properties degenerate over the next 6 weeks. This result indicates that the pulse of light rapidly triggers development and that continued visual input is necessary to maintain normal receptive fields. Once the cells have degenerated in darkness, they are no longer capable of developing normal response properties (*8, 9*).

Visual input seems to act as a switch that rapidly activates the underlying biochemical events that control neural plasticity in the visual system.

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6. It is not clear why there was a loss of binocular cells and a high incidence of unresponsive cells in cats who received 12 hours of visual experience during dark rearing. The 12-hour condition resulted in more severe abnormalities than the 6-hour condition [χ^2 (5) = 28.4, $P < .001$]. The difference between these conditions could therefore be related to the longer visual exposure or to a consolidation effect because vision was experienced on two successive days in the 12-hour condition. Additionally, since the eyes of dark-reared cats are frequently misaligned, effects of strabismus may be more manifest in the 12-hour condition.
7. Observations of visuo-motor behavior indicated differences between the DR and DR(6, 12) rearing conditions that were consistent with the physiological data. After several weeks of monocular vision, DR → MD cats showed clear signs of visuo-motor behavior [J. Van Hof-Van Duin, *Brain Res.* **104**, 233 (1976); G. D. Mower, C. J. Caplan, G. Letsou, *Behav. Brain Res.* **4**, 209 (1982)] and became indistinguishable from cats who experienced MD from birth on a variety of simple tasks (placing, jumping, tracking, obstacle avoidance). DR(6, 12) → MD cats, on the other hand did not show such behavioral recovery and still failed these tasks at the end of the period of MD.
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10. Supported by NIH grants EY 03335 and HD 06276.

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Inhibitory Influence of Unstimulated Rods in the Human Retina: Evidence Provided by Examining Cone Flicker

Abstract. *In the parafoveal retina of human observers, cone-mediated sensitivity to flicker decreases as rods become progressively more dark-adapted. This effect is greatest when a rod response to flicker is precluded. These results indicate that rods tonically inhibit cone pathways in the dark.*

Several different mechanisms permit rod- and cone-related signals to interact within the visual system (*1, 2*). Rods as well as cones contribute to color vision (*3, 4*), and separate rod and cone responses interact in determining threshold (*5*) and the sensation of flicker (*6*). In the most thoroughly studied psychophysical examples (*3, 5, 6*), the underlying process always has two features. (i) Rod- and cone-related signals interact most strongly when both types of photoreceptors are photically stimulated. This is surprising since both rods and cones release a neurotransmitter most rapidly in the dark (*7*), and dark-release of both an excitatory and an inhibitory neurotransmitter is documented in the vertebrate retina (*8*). (ii) Photic stimulation of both rods and cones leads to a response of similar effect (whether inhibitory or excitatory) at some common neural locus. In fact, with only one well-documented exception (*9*), intracellular neurophysiological studies have shown rod-cone interaction to involve summation.

We now demonstrate a type of rod-cone interaction in the human visual system with a different mechanism. We have found that unstimulated, dark-adapted rods maximally inhibit cone-mediated sensations of flicker.

A sinusoidally flickering light 2°20′ in diameter was presented 7° from fixation in the temporal field (*10*). Modulation depth was fixed at 87 percent, and the observer adjusted illuminance so that the light appeared to barely flicker (*11*). Although we have obtained similar data from 20 observers, including 15 with no psychophysical training, Figs. 1 and 2 represent data from S.H.G., an experienced psychophysical observer with normal vision.

We found that flicker sensitivity was determined by three variables: the extent of dark adaptation, the spectral distribution (*10*) of the stimulus, and flicker frequency. For example, after being exposed to a bleaching field of 46,000 trolands, the observer tracked sensitivity to flicker throughout the time course of dark adaptation. Data obtained with a 5-Hz flickering green stimulus (Fig. 1) resembles usual dark-adaptation curves (*12*). The upper limb (less than 6 minutes in the dark) can be attributed to the adaptational properties of cones, and the lower limb to those of rods. Such an increase in sensitivity throughout the entire time course of dark adaptation was obtained only with green or yellow stimuli flickering at frequencies below 7 Hz. The sensitivity to red flicker also increased during the cone recovery stage of dark adaptation but, during the rod recovery period of dark adaptation, sensitivity decreased (Fig. 1). This phenomenon was most pronounced with higher flicker frequencies: using other response measures, we have obtained a similar suppression in sensitivity to flicker with frequencies up to 40 Hz (*11*).

