Sympathetic Nervous System Plays a Role in Postnatal Eyeball Enlargement in the Rabbit

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PURPOSE. To examine the role of ocular sympathetic activity in the enlargement of the rabbit eyeball during postnatal growth.

METHODS. Fourteen New Zealand albino rabbits aged 5 weeks underwent unilateral surgical transection of the cervical sympathetic trunk caudal to the superior cervical ganglion. Postoperative enlargement of both eyeballs was monitored by measuring the axial length and corneal diameters every 2 weeks for 22 weeks (7–27 weeks of age). Rabbits were housed under a 12-hour light/12-hour dark cycle, and the measurements were made in the middle of the light period. At a final age of 30 to 31 weeks, the refractive state of the whole eye was determined on both sides by measurement through the central cornea with a refractometer. Rabbits were then killed, eyeballs enucleated, and their ocular volumes determined.

RESULTS. From 9 weeks of age the axial length and corneal diameters were significantly shorter (P < 0.05) in the decentralized eye (surgical side) compared with the intact eye. This reduction remained statistically significant throughout the study period. However, the final refractive states of the two eyes were found not to be different. The mean ocular volume determined after postmortem enucleation was 4.5% less in the decentralized eye than in the intact eye (P < 0.05).

CONCLUSIONS. Sympathetic nervous system activity is involved in the normal enlargement of the rabbit eyeball during postnatal growth. However, removal of the ocular sympathetic tone at the age of 5 weeks does not significantly alter the refractive state of the eye when measured in young adulthood. (Invest Ophtalmol Vis Sci. 2000;41:2684–2688)

When young adult rabbits were entrained under a 12-hour light/12-hour dark cycle, a consistent 24-hour variation in axial length (the distance between the center of the cornea and the posterior pole of the retina) was observed.1 This variation of axial length was endogenous and independent of the level of illumination because these data were collected during an acute constant dark period. The physiological basis for the 24-hour variation in axial length is largely unknown, except that ocular sympathetic activity probably plays a role. When the eye’s sympathetic tone was removed by surgical transection of the cervical sympathetic trunk in young adult rabbits, the magnitude of the nocturnal elongation of axial length was smaller on the operated side than the unoperated, control side.1

The eye, in concert with the body, grows considerably during postnatal development. Given that ocular sympathetic activity is probably involved in the nocturnal elongation of axial length,1 we hypothesized that ocular sympathetic activity might play a role in the postnatal enlargement of the rabbit eyeball. Such a positive relationship between the sympathetic nervous system and eyeball enlargement has not been previously observed in humans or any other animal species. Human patients with congenital Horner’s syndrome, in which sympathetic signals to the head (including the eye) are compromised early in life, have not been described as having a gross abnormality in eyeball size.2 Experimental data from chicks, which are frequently used to study eye growth, indicate a negative correlation between ocular sympathetic activity and eyeball enlargement. Superior cervical ganglionectomy in neonatal chicks (2 days post hatching) potentiates the developmental increases in eye weight3 and axial length.4

In the present study, we examined the enlargement of the rabbit eyeball for approximately 6 months after surgically removing its sympathetic tone at 5 weeks of age. Postoperatively, the sizes of the eyeballs on the operated side and on the unoperated side were compared at regular intervals until the rabbit grew to young adulthood. Eyes were refracted before euthanasia, and ocular volumes were measured after postmortem enucleation.

METHODS

New Zealand albino rabbits were used in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Fourteen rabbits, 4 weeks of age, were purchased from a local supplier. At this time they were approximately 0.7 kg in body weight and were weaned from their mothers. Rabbits were housed in individual cages in a room kept at a constant temperature (21°C). The lighting was adjusted to go on at 6 AM and off at 6 PM providing a daily cycle of 12 hours of light and 12 hours of dark. Food and water were available ad libitum.
Approximately 1 week after arrival, each rabbit underwent unilateral transection of the cervical sympathetic trunk. The cervical sympathetic trunk was sectioned caudal to the superior cervical ganglion in the neck area. The postganglionic nerve fibers remained intact while not receiving their normal preganglionic drive from cells of the intermediolateral column of the thoracic spinal cord (decentralization). The transection was performed on the right side in 8 rabbits and on the left side in 6 rabbits. Rabbits were allowed to recuperate postoperatively in the accustomed daily light–dark cycle. Between 1 to 2 weeks after surgery the rabbits were examined for the appearance of miosis and ptosis on the operated side to verify the loss of sympathetic tone. In addition, decentralization of the ocular sympathetic nerves was verified by two tests involving either topical hydroxyamphetamine or topical cocaine. These tests are a way of confirming that the sympathetic nerves (i.e., postganglionic fibers) were intact in both eyes, but that the sympathetic tone was lost in the decentralized eye.

