Experimental Eye Research 115 (2013) 123–130

Contents lists available at SciVerse ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Comparison between carbachol iontophoresis and intravenous pilocarpine stimulated accommodation in anesthetized rhesus monkeys

Mark Wendt, Lin He, Adrian Glasser*

College of Optometry, University of Houston, 505 J. Davis Armistead Building, Houston, TX 77204, USA

ARTICLE INFO

Article history:
Received 12 November 2012
Accepted in revised form 30 June 2013
Available online 11 July 2013

Keywords:
accommodation
lens
monkey
pilocarpine

ABSTRACT

Rhesus monkeys are an animal model for human accommodation and presbyopia and consistent and repeatable methods are needed to stimulate and measure accommodation in anesthetized rhesus monkeys. Accommodation has typically been pharmacologically stimulated with topical pilocarpine or carbachol iontophoresis. Intravenous (i.v.) pilocarpine has recently been shown to produce more natural, rapid and reproducible accommodative responses compared to topical pilocarpine. Here, i.v. pilocarpine was compared to carbachol iontophoresis stimulated accommodation. Experiments were performed under anaesthesia on five previously iridectomized monkeys aged 10–16 years. In three monkeys, accommodation was stimulated with carbachol iontophoresis in five successive experiments and refraction measured with a Hartinger coincidence refractometer. In separate experiments, accommodation was stimulated using a 5 mg/kg bolus of i.v. pilocarpine given over 30 s followed by a continuous infusion of 20 mg/kg/hr for 5.5 min in three successive experiments with the same monkeys as well as in single experiments with two additional monkeys. Refraction was measured continuously using photorefraction with baseline and accommodated refraction also measured with the Hartinger. In subsequent i.v. pilocarpine experiments with each monkey, accommodative changes in lens equatorial diameter were measured in real-time with video-image analysis. Maximum accommodation of three monkeys with carbachol iontophoresis (five repeats) was (mean ± SD; range) 14.0 ± 3.5; 9.9–20.3 D and with i.v. pilocarpine stimulation (three repeats) was 11.1 ± 1.1; 9.9–13.0 D. The average of the standard deviations of maximum accommodation from each monkey was 0.8 ± 0.3 D from carbachol iontophoresis and 0.3 ± 0.2 from i.v. pilocarpine. The average latency to the start of the response after carbachol iontophoresis was 2.5 ± 3.9; 0.0–12.0 min with a time constant of 12.7 ± 9.5; 2.3–29.2 min. The average latency after i.v. pilocarpine was 0.31 ± 0.03; 0.25–0.34 min with a time constant of 0.19 ± 0.07; 0.11–0.31 s. During i.v. pilocarpine stimulated accommodation in five monkeys, lens diameters decreased by 0.54 ± 0.09; 0.42–0.64 mm with a rate of change of 0.052 ± 0.002; 0.050–0.055 mm/D. Accommodative responses with i.v. pilocarpine were more rapid, consistent and stable than those with carbachol iontophoresis. The accommodative decrease in lens diameter with i.v. pilocarpine as a function of age was consistent with previous results using constant topical pilocarpine. Intravenous pilocarpine stimulated accommodation is safe, more consistent and more rapid than carbachol iontophoresis and it requires no contact with or obstruction of the eye thus allowing continuous and uninterrupted refraction and ocular biometry measurements.

1. Introduction

Rhesus monkeys have been widely used as an appropriate animal model for human accommodation and presbyopia (Bito et al., 1982; Croft et al., 2006). The anatomy of the eye, the accommodative mechanism and the age-course of the progression of presbyopia relative to life span in rhesus monkeys are all similar to humans (Bito et al., 1982; Bito et al., 1987; Glasser and Kaufman, 1999; Lütjen-Drecoll et al., 1988a; Lütjen-Drecoll et al., 1988b). Approaches to restore accommodation have also been investigated in rhesus monkeys (Haefliger and Parel, 1994; Koopmans et al., 2006). Accommodation has been induced in anesthetized

* Corresponding author. Tel.: +1 713 743 1842; fax: +1 713 743 2053.
E-mail addresses: mwendt@uh.edu (M. Wendt), aglasser@uh.edu (A. Glasser).

0014-4835/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.exer.2013.06.028

monkeys in a variety of ways, including with Edinger-Westphal (EW) stimulation, application of muscarinic agonists to the eye or with systemic (intramuscular (i.m.) or intravenous (i.v.)) pilocarpine (Bito et al., 1982; Chin et al., 1968; Crawford et al., 1989; Croft et al., 1998; Haefliger and Parel, 1994; Jampel and Mindel, 1967; Koopmans et al., 2006; Koretz et al., 1987; Neider et al., 1990; Nishi and Nishi, 1998; Tornqvist, 1965, 1966; Vilupuru and Glasser, 2002; Wendt and Glasser, 2010, 2012).

Carbachol iontophoresis or topical pilocarpine are the most common pharmacological methods to stimulate accommodation in anesthetized rhesus monkeys (Haefliger and Parel, 1994; Koopmans et al., 2006; Nishi and Nishi, 1998; Tornqvist, 1964; Vilupuru and Glasser, 2002; Wendt and Glasser, 2010, 2012). Although topical pilocarpine may be the most approachable and straightforward of these, it has been shown to be unreliable (Wendt and Glasser, 2010). Carbachol iontophoresis has been routinely and widely used (Koopmans et al., 2006; Koretz et al., 1987; Vilupuru and Glasser, 2002; Wendt and Glasser, 2012). However, carbachol iontophoresis stimulated accommodative amplitudes are variable in the same monkey (Wendt and Glasser, 2012) and slow (taking 20–30 min to asymptote) (Ostrin and Glasser, 2005; Vilupuru and Glasser, 2002; Wendt and Glasser, 2012). Further carbachol produces an unnatural accommodative response in that although initially the accommodative response is normal with anterior movement of the anterior lens surface and posterior movement of the posterior lens surface, following that the entire lens is translated forward (Ostrin and Glasser, 2005; Vilupuru and Glasser, 2002). It is unclear why the delayed, unnatural forward lens translation occurs, but it may be that high carbachol concentrations delivered into the anterior chamber cause a supra-maximal contraction of the ciliary muscle.

