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Reproducibility of carbachol stimulated accommodation in rhesus monkeys

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ABSTRACT

Approaches are being explored to restore accommodation to the presbyopic eye. Some of these approaches can be tested in monkeys by stimulating accommodation in various ways including using carbachol iontophoresis. Knowledge of the repeatability of carbachol iontophoresis stimulated accommodation in the monkey phakic eye is necessary to understand the variability of this method of evaluating accommodation. Data from 9 to 10 separate carbachol iontophoresis experiments performed on phakic eyes from 8 monkeys were retrospectively analyzed. For each experiment, carbachol was applied iontophoretically to the eyes of anesthetized monkeys and refraction generally measured every two minutes until accommodation reached a plateau. Repeated experiments were performed in each monkey over periods ranging from 10 to 18 months. Maximum accommodation measured for each monkey ranged from 11.1 D to 18.3 D with standard deviations from 0.8 D to 2.1 D and differences in accommodative amplitude varying from 2.2 D to 7.5 D. Time to reach maximum accommodation ranged from 18 to 64 min in individual experiments. Averaged time-courses indicate that maximum accommodation is generally achieved between 10 and 20 min after carbachol administration. Although carbachol iontophoresis is considered a reliable method to stimulate maximum accommodation in anesthetized monkeys, the amplitude achieved typically varies by more than 2 D. Presbyopia treatments evaluated in this way in phakic monkeys would need to show an increase in accommodation of over 2 D to clearly demonstrate that the treatments work when being tested with carbachol iontophoresis stimulation.

1. Introduction

Numerous approaches are being investigated to restore accommodation to the presbyopic eye (Ben-Nun and Alió, 2005; Eskridge, 1972; Glasser, 2008; Koopmans et al., 2006; Krueger et al., 2005; Lubatschowski et al., 2010; Nishi et al., 2008, 2009; Reggiani Mello and Krueger, 2011; Ripken et al., 2008; Schor, 2009; Schumacher et al., 2009; Sheppard et al., 2010). The effectiveness of such procedures can be tested in anesthetized rhesus monkeys by stimulating and objectively measuring the accommodative response. In the case of an accommodative intraocular lens (AIOL), a successful outcome would be achieving significant objectively measurable accommodation. However, if testing is done in monkeys of an intervention which is directed at restoring or enhancing the accommodative ability of the phakic eye, such as femtosecond laser photodisruption of the crystalline lens, it is imperative to know the variance of the accommodative response that is stimulated and measured so it is clear if the treatment is effective or not. Efforts aimed at restoring or enhancing phakic accommodation may only show modest gains. To understand if these procedures increase accommodative amplitude when tested in rhesus monkeys, it is important to know by how much the accommodative amplitude must be increased to unequivocally conclude that the treatment was effective. This depends on the reliability of both stimulating and measuring accommodation in anesthetized monkeys and the variance of the measured accommodative responses.

Accommodation can be produced in anesthetized rhesus monkeys by central stimulation of the Edinger-Westphal (EW) nucleus. However, surgically implanting EW stimulating electrodes and performing the experiments is demanding, costly and requires specialized dedicated laboratory equipment. Accommodation can be more readily produced in anesthetized monkeys with pharmacological stimulation using cholinergic agonists such as pilocarpine and carbachol. Topical application of pilocarpine eye drops produces widely variable accommodative responses (Wendt and Glasser, 2010). The reasons for this variability are unclear, but likely include variable absorption of the drug through the cornea. Although this method has been used for testing accommodation restoration concepts (Ben-Nun and Alió, 2005; Haefliger and Parel,

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1994; Nishi et al., 1992, 1993; Nishi and Nishi, 1998), because of the variability it is ill suited for assessing accommodative amplitude. Carbachol stimulated accommodation has been used extensively in anesthetized monkeys (Koretz et al., 1987; Vilupuru and Glasser, 2002). Carbachol is applied iontophoretically by touching agar gel containing the drug directly to the cornea and applying a current to carry the charged carbachol ions into the anterior chamber. Carbachol application is followed by regular and repeated measurement of the accommodative response over time; once accommodation reaches a plateau, carbachol is delivered again and refraction measured repeatedly over time until no further increase in accommodative response is observed. This method is designed to ensure that maximum accommodation is achieved.

If accommodation restoration procedures are tested in rhesus monkeys, an enhancement in maximum accommodative amplitude due to the interventions must be measurable and detectable. Such testing in monkeys may require performing the same intervention on many monkeys or doing repeated tests on fewer monkeys to collect data suitable for statistical analysis. However, due to ethical considerations and the relatively high costs involved, it is desirable to use as few monkeys as possible. Determining the efficacy of an intervention in relatively few monkeys might take many weeks because several accommodation experiments may be necessary both pre-treatment and post-treatment to get data suitable for statistical analysis. Carbachol iontophoresis stimulated accommodation experiments provide only a single response because the eye will not return to the unaccommodated state until the carbachol is naturally washed out of the eye (or pharmacologically reversed). The carbachol stimulated accommodative response may take 30 min to plateau, may endure for an hour or more and may take several hours to wash out completely. Once such an accommodation experiment has been performed, it is necessary to wait at least several days before the eye can be considered to be completely drug free before the experiment can be repeated. The time interval between experiments is also constrained by how frequently monkeys can be anesthetized. Despite the drawbacks associated with being able to induce only a single accommodative response and the lengthy time required for multiple experiments, carbachol iontophoresis remains one of the most accessible methods to stimulate accommodation in anesthetized rhesus monkeys.