Selected parameters of the eyeball (axial length, anterior chamber depth, lens thickness, vitreous chamber depth, and horizontal and vertical corneal diameters) were determined before surgery (i.e., at 5 weeks of age) and subsequently every 2 weeks (±1 day) for 22 weeks postoperatively. At each measurement session, the individual rabbit was removed from its cage at noon (±1 hour), weighed, and placed in a shallow tray in a designated area with constant illumination. No further restraint was necessary. In all cases the right eye was measured first. One or two drops of 0.1% proparacaine (a 1:5 dilution of 0.5% commercial preparation) were applied to the eye. An ultrasonic sensor probe (Humphrey Ultrasonic Biometer, model 810; San Leandro, CA) was gently placed on the corneal surface with care taken to measure from the central-most region and to hold the probe perpendicular to the surface. Values of axial length, anterior chamber depth, and lens thickness were obtained from the Ultrasonic Biometer. Three measurements were taken from each eye, and the average was used in data analyses. The depth of the vitreous chamber was calculated by subtracting the anterior chamber depth and lens thickness from the axial length. The horizontal and vertical corneal diameters were measured manually with a Castroviejo caliper. After data had been collected from each rabbit, the values of the decentralized eyes and the intact eyes were grouped and comparisons were made using the paired t-test. A difference of \( P < 0.05 \) was regarded as statistically significant.

It is known that sympathetic decentralization reduces the nocturnal elongation of axial length and the nocturnal elevation of intraocular pressure (IOP) at 3 to 4 weeks after the operation. However, the long-term effects of sympathetic decentralization on axial length and IOP have not been determined. For 12 of the 14 postoperative rabbits in the present study, light–dark variations in axial length and IOP were studied at weeks 23 to 24 postoperatively (28–29 weeks of age). To eliminate any influence of environmental light, the measurements were performed in constant darkness. All light in the rabbit holding room was extinguished at 11 AM, and the first set of measurements was made at noon. Subsequent readings were made every 4 hours, on the hour (±15 minutes), until 8 AM the next day. A dim red photograph-safe light (wavelength > 600 nm; intensity < 5 lux) was turned on as needed to assist with the measurements. For each measurement session, axial length was determined first using the Ultrasonic Biometer as described previously, and then the IOP was measured with a modified pneumatonometer previously calibrated for the rabbit eye. The average axial length and average IOP in the “subjective” light period (pooled from the 3 measurements of 8 AM, noon, and 4 PM) and the dark period (pooled from 8 PM, midnight, and 4 AM) were calculated for each eye. The changes in the average axial length from the subjective light period to the dark period were compared using the paired 2-sample t-test (\( n = 12 \)). Similarly, elevations of average IOP from the subjective light period to the dark period were compared between the two eyes.

All 14 rabbits were killed at a final age of 30 to 31 weeks, by which age we were having difficulty performing ultrasonography in a timely manner due to frequent movements of the rabbit’s head. Immediately before euthanasia the refractive state of each eye was measured in the rabbits while conscious. Readings were taken through the central cornea using a Jena Coincidence Refractometer (model 110; Seiler Instrument & Manufacturing, St. Louis, MO). At least 3 consecutive measurements were made for each eye, and the average was calculated. Rabbits were then killed by intravenous injection of 1 ml Beuthanasia-D (Scherer–Plough, Kenilworth, NJ). The eyes were enucleated, the right eye first, and rinsed thoroughly with saline. The extraocular muscles and connective tissue were carefully removed from the globe, and the optic nerve was cut as close to the sclera as possible. The globe was cleared of excess fluid by dabbing with filter paper, and it was carefully immersed in a container (approximately 30 mm in diameter) half-filled with saline. The height of the fluid column in the container was measured (to an accuracy of 0.01 mm) using the Ultrasonic Biometer sensor attached to a stereotaxic micromanipulator (model 1460; David Kopf Instruments, Tujunga, CA). The sensor was slowly lowered until it touched the surface of the fluid. The height of the fluid column was determined before and after immersion of the eyeball. The change in the height of the fluid column was converted to the volume of the eyeball according to a preestablished relationship between the height of fluid column and the volume of saline in the container. Ocular volumes of the decentralized eyes and the intact eyes were compared using the paired t-test.

