Form Deprivation Myopia in Mature Common Marmosets (Callithrix jacchus)

David Troilo, Debora L. Nickla, and Christine F. Wildsoet

PURPOSE. Experimental manipulations of visual experience are known to affect the growth of the eye and the development of refractive state in a variety of species including human and nonhuman primates. For example, it is well established that visual form deprivation causes elongation of the eye and myopia. The effects of such manipulations have generally been examined in neonatal or juvenile animals. Whether adolescent common marmosets (a new world primate) are susceptible to form deprivation myopia was studied.

METHODS. Five adolescent marmosets were used in this study. Monocular form deprivation was induced by lid closure for 12 to 20 weeks, starting between 299 and 315 days of age. The effects of deprivation were assessed with keratometry, A-scan ultrasonography, and cycloplegic refractions. Both eyes (treated and fellow control) were measured before lid-closure, at the end of the deprivation period, and several times over the following 8 to 12 weeks.

RESULTS. Adolescent marmosets are susceptible to visual form deprivation myopia. The experimental eyes showed significant axial elongation and myopia relative to the fellow control eyes. These changes were smaller, however, than those observed in younger eyes deprived for comparable periods. Like juvenile animals, the adolescent marmosets did not show recovery from myopia over the period monitored.

CONCLUSIONS. The period for susceptibility to form deprivation myopia in the marmoset monkey extends beyond the early developmental period when ocular growth is rapid and emmetropization normally takes place. Visual form deprivation in adolescent marmosets with adult-sized eyes results in increased ocular growth and myopia. These data suggest that visual factors may influence the growth and refractive development of the human eye after puberty and may be involved in late-onset myopia. (Invest Ophthalmol Vis Sci. 2000;41:2043-2049)

Research using animal models has shown, beyond doubt, that visual experience affects eye growth and refractive development (for review see Refs. 1–3). The original work in this field established the fact that visual form deprivation produces excessive axial elongation and myopia in a variety of species studied, including primates.4–7 Pathology-related form deprivation in human infants has also been associated with the development of axial myopia.8–15

The development of form deprivation myopia is presumably a consequence of the visually guided eye growth control system running open-loop in the absence of normal visual experience. Evidence for visual regulation of ocular growth comes from the observation that after the termination of the deprivation treatment young eyes generally recover to emmetropia.14–17 Even stronger evidence for visual control of eye growth is the compensatory growth response elicited in several different species by spectacle lens-imposed defocus.18–25

Common to nearly all these studies is the fact that the subjects are typically neonates or young juveniles whose eyes are still rapidly elongating and growing toward emmetropia.7,14,24–26 Although myopia can be detected in human infants and young juveniles, it typically first presents in childhood from 9 to 10 years of age or later,27,28 well after the ocular growth rate has slowed and the eye has approached adult dimensions.29 This has led some clinical researchers to question the validity of experimental models of myopia,30,31 most of which use neonatal animals, for studying the development of myopia in humans.

There are a few studies that show age-dependent effects on form deprivation myopia,6,14,25,26,27 or compensation for negative power spectacle lenses.33 Mature animals generally show a reduced response to form deprivation relative to those of younger animals (chicks,32,33 macaques,34 and quokka wallabies35), raising the possibility that there is a sensitive period for the visual control of eye growth.

Our study examines the effect of lid suture-induced form deprivation in a new world primate, the common marmoset, at an age specifically selected for a comparison to the age in humans when myopia typically first presents. Several studies using neonatal and juvenile marmosets have reported experimentally induced myopia.7,22,56–58 We find that adolescent marmosets remain susceptible to form deprivation and develop axial myopia; the response is reduced, however, compared with that found in similarly treated younger animals. Furthermore, as in younger marmosets, form deprivation myopia from lid suture persists long after vision is restored. The subjects in this study were also part of a larger study examining marmoset...
scleral extracellular matrix changes associated with form deprivation myopia.\textsuperscript{39}

\section*{METHODS}

\subsection*{Subjects}

Five common marmosets (\textit{Callithrix jaccus}) were used as experimental subjects in this study. All marmosets were housed in family groups in our marmoset breeding facility. Artificial lighting was provided using daylight-balanced fluorescent lamps (Vita-Light, Fairfield, NJ) on a 12 hour light/12 hour dark diurnal cycle. Temperature was maintained at 75 ± 2°F and humidity at 45% ± 5%. Food and water were provided ad libitum. Food consisted of formulated dry pellets (Marmoset Lite; Animal Spectrum, North Platte, NE), with fresh fruit and protein supplements. Vitamin D\textsubscript{3} supplement was provided twice weekly.