Systemically administered pilocarpine has also been widely used to stimulate accommodation in anesthetized monkeys (Erickson-Lamy et al., 1987; Kaufman and Bárány, 1975; Tornqvist, 1964, 1965, 1967). Tornqvist performed comprehensive studies of i.m pilocarpine stimulated accommodation in monkeys (Tornqvist, 1964, 1965, 1967). However, Tornqvist studies were not on rhesus macaques (*Macaca mulatta*), did not include continuous measurements of accommodation and did not measure accommodative biometric changes that are inherently part of the accommodative response. Tornqvist also identified side effects of systemic pilocarpine which might suggest that it cannot be used safely and effectively. Recently, i.v. pilocarpine stimulation in conjunction with ocular biometry measurements has been shown to produce accommodative responses which are more natural, rapid and reproducible compared to topical pilocarpine (Wendt and Glasser, 2010). The primary objective of this study was to compare the utility, safety, time-course, stability and repeatability of i.v. pilocarpine and carbachol iontophoresis stimulated accommodative refractive changes from repeated experiments in the same monkeys. The secondary objective of this study was to compare the accommodative refractive changes with the accommodative changes in lens diameter during successive i.v. pilocarpine stimulation experiments in five monkeys.

2. Methods

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, iridectomized, eyes of five rhesus monkeys (*M. mulatta*), between 10 and 17 years of age (monkey numbers 66, 73, 99, 112, 115 with ages in years of 16.6, 11.5, 10.6, 12.6, 13.6 respectively). Throughout the text, the term experiment is referred to as a single procedure or trial in a single experimental session in which one accommodative response was stimulated. Direct comparisons of carbachol and i.v. pilocarpine were made on three monkeys (73, 99, and 112). Monkeys were initially anesthetized with intramuscular 15 mg/kg ketamine and experiments were performed under i.v. propofol (PropoFlo, Abbott Laboratories, North Chicago, IL) anesthesia with an initial bolus of 1.5 mg/kg and a continuous infusion at 0.5 mg/kg/min. For i.v. pilocarpine experiments, monkeys were intubated and respirated, maintained prone on a table with the head held upright and facing forward in a head holder with the eyelids held open with a lid-speculum and a clear plano contact lens on the cornea. Pulse rate, SpO_2, and temperature were monitored. Sutures were tied beneath the lateral and medial rectus muscles to prevent eye movements. Prior to the i.v. pilocarpine stimulation experiments, 0.025 mg/kg i.m. dexmedetomidine (Pfizer, New York, New York) was administered to further reduce eye movements. At the end of the experiments, dexmedetomidine was reversed with 0.25 mg/kg i.m. atipamezole (Pfizer, New York, New York).

2.2. Carbachol iontophoresis stimulated accommodation

In five separate experiments, at least one week apart, carbachol iontophoresis experiments were performed on each of three monkeys to determine repeatability. Carbachol was prepared and delivered iontophoretically for 8 s each on the nasal and temporal sides of the cornea during the initial application and 4 s each in subsequent applications (Koopmans et al., 2006; Koretz et al., 1987; Vilupuru and Glasser, 2002; Wendt and Glasser, 2012). The contact lens was then replaced on the cornea. Static measurements of accommodation were made with a Hartinger coincidence refractometer (Carl Zeiss Meditec, Jena, Germany) from immediately before until up to 100 min following carbachol delivery with three repeated measurements in quick succession at each 2 min interval. Measurements continued until no further increase was observed after three successive 2 min time intervals at which time carbachol iontophoresis was applied a second time. Accommodation measurements continued until a final asymptote was reached.

2.3. Intravenous pilocarpine stimulated accommodation

Three separate i.v. pilocarpine experiments were performed on each of five monkeys, each at least one week apart, to determine repeatability. Accommodation was measured simultaneously using real-time dynamic photorefraction and the Hartinger using a hot mirror beam splitter placed 3 cm in front of the eye at a 45° angle (He et al., 2012; Vilupuru and Glasser, 2002). Photorefraction images were analyzed real-time in a Matlab (The MathWorks, Inc., Natick, MA) program at approximately 15 Hz.

To prevent systemic side effects of the i.v. pilocarpine an initial i.m. dose of 0.015 mg/kg glycopyrrolate was administered (Tornqvist, 1967; Wendt and Glasser, 2010). All subsequent drug administrations and measurements were timed with respect to this initial glycopyrrolate delivery (Fig. 1). Photorefraction measurements started 25 min after glycopyrrolate and five baseline Hartinger measurements were made through the beam splitter at 27 min. At 30 min, pilocarpine was administered via an i.v. catheter in the other leg from the propofol catheter using a syringe infusion pump (KDS210, KD Scientific, Boston) controlled via a Matlab program. An initial i.v. pilocarpine bolus of 5 mg/kg was administered over 30 s immediately followed by a constant infusion of 20 mg/kg/hr for 5.5 min. Another five Hartinger measurements were performed immediately after the pilocarpine constant infusion was completed (at 36 min) when the eye was maximally accommodated.

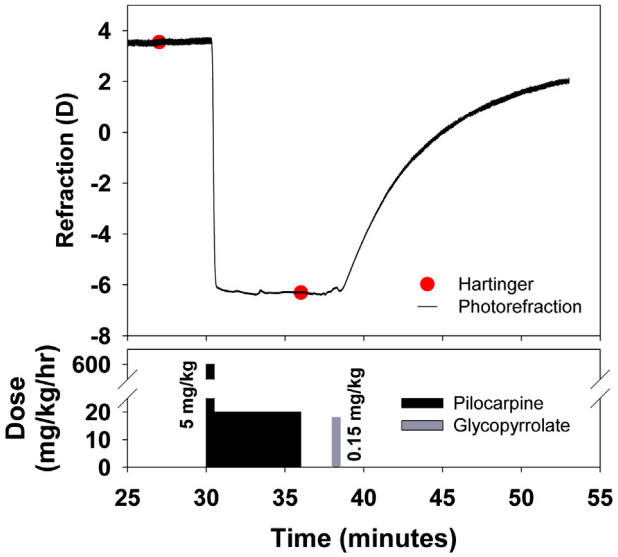

Fig. 1. One i.v. pilocarpine stimulation experiment in monkey 112 showing accommodation measured dynamically with photorefraction (black line) and statically with a Hartinger (mean ± SD, $n = 5$) at 3 min before and 6 min after pilocarpine administration began (red circle symbols). Pilocarpine (black) and glycopyrrolate (gray) doses are shown below. The time scale starts at 25 min with respect to the initial i.m. protective glycopyrrolate dose which is not shown since it has no effect on refraction or accommodation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Two minutes later (38 min), 0.15 mg/kg i.v. glycopyrrolate was administered over 30 s to reverse the effects of pilocarpine. Photorefraction measurements continued for 15 min as accommodation gradually returned towards baseline. Photorefraction slopes were calibrated to refraction from a linear regression line fit to the means of the two sets of Hartinger measurements.

