The sensitivity of rat rod outer segment disc shedding to light

Arnold I. Goldman

Albino rats were raised in cyclic lighting and subjected to either constant light inhibition of the shedding peak or a 10 hr phase advance in the lighting schedule, with lighting levels of varied intensity. All responses followed sigmoid curves of shedding vs. intensity. For constant light inhibition the curve had a shallow slope with a 50% peak at 0.85 ft-cd. Shedding after a phase shift showed a steep curve with a 50% peak at 0.3 ft-cd when animals were observed at 9:30 P.M. and a shallow slope with a 50% peak at 0.003 ft-cd when observed at 7:30 A.M. It is concluded, that the mechanisms of shedding inhibition and phase shifts in the shedding rhythm differ with respect to the role of light. (INVEST OPHTHALMOL VIS SCI 22:695-700, 1982.)

Key words: Disc shedding, retina, light inhibition, phase shifts, circadian rhythms, light intensity, rat

It is well established that photoreceptor outer segment disc shedding follows a circadian rhythm in the rat\(^1\)-\(^3\) and at least a diurnal rhythm in amphibians.\(^4\)-\(^5\) Furthermore, it is known that when animals are kept under conditions of constant illumination, the peak of disc shedding is suppressed\(^2\)-\(^3\),\(^6\) and that exposure of rats to different lighting sched-

From the Departments of Ophthalmology and Anatomy, Medical College of Wisconsin, Milwaukee. Supported by National Eye Institute grants EY 02975 and EY 01931, an unrestricted grant from Research to Prevent Blindness, Inc., and Institutional PHS-BSRG 402.
Submitted for publication July 17, 1981.
Reprint requests: Arnold I. Goldman, Ph.D., Medical College of Wisconsin, 8700 West Wisconsin Ave., Milwaukee, Wis. 53226.
Fig. 1. Effect of light intensity on constant light inhibition of shedding. Animals were placed in constant light of the indicated intensity overnight and were sacrificed in light at the time of the expected shedding peak. Each point represents the mean and S.E.M. for four animals.

holding facility ranged between 10 and 16 ft-cd, measured at cage-top level.

Animals were exposed to the experimental lighting conditions in ventilated chambers illuminated by rectangular safelights with 15 W bulbs. The safelight lenses were replaced by black construction paper that was perforated to control the amount of light output. The holes faced the ceiling of the chambers, ensuring that the animals received diffuse, uniform lighting. Small changes in the level of illumination were made by varying the supply voltage to the safelights with a solid-state dimmer. The use of perforated construction paper as the primary means of adjusting light intensity permitted a wide range of lighting levels while keeping the spectral distribution of the lamps nearly uniform. The presence or absence of light within each chamber was monitored by an amplified light sensor connected to a chart recorder. These sensors were mounted within the chamber unless levels below the sensitivity of these devices (0.05 ft-cd) were being investigated; in that case, the sensors were placed inside the safelight fixture.

Lighting levels within the chambers were measured by a probe inserted through a port near the floor. A Gossen Panlux footcandle meter was used for levels above 0.1 ft-cd. Levels below 0.1 ft-cd were measured by a Besseler PM2L enlarging meter that was calibrated against the Panlux before each set of measurements. This extended the sensitivity to 0.0003 ft-cd, the lowest reading to be sensed by the Besseler. Accordingly, darkness was considered equivalent to 0.0003 ft-cd for purposes of curve fitting.