\subsection*{Treatments and Measurements}

All marmosets used in this study were monocularly form deprived by lid suture; the fellow eyes were untreated and served as controls. The age of onset of the form deprivation treatment was between 299 and 315 days of age (mean, 306 days). Marmosets at this age are reaching sexual and physical maturity (puberty),\textsuperscript{40} and we consider them to be equivalent to human adolescents. The duration of form deprivation in the present study was between 79 and 133 days (mean, 108 days). Animals were measured before the lid-suture surgery, immediately after the lids were reopened, and at various times over the following 14 to 78 days. Additional comparisons were made with 5 animals from a previous study\textsuperscript{7} in which the animals were

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Development of refractive state (top) and growth of vitreous chamber (bottom) in untreated marmoset eyes. The period of deprivation for the older experimental animals (adolescents) in the present study is shown by the black vertical bars to the right (mean duration, 105 days). Data from these animals are compared with data from comparably treated younger animals (juveniles), from a previous study.\textsuperscript{7} The deprivation period for the juvenile subjects is shown by the black bars to the left (mean duration, 84 days). Note that the deprivation period for the adolescent marmosets is well after the rapid juvenile growth phase (bottom) and after emmetropia had been attained (top).}
\end{figure}
treated in the same way but for a slightly shorter period (84 days) and beginning at an earlier age (21 days). For a comparison to normal growth, the untreated eyes of 54 additional marmosets ranging in age from birth to 2 years were used.

Figure 1 shows the mean age and duration of form deprivation in this study (black bars on right) compared with that for a younger (juvenile) group of animals from a previous study (black bars on left). Notice that the juvenile group was deprived of form vision during the period of active emmetropization from neonatal hyperopia (Fig. 1, top) when the eyes were in the rapid juvenile growth phase (Fig. 1, bottom). In contrast, the adolescent marmosets used in this study were form-deprived after eye growth had slowed, the eyes were nearly adult size, and refractions had stabilized around emmetropia (or slight myopia, as typically seen in untreated marmosets, see Troilo and Judge). In humans, the comparable period is usually around 13 years of age, approximately at the beginning of puberty.

Lid-suture surgery was performed while the animals were anesthetized by intramuscular injection of Saffan (0.2 mL/100 g; Pittman-Moore, UK). Briefly, the upper and lower outer lid margins were trimmed away, and the tarsal plates were separated from the lids. The upper and lower tarsii were sutured together with 7-0 vicryl. The lid margins were then sutured together with 5-0 silk. A small (<2 mm) drainage opening was left at the nasal canthus. The outer sutures were removed 5 to 7 days later. At the end of the treatment period, the marmosets were anesthetized and the lids were reopened along the suture line.

Measurements of refractive state, axial ocular dimensions, and corneal curvature were made after cycloplegia was induced in the animals with two drops of 1% cyclopentolate, given 5 to 10 minutes apart. Measurements were made 60 minutes later. This protocol reliably produces maximal cycloplegia in marmosets. Animals were anesthetized with Saffan for tractability. A lid retractor was used to hold the lids open for measurement. Refractive errors were measured by retinoscopy and refractometry (Hartinger Coincidence Refractometer; Zeiss, Oberkochen, Germany), in that order. These two measures were always performed independently by the same two investigators. In each case, the refractive errors of the two principal meridia were averaged to obtain spherical equivalent data; the two sets of refractive error data were then averaged. To measure ocular dimensions (anterior chamber depth, lens thickness, vitreous chamber depth, and choroid thickness), high-frequency (30 MHz) A-scan ultrasonography was used. Infrared videokeratometry was used to measure the radius of corneal curvature.

Additional details of the surgical and measurement methods are given in previous studies. All animal use in this study was in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. An early report of this study was presented at the annual meeting of the Association of Research in Vision and Ophthalmology.

