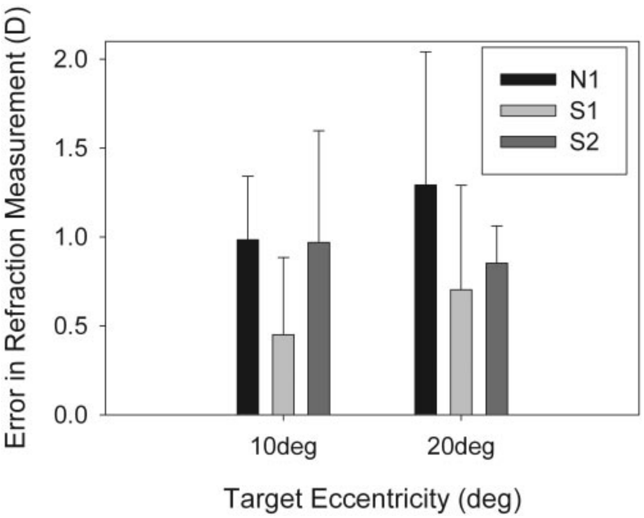

FIGURE 6. Summary data from the normal and strabismic monkeys showing differences in refractive error measurements due to misalignment between the photorefractor Purkinje image and the center of the pupil. Data was obtained when the monkey was fixating eccentric targets with a 0.12 D lens placed over the covered eye. Pixel intensity slope measurements were converted to dioptric refractive error values by using calibration coefficients determined from straight-ahead fixation data. Off-axis measurements of 10° resulted in values for refractive error of approximately 1 D. Off-axis measurements of 20° tended to be larger.

same over the different experimental days. During our evaluation, we found that on occasion the range of mean pixel intensity slopes for the different lenses used for calibration was small yielding calibration curves with slopes close to zero. It was not immediately apparent what might have happened on those particular days, but such poor calibration curves were readily identified and could be excluded from analysis. We also found that the calibration curves, though consistent for the same monkey, were different across the monkeys. This result confirms data presented by Vilupuru and Glasser[11] that showed that calibration curves generated in different anesthetized monkeys are different. Data from human subjects also show variation in calibration curves across individual subjects.[14] Schaeffel and colleagues[14] suggested that these calibration differences may be a function of difference in pupil size across subjects and/or difference in reflectivity of the retina for the IR illumination. The same may be true in our monkeys. We did not try to pursue the source of these calibration coefficient differences across the monkeys because developing a calibration curve specific for each monkey was relatively simple to do. We also observed that the inter-day variability of calibration coefficients appears to be higher in one of the strabismic monkeys (S2) compared to the other monkeys. It is not clear why this should be the case, but imprecise moment-by-moment control of accommodative state (i.e., greater variability in accommodative state in the viewing eye) could be a reason. For the current experiments, we did not monitor the accommodative state in the viewing eye and so it is unclear if this is the source of the variability in this monkey. Examining the corresponding changes in vergence angle could be used as an indirect method to assess whether fluctuations in accommodation observed were due to true variations in accommodative state in the fixating eye, but this method may not be appropriate for use in the strabismic monkeys because the link between vergence and accommodation may be disrupted.

Calibration Errors Due to Off-Axis Measurement of Refraction

A specific issue that we addressed in this study was errors in calibration due to misalignment between the photorefractor Purkinje image and the center of the pupil. This issue is relevant to our research because of the eccentric position of covered eye in the strabismic monkeys. It is often difficult to maintain precise alignment between the Purkinje image and the center of the pupil in strabismic subjects (be they animal or human) because of a number of factors including small drifts in the strabismic eye, possible nystagmus, and changes in the strabismus angle that may occur over time or possibly even during the course of an experiment. Fortunately, our data suggest that calibration curves are relatively consistent within 10° of misalignment between the Purkinje image and the center of the pupil. Misalignment of even 20° may be tolerated, but we found that, in the monkey, we ran into other problems such as the Purkinje image being too close to the edge of the pupil or that the eyelids covered a significant portion of the pupil.

Measurement Errors Due to Off-Axis Measurement of Refraction

Even though calibration errors for small misalignment between the Purkinje image and the center of the pupil may be small, errors due to off-axis measurement of refraction may be an issue.[9] Once again the strabismic monkey poses specific problems (such as drifts or nystagmus for example), that a normal monkey or human may not generate. Off-axis measurement errors may also present a problem when attempting to study accommodation changes during eye movements. To avoid this error and maintain precise alignment between the Purkinje image and the center of the pupil during eye movements, one could use current eye position information to dynamically change camera position or the calibration function applied. However our data show that for small magnitudes of off-axis measurement (i.e., $<10°$), the likely errors in refraction measurements is relatively small (on the order of ~1 D to 2 D). Certainly this difference may not be a measurement error per se; it may simply reflect the actual refractive error (may be related to eye optics) when measured off the principal axis of the eye. However for the purposes of assessing accommodative control in the normal or strabismic monkey, we can conclude that off-axis measurements produce relatively small changes in the refractive error measurement.

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