No prior studies known to the authors have demonstrated the repeatability (precision) of the maximum carbachol stimulated accommodation measured in anesthetized monkeys. In this study, a retrospective analysis has been performed of data from multiple experiments from phakic eyes of eight monkeys used in repeated carbachol stimulated accommodation experiments. Accommodative response amplitudes from four of these monkeys have been reported previously in a study assessing polymer refilling for restoring accommodation (Koopmans et al., 2006). The retrospective analysis described here was undertaken to determine the precision of carbachol iontophoresis stimulated maximum accommodation from repeated experiments in the same eyes.

2. Methods and materials

2.1. Animal preparation

All experiments conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were performed in accordance with institutionally approved animal protocols. Experiments were performed on the right eyes of eight rhesus monkeys (*Macaca mulatta*) ranging in age from four to eight years with an average age of 5.2 ± 0.9 (mean ± SD) at the time of the first experiment on each monkey. Each monkey had previously undergone total iridectomy. Monkeys were sedated with 15 mg/kg ketamine and 0.5 mg/kg acepromazine (Phoenix Pharmaceutical, St. Joseph, MO) and then anesthetized with either repeated boluses of 10 mg/kg ketamine or an initial intravenous bolus of 1.5 mg/kg propofol (PropoFlo, Abbott Laboratories, North Chicago, IL) followed by continuous i.v. propofol infusion at a rate of 0.5 mg/kg/min. Anesthetized monkeys were held prone with the head held upright and facing forward in a head-holder. Pulse rate, SpO_2, and temperature were monitored and the monkey was wrapped in a water heated blanket to maintain body temperature. Eyelids were held open with a speculum and a contact lens was placed on the cornea. The contact lenses were not intended to correct for refractive errors in these experiments, but were used solely to prevent dehydration of the cornea. When performing accommodation experiments on anesthetized monkeys, the baseline refraction is relatively unimportant. Contact lenses were 11 mm in diameter, rigid PMMA with base curves of 6.5 or 6.75 mm. These two base curvatures are generally suitable to fit all the monkeys in the colony. With the contact lenses on, different monkeys will have slightly differing refractions. These contact lenses had a range of powers from −3 to 0 D to meet the requirements of unrelated experiments for which the contact lenses were originally designed.

2.2. Carbachol iontophoresis

40% Carbachol (Sigma, St. Louis) was pre-prepared in 2% agar gel, in 0.5 ml microcentrifuge tubes, and stored frozen until the day of each experimental session. For each experiment, a tube of carbachol was thawed and sliced into 3 cylindrical sections using razor blades in a purpose designed cutter. The agar gel in a section was pushed to protrude slightly out of its tube to allow good clean contact of the gel with the cornea and pinned in this position with an 18G needle through the tube wall and the agar. A 22G needle was inserted through a pinch of skin above the brow of the monkey. The cathode and anode, respectively, of a battery source designed to deliver 80 μA of current were attached to the two needles. The contact lens was removed from the eye. The initial carbachol dose was delivered by touching the agar to the nasal and temporal sides of the cornea with the current turned on for eight seconds each. After the accommodative response reached an asymptote (see below), a second dose of carbachol was delivered for four seconds on each side of the cornea. After each carbachol delivery, the eye was irrigated with saline and the contact lens replaced. All measurements were performed with the contact lenses on the eyes.

2.3. Carbachol time-course

Refraction was measured with a Hartinger coincidence refractometer (Zeiss, aus JENA) which is self-calibrated requiring no external calibration and has been verified to be accurate over the full range of +18 D to −38 D. Measurements were made three times in rapid succession (over a 10–15 s period) for the baseline and repeatedly three times each in rapid succession for each subsequent time-point after carbachol was administered. After each individual measurement, the Hartinger refraction dial was arbitrarily adjusted to purposefully misalign the Hartinger mires so as to ensure independent measurements. After carbachol delivery, refraction measurements were generally repeated at two minute intervals until refraction stopped changing, at which point the second carbachol dose was delivered. Measurements resumed every two minutes until no further change in refraction for three successive sets of three repeated two minute measurements (i.e.,

over a period of approximately 6 min). At this last time-point, maximum accommodation in each experiment was assumed to have been reached. A single mean refraction was calculated from each set of three successive measurements at each time-point. Maximum accommodation was calculated as the average baseline refraction minus the average refraction from the means of the three most accommodated time-points. Due to variations in measured refraction, these three most accommodated measurements are not necessarily the last three time-points. Experiments were repeated nine or ten times for each monkey over periods ranging from 10 to 18 months.