To ascertain the mechanism underlying these data, we first determined the types of photoreceptors involved. The experiment with 20-Hz red flicker was repeated with green or yellow flickering stimuli (*10*). When data were plotted in photopic units (reflecting the sensitivity of human cones), results were uninfluenced by stimulus wavelength, indicating that cones were detecting flicker. Additionally, the bleaching field used prior to dark adaptation was varied in wavelength; scotopic trolands illuminance (influence on human rods) was fixed, but photopic illuminance was varied. Regardless of the bleaching wavelength, data corresponding to the rod recovery stage of dark adaptation remained unaltered (*13*).

The controls described above show that the data reflect rod-cone interaction but do not specify mechanism. Rod- and cone-related flicker signals can sum in the retina (*6, 14*). Since the latency of rods is longer than that of cones, rod and cone responses can either enhance or cancel each other depending on flicker frequency. We rule out this explanation for our data for two reasons. (i) We have collected similar data with red stimuli of many different frequencies and have always observed flicker sensitivity to decrease monotonically during rod adaptation (*15*). (ii) We used a control procedure throughout the entire study, but data are shown only for 20-Hz flicker in Fig. 1. Red and green flickering stimuli were presented 180° out of phase: the time-averaged scotopic illuminance of

both red and green stimuli were made equal, but the photopic illuminance of the red stimulus was 1.6 log units (×40) greater. Thus, the combined red and green stimulus provided rods with a steady stimulus but presented cones with flicker. Sensitivity to 20-Hz red flicker was negligibly influenced by adding the green flicker in counterphase (the inverted open triangle in Fig. 1). Since doubling the time-average photon catch of the rods did not significantly affect the results, the direct scotopic effect of the test light was negligible in determining sensitivity changes in the dark. We conclude that the sensitivity of cones to flicker depends directly on the state of rod dark adaptation.

Sensitivity of cones to flicker also depends on the prevailing level of rod light adaptation. In collecting the data of Fig. 2, sensitivity to flicker was obtained as a function of the illuminance of a continuous 512-nm adapting field 28° in diameter. As background illuminance increased, sensitivity to flicker also increased (*16*), an effect most pronounced at the higher frequencies. When the abscissa is plotted in terms of scotopic illuminance, the result is independent of wavelength; we have recently found that this effect is relatively independent of stimulus diameter and nonfoveal retinal position (*17*). The effect cannot be obtained with scotopic flicker (a green flickering stimulus dimmer than −1 log troland).

Although our data seem to indicate a relation between the sensitivity of cones to flicker and the adapted state of rods, one reservation still remains because the scotopic illuminance of the red flickering stimulus used in the experiments shown in Fig. 2 is generally greater than that of the adapting field (*18*). This suggests that the adapting field has little direct effect on rods underlying the test stimulus and that our results are largely determined by the adapted state of rods spatially adjacent to this stimulus. A final experiment indicates this to be the case. We repeated the experiment illustrated by the filled triangles in Fig. 2 with an adapting field consisting of an annulus with an inner diameter contiguous with the flickering test but with an otherwise identical spatial configuration. These two sets of data were indistinguishable from each other.

Our data show that as rods become more dark-adapted, and less subjected to photic stimulation, sensitivity of the cones to flicker decreases. A similar observation regarding photopic flicker sensitivity was also reported on the basis of electroretinographic measurements in the frog (*19*); several other behavioral (*13, 20*) and electrophysiological (*21*) observations show cone sensitivity to be slightly depressed during rod dark adaptation. Our data emphasize the magnitude of the effect, and our controls enable us to state that cones are most sensitive when rods are both light-adapted and stimulated.

Two underlying mechanisms seem possible. (i) Light-adapted, stimulated rods could facilitate cone flicker sensitivity. Since many types of pathological conditions in which rods malfunction are characterized by normal, light-adapted cone vision (*22*), this explanation seems unlikely. (ii) Dark-adapted, unstimulated rods could inhibit cones. A growing body of evidence shows that inhibitory neurotransmitters are released by various retinal amacrine and horizontal cells in all vertebrate classes (*8*). Since many of these neurons are excited by dark-adapted rods, their influence should be greatest when rods are least influenced by light. If this is the underlying explanation for our data, several questions will need to be addressed by further research. What advantage might this type of rod-cone interaction bring to the behaving organism? Why has this large effect never been reported by other investigators performing similar experiments, and why is it more pronounced with high-frequency flicker? Finally, what specific cellular mechanism permits this type of rod-cone interaction? If rod-cone interactions obtained in the electroretinogram of the frog (*19*) and our behavioral results are manifestations of the same mechanism, a distal retinal locus is indicated. This possibility is attractive since, in the cat, Nelson has found cone-driven horizontal and bipolar cells whose response is enhanced by light adaptation of rods (*23*).