2.4. Intravenous pilocarpine lens diameter measurements

In separate experiments, at least one week apart, additional i.v. pilocarpine stimulated accommodation experiments were performed during which the accommodative change in lens diameter was measured in all five monkeys. Lens diameter serves as an independent biometric measure of accommodation and has been shown to be linearly correlated with the accommodative refractive change (Glasser et al., 2006). Lens diameters were measured by analysis of video images captured through a custom designed ocular perfusion lens (Wendt et al., 2008). Imaging was performed with a video camera (COHU, San Diego, CA) attached to a slit-lamp microscope (Carl Zeiss Meditec, Jena, Germany, Model 110). The slit-lamp light beam was adjusted to a circle so that the perimeter of the monkey lens was clearly illuminated with a 'halo' (Fig. 2). Video images calibrated to millimeters were analyzed in real-time using a Matlab program at approximately 25 Hz which tracked the brightly illuminated lens edge and determined the least squares circular fit to the lens.

2.5. Statistical analyses

Average accommodative amplitudes and standard deviations were determined for the two accommodation stimulation methods from the repeated experiments. Averages and standard deviations were compared using *t*-tests. The time-courses of the two accommodation stimulation methods were compared by fitting exponential functions to the averaged responses (see below). Orthogonal regressions were used to compare the slopes of the refraction versus

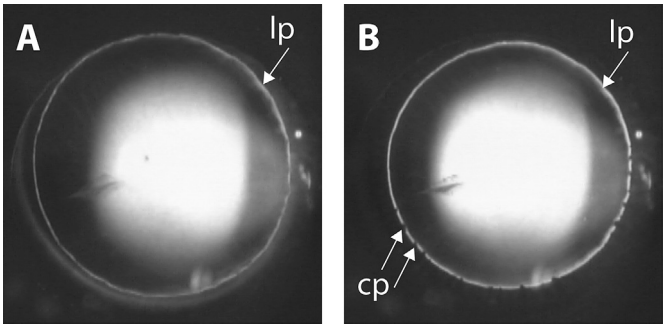

Fig. 2. Video images of the lens from the iridectomized eye of monkey 115 in the unaccommodated (A) and maximally accommodated (B) states. In the maximally accommodated state, several discontinuities are observed in the lower half of the lens perimeter (lp) where the tips of individual ciliary processes (cp) have encroached on the lens perimeter due to maximal ciliary muscle contraction.

lens diameter relationships from the i.v. pilocarpine experiments. Root-mean-square (RMS) deviations of the accommodative responses from the mean accommodative response were used to assess variability of the individual experiments.

3. Results

The amplitudes and rates of carbachol iontophoresis stimulated accommodation (Fig. 3A–C) were more variable than the i.v. pilocarpine stimulated accommodative responses (Fig. 3D–F) for each monkey. Maximum carbachol stimulated accommodation for each experiment was defined as the average of the last four time-point measurements. For i.v. pilocarpine, maximum accommodation was defined as the average from the final minute of the pilocarpine infusion (minute 5–6 in Fig. 3D–F). Maximum change in lens diameter from the i.v. pilocarpine experiments was defined as the difference between the average from one minute immediately preceding the i.v. pilocarpine infusion and the average from the final minute of pilocarpine infusion. The average maximum accommodation for each monkey with carbachol iontophoresis and i.v. pilocarpine are shown in Table 1.

For monkey 99, the i.v. pilocarpine and carbachol iontophoresis accommodative amplitudes were not statistically different ($p = 0.341$). Monkeys 73 and 112 had significantly higher accommodative responses to carbachol iontophoresis than i.v. pilocarpine ($p < 0.001$). For monkey 112, the carbachol response ensued over a substantially longer duration with an initial rise to approximately 9 D and a slower progression to a maximum at approximately 90 min after the initial dose of carbachol. This progressive increase was not noticed during the first carbachol experiment on this monkey so measurements were stopped after 40 min and because of this, subsequent analyses of amplitudes and standard deviations for this monkey exclude this first response. The average accommodative response of the last four carbachol experiments in this monkey was 19.17 ± 1.18 D. Standard deviations from carbachol iontophoresis experiments in the different monkeys ranged from 0.5 to 1.1 D (mean ± SD: 0.8 ± 0.3 D). These were higher than, although not significantly different from the standard deviations for i.v. pilocarpine which ranged from 0.13 to 0.44 D (mean ± SD: 0.45 ± 0.49 D) ($p = 0.125$, paired *t*-test).

To quantitatively compare time courses between carbachol iontophoresis and i.v. pilocarpine induced accommodative responses, each individual response was fit with an exponential equation, $y = A \times \left(1 - e^{\left(\frac{b-x}{\tau}\right)}\right)$, where A is the amplitude, b is the latency, and τ is the time constant. As shown in Fig. 4A and B, this equation was not an ideal fit to all responses, but it provided a consistent method to quantify latencies and time constants. The