3. Results

The intra-experiment rates of carbachol stimulated accommodative responses were variable as were the shapes of the time-course curves (Fig. 1). Most time-course plots show similar trends with accommodation rising initially and reaching a plateau at between 10 and 20 min after carbachol iontophoresis and then either leveling off or increasing at a much slower rate thereafter. Time to reach maximum accommodation varied from 18 to 64 min. In four experiments, the initial carbachol dose resulted in only a small accommodative response indicating ineffective application of carbachol to the cornea or poor delivery of the drug into the eye. In these cases, second carbachol doses achieved accommodative responses between 81% and 103% of all other experiments from the respective monkey. The second dose significantly increased the accommodative response in 60.7% of all experiments (unpaired student's t-test, $p < 0.05$). In 10.1% of the experiments, accommodation paradoxically showed a significant decrease after the second dose of carbachol.

For each monkey, the time-course of each carbachol stimulated accommodative response was variable (Fig. 1). While most Hartinger measurements were recorded at 2 min intervals, there were 4 early experiments in which measurements were recorded at odd minutes (3 sessions with monkey 112 and one with monkey 114). These-time series data were linearly interpolated using Matlab (Fig. 1C and D) to obtain values at even minutes which could then be averaged in the same way as the rest of the data. The number of data points used to calculate the average response shown in Fig. 1C and D at each time-point is shown in Table 2. Later portions of the average time-courses are derived from fewer experiments because not all experiments lasted the same duration and the termination point of each experiment was determined by when the response reached an asymptote. The average time-course plots all show similar trends with accommodation rising initially and reaching a plateau at between 10 and 20 min after carbachol iontophoresis and then either leveling off or increasing at a much slower rate thereafter.

Average baseline refraction through the contact lenses for individual monkeys ranged from −0.9 D to +7.0 D while average maximum accommodation for individual monkeys ranged from 11.1 D to 18.3 D (Table 1). Baseline refraction measurements from one experiment to the next generally showed less variability than maximum accommodation or most myopic refraction as indicated by the standard deviations and ranges in Table 1. For baseline refraction measured through the contact lenses, standard deviations in individual monkeys ranged from 0.6 D to 1.5 D with differences of baseline refractions for individual monkeys varying from 2.1 D to 5.2 D. ANOVA analysis for each individual monkey determined that the maximum accommodation achieved across repeated measurements for each monkey showed significant

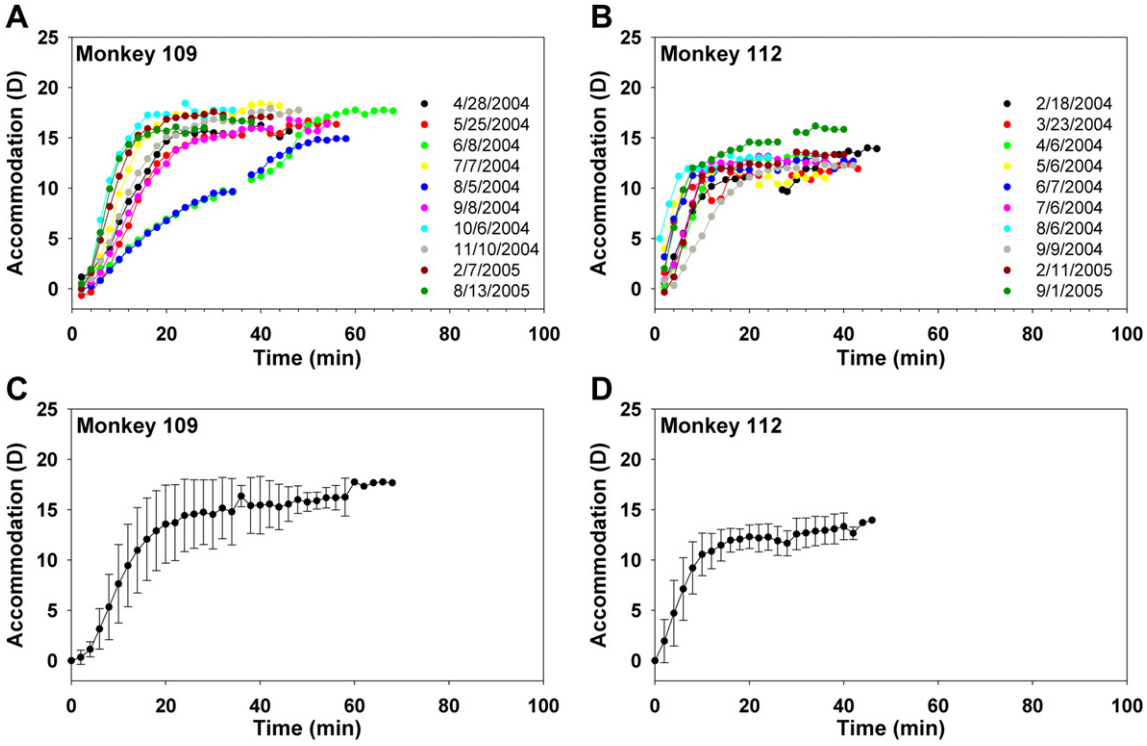

Fig. 1. (A and B) Individual carbachol time-course data for two monkeys. Some time-points were missed because the second carbachol doses were being administered. Data for different experiments are in different colors. (C and D) Average time-courses for the same two monkeys. Error bars are ±1 standard deviation at each time-point. No error bars are present where a single data point contributed to the average time-course. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Mean, standard deviation, and range of baseline refractions (with contact lenses on) and of maximum accommodative amplitude from each monkey over the course of all experiments.