Animals were transferred in groups of four to the exposure chambers during the light phase of the cycle. Animals were maintained in the chambers overnight and were sacrificed at 7:30 A.M. the next morning under illumination conditions equivalent to that in the chamber. Phase-shifted animals experienced the following light regimen: On the first day the lights were extinguished at 6 P.M. and turned on again at 8 P.M. On days two through six the lights were turned off at 8 A.M. and on at 8 P.M. On day seven, the lights were turned off at 8 A.M. and remained off until sacrifice in the dark, either at 9:30 P.M. or 7:30 A.M. the next day. Since this schedule has been shown to produce a 10 hr phase advance in the shedding rhythm when eyes were taken over a 24 hr period, only those times for which a peak would be expected were sampled.\(^2\) The final day of darkness was to distinguish circadian from light-triggered responses. When it was necessary to open the chambers to feed and water the animals, the room was illuminated only by a safelight with a 15 W bulb and a Kodak No. 1A safelight (less than 0.1% transmission below 605 nm) at a distance of no less than 2 to 3 ft. Exposure times were kept to the minimum required for animal maintenance.

Animals were sacrificed by overdose of sodium...
Sensitivity of rat ROS disc shedding to light 697

Figs. 2A and 2B. Effect of light intensity on 10 hr phase advance. Animals raised on a 6AM : 6PM lighting schedule were shifted to a 8PM : 8AM schedule for 6 days and were sacrificed after 1 day in darkness. Points defined as in Fig. 1.

Fig. 2A. Sacrifice at 9:30 P.M., the time of the shedding peak if the phase shift was complete.

Results

Light inhibition of the 7:30 A.M. peak of shedding (Fig. 1) followed a sigmoid curve ($r^2 = 0.98$) of shedding vs. log intensity. Inhibition was essentially complete at levels above 4 ft-cd (7.3 ± 0.8) and no inhibition was observed at 0.1 ft-cd or less (28.6 ± 1.5); these shedding rates were identical to those of rats maintained overnight in total darkness. A 50% inhibition of shedding occurred at 0.85 ft-cd.

In contrast, the sensitivity of the shedding rhythm to a phase shift was greater than that of shedding inhibition. When animals were examined at 9:30 P.M., the time expected for a shedding peak after a successful 10 hr shift, the data fit a steeper sigmoid curve ($r^2 = 0.95$) than that for shedding inhibition (Fig. 2A). Animals receiving levels below 0.3 ft-cd showed only baseline shedding at this time (5.6 ± 0.3), whereas animals receiving 0.4 ft-cd or greater displayed a full shedding peak (26.4 ± 2.0). This curve appeared sharply bi-phasic; with the exception of the group exposed at 0.3 ft-cd (14.3 ± 3.2), the response appeared either in full or not at all. These levels were significantly different ($p < 10^{-6}$ by Student’s t test).

Data from animals subjected to the 10 hr phase shift and sacrificed at 7:30 A.M. (Fig. 2B) revealed a loose fit to a sigmoid curve ($r^2 = 0.73$). Animals maintained under conditions of darkness for the entire shift period exhibited a complete shedding peak (25.5 ± 2.2). At 0.001 ft-cd there was still significant shedding (17.4 ± 2.4); but the levels were lower than that seen in constant darkness. The level for a 50% inhibition of shedding predicted by the sigmoid curve occurred at 0.003 ft-cd. All shedding at levels above 0.01 ft-cd was significant ($p < 10^{-6}$).
ft-cd was indistinguishable from baseline levels (6.67 ± 0.46).

Discussion

These data indicate that the inhibition of shedding by constant light requires higher levels of illumination than does a phase shift in the shedding rhythm. Other studies have demonstrated that such inhibition is local to the eye and that the effect of constant light is to mask rather than eliminate the shedding rhythm. All of this information suggests that the mechanisms for constant light inhibition of shedding are different than those that shift the phase of the rhythm. Although the processes that occur during the dark period required for shedding are as yet unknown, it appears certain that they are separate from those regulating the circadian pacemaker that controls this function.