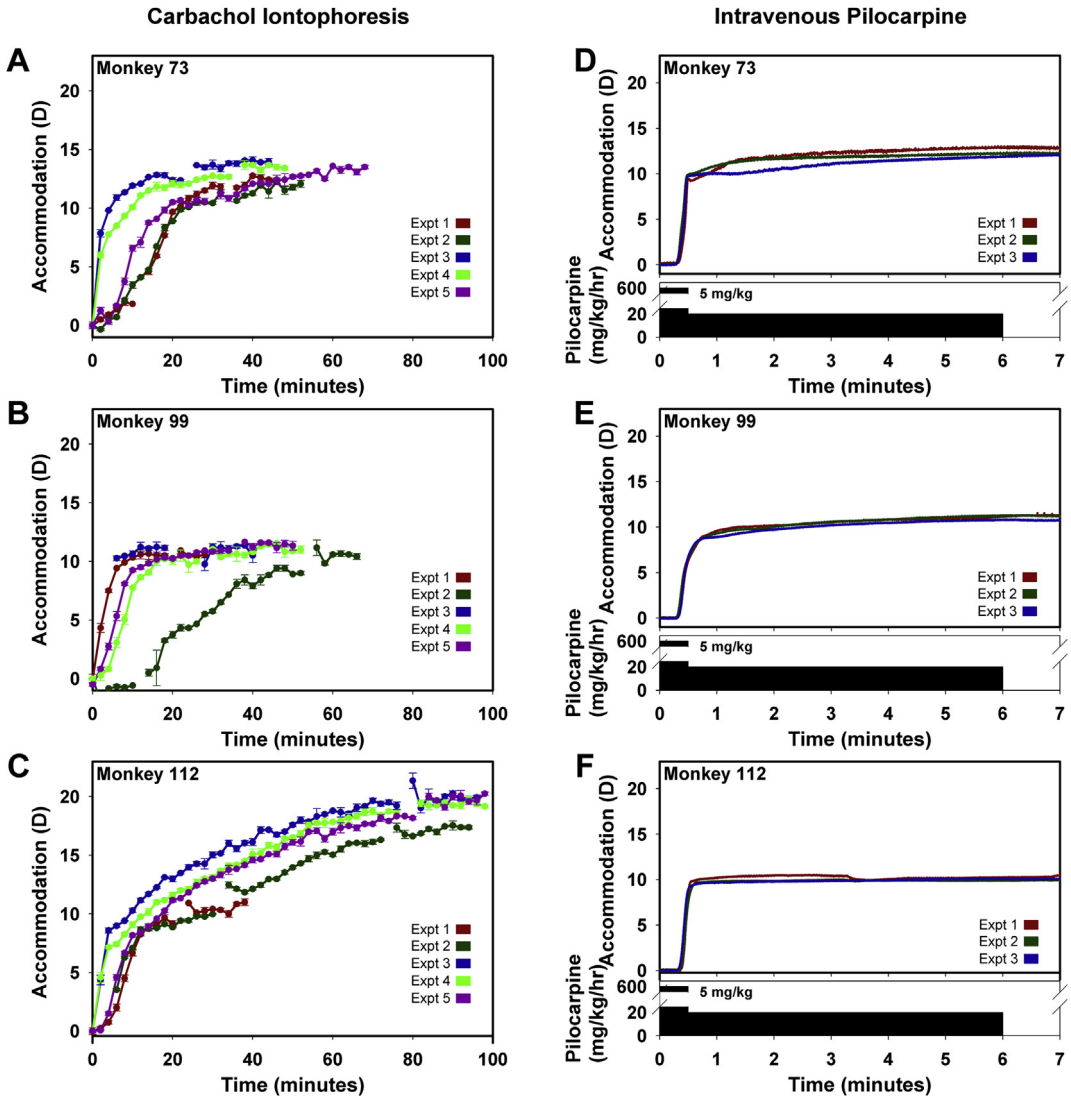

Fig. 3. Five repeated carbachol iontophoresis (A–C) and three repeated i.v. pilocarpine (D–F) stimulated accommodation experiments (Expt.) in three monkeys. Each experiment represents a repeat of the same protocol. Pilocarpine infusion rates and durations (6 min) are indicated below the time-course graphs (D–F).

latency and time constant for i.v. pilocarpine was 0.31 ± 0.03 min (range: 0.25–0.34 min) and 0.19 ± 0.07 min (range: 0.11–0.31 min), respectively compared to 2.50 ± 3.90 min (range: 0.0–12.0 min) and 12.70 ± 9.50 min (range: 2.30–29.2 min) for carbachol iontophoresis (Table 1).

Average time course of accommodation was calculated for each monkey and each stimulation method, two examples of which are shown in Fig. 4C and D. For carbachol iontophoresis, measurements made at two minute intervals for all five experiments were averaged to create a single average curve. Measurements were not made at every 2 min time point (such as when additional carbachol doses were administered) for all five experiments, so numbers of data points contributing to the average varied. For i.v. pilocarpine, the average time-course was calculated from data linearly interpolated to equal time points. The mean deviation of all data points from each average curve was calculated as a single root-mean-square (RMS) value for each monkey and stimulation method. The average RMS from intravenous pilocarpine was 0.13 ± 0.17 D (range: 0.21–0.51 D) and for carbachol iontophoresis was 2.03 ± 0.58 D (range: 1.40–2.54 D).

Accommodative changes in lens diameter were measured at 25 Hz in single i.v. pilocarpine stimulated accommodation experiments in the same five monkeys. These lens diameter time-courses were compared to the average accommodative photorefraction time-courses from the three prior experiments on the same monkeys (Fig. 5). Lens diameter throughout the time-course was plotted against mean refraction (Fig. 5F). Orthogonal regression fits to these data, which are heavily weighted by data from the baseline and maximally accommodated periods, have similar slopes in each case. The average decrease in lens diameter per diopter of accommodation was 0.052 ± 0.002 mm/D (range: 0.050–0.055 mm/D). All the individual accommodative responses for the five monkeys are plotted in Fig. 6A. The accommodative decrease in lens diameter ranged from 0.42 to 0.64 mm (Fig. 6B) and the average total decrease in lens diameter was 0.54 ± 0.09 mm. Maximum change in lens diameter and maximum accommodation were calculated as the mean and standard deviation of all measurements during the last minute of i.v. pilocarpine infusion (minute 5 to 6 in Fig. 6A and B). There is a linear relationship between the change in lens diameter and maximum i.v. pilocarpine stimulated accommodation (Fig. 6C). The accommodative changes in lens diameter from i.v. pilocarpine stimulation were appropriate for the ages of the monkeys compared to accommodative changes in lens diameter from a prior study in

Table 1
Comparison of responses from carbachol iontophoresis and i.v. pilocarpine.

Experiments per monkey	Carbachol iontophoresis[a]		Intravenous pilocarpine[b]	
	5		3	
	Mean ± SD	Range	Mean ± SD	Range
Maximum accommodation monkey#73	13.0 ± 0.8	11.5–14.1	12.3 ± 0.5	11.6–13.0
Maximum accommodation monkey#99	10.8 ± 0.5	9.9–11.6	11.0 ± 0.2	10.7–11.3
Maximum accommodation monkey#112	19.2 ± 1.1	17.4–20.3	10.1 ± 0.2	9.9–10.3
Mean maximum accommodation (D)	14.0 ± 3.5	9.9–20.3	11.1 ± 1.1	9.9–13.0
Standard deviations of maximum accommodation (D)	0.8 ± 0.3	0.5–1.1	0.3 ± 0.2	0.2–0.5
Latency (min)[c]	2.5 ± 3.9	0.0–12.0	0.31 ± 0.03	0.25–0.3
Time constant (min)	12.7 ± 9.5	2.3–29.2	0.19 ± 0.1	0.11–0.3
RMS from average curve (D)[d]	2.03 ± 0.6	1.40–2.5	0.32 ± 0.2	0.20–0.5

[a] Maximum accommodation from carbachol iontophoresis was determined as the average of the final four individual measurements for each experiment.
[b] Maximum accommodation from i.v. pilocarpine was determined as the average of all values from 5 to 6 min after infusion of pilocarpine began.
[c] Latency and time constant were calculated as described in Fig. 2.
[d] Root-mean-square (RMS) values were calculated as the square root of the mean of the square of the distances from all data points to the average curves as shown in Fig. 4C and D.

which accommodation was achieved with constant topical 2% pilocarpine applied using the perfusion lens (Wendt et al., 2008).

