Pupillary size regulates the amount of light received in the retina. As light intensity in the environment changes, pupillary size changes accordingly. It is well understood that pupillary size increases when the intensity of environmental light decreases. However, whether there is an endogenous change in pupil size, parallel to the daily light--dark cycle, remains unsolved. Although a circadian change in human pupil size has been reported, supporting data are inconsistent and unconvincing (see review). There is no other information in the literature indicating the existence of an endogenous pupillary rhythm in nonhuman vertebrates.

In vertebrates, the autonomic nervous system plays an important role in regulating pupil size. Activation of the ocular parasympathetic nerves by light illumination of the retina causes miosis because of contraction of the iris sphincter muscles. Stimulation of the iris dilator muscles by the ocular sympathetic nerves or circulating catecholamines causes mydriasis. Under light conditions, changes in the intensity of illumination and visual accommodation rapidly affect the parasympathetic input to the iris, making the pupil size more variable. The pupil size in a totally dark environment (termed here as the basal pupil size) is more stable and can be measured by infrared video pupillometry.

Using infrared video technology, the 24-hour change in rabbit basal pupil size was examined in the current study. Basal pupil size in light--dark entrained rabbits clearly showed a circadian rhythm. The pattern of this circadian rhythm was compared with the circadian rhythms of intraocular pressure (IOP) and body temperature, two previously characterized circadian rhythms in the laboratory rabbit.

METHODS. Adult male New Zealand albino rabbits, ranging in weight from 3.6 to 4.2 kg, were used in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Rabbits were housed in individual cages at constant temperature (21°C) and humidity under a 12-hour light--12-hour dark cycle. Food and water were given ad libitum. Light came from the fluorescent ceiling lamps, and the light intensity in the rabbit cages was 150 to 300 lux. Lamps were on at midnight, defined as the 0 hour of the circadian time (CT). Rabbits were kept in this light--dark condition for at least 3 weeks. An expected increase of body temperature. Invest Ophthalmol Vis Sci. 1996;37:2345–2349.

### References

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circadian elevation of IOP at 2 hours into the dark phase6 was used to confirm the entrainment.

**Measurements of Pupil Diameter, Intraocular Pressure, and Body Temperature.** Basal pupil size was videotaped in the constant dark environment. Each rabbit was removed from its cage, put on a shallow tray (16 × 11 × 4 inches), and placed on a table. After transport, the rabbit’s eyes were always open and its head was allowed to move freely. A near infrared 880 nm light source (Strip-Lite; International Engineering, Virginia Beach, VA) was placed at a distance of 15 to 20 cm from the rabbit eye. An infrared-sensitive Sony SSC-M354 video camera equipped with a Nikon 80–200 mm zoom lens was set at approximately 3 m distance and at the same height as the rabbit eye. A real-time pupil image was displayed continuously on the on-line monitor and viewed by the researcher, but it was shielded from the rabbit. A commercial video recorder was used to tape the image. By slightly adjusting the position of the pupil tray, a clear, flat pupil image was obtained. The rabbit was then returned to its cage. Transport of the rabbit in the dark was assisted by a photo-safe dim red light (wavelength >600 nm; intensity <5 lux) because rabbits lack photoreceptors to detect this light spectrum.7 As a precaution, the on-line monitor and the video recorder were covered by a high-pass red filter (wavelength >600 nm).

A reference ruler, sitting vertically at the position of the rabbit eye, was also videotaped under the infrared light. The best images of pupil and reference ruler were selected from the tape later and digitized for off-line calculation of vertical pupil diameters with a customized computer program. The vertical pupil diameter image, unlike the horizontal pupil diameter image, was not affected by the change in the angle between the pupil and the optical axis of the camera (because of the movement of the rabbit head). When the pupil image was in focus, the distance between the camera and the rabbit eye was close to a fixed number (3 m). The deviation was determined to be less than 3 cm. According to simple geometry (3 cm/3 m), the maximal artifact in the calculated pupil diameter from the deviation in distance was less than 1%.

Intraocular pressure was measured after the administration of 1 drop of 0.1% proparacaine (Alcon, Fort Worth, TX), with a pneumotonometer system previously calibrated for the rabbit eye.8 Rectal temperature was measured using a YSI thermometer (Yellow Springs, OH) with the 402 probe inserted 7.5 cm into the rectum for approximately 1 minute. Before the start of a series of experiments, each rabbit was acclimated to handling, confinement in the tray, and measurements of pupil size, IOP, and rectal temperature.

