

A Circadian Pacemaker for Visual Sensitivity?^a

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INTRODUCTION

Light input to the mammalian eye is necessary both for visual signal detection and circadian rhythm entrainment. These two functions are often assumed to be independent of each other. An animal blinded by ablation of the optic tract, for example, can still entrain its circadian rhythms to daily light-dark schedules.¹ On the other hand, an animal with a lesion of the suprachiasmatic nucleus (SCN) loses such rhythms,² but retains its ability to detect visual signals.³ Separate visual pathways mediate these contrasting effects: the classical visual sensory system, which ascends past the optic chiasm to thalamic and cortical centers, subserves detectability; and a direct retinohypothalamic pathway, which terminates at the level of the optic chiasm in the SCN, subserves circadian entrainment.⁴ Though the eye is necessary for the two systems to operate normally, even extensive degradation of the photoreceptor apparatus does not eliminate detectability,^{5,6} entrainability,^{7,8} or light-induced circadian phase responses.⁹ Orbital enucleation, however, does, of course, eliminate detectability, and causes an animal's circadian rhythms to free-run at a period (τ) close to 24 hours, but independent of external light-dark cycles, just as an intact animal free-runs in continuous darkness (DD).¹⁰

In this chapter we begin an exploration of possible interactions between visual sensory and circadian functions. It seems quite possible that the two systems share some front-end apparatus: luminosity functions for detectability and entrainability both follow that of rhodopsin absorption, the dominant mode of photoreception in the rat retina.^{11,12} In addition to the retinohypothalamic route, visual information reaches the SCN and affects circadian entrainment by way of afferents from the lateral geniculate nucleus,^{13,14} a sensory projection area. Circadian rhythms have also been found within the eye, as exemplified by the daily shedding of photoreceptive disks from the rod outer segments.¹⁵ That such rhythmicity persists in DD,¹⁶ and after optic nerve transection¹⁷ and pinealectomy,¹⁸ strongly suggests the existence of a mammalian circadian pacemaking mechanism within the eye. This is not a radical proposition: experiments with invertebrate eye and optic nerve show circadian organization in neural activity exclusive of, though often modulated by, central nervous system events.¹⁹⁻²¹ In mammals, however, the functional significance of such clockwork, or relation to SCN circadian organization, has been barely explored.

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Our experiments exploit the ability of the behaving rat to perceive and report accurately the presence or absence of dim visual signals in much the same manner as a human psychophysical observer. The rat, however, can be maintained indefinitely in continuous testing, which offers the opportunity to measure detection performance, and to infer changes in visual sensitivity, around the clock. We have previously reported free-running circadian rhythmicity in scotopic sensitivity measured in DD, as well as light-adapted sensitivity.²²⁻²⁴ Here we attempt to deepen the analysis by entraining and phase-shifting the rhythm by external light-dark cycles, and studying SCN-lesioned animals whose behavioral-activity repertoire has been made arrhythmic. If mammalian visual sensitivity were to show circadian organization in the absence of SCN involvement, we reasoned that our psychophysical data could serve to define the operating characteristics of a putative visual system pacemaker, suggest functional links to the SCN pacemaker, and motivate the search for its anatomical locus and physiological substrate.

METHOD

Subjects and Apparatus

Adult male Long-Evans rats were trained as psychophysical observers. Before experimentation they were individually housed in a colony room under LD 12:12 (Vita-Lite, daytime illuminance range within the cage approximately 35 lux), with unlimited food (Purina Lab Chow) and water. Each animal received a chronic bipolar stainless steel electrode implant (Plastic Products MS303) aimed at the lateral hypothalamus, used for delivery of reinforcing brain stimulation within the detection task. Level-skull coordinates were 3.3 mm posterior to bregma, 1.5 mm to the right of the midline, and 9.0 mm below the skull. In addition, some animals received bilateral SCN lesions (Grass LM3 lesion maker, stainless steel electrode) 0.2 mm posterior to bregma, \pm 0.2 mm to the midline, and 8.8 to 9.0 mm below the skull. Sodium pentobarbital anesthesia was used. After experimentation, lesioned animals were sacrificed and brains perfused with neutral-buffered formalin. Coronal sections of 40 μ were stained with cresyl violet. Animals judged to have a complete lesion showed no evidence of SCN cells within the extent of the lesioned area, $>800 \mu$ in the A-P direction in all cases.