4. Discussion

Reliable and consistent methods to stimulate accommodation in anesthetized monkeys are necessary if repeated accommodation experiments are to be performed in the same monkey over time to determine the effects of a surgical intervention, or to determine age-related changes in any aspect of the accommodative apparatus, or to evaluate accommodation restoration procedures performed on monkeys. Although systemic (i.v. or i.m.) pilocarpine stimulated accommodation has been used previously (Hubbard et al., 1996; Kaufman and Bárány, 1976; Tornqvist, 1964, 1965, 1967), only recently has a consistent i.v. pilocarpine protocol been developed to stimulate maximum accommodation (Wendt and Glasser, 2010). Early studies of systemically administered pilocarpine demonstrated considerable variability due to the systemic effects of the pilocarpine (Tornqvist, 1964, 1965, 1967). More recently, i.v. pilocarpine has been demonstrated to be a more reliable method to stimulate accommodation than topical pilocarpine and i.v. pilocarpine was shown to produce a response that mimics the naturally occurring or EW stimulated accommodative response (Wendt and Glasser, 2010). In the experiments reported here i.v. pilocarpine has been demonstrated to achieve a rapidly occurring, reliable and reproducible drug stimulated accommodative response compared to carbachol iontophoresis.

Although carbachol iontophoresis has often been used for pharmacological stimulation of accommodation in rhesus monkeys (Crawford et al., 1990; Koretz et al., 1987), only recently has its reproducibility has been investigated (Wendt and Glasser, 2012). Carbachol stimulated accommodative responses can vary by as much as 7.5 D within a single monkey over a period of 17 months (Wendt and Glasser, 2012). Carbachol iontophoresis stimulated accommodation is slow (20–30 min), unreliable in terms of the time-course or the amplitude achieved, it cannot readily be reversed and most importantly, does not produce a natural accommodative response (Ostrin and Glasser, 2005) and further, as documented here in monkey 112 can produce abnormally high accommodative responses. Unlike i.v. pilocarpine, it does allow monocular accommodation stimulation to be performed.

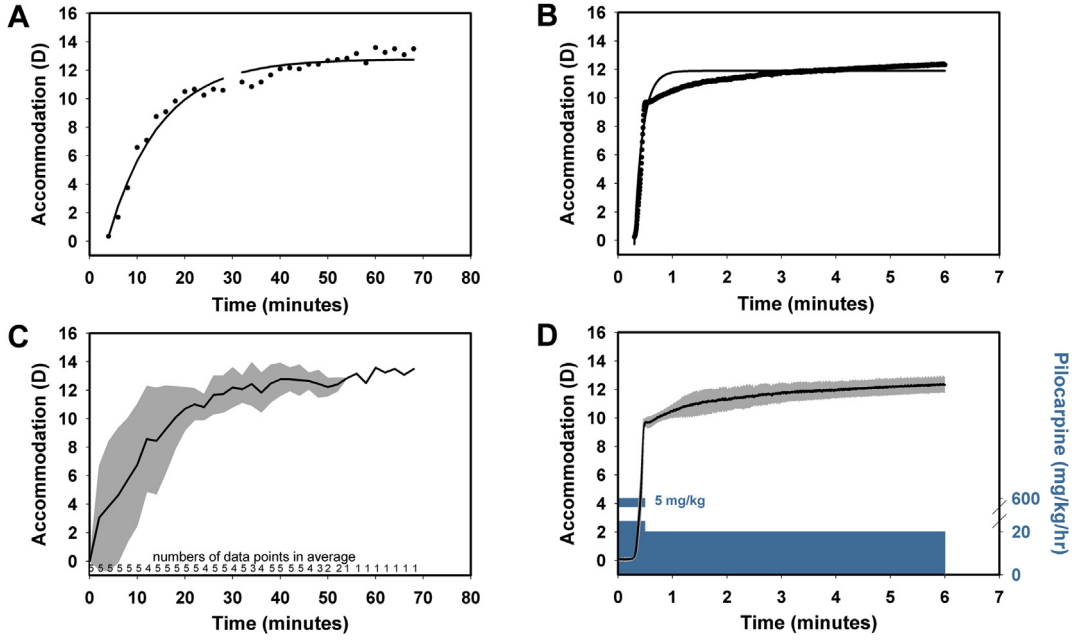

Fig. 4. Calculations for comparisons of the latency, time constant and variance of carbachol iontophoresis (A) and intravenous pilocarpine (B) experiments from monkey 73. Each individual curve shown in Fig. 3 was fit with an exponential equation as described in the text (C & D). Average time courses are shown as black lines and standard deviations as gray regions. For carbachol iontophoresis, the average is calculated from all data recorded at two minute intervals (C). The numbers of data points used to calculate the average at each time point are indicated on the graph. For i.v. pilocarpine, the average is calculated from data interpolated to equal time points from three experiments (D). Accommodation analysis continued for six minutes after the initial pilocarpine infusion. Pilocarpine infusion rates are indicated in blue with the infusion scale on the right axis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