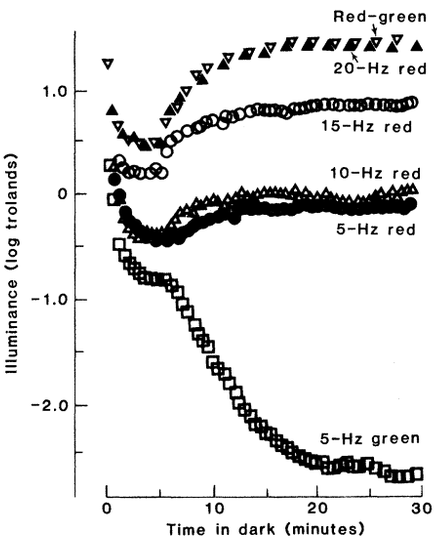

Fig. 1. Illuminance of the flickering test stimulus necessary for just-perceptible flicker as a function of time in the dark. Flicker was generated by a green stimulus of 5 Hz or by a red stimulus of 5, 10, 15, or 20 Hz. Data represented by inverted open triangles were obtained with red and green flicker presented in counterphase and matched in scotopic illuminance.

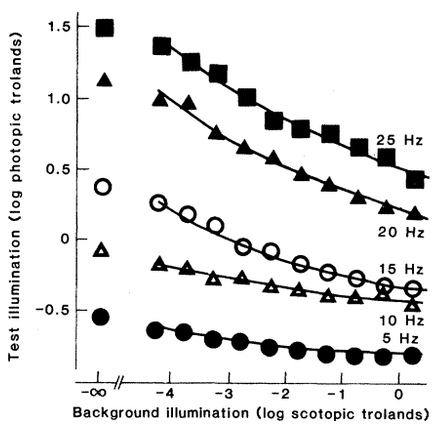

Fig. 2. Illuminance of the flickering red test disk necessary for just-perceptible flicker as a function of the illuminance of a 28° continuously exposed adapting field of 512 nm wavelength. For the test stimulus, 1 photopic troland = −1.3 log scotopic trolands.

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9. In the mud puppy, most postreceptor neurons receive a qualitatively similar input from both rods and cones (*2*). However, type c horizontal cells either hyperpolarize or depolarize depending on whether the photic stimulus maximally influences rods or cones.
10. Stimuli were presented by a five-channel Maxwellian view optical system. One channel had a tungsten source and provided a continually exposed field 28° in diameter with wavelength determined by interference filters with half-

bandwidths of 6 to 12 nm. This was used for photopigment bleaching prior to dark-adaptation experiments (Fig. 1), or as an adapting field in light-adaptation experiments (Fig. 2). Flickering stimuli as well as the fixation target were provided by channels with separate light-emitting diode sources. These were either red-orange (General Instruments type MV5152), yellow (General Instruments type MV5352), or green (Stanley Electric type EBG5504) with respective peak wavelengths of 630, 585, and 540 nm and with half-bandwidths of about 40 nm.

11. Three indices were used to assess sensitivity to flicker. That used in the experiments shown in Figs. 1 and 2, the illuminance of a stimulus which causes it to appear to just flicker, is inversely proportional to sensitivity. We also fixed the time-averaged illuminance of a flickering stimulus, and the observer either varied its modulation depth for threshold or varied its frequency to achieve critical flicker frequency (CFF). Modulation depth varied inversely with sensitivity, whereas an increased CFF corresponded to an increase in sensitivity.
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Physiological Correlates of Prolonged Sleep Deprivation in Rats

Abstract. *The issue of whether sleep is physiologically necessary has been unresolved because experiments that reported deleterious effects of sleep deprivation did not control for the stimuli used to prevent sleep. In this experiment, however, experimental and control rats received the same relatively mild physical stimuli, but stimulus presentations were timed to reduce sleep severely in experimental rats but not in controls. Experimental rats suffered severe pathology and death; control rats did not.*