**Results**

Hydroxyamphetamine and cocaine tests confirmed that the unilateral sympathetic decentralization performed at 5 weeks of age was successful in all 14 rabbits. All rabbits continued growing during the course of the study with an increase of body weight from 0.7 ± 0.1 kg (mean ± SD) initially to 4.5 ± 0.4 kg at the time of euthanasia. Based on the steady increase in individual body weight and normal cage behavior, we ascertained that all rabbits recovered from the surgery normally and remained healthy.

The mean values of axial length, horizontal corneal diameter, lens thickness, and anterior chamber depth in the decentralized eye and in the intact eye during the study period are summarized in Figure 1. Differences between the decentralized eye and the intact eye are presented in Figure 2. Beginning at 9 weeks of age, the axial length of the decentralized eye was significantly less than that of the intact eye. Horizontal and vertical corneal diameters were also significantly less in the decentralized eye from 9 weeks of age. The reduction of axial length in the decentralized eye was from both the anterior and
vitreous chamber components. Anterior and vitreous chamber depths were both less in the decentralized eyes, although a consistent reduction was not apparent until slightly different ages (i.e., 13 and 11 weeks, respectively). The effect of sympathetic decentralization on lens thickness was relatively small. However, the lens was consistently thicker in the decentralized eye from 15 weeks of age. These differences in eyeball parameters between the operated and control sides remained statistically significant until at least 27 weeks of age.

The light–dark variations in axial length and IOP appeared in 12 rabbits postoperatively at 28 to 29 weeks of age. The pattern of variation in axial length, as well as IOP, with time was generally similar in both eyes (Table 1); it troughed in the subjective light period and peaked in the dark period. At all 6 time points, the mean value of either axial length or IOP was less in the decentralized eye than in the contralateral, intact eye. For both eyes, the average axial length and IOP in the dark period were greater than their respective values in the subjective light period. The elongation of average axial length from the subjective light period to the dark period in the decentralized eye, $0.13 \pm 0.02$ mm (mean $\pm$ SEM, $n = 12$), showed no statistical difference from that of the intact eye, $0.14 \pm 0.05$ mm. However, the light–dark elevation of average IOP in the decentralized eye ($2.0 \pm 0.4$ mm Hg) was significantly less ($P < 0.05$) than in the intact eye ($3.6 \pm 0.7$ mm Hg).

The final refractive state of each eye was successfully determined for 13 of the 14 rabbits (1 rabbit presented no clear refractive images). Measurements were performed at 30 to 31 weeks of age, immediately before euthanasia. Mean refractive power was $-0.1 \pm 0.3$ D (mean $\pm$ SEM, $n = 13$) for the decentralized eye and $0.4 \pm 0.4$ D for the intact eye. The difference between the two eyes was neither consistent nor statistically significant (paired $t$-test). The mean ocular volume determined postmortem was $2.69 \pm 0.06$ cm$^3$ ($n = 14$) for the decentralized eyes, which was significantly less ($P < 0.05$) than that for the intact eyes ($2.81 \pm 0.05$ cm$^3$). The average reduc-

![Figure 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933219/) shows the parameters of axial length ($E$), anterior chamber depth ($\dagger$), vitreous chamber depth ($L$), and lens thickness ($M$). Differences that reached statistical significance with the paired $t$-test are indicated by asterisks (*$P < 0.05$; **$P < 0.01$).

![Figure 2](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933219/) The developmental increase of eyeball parameters in the postnatal rabbit. The mean derived from 14 rabbits is plotted for the control eye (nonsurgical side; closed circles), and the eye underwent decentralization of the ocular sympathetic nerves at 5 weeks of age (open circles). Three parameters (axial length, horizontal corneal diameter, and anterior chamber depth) showed a difference between the decentralized eye and the control eye, with the former consistently being smaller. Only for lens thickness was the decentralized eye larger than the control eye.
tion in the decentralized eye was 4.5% compared with the intact eye.

**DISCUSSION**

**Effects on Eyeball Parameters and Refractive State**

Previous studies in rabbits have demonstrated that postnatal eyeball enlargement can be retarded by damaging ocular structures using transcleral cryotherapy of the ciliary body and laser photocoagulation or transcleral cryotherapy of the anterior choroid and retina. When an eye is injured it is difficult to evaluate only the direct effects of reducing ocular sympathetic tone that would lead to damage of other ocular tissues, although there might eventually be some anterograde, transsynaptic degeneration of the postganglionic sympathetic nerves. A transsynaptic degeneration seems unlikely to develop within a few weeks after the sectioning of the cervical sympathetic trunk, when the reductions of eyeball parameters begin to appear. The likelihood that transsynaptic degeneration of the ocular sympathetic nerves would lead to damage of other ocular tissues is low.