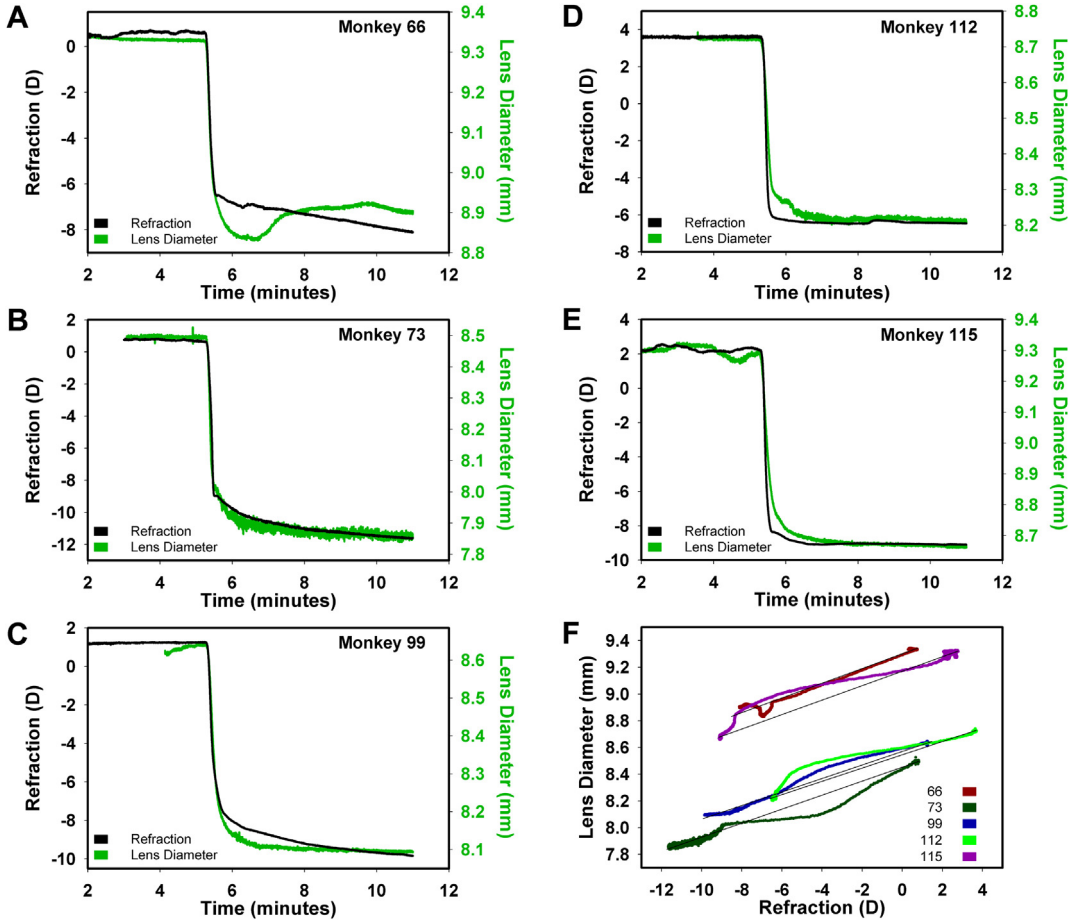

Fig. 5. Accommodative refractive changes (black lines; left axis) and lens diameter changes (green; right axis) as measured in subsequent i.v. pilocarpine stimulation experiments (A–E). Changes in lens diameter were similar to changes in the average refraction measured from the same monkey in three different photorefraction experiments (F). Orthogonal regression fits of lens diameter versus average refraction are heavily weighted by baseline and maximally accommodated data. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Although systemic pilocarpine can produce salivation, sweating, urination, defecation and in extreme cases emesis (Tornqvist, 1965, 1967), the doses of i.v. pilocarpine used here in conjunction with a 30 min pre-pilocarpine, systemically protective, low-dose of a muscarinic antagonist (glycopyrrolate) had no adverse systemic effects in any of the monkeys other than mild salivation and mild sweating of the palms and feet. Even high carbachol iontophoretic doses to the eye can produce systemic effects. The i.v. pilocarpine stimulation and glycopyrrolate reversal protocols described and used here are safe, effective and reproducible.

The benefits of i.v. pilocarpine include that it produces a rapid accommodative response, it is reliable, the accommodative response achieved is normal, it is safe and it can be readily reversed. It also requires no contact with the eye and therefore the ocular accommodative response can be measured continuously, unobstructed from before the response begins through to after the response is reversed (Fig. 1). Since pilocarpine is administered systemically, it will also produce a binocular accommodative response. It is of interest to know if maximum accommodation is achieved with the i.v. pilocarpine protocol described here. The bolus doses (5.0 mg/kg) administered were considerably higher than the maximum bolus (2.0 mg/kg) described in a prior study (Wendt and Glasser, 2010). High doses were used to ensure that maximum accommodation was achieved and the relatively repeatable and stable plateaus that resulted suggests that it is. In addition, the similarity of the relationship between accommodation and change in lens diameter to that reported previously in which an asymptote in the accommodative response was achieved (Glasser et al., 2006; Wendt et al., 2008), also suggests that maximum accommodation was achieved. The magnitude of the i.v. pilocarpine stimulated accommodative changes in lens diameter achieved with this protocol are age-appropriate compared to the responses achieved in monkeys of a range of ages in a prior study (Wendt et al., 2008) (Fig. 7). The 0.052 $\pm$ 0.002 mm/D decrease in lens diameter reported here is similar to the 0.060 $\pm$ 0.01 reported previously in 2 monkeys aged 5 and 6 years using EW stimulation (Glasser et al., 2006). It is likely that the i.v. pilocarpine doses used here are higher than what is required to achieve maximum accommodation in all monkeys, however variable susceptibility of individual monkeys to i.v. pilocarpine requires that a high dose is used in all monkeys to ensure that maximum accommodation is achieved using a single consistent protocol. However, high doses can cause paradoxical accommodative responses due to systemic effects (Tornqvist, 1967), so there is some inter-individual variability in dose/response relationship. In this study, the accommodative responses from i.v. pilocarpine are in general lower than those achieved with carbachol iontophoresis, but this is likely because carbachol iontophoresis produces an accommodative response that is greater than what would be considered physiologically normal. Carbachol iontophoresis has been shown to produce as much as 7 D more accommodation than EW stimulation (Crawford et al., 1989; Crawford et al., 1990).