RESULTS

We found that the eyes of adolescent marmosets remain susceptible to form deprivation; the vitreous chamber becomes longer than normal and myopia develops. Figure 2 shows the refractive errors and vitreous chamber depth in both the experimental (black bars) and control (white bars) eyes before (pre) and at the end (post) of the deprivation period. Refractive error and vitreous chamber depth were not significantly different between the experimental and control eyes before the deprivation. After the period of deprivation, the refractive errors (Fig. 2, top) were significantly more myopic in the experimental versus the control eyes (mean ± SE; -4.29 ± 1.2 versus -1.29 ± 1.2 diopters [D]; paired t-test, P < 0.05). This myopia is axial in nature: The vitreous chamber depth of the experimental eye becomes significantly larger than that of the control eye over the period of deprivation (Fig. 2, bottom). The form deprivation did not produce any significant change in corneal radius of curvature (3.49 ± 0.03 versus 3.47 ± 0.06 mm; P = 0.67), anterior chamber depth (1.86 ± 0.03 versus 1.86 ± 0.02 mm; P = 0.76), lens thickness (1.82 ± 0.04 versus 1.81 ± 0.03 mm; P = 0.74), or choroid thickness (136 ± 12 versus 138 ± 4 μm; P = 0.83).

All the individual animals deprived of form vision in this study showed a susceptibility to form deprivation myopia, but
they differed in the magnitude of their response. To indicate the variability in the refractive errors and axial growth responses between animals, Figure 3 shows the change in both the experimental and the control eyes for the five individual subjects plotted as arrows; the tails represent the measurement at the onset of deprivation, and the heads indicate the measurement at the end of deprivation. Although there is some variability in the magnitude of the response, in all cases the form-deprived eyes were more myopic at the end of the deprivation period relative to the beginning, and became more myopic relative to their untreated fellow eyes (Fig. 3, top). Similarly, the vitreous chamber depths of the treated eyes increased more than those of their fellow eyes (Fig. 3, bottom) over the period of deprivation. Note that at this age there is still some elongation in the untreated fellow eyes, but the changes are significantly smaller than those for the experimental eyes (paired t-test, \( P < 0.05 \)).

Although form deprivation in our adolescent marmosets reliably produced significant axial elongation and myopia, the magnitude of the effect was reduced compared with similarly deprived younger marmosets (Figs. 4 and 5). In the older adolescent animals the change in refractive error at the end of the deprivation resulted in approximately half as much myopia as seen in younger animals, although this difference did not reach statistical significance (Fig. 4, top: \(-3.00 \pm 1.49 \) D, older, versus \(-7.35 \pm 1.94 \) D, younger; unpaired \( t \)-test, \( P = 0.11 \)). Similarly, the mean interocular difference in vitreous chamber depth in the older group is significantly smaller than in the younger group (Fig. 4, bottom: \( 0.18 \pm 0.06 \) mm, older, versus \( 0.68 \pm 0.15 \) mm, younger; unpaired \( t \)-test, \( P < 0.01 \)). Although the reduced myopia can be at least partially explained by the fact that refractive error corresponding to a given change in axial length is reduced in larger eyes due to optical scaling effects,\(^\text{14,16} \) this does not explain the reduced amount of elongation in the experimental eyes of older animals. The reduced response to form deprivation in older marmosets occurs despite these animals having been deprived for a longer duration than the younger animals (means, 108 versus 83 days). Thus, the age-related decrease in response might be even greater than suggested by our data.

It has previously been reported that juvenile marmosets do not recover from the form deprivation myopia induced by

![Figure 3](image3.png)

**Figure 3.** The changes in refractive error (top) and vitreous chamber (bottom) of experimental (solid black arrows) and control (dashed white arrows) eyes over the deprivation period are shown for individual marmosets. The tails of the arrows represent the values before deprivation, the heads of the arrows represent the values at the end of the deprivation period. The duration of deprivation in days for each individual is shown in brackets in the bottom panel. In all cases the form-deprived eyes grew longer than their fellow eyes during the treatment period and became relatively more myopic, although the magnitude of the response varied among animals.