	Monkey	64	73	99	109	112	114	115	119
Baseline Refraction	Mean (D)	−0.9	+3.1	+1.6	+5.3	+4.1	+7.0	+5.1	+4.5
	Standard Deviation (D)	0.8	1.3	1.1	0.8	0.6	0.6	1.5	1.2
	Range (D)	3.0	4.0	3.8	2.9	2.1	2.1	5.2	4.4
Maximum Accommodation	Mean (D)	18.3	15.5	17.2	17.0	13.3	11.5	15.5	11.1
	Standard Deviation (D)	2.1	1.6	0.8	1.0	1.1	1.1	1.7	1.2
	Range (D)	7.5	4.8	2.2	3.4	3.9	3.3	5.6	4.3

differences ($p < 0.0001$ for all monkeys). Standard deviations of maximum accommodation achieved in individual monkeys ranged from 0.8 D to 2.1 D with differences in accommodation within individual monkeys varying from 2.2 D to 7.5 D. There was no systematic variation in accommodation, baseline refraction or minimum refraction over time between experimental sessions (Fig. 2). It is clear that some of the variations in accommodative response were due to variations in baseline refraction (see for example the third session with Monkey 119 in Fig. 2).

Since two different anesthetics (propofol and ketamine) were used, the data for each monkey were analyzed for differences between the anesthetics. Data points from experiments in which propofol was used are circled in Fig. 2. The choice of anesthetic was not distributed systematically. Four monkeys had at least two experiments with each anesthetic making *t*-tests possible. An unpaired student's *t*-test was used because the experiments were not inherently paired in any way resulting in unequal numbers of experiments in most cases. The *p*-values were $p = 0.319$ for monkey 73, $p = 0.574$ for 99, $p = 0.014$ for 114, and 0.744 for monkey 119. Only the response amplitudes from monkey 114 showed a significant difference between anesthetics ($p < 0.05$).

Because the time-course for each experiment varied, the data were re-calculated with time normalized to a maximum of one (Fig. 3). The time normalized data from each experiment for each monkey were resampled using linear interpolation so all experiments for each monkey had the same number of data points at the same normalized time intervals. The interpolated accommodative values at each interval were then averaged to create an average normalized time-course graph for each monkey. The mean maximum response in these graphs (Table 3) is the average of all the individual asymptotes and therefore represents more robust average maximum accommodation and standard deviations than those obtained from the raw data. The average time-course data were fit with the Chapman equation, $y = A(1 - e^{-bx})^c$, where A is maximum response amplitude, b is the time constant, x is time and c is a constant. This equation was visually verified by examination of the residuals to fit the data well for all monkeys and yielded r^2 values between 0.995 and 0.999. The amplitude, A, from this equation provides an alternative measure of maximum accommodative amplitude for each monkey that can be compared to the three maximum values recorded in each experiment shown in Fig. 2 and Table 1. However, the amplitude from the Chapman equation provides only a single amplitude from all experiments as opposed to a maximum amplitude for each experiment. A way to estimate the variance of the individual experiments is to calculate the RMS difference of each data point from the Chapman equation (Table 3). These RMS values ranged from 1.27 to 2.31.

Since the experiments were conducted over a maximum time period of 18 months, possible effects of aging on accommodative response amplitude over this time period were explored. The maximum accommodative response data were plotted as a function of age using two different methods. All of the data from each monkey were plotted as a function of age at the time when each experiment was performed (Fig. 4A) and the accommodative responses and age of each monkey over all experiments were averaged for each monkey (Fig. 4B). In neither case was there a significant relationship between maximum accommodation and age ($p = 0.158$ for all data and $p = 0.533$ for the averaged data).

4. Discussion

This analysis was conducted on normal phakic eyes of adolescent monkeys to get an estimate of the variance of the maximum accommodation measurements from carbachol iontophoresis experiments. The variability determined from these experiments is likely to represent a best-case scenario because near-ideal conditions exist in the normal phakic eyes. In eyes in which accommodation restoration procedures or treatments have been performed, optical clarity may be reduced from secondary capsular opacifications or from laser treatments of the lens, for example, which could add further variability to the measurements. When procedures are ultimately tested on conscious humans, the strength of the accommodative response will be affected by neural feedback to the ciliary muscle, which will add another level of variability.