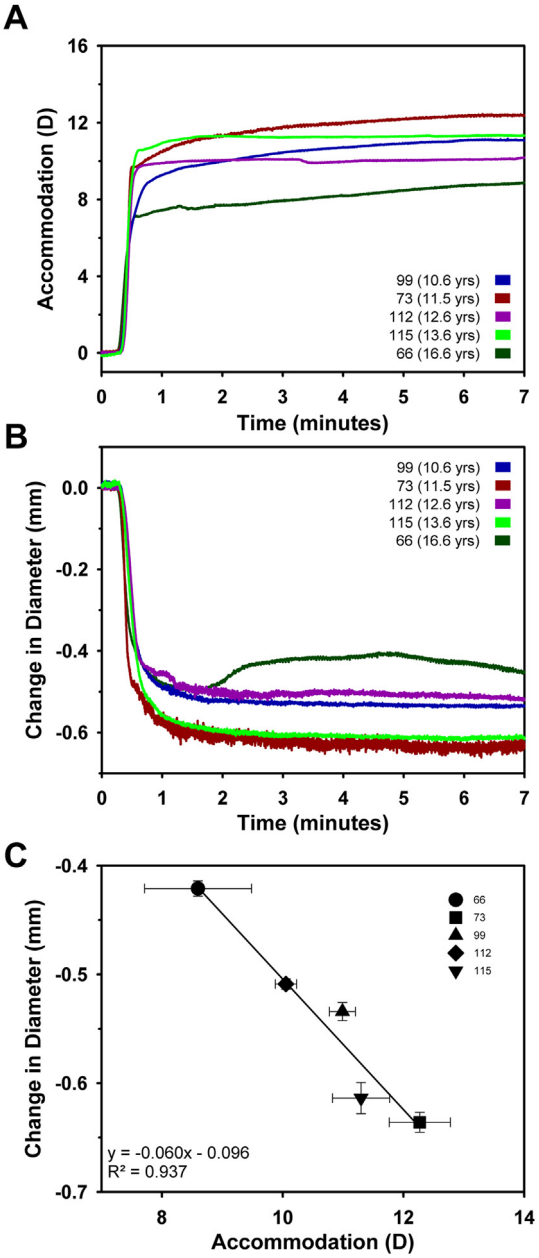

Fig. 6. The average accommodative responses from each of the three repeated i.v. pilocarpine experiments (A) and the change in lens diameter (B) for each of the five monkeys. Change in lens diameter decreases as a function of average accommodative response (C). The error bars show standard deviations. The line indicates an orthogonal fit to the data.

The i.v. pilocarpine stimulated accommodative amplitudes were more consistent for each monkey than those achieved with carbachol iontophoresis as indicated by the smaller standard deviations from multiple experiments. Intravenous pilocarpine also had smaller standard deviations for latency and time constants and smaller RMS errors compared to carbachol iontophoresis. For i.v. pilocarpine, the average time constant was 63 times faster than that from carbachol iontophoresis while the average latency was 9 times faster. The relatively rapid, reproducible and more normal accommodative responses achieved with i.v. pilocarpine mean that this is a better option for pharmacologically stimulating accommodation than carbachol iontophoresis.

The lens diameter measurements were performed in conjunction with the refraction measurements to demonstrate that

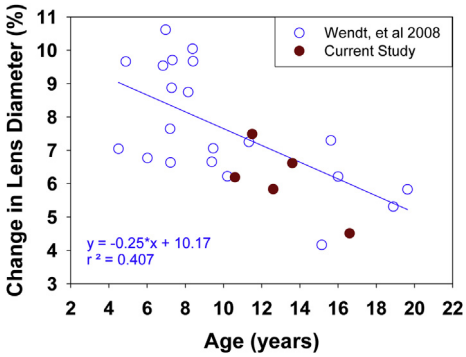

Fig. 7. The accommodative change in lens diameter as a function of age from the current study (solid symbols) plotted together with the change in lens diameter from a prior study (Wendt et al., 2008) (open symbols, linear regression and equation) in which accommodative changes in lens diameter were stimulated with topical pilocarpine maintained on the cornea in the same perfusion lens as was used with saline in the current study.

accommodative changes other than refraction can be effectively measured during i.v. pilocarpine stimulated accommodation. The lens diameter measurements provide a physical biometric measure of the accommodative changes in the lens. Although performed in independent experiments, the accommodative refractive and the lens diameter changes are relatively consistent in their time-course. Prior studies have shown that lens diameter is linearly correlated with accommodative refractive changes over the full range of accommodation in rhesus monkeys (Glasser et al., 2006). Similar relationships are seen here from five monkeys even though the refractions and lens diameters were measured on different days in successive experiments. These measurements cannot be performed simultaneously in the same eye because the lens diameter measurements require optical neutralization of corneal refractive power. The short time-course of the i.v. pilocarpine stimulated accommodative response and the fact that the eyes are not obstructed means that any other aspects of accommodation could be measured with i.v. pilocarpine stimulated accommodation including changes in lens surface curvature (Rosales et al., 2008), ciliary process movements (Ostrin and Glasser, 2007), axial accommodative biometric changes (Vilupuru and Glasser, 2002), changes in wavefront aberrations (Vilupuru et al., 2004) ultrasound biomicroscopic changes in the ciliary muscle (Croft et al., 2009). Any of these parameters could be measured dynamically from the unaccommodated state throughout the accommodative response without interruption since there is no need to touch or obscure the eyes to stimulate accommodation as there is with carbachol iontophoresis. Further, unlike EW stimulation which also stimulates convergence eye movements, i.v. pilocarpine does not produce any systematic eye movements, thereby allowing for better ocular stability. This protocol offers a non-surgical method to stimulate accommodation in any iridectomized monkey for studying aspects of accommodation and presbyopia with significant advantages over other pharmacological accommodation stimulation methods described previously.

Acknowledgments

Thanks to Chris Kuether for constructing the perfusion cell lens.

References

Bito, L.Z., DeRousseau, C.J., Kaufman, P.L., Bito, J.W., 1982. Age-dependent loss of accommodative amplitude in rhesus monkeys: an animal model for presbyopia. Invest. Ophthalmol. Vis. Sci. 23, 23–31.