![Figure 4](image4.png)

**Figure 4.** Comparison of the mean (±SE) interocular difference (experimental − control) for refractive error (top) and vitreous chamber depth (bottom) after form deprivation. Data from the older adolescent group (black bars) of form-deprived marmosets are compared with the younger juvenile group (gray bars) of marmosets comparably treated (data from Troilo and Judge\(^\text{7} \)). For refractive errors, negative values indicate more myopia in the experimental eye. For vitreous chamber depth, positive values indicate that the experimental eyes have deeper (longer) chambers. There was significantly less vitreous chamber elongation and generally less myopia in the adolescent group compared with the juvenile group.
lid suture. We found in the present study that animals deprived later in life similarly do not show recovery from the induced myopia (Fig. 5). After the end of lid suture the treated eyes of both the younger and older groups of marmosets remained longer than their fellow controls (Fig. 5, bottom) and, as a consequence, the myopia persisted (Fig. 5, top). The interocular differences at lid opening did not differ significantly from the interocular differences at the last measurement taken (Fig. 5, bar insets). Figure 5 also again shows the relatively reduced response to lid suture in the older marmosets (right) compared with the younger ones (left).

**DISCUSSION**

Our results show conclusively that marmosets remain susceptible to form deprivation myopia during puberty, after the eyes have slowed their growth rate and are nearly adult size. During the comparable period in humans, most juvenile myopia has already presented. The response to form deprivation in our older marmosets was reduced compared with that in similarly treated younger juvenile eyes; the older eyes elongate less and become less myopic. These findings are consistent with, and extend, reports from previous studies of older animals in several other species, including chicken, tree shrew, tree shrew, quokka wallabies, and macaques. Analysis of available data from macaques deprived at a variety of ages for variable durations suggests that the susceptibility to form deprivation declines exponentially, leaving open the possibility that even late in adulthood there remains some degree of responsiveness to visual signals that influence the size and refractive state of the eye.

Several possibilities might explain the reduction in response with age. It is possible that there are age-related changes in the sensory processing of stimuli driving the eye growth control system. We speculate, however, that the reduced responses are more likely to involve changes in the efferent side of the growth control system: Because the eye is larger and slower growing, there may be limits on the ability of the sclera to respond to the deprivation. In both animals and humans axial myopia is associated with changes in scleral thickness, cell proliferation, and extracellular matrix synthesis. Such changes have been described for chicks, tree, and tree.
shrews,48–50 and marmosets.39,51 In juvenile tree shrews, form deprivation causes a decrease in proteoglycan synthesis, presumably related to the restructuring of the sclera.50 We find that the scleras of adolescent marmosets with induced myopia also show reduced proteoglycan synthesis.39 These results imply that similar processes are involved in elongating the eye at all ages, suggesting that an attenuation of the response, rather than an inability of the sclera to respond, is the mechanism for the age-related decline in form deprivation myopia.

Similar to what is found in juvenile marmosets,7 we found that the axial elongation and myopia produced by lid suture in adolescent marmosets persist well after the end of the deprivation. This is in marked contrast to the recovery from form deprivation myopia found in young chicks,14,16 tree shrews,13,14,21,26 and macaques.5,17 To date, there have been no studies looking at recovery from form deprivation in older animals. However, the absence of recovery from lid-suture myopia in the marmoset is not related to age per se because it is also seen in young animals. It is also presumably not a consequence of a species difference in the response to form deprivation but, rather, to a procedural effect: Preliminary evidence shows that marmosets deprived of form vision using plastic diffusers do, in fact, show signs of recovery.5,2 We speculate that the inability to recover from the lid suture–induced myopia may be due to permanent changes caused by having the lids closed for a long period; these changes could be related to factors such as alterations in corneal oxygen tension or temperature, for instance.

We conclude that visual form deprivation has the ability to alter ocular growth well past infancy and into maturity. Our results support the notion that emmetropization remains active throughout life53 and can be perturbed by abnormal visual experience. These findings have clinical relevance in that they imply that visual experience may be involved in the development of both late-onset and juvenile-onset human myopia and lend further validity to the use of animal models for myopia research.

Acknowledgments

The authors thank Margo Cummings, Heidi Denman, and Jason Griffith for their assistance with marmoset care.

References