The precise sources of the variability in this study cannot be discerned with certainty. The alignment of the Hartinger with the eye, physiological variability from the monkey that might affect refraction and accommodation, movement of the contact lenses, and the Hartinger too can all introduce variability. The Hartinger can contribute to the variability because the Hartinger

Table 2
Number of data points (n) used to create average time-course for each monkey at each time-point from 30 min after the start of each experiment.

	Time interval																			
Monkey	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60	62	64	66	68
64	9	7	10	8	8	6	7	4	3	2	2	1								
73	9	6	9	6	6	5	5	6	4	4	2									
99	7	9	8	7	5	4	3	2	1	1										
109	8	10	8	4	8	7	8	6	6	5	4	4	4	3	2	1	1	1	1	1
112	8	8	9	9	6	6	4	1	1											
114	9	9	9	9	6	7	6	4	4	4	3	1	1							
115	7	7	8	9	6	8	7	5	3	1	1	2	2	1	1					
119	7	7	6	4	3	2														

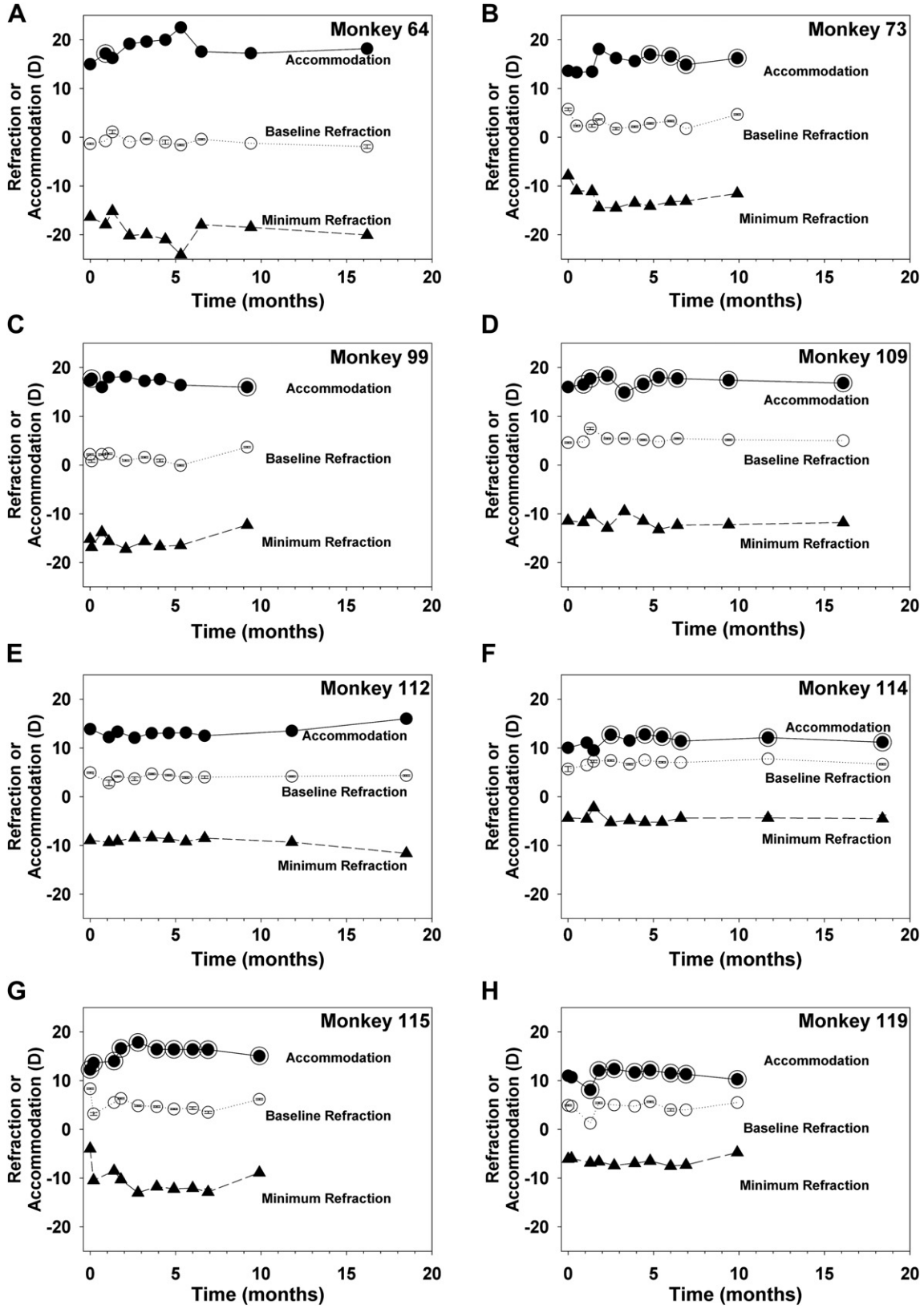

Fig. 2. Pre-carbachol baseline refraction (dotted lines and open circles), minimum refraction (dashed lines and triangles), and accommodation (solid lines and filled circles) plotted as a function of time in months. Accommodation was calculated as the difference between baseline refraction and the minimum refraction achieved. Error bars showing standard deviations from each experiment are smaller than the symbols. Circled symbols for accommodation are from those experiments where propofol anesthesia was used.