Bito, L.Z., Kaufman, P.L., DeRousseau, C.J., Koretz, J., 1987. Presbyopia: an animal model and experimental approaches for the study of the mechanism of accommodation and ocular aging. Eye 1 (Pt 2), 222–230.
Chin, N.B., Ishikawa, S., Lappin, H., Davidowitz, J., Breinin, G.M., 1968. Accommodation in monkeys induced by midbrain stimulation. Invest. Ophthalmol. 7, 386–396.
Crawford, K., Terasawa, E., Kaufman, P.L., 1989. Reproducible stimulation of ciliary muscle contraction in the cynomolgus monkey via a permanent indwelling midbrain electrode. Brain Res. 503, 265–272.
Crawford, K.S., Kaufman, P.L., Bito, L.Z., 1990. The role of the iris in accommodation of rhesus monkeys. Invest. Ophthalmol. Vis. Sci. 31, 2185–2190.
Croft, M.A., Glasser, A., Heatley, G., McDonald, J., Ebbert, T., Dahl, D.B., Nadkarni, N.V., Kaufman, P.L., 2006. Accommodative ciliary body and lens function in rhesus monkeys, I: normal lens, zonule and ciliary process configuration in the iridectomized eye. Invest. Ophthalmol. Vis. Sci. 47, 1076–1086.
Croft, M.A., Kaufman, P.L., Crawford, K.S., Neider, M.W., Glasser, A., Bito, L.Z., 1998. Accommodation dynamics in aging rhesus monkeys. Am. J. Physiol. 275, R1885–R1897.
Croft, M.A., McDonald, J.P., Nadkarni, N.V., Lin, T.L., Kaufman, P.L., 2009. Age-related changes in centripetal ciliary body movement relative to centripetal lens movement in monkeys. Exp. Eye Res. 89, 824–832.
Erickson-Lamy, K.A., Polansky, J.R., Kaufman, P.L., Zlock, D.M., 1987. Cholinergic drugs alter ciliary muscle response and receptor content. Invest. Ophthalmol. Vis. Sci. 28, 375–383.
Glasser, A., Kaufman, P.L., 1999. The mechanism of accommodation in primates. Ophthalmology 106, 863–872.
Glasser, A., Wendt, M., Ostrin, L., 2006. Accommodative changes in lens diameter in rhesus monkeys. Invest. Ophthalmol. Vis. Sci. 47, 278–286.
Haefliger, E., Parel, J.M., 1994. Accommodation of an endocapsular silicone lens (Phaco-Ersatz) in the aging rhesus monkey. J. Refract. Corneal Surg. 10, 550–555.
He, L., Wendt, M., Glasser, A., 2012. Full-field accommodation in rhesus monkeys measured using infrared photorefraction. Invest. Ophthalmol. Vis. Sci. 53, 215–223.
Hubbard, W.C., Kee, C., Kaufman, P.L., 1996. Aceclidine effects on outflow facility after ciliary muscle disinsertion. Ophthalmologica 210, 303–307.
Jampel, R.S., Mindel, J., 1967. The nucleus for accommodation in the midbrain of the macaque. Invest. Ophthalmol. 6, 40–50.
Kaufman, P.L., Bárány, E.H., 1975. Subsensitivity to pilocarpine in primate ciliary muscle following topical anticholinesterase treatment. Invest. Ophthalmol. 14, 302–306.
Kaufman, P.L., Bárány, E.H., 1976. Loss of acute pilocarpine effect on outflow facility following surgical disinsertion and retrodisplacement of the ciliary muscle from the scleral spur in cynomolgus monkey. Invest. Ophthalmol. Vis. Sci. 15, 793–807.
Koopmans, S.A., Terwee, T., Glasser, A., Wendt, M., Vilipuru, A.S., van Kooten, T.G., Norrby, S., Haitjema, H.J., Kooijman, A.C., 2006. Accommodative lens refilling in rhesus monkeys. Invest. Ophthalmol. Vis. Sci. 47, 2976–2984.
Koretz, J.F., Bertasso, A.M., Neider, M.W., True-Gabelt, B., Kaufman, P.L., 1987. Slit-lamp studies of the rhesus monkey eye. II Changes in crystalline lens shape, thickness and position during accommodation and aging. Exp. Eye Res. 45, 317–326.
Lütjen-Drecoll, E., Tamm, E., Kaufman, P.L., 1988a. Age changes in rhesus monkey ciliary muscle: light and electron microscopy. Exp. Eye Res. 47, 885–899.
Lütjen-Drecoll, E., Tamm, E., Kaufman, P.L., 1988b. Age-related loss of morphologic responses to pilocarpine in rhesus monkey ciliary muscle. Arch. Ophthalmol. 106, 1591–1598.
Neider, M.W., Crawford, K., Kaufman, P.L., Bito, L.Z., 1990. In vivo videography of the rhesus monkey accommodative apparatus: age-related loss of ciliary muscle response to central stimulation. Arch. Ophthalmol. 108, 69–74.
Nishi, O., Nishi, K., 1998. Accommodation amplitude after lens refilling with injectable silicone by sealing the capsule with a plug in primates. Arch. Ophthalmol. 116, 1358–1361.
Ostrin, L.A., Glasser, A., 2005. Comparisons between pharmacologically and Edinger-Westphal-stimulated accommodation in rhesus monkeys. Invest. Ophthalmol. Vis. Sci. 46, 609–617.
Ostrin, L.A., Glasser, A., 2007. Edinger-Westphal and pharmacologically stimulated accommodative refractive changes and lens and ciliary process movements in rhesus monkeys. Exp. Eye Res. 84, 302–313.
Rosales, P., Wendt, M., Marcos, S., Glasser, A., 2008. Changes in crystalline lens radii of curvature and lens tilt and decentration during dynamic accommodation in rhesus monkeys. J. Vis. 8, 1–12.
Tornqvist, G., 1964. Comparative studies of the effect of pilocarpine on the pupil and on the refraction in two species of monkey (Cercopithecus ethiops and Macaca irus). Invest. Ophthalmol. 3 (4), 388–398.
Tornqvist, G., 1965. Effect on refraction of intramuscular pilocarpine in two species of monkey (Cercopithecus aethiops and Macaca irus). Invest. Ophthalmol. 4 (2), 211–215.
Tornqvist, G., 1966. Effect of topical carbachol on the pupil and refraction in young and presbyopic monkeys. Invest. Ophthalmol. 5 (2), 186–195.
Tornqvist, G., 1967. Accommodation in monkeys: some pharmacological and physiological aspects. Acta Ophthalmol. 45, 429–460.
Vilupuru, A.S., Glasser, A., 2002. Dynamic accommodation in rhesus monkeys. Vis. Res. 42, 125–141.
Vilupuru, A.S., Roorda, A., Glasser, A., 2004. Spatially variant changes in lens power during ocular accommodation in a rhesus monkey eye. J. Vis. 4, 299–309.
Wendt, M., Croft, M.A., McDonald, J., Kaufman, P.L., Glasser, A., 2008. Lens diameter and thickness as a function of age and pharmacologically stimulated accommodation in rhesus monkeys. Exp. Eye Res. 86, 746–752.
Wendt, M., Glasser, A., 2010. Topical and intravenous pilocarpine stimulated accommodation in anesthetized rhesus monkeys. Exp. Eye Res. 90, 605–616.
Wendt, M., Glasser, A., 2012. Reproducibility of carbachol stimulated accommodation in rhesus monkeys. Exp. Eye Res. 99, 89–97.