In addition to signals to the eye, unilateral transection of the cervical sympathetic trunk removes sympathetic signals to other parts of the head. For example, the pineal gland receives bilateral postganglionic innervation from the superficial cervical ganglia. Unilateral superior cervical ganglionectomy reduces pineal function. Therefore, unilateral sectioning of the cervical sympathetic trunk may lead to a centrally mediated change affecting the whole body including the eyes. By comparing eyeball parameters between the two eyes, we are able to evaluate only the direct effects of reducing ocular sympathetic activity. Whether there is a centrally mediated postoperative influence on eyeball enlargement cannot be evaluated in an individual rabbit because the effect would be similar in both eyes.

Our results support the hypothesis that sympathetic input to the eye plays a role in the postnatal enlargement of the rabbit eyeball. Decentralization of the ocular sympathetic nerves at the age of 5 weeks significantly slowed the normal postnatal, developmental increases in axial length and corneal diameters by 9 weeks of age. The magnitude of the reduction of these parameters was relatively stable after 11 to 13 weeks of age. It is generally believed that at an early age the rabbit eyeball is more susceptible to induced growth change than in adulthood. Exogenous forces (an increase of IOP plus a rise of body temperature) can change the refractive state of the eye (a reflection of ocular size) in 5- to 6-week-old rabbits but not in 6- to 8-month-old rabbits. In the present study, the physiological changes brought about by the sectioning of the cervical sympathetic trunk at the age of 5 weeks were translated into differences in axial length and corneal diameters at the age of 9 weeks. But, the same surgical procedure performed in young adult rabbits in a previous study did not cause a change in axial length, measured in the subjective light period from 3 to 4 weeks postoperatively.

Although the rabbit eyeball grows considerably during the age interval of 5 to 31 weeks, it maintains a relatively constant refractive state. The refractive state is determined by the axial length and the refractive powers of the cornea and lens. In the present study, both the decentralized and intact eyes had a normal final refractive state. This suggests that emmetropization took place in the decentralized eye despite the loss of sympathetic tone. However, it should be noted that our refractive data were obtained through the central cornea. We cannot rule out the possibility that loss of sympathetic tone affects the refractive state through the nasal, peripheral cornea that is used by rabbits for near vision.

**How Sympathetic Tone Might Influence Eyeball Enlargement**

One possible explanation for our finding that removal of sympathetic tone slows the normal enlargement of the rabbit eyeball involves the daily fluctuation of IOP. Light–dark entrained laboratory rabbits exhibit circadian variation in IOP, with it being high in the dark period. Although several physiological factors are likely to be involved in this circadian IOP fluctuation, the nocturnal increase of ocular sympathetic activity is the most important factor. A circadian IOP fluctuation of several millimeters of mercury is within the range for a reversible stretch of the rabbit eyeball. It is possible that the decrease in the magnitude of daily IOP fluctuation brought about by the sympathetic decentralization leads to less dy-
dynamic stretch on the elastic ocular coats and less eyeball enlargement. Previously, a 24-hour variation of IOP was suggested to play a role in normal eye enlargement in chicks.\(^1\)

Clinical observations suggest that excessive ocular parasympathetic tone during visual accommodation may lead to myopia,\(^2\) which is usually accompanied by an increase of axial length. Visual accommodation in humans is an active process determined by the balance between parasympathetic and sympathetic inputs to the eye. In the present study, sympathetic inputs to the rabbit eye are irreversibly lost after sectioning of the cervical sympathetic trunk. Although the rabbit’s eye has little ability to accommodate,\(^2\) it is possible that the effect of sympathetic decentralization on eyeball enlargement is related to the now unopposed parasympathetic tone, independent from accommodation. However, this unopposed parasympathetic tone causes a decrease in axial length.

Besides the mechanical factor of IOP fluctuation, changes in biochemical processes in the eye, such as the process of growth signals, also need to be considered as causative in the retardation of eyeball enlargement after sympathetic decentralization. Removal of ocular sympathetic tone in young adult rabbits leads to a rapid and long-lasting reduction in tyrosinase activity in the choroid and iris, and this affects iris color in pigmented rabbits.\(^2\)\(^-\)\(^2\) Whether or not there is a correlation between postnatal eyeball enlargement and iris pigmentation and whether or not tyrosinase or any other biochemical process plays a role in the enlargement of rabbit eyeball warrant further investigation. In chicks, eyeball enlargement is correlated with biosynthetic activity of the sclera,\(^2\)\(^-\)\(^2\)\(^5\) and the ocular growth may be regulated by various neural mechanisms.\(^2\)\(^6\)

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**References**