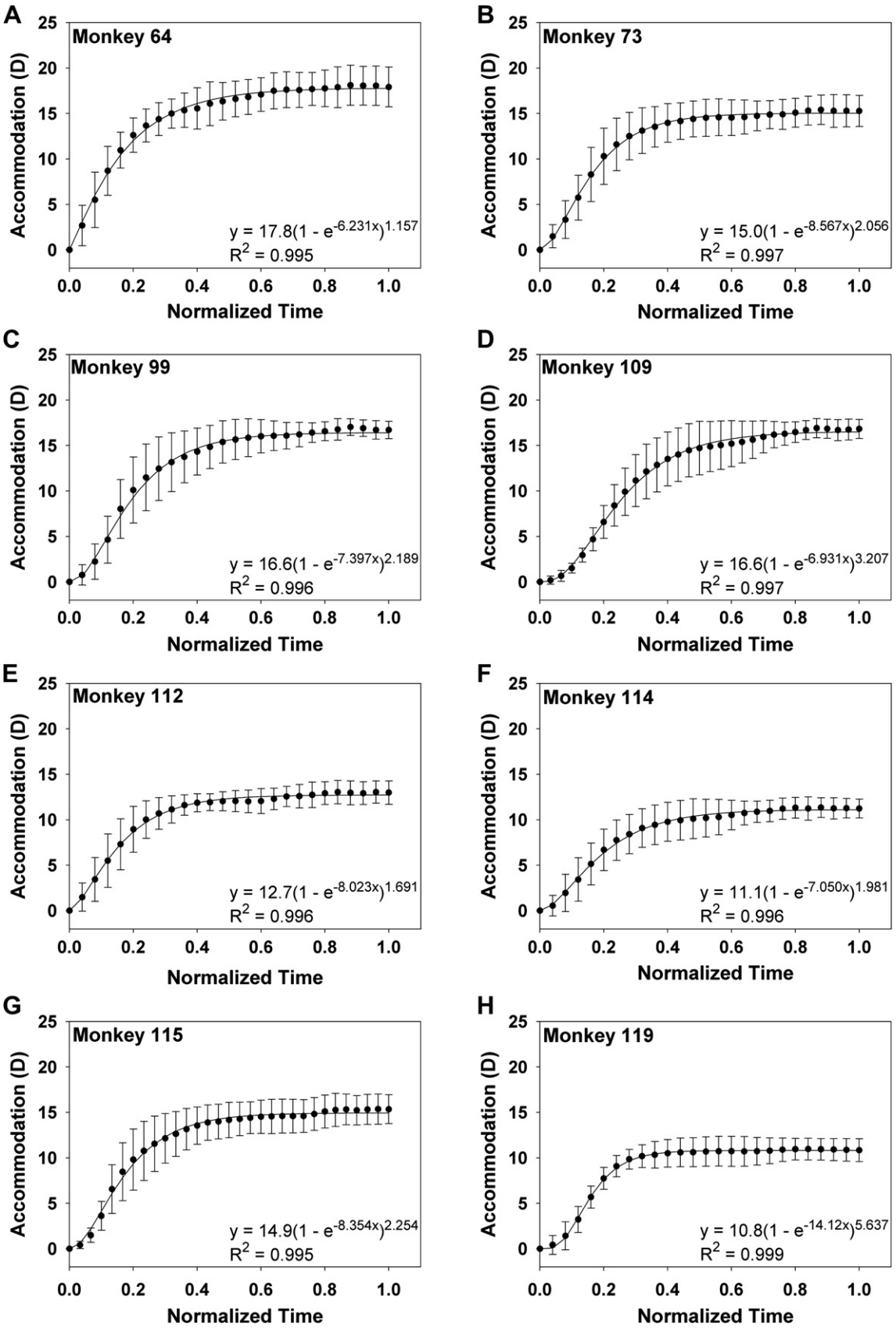

Fig. 3. Average carbachol time-course graphs for each monkey with time normalized to 1 and accommodation values resampled to regular intervals. Data are fit with the Chapman equation (solid line). Error bars are ±1 standard deviation.

Table 3
Accommodative amplitudes calculated from the Chapman equation applied to averaged data from each monkey using the normalized time-course. RMS is the difference of all individual data points from the Chapman curve fit for each monkey.

Monkey	64	73	99	109	112	114	115	119
Accommodation (D)	17.8	15.0	16.6	16.6	12.7	11.1	14.9	10.8
RMS (D)	2.08	2.15	2.31	2.06	1.63	1.82	2.02	1.27

measurements are based on the operator's ability to align the mires and because the Hartinger scale is limited to 0.25 D increments. When used on a calibrated model eye under ideal conditions, the Hartinger is accurate and it is used because it is perhaps the most robust and reliable instrument for these studies. Other instruments have different benefits and drawbacks, and clearly if less precise instruments are used, this would make it even more challenging to determine an improvement after an accommodation restoration procedure.