If sleep serves an important physiological function, sleep deprivation should produce severe physiological impairment. Literature reviews (*1*) have emphasized the lack of such impairment, however. Older animal studies (*2*) that reported pathological changes or death following prolonged sleep deprivation have been either neglected or discounted for their failure to control for stimulus effects. When sleep is blocked by unrelenting, noxious stimulation, such as continuous enforced locomotion, it is unclear whether subsequent pathology is mediated by sleep loss or by other effects of the stimulation, such as stress or fatigue. Our procedure delivered the same relatively mild physical stimuli to experimental and control rats, but timed their delivery to limit sleep severely in experimental rats but not in controls. The result was severe debilitation and death in experimental but not in control rats.

A deprived rat and a yoked control rat were housed in separate clear plastic cages; a single fiber glass disk formed a partial floor for both cages (*3*). Beneath each side of the disk and extending beyond it to the walls of each cage was a tray containing water to a depth of 3 cm (Fig. 1). Whenever the disk was rotated, both rats had to walk in the direction opposite disk rotation to avoid being forced into the water.

Each rat's electroencephalogram (EEG), electromyogram (EMG), and theta activity were continuously recorded and later scored by computer for wakefulness (W), high-amplitude non-REM sleep (HS), low-amplitude non-REM sleep (LS), paradoxical sleep (PS), and total sleep (TS) (*4*). Upon recognizing sleep onset in the deprived rat (usually within 5 seconds), a microcomputer activated disk rotation at a moderate speed of 3.5 rev/min until the deprived rat had been awake for 6 seconds. (Rats did not sleep in the water.) Direction of rotation was varied randomly. Thus, both rats were subject to the same environment and disk rotation, but deprived rats could not accumulate much sleep, whereas control rats could sleep whenever deprived rats were spontaneously awake.

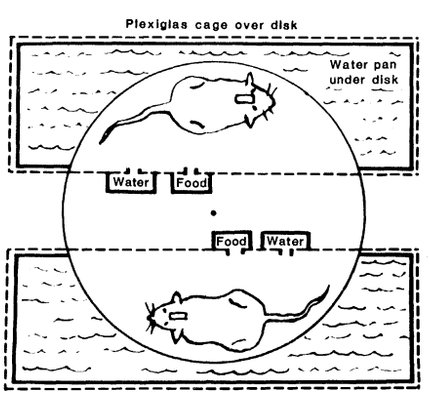

Fig. 1. Schematic diagram (top view) of the experimental apparatus.

After 4 days of stable baseline recording with the disk stationary, eight yoked pairs of age-matched Sprague-Dawley male rats, 6 to 16 months old and adapted to constant light, were run for 5.7 to 33.4 days in constant light. Food and water were freely available. Cage air temperature was held near 29°C. Criteria for disk rotation provided by EEG, EMG, and theta were occasionally varied within a small range to maximize wakefulness in deprived rats and sleep in control rats. Extreme criteria for sleep onset could theoretically produce 100 percent sleep deprivation in deprived rats, but rotations might be so frequent as to severely limit sleep in control rats as well. The procedure produced a mean of 109 rotations per hour, but the disk rotated only 23 percent of total time. We estimate that the rats were forced to walk an average of 0.9 mile a day; rats may voluntarily run 30 miles a day on a wheel (*5*).

Percentages of total recording time spent in sleep states are shown in Table 1. From baseline to experiment, TS was reduced by 87.4 percent in deprived rats and 30.6 percent in control rats. Thus, this study is best viewed as a comparison between severe and moderate sleep deprivation. Brief sleep episodes in deprived rats resulted from apparatus failures and difficulty in blocking LS without markedly increasing rotation frequency (*6*).

Apart from modest weight loss and minor skin lesions, no control rat showed outward signs of pathology or any observable indication that it could not have continued in the experiment indefinitely. All control rats appeared groomed, motorically active, and responsive to stimuli.

At least two of the following pathological signs became obvious in each deprived rat: debilitated appearance, including yellowed and apparently ungroomed fur, various skin lesions, and swelling of the paws (eight rats); ataxia or severe motor weakness, manifest by difficulty in maintaining balance and staying on or remounting the disk (seven rats); loss of EEG amplitude to less than half of normal waking values (seven