Bilateral pupil diameters were recorded every hour on the hour (±10 minutes) throughout a complete 24-hour period in seven light–dark entrained rabbits. On the days of data collection, lamps were kept off during 0 to 12 hours CT (subjective light phase), and measurements were started at 8 to 10 hours CT. The 24-hour recording was accomplished in general by two researchers, with a shift change between 2 to 3 hours CT the next day.

Circadian rhythms of IOP and body temperature in these seven rabbits were documented. Measurements of bilateral IOPs and rectal temperature were performed in four separate sessions under the regular 12-hour light–12-hour dark cycle and in constant dark. Rabbits were removed from their cages and placed in the tray for hourly measurements of IOP or rectal temperature for 24 hours. Under the regular light–dark cycle, measurements at 12 hours CT were performed within 10 minutes before the onset of dark, and measurements at 24 hours CT were performed within 10 minutes before the onset of light. In the constant dark environment, measurements were taken following the same schedule as the video recording of the pupil. Measurements of IOP and rectal temperature in the dark were performed under dim red light.

**Sympathetic Decentralization.** Unilateral transection of the cervical sympathetic trunk was performed in 10 light–dark entrained rabbits under aseptic conditions. Rabbits were anesthetized with ketamine (50 mg/kg subcutaneously; Aveco, Fort Dodge, IA), chlorpromazine (10 mg/kg subcutaneously; Schein Pharmaceutical, Port Washington, NY) and pentobarbital (15 mg/kg intravenously; Abbott, Chicago, IL). A 1 cm section of the cervical sympathetic trunk proximal to the superior cervical ganglion, on the left side, was removed. Surgical procedures always were performed in the light phase. After surgery, rabbits were allowed to recover and then were maintained in the regular 12-hour light–12-hour dark cycle. Three weeks after the procedure, the sympathetic decentralization was confirmed by observations of unilateral miosis and ptosis in the left eye.

Basal pupil sizes in both eyes of the rabbits after surgery were videotaped hourly for 24 hours in the constant dark environment. In a separate experiment, bilateral measurements of IOP were performed hourly for a 24-hour period in constant dark.

**Data Analysis.** In normal rabbits, values of pupil diameter and IOP from both eyes were averaged and used for data entry. In sympathetic decentralized rabbits, values of pupil diameter and IOP from the decentralized eye and the intact eye were grouped separately. The differences in pupil diameter, as well as the IOP differences, between the two eyes also were calculated.

**RESULTS.** All the pupil sizes were videorecorded in the constant dark environment. Seven normal rabbits displayed smaller pupil diameters in the subjective
light phase and consistently larger pupil diameters in the subjective dark phase (Fig. 1, upper panel). Mean pupil diameter was minimal (8.2 ± 0.3 mm; mean ± SEM) at 5 hours CT (5 hours after the accustomed lights-on) and gradually increased afterward. It increased sharply during the first hour in the subjective dark phase (12 to 15 hours CT). The peak of pupil diameter (10.8 ± 0.1 mm) appeared at the beginning of the subjective dark phase. The average oscillation (peak minus trough) of pupil diameter translates to an enlargement of pupil size by 73%. Although pupil diameter fell gradually toward the end of the subjective dark phase, it was still larger than all the pupil diameters recorded in the subjective light phase.

A circadian rhythm of IOP was observed in the same rabbits under both the regular 12-hour light–12-hour dark cycle and the constant dark environment (Fig. 1, middle panel). The minimal IOP appeared between 4 and 6 hours CT, and an IOP peak appeared at the beginning of the subjective dark phase (14 hours CT). There was a second IOP peak at approximately 18 to 21 hours CT. As did the circadian pupillary change, IOP increased sharply during the first hour in the subjective dark phase and decreased gradually toward the end of the subjective dark phase. Average amplitudes of the IOP oscillation in the two experimental conditions were close (12 to 14 mm Hg).

Under the regular light–dark cycle and constant dark, rectal temperature was at the lowest at 6 to 8 hours CT. Rectal temperature increased continuously in the late subjective light phase and early subjective dark phase and peaked at 19 to 21 hours CT (Fig. 1, bottom panel). Unlike the circadian rhythms of pupil size and IOP, there was no sharp increase of rectal temperature at the beginning of the subjective dark phase. The average oscillation of rectal temperature was 0.5°C to 0.6°C.