This was a retrospective analysis of data from experiments conducted for other purposes and so some inconsistencies exist. Measurements were not performed at two minute intervals in the earliest experiments from monkeys 112 and 114. Ideally, the time-points in all experiments should be standardized so data from repeated experiments can be averaged without need for interpolation. However, even with this standardization, the duration of each experiment will vary depending on how long it takes to achieve an asymptote. Experiments could be run for equally long, pre-determined durations, but this would not guarantee that maximum accommodation is achieved at the end-point in all experiments, or it would require that all experiment be run for unnecessarily long durations. Here, up to 10 repeated experiments were performed in some monkeys, whereas normally due to time constraints, perhaps only three repeats or less might be practical. With repeated experiments, interpolation and normalizing may be required to get reliable estimates of standard deviations. Increasing the number of experiments does not reduce standard deviation, but it does result in a more robust estimate of the standard deviation and therefore provides a better indication of the variance. A more robust measure of the variance would allow a more sensitive measure of whether an accommodation restoration treatment increased accommodative amplitude.

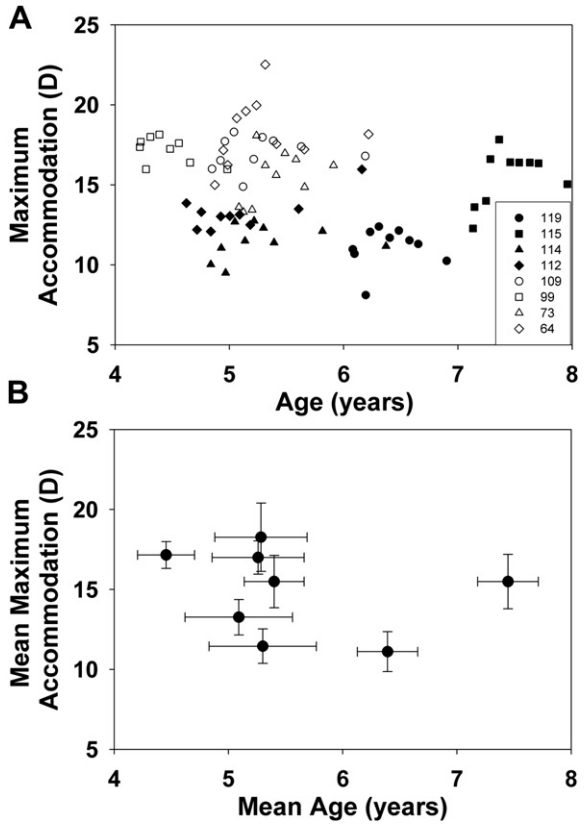

Fig. 4. (A) Maximum accommodation from each experiment with data from each monkey plotted as a function of the monkey age at each experiment. Different symbols indicate the different monkeys used. (B) All maximum accommodative data from each monkey and the age of each monkey was averaged over the set of experiments. These average data are plotted as a function of the age of the monkeys. In neither case was there a significant linear age-related trend ($p = 0.158$ and $p = 0.533$).

While the average time-course plots all reached a plateau by 20 min, there were individual experiments which took longer to reach a maximum. Since the goal of these experiments is to measure maximum accommodative response amplitude, the time to reach this end-point is unimportant and therefore these experiments were not excluded from the analysis. However, responses that took longer than 20 min may indicate that the carbachol delivery was less effective. This could be due to variations in the particular batch of laboratory prepared carbachol/agar, inexact placement of the carbachol in contact with the cornea, poor contact between the agar and the cornea, or short circuiting of the iontophoresis current by inadvertent contact between the electrode and the eyelid or the speculum. Since such variations in the responses clearly do occur and the causes of these variations are not certain, these occurrences cannot be prevented and they represent a real source of variance in assessing accommodation in this way and therefore these experiments were included in the analysis. These results suggest that it would be prudent to give a second dose of carbachol at 20 min regardless of whether the response has reached a plateau or not to ensure that maximum accommodation is reached. If the first dose appears to be ineffective, a third dose should be given to ensure that the maximum is reached.

These experiments were conducted at a time when ketamine was being replaced with propofol as the preferred anesthetic in the laboratory. In only one monkey were the accommodative results significantly different for the two different anesthetics at 95% confidence but not at 99% confidence. The choice of anesthetic (Crawford et al., 1989), as well as the level of anesthesia can affect the accommodative response. The reasons why accommodation may be affected by anesthesia are potentially many and varied, but may include the effect of anesthetics on level of central nervous system activity, muscle tonus, bradycardia, blood pressure and intraocular pressure among many other factors that anesthetics are well known to affect.

Within a single experimental session, the eye may move while the monkey is under anesthesia. Eye movements tend to be rapid saccadic "twitch" movements under ketamine and much slower drifting oscillations over 2–5 min under propofol. Eye position can affect the refraction value measured with the Hartinger coincidence refractometer. Ideally measurements would be made along the optical axis of the iridectomized eye. The degree of off-axis movement was not measured in these experiments. However, extreme eye movements of approximately 7.2° have been recorded (unpublished data) which could cause refractive variations of 1.5 D (He et al., 2011), although most eye movements would be of a smaller magnitude than this.