The present study is consistent with the hypothesis that the circadian rhythm of shedding may be regulated by a neurally processed signal rather than by direct effects of light, since such a shift appears to be more sensitive to low levels of illumination than does the inhibition of shedding. Although the duration of light stimulus differs between the shedding-inhibition and phase-shift experiments, both represent the minimum duration of such a stimulus to provide a complete expression of the modified shedding response. A complete phase shift in the shedding rhythm requires only 0.4 ft-cd of entraining light. This response is very sharp; when shedding at 9:30 A.M. is examined, 0.39 ft-cd gives a complete shedding response (25.9 ± 4.0), 0.30 ft-cd gives a partial response (14.3 ± 3.2), and 0.1 ft-cd gives baseline shedding (7.0 ± 1.0). The data from 7:30 A.M., however, indicate that only those animals kept in total darkness (less than 0.0003 ft-cd) for the period of the shift exhibited a complete shedding peak at the time predicted by the original entrainment schedule. Levels below 0.01 ft-cd resulted in very small amounts of shedding (between 11 and 17 phagosomes/180 μm), and animals cycled above that level exhibited only baseline shedding. This conclusion is analogous to that reached in a study of the time required to achieve the 10 hr phase advance. In that study, the 7:30 A.M. peak disappeared within 3 days, even though the 9:30 P.M. peak had not appeared until the fifth day. The investigators concluded that on the fourth day, the peak had migrated to an undetermined, intermediate time.

The ability of the rat to entrain to such low light levels is not without precedent. Cardi-
nali et al.\textsuperscript{14} used lights of 65 $\mu$W/cm\(^2\) at various wavelengths and determined that red light had no effect on hydroxyindole-O-methyl transferase, whereas other wavelengths inhibited this enzyme (for purposes of comparisons, my entrainment lights produce approximately 10 $\mu$W/cm\(^2\) per ft-cd at the voltages used in the current experiment). McGuire et al.\textsuperscript{15} could entrain the circadian rhythm in body temperature with 0.1 $\mu$W/cm\(^2\) of red light. McCormack and Sontag\textsuperscript{16} studied circadian rhythms in both running activity and ovulation in rats and obtained complete entrainment at levels of 4.7 $\mu$W/cm\(^2\) of red light (0.08 ft-cd) and entrainment in some of their rats at levels down to 0.003 $\mu$W/cm\(^2\) of red light. Such studies suggest that the enzyme inhibition studies of Cardinali et al.\textsuperscript{14} may be analogous to my shedding inhibition experiments, since neither deal with a change in the phase of the circadian rhythm and both required comparatively high levels of illumination. The sensitivity of the circadian rhythm in running activity appears to be much more sensitive to light than does the shedding rhythm. This may reflect differences in the control of such rhythms; activity rhythms are thought to be regulated by the suprachiasmatic nucleus,\textsuperscript{17} whereas current evidence suggests an intraocular oscillator for the shedding rhythm.\textsuperscript{2, 3}

Previous studies have demonstrated that a phase shift in the disc shedding rhythm involves the passage of an entrainment signal through the optic nerves.\textsuperscript{3} A possible anatomic basis for such a pathway has recently been established,\textsuperscript{18} although it has not yet been implicated in any aspect of photoreceptor renewal. The sharp response and high sensitivity of this rhythm to low levels of light further support the concepts of a neurally processed signal that controls the entrainment of shedding. The signal appears to be amplified when compared with constant light inhibition of shedding.

Both the sharpness and the sensitivity of the light-mediated phase shift in the shedding rhythm differentiate it from the response to constant light inhibition of the shedding peak. Where the former most likely involves some degree of neural processing, the latter appears to be a direct effect of light on specific metabolic processes within the retina. In addition to the theoretical implications of these findings, some practical information can be gained as well. The shedding rhythm is extremely sensitive to perturbation by a regimen of exposure to very low levels of illumination, provided that these levels are synchronized to reinforce the new rhythm. Although such a protocol may not produce a complete shift in the shedding rhythm, it is very likely to profoundly disturb the original rhythm.

Thanks are given to Ms. Theresa Davis for technical assistance. Special thanks are given to Dr. Delwin T. Lindsey for the design and construction of the exposure chamber light monitors.

\textbf{REFERENCES}