Ten light-dark entrained rabbits underwent surgical decentralization of the ocular sympathetic nerves on one side. After 3 weeks of recuperation in the regular light–dark cycle, all rabbits exhibited unilateral miosis and ptosis, indicating a successful sympathetic decentralization. In the constant dark environment, a circadian pupillary enlargement in the subjective dark phase was present in the intact eye but not in the decentralized eye (Fig. 2). Pupil size in the decentralized eye was at least 1.5 mm smaller than in the intact eye and was relatively stable throughout the circadian cycle. When the IOP in these rabbits was measured in constant dark, the circadian IOP elevation in the intact eye was unaffected by the surgical procedure (Fig. 3). In the decentralized eye, a significant reduction of the circadian IOP elevation occurred. In addition to an IOP peak in the early subjective dark phase, a second IOP peak was noticed at the end of the subjective dark phase in both eyes. The difference in IOP between the two eyes, plotted as the bottom curve in Figure 3, showed a circadian pattern with a single peak at the beginning of the subjective dark phase. This pattern was similar to the circadian pattern of the difference in pupil size between the two eyes in the same rabbits (bottom curve, Fig. 2) or the circadian pupillary pattern in the normal rabbits.

**DISCUSSION.** The current study provides direct evidence that a circadian rhythm of pupil size exists in rabbits housed in a constant dark environment.
Transection of the cervical sympathetic trunk completely eliminated this circadian pupillary rhythm, indicating that the pathway mediating the circadian pupillary enlargement is through the ocular sympathetic nerves. Regulatory mechanisms from other pathways, including the ocular parasympathetic nerves, play a minimal role in this pupillary rhythm. It is unclear whether a similar circadian pupillary rhythm can be detected under a light condition.

Ocular sympathetic decentralization also significantly reduced the circadian IOP elevation in the subjective dark phase, which has been reported by others. Although there seemed to be more than one factor contributing to the circadian IOP rhythm, the major factor is mediated by the ocular sympathetic nerves. In unilaterally decentralized rabbits, the IOP difference between the decentralized eye and the intact eye should represent the portion contributed by the ocular sympathetic nerves. Figure 3 shows that this contributing factor appears to have a circadian pattern, which is almost identical to the pattern observed in the circadian change of basal pupil size (Fig. 1).

Another characteristic in the circadian pupillary rhythm also appeared in the circadian IOP rhythm—namely, there is a sharp increase in pupil size and IOP at the beginning of the subjective dark phase. These observations suggest that a relationship exists between these two physiological rhythms, which probably involve a common causal factor, the uninterrupted sympathetic signals. In a previous study, it was shown that a moderate frequency (5 Hz) electric stimulation of the ocular sympathetic nerves can enlarge pupil size and raise IOP before the onset of dark.

In addition to local sympathetic stimulation, an increase in humoral hormones related to physiologi-
We evaluated the argument that the circadian enlargement of rabbit pupil size is the consequence of an increase in physiological activities, working in concert with the ocular sympathetic signals, in the dark phase. It has been demonstrated that laboratory rabbits can be nocturnal, diurnal, or crepuscular active in locomotion, depending, in part, on laboratory noise and the feeding schedule. Therefore, the relevant physiological activities have to be determined in the same rabbits used for the pupillary study. In a totally dark environment, the rabbits' physiological activities were difficult to observe. However, judging by the noise level generated from the rabbit cages, it was our impression that rabbits were more active in the late dark phase. For a quantitative analysis, we measured the rabbit's deep rectal temperature, which is probably a useful indicator for its general physiological activities. Data showed that the average rectal temperature rose gradually from the middle of the subjective light phase and reached its peak in the late subjective dark phase. This circadian pattern is different from the circadian rhythm of basal pupil size. Thus, the nocturnal increase of physiological activities in these rabbits is unlikely to be the major cause for the circadian enlargement of pupil size, at least in the early dark phase. This observation indicates that the circadian IOP elevation in the early dark phase is probably independent from the rabbit's general physiological activities. A similar conclusion was derived using circulating catecholamines as the indicator for the rabbit's physiological activities. Whether the rise in physiological activities contributed to the second IOP peak in the dark phase cannot be concluded based on data in the current study.

Circadian rhythms are important biologic forces affecting body functions and behavior. Endogenous circadian rhythms are synchronized to the environmental light–dark cycle through neuroendocrinological changes in the brain. This entrainment process is thought to be accomplished by light stimulation in the subjective dark phase. In rabbits, there seems to be an endogenous adjustment to the entrainment by enlarging the basal pupil size in the subjective dark phase. Whether a similar endogenous pupillary enlargement exists in other species remains to be determined.

**Key Words**

body temperature, circadian rhythm, intraocular pressure, pupil, sympathetic nerve

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**References**