If eye movements were obviously noticeable, sutures were tied under the lateral and medial rectus muscles to restrain the eye. Care was taken to ensure that tension in the sutures was just sufficient to restrain eye movements, but light enough to not influence the refraction. Vertical eye movements were still observed in some experiments even after medial and lateral sutures had been placed.

If eye movements result in pulling against the sutures, this could influence zonular tension and thereby alter refraction, although no studies known to the authors have quantified this.

Even if eye movements are controlled within an experimental session, there are likely to be variations in eye alignment due to head position between sessions. Although the head is restrained in a head-holder, the head position may vary in any orientation. Precise positioning of the head and eyes is challenging because the eyes are iridectomized and so the line of sight of the eyes can be difficult to judge without the presence of a pupil. These factors are challenging to control and they necessarily represent possible sources of variability.

Variability in baseline refraction can occur even within in a single experiment when the position of the Hartinger has not moved. While much of this is likely due to eye movements, it is possible that variations in accommodative tonus may occur under anesthesia, and especially so under ketamine anesthesia which might result in lighter levels of anesthesia than propofol. Ultimately, all conditions cannot be maintained between baseline and subsequent measurements because the contact lens must be removed prior to carbachol administration and then replaced afterward. Variations in the position of the contact lens on the cornea can result in refractive variations. When baseline and most accommodated refraction show differences, variations in accommodative amplitude may indicate variations in either eye position within the same experimental session, differences in carbachol delivery, weekly differences in the monkey's physiological response to carbachol or anesthesia, or due to other unknown factors.

The maximum accommodative response achieved in anesthetized rhesus monkeys has been shown to be greater from carbachol iontophoresis than from EW stimulation (Crawford et al., 1989). This is suggested to be due to the ciliary muscle undergoing a greater contraction in response to carbachol stimulation. Variations in accommodative response over time may result from variations in ciliary muscle contraction just as other muscles vary in maximum response. Carbachol stimulated accommodation also produces a delayed forward shift of the lens which is not seen with EW stimulated accommodation (Ostrin and Glasser, 2005). Similar differences have been shown between visual stimulated and pilocarpine stimulated accommodation in humans (Koeppl et al., 2005) where pilocarpine induced an "unphysiological" forward shift of the lens. Clearly, if evaluating a treatment to restore accommodation, responses from the same type of stimulus must be compared pre- and post-treatment. In addition, the greater accommodative response that results from carbachol iontophoresis may exaggerate the effectiveness of a treatment. The forward shift of the lens that occurs with carbachol iontophoresis that does not occur with EW stimulated accommodation (Ostrin and Glasser, 2005) could also affect the interpretation of the efficacy of a treatment.

Carbachol iontophoresis stimulated accommodative amplitude has been compared with amplitudes from other methods of stimulating accommodation (Baumeister et al., 2008; Ostrin and Glasser, 2005, 2007; Vilupuru and Glasser, 2002) or with responses to carbachol iontophoresis subsequent to experimental treatments for presbyopia (Koopmans et al., 2006; McDonald et al., 2003). When accommodation is measured only once in a single experimental session or only once either before or after a treatment, it is difficult to determine what the true accommodative amplitude is. If multiple carbachol iontophoresis sessions are carried out both before and after treatment there is more certainty in the accommodative amplitude recorded. With repeated experiments, statistical analysis can show the similarity or difference between pre- and post-treatment measurements. For this reason, ideally a range of 3–5 carbachol experiments should be performed both before and after treatment. The multiple pre-treatment experiments are important for reliably identifying the pre-treatment accommodative response and associated variance and the multiple post-treatment sessions are necessary for identifying if the treatment has improved the accommodative response. The standard deviations in Table 1 indicate that treatments would likely need to increase accommodation by more than 2 D to achieve statistical significance. For example, 10 repeated accommodative measurements for monkey 112 had a standard deviation of 1.105 D. If these data are compared to another set of data of equal size and standard deviation, then the difference between the means would need to exceed 1.038 D to achieve statistical significance ($p < 0.05$). In the worst case, monkey 64 had a standard deviation of 2.141 D. In this case, the difference in means would need to exceed 2.011 D to achieve statistical significance ($p < 0.05$). It has been suggested that an effective treatment for presbyopia should restore 3 to 5 diopters of accommodation to a truly presbyopic eye (Glasser, 2008; Schor, 2009; Sheppard et al., 2010). Improvements in accommodative amplitudes of this magnitude would be detectable with carbachol iontophoresis induced accommodation in monkeys. However, efforts to increase accommodative amplitude in the phakic eye using pharmacological interventions or with femtosecond laser treatments of the lens are likely to only increase the accommodative response by 0.5–1 D. To detect changes of this magnitude with carbachol iontophoresis stimulated accommodation in anesthetized monkeys would require many pre- and post-treatment experiments to attain statistical significance.

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