The psychophysical testing chambers were made from 30-cm diameter Plexiglas cylinders, placed inside individual sound- and light-attenuating compartments within a darkened temperature- and humidity-stabilized room. Snout penetration through an observation hole, which interrupted an infrared photobeam, triggered 0.5-second signal presentations. The signals were produced by a 0.5 mm-diameter light-emitting diode (LED) located approximately 3 cm in front of the eyes. A lever on one side of the hole was used to register positive detections of the signal, and one on the other side was used to register rejections. At the rear of the chamber, a third lever was used to deliver 45-mg food pellets (BioServ dustless precision) from an external dispenser to a food cup; water was obtained by licking a tube with an electronic contact sensor. A ceiling-mounted pulley-swivel system transmitted 0.5-second electrical brain stimulations from a constant-current 60 Hz AC source to a flexible lead attached to the skull electrode cap. Current level ranged from 0.08 mA to 0.20 mA (peak to peak), individually adjusted to maintain high self-paced trial rates. The experimental events and data collection were controlled by microcomputers assigned to each of four chambers. (One animal [FIGURE 1] used an earlier version of the apparatus²³ in which signals were projected on a translucent screen from a grating monochrometer; the

experiment was controlled by solid-state logic modules with data written to a multichannel event recorder.)

General Procedure

Signal Detection Task

The animals were trained to indicate the presence or absence of dim photic signals of approximately 0.001 lux from a green LED, sufficient to maintain moderate levels of dark-adapted detectability. A 0.5-second test flash occurred on half the trials, randomly assigned, when the rat placed its snout in the observation hole. A 5-second choice interval followed in which the animal could retract its head and report a positive detection (left lever) or a rejection (right lever). Correct detections (hits and correct rejections) yielded immediate 0.5-second brain stimulation reinforcement, whereas errors (false alarms and misses) yielded a corresponding empty interval. A two-second intertrial interval followed before the next signal could be presented. These reinforcement contingencies maintained hundreds of self-paced trials within a day's testing, with drinking, feeding, and quiet intervals interspersed.

The animals were tested continuously for several months, in both free-running and entrained conditions. The relative proportions of hits (positive detections given signal) and false alarms (positive detections given no signal), taken in one-hour intervals, provided the data for specifying the level of visual sensitivity, d' , on a bias-free psychophysical metric. This simple transform of the choice data provides an estimate of the signal's sensory effect in z-score units, and corrects for any variability in the observer's criterion for reporting a positive detection due to motivational fluctuations.²⁵

Free-runs and Entrainment

For free-runs, the test chamber was completely darkened except for the dim LED signal flashes. In order to test photic entrainment of the dark-adapted visual sensitivity function across day and night, we used a skeleton photoperiod²⁶ in which two bright fluorescent pulses (Vita-Lite, approximately 350 lux) of 15-minute duration were presented at times of day analogous to the onset and termination of light-exposure under a standard LD 12:12 cycle.

RESULTS AND DISCUSSION

The Sensitivity Datum

There are various definitions of "sensitivity" when physiologists and psychologists speak of visual responsiveness to photic stimulation. An experimenter may search for the lowest levels of light that produce an electrophysiological retinal response, an evoked cortical response, suppression of plasma melatonin, or a circadian phase response. The results, of course, contrast with one another because they involve different, if overlapping, subsets of the visual system, as well as nonvisual components. The retina, for example, shows sensitivity to light many orders of magnitude lower than that required to elicit a circadian phase response in mammals.²⁷ In order to determine psychophysical visual sensitivity, we engage the animal or human observer in "report-

ing" behavior. The process involves peripheral sensory reception and transmission to the projection areas, as well as judgmental and response-output factors that are less well understood in physiological terms. The psychophysical sensitivity datum is unique in that it specifies the minimal conditions for visual perception by the whole organism. The sensory and judgmental (reporting criterion, bias, expectation) factors can be analyzed by signal-detection methods.²³ The psychophysicist thereby has a way to show that the "pure" sensory effect of stimulation (as measured in units of d') is unaffected by random variability (or systematic variation) in the judgmental factors. The framework is well-suited to long-term, continuous tests of visual sensitivity that must partial out potential confounders such as activity level, hunger and thirst, sleepiness, and susceptibility to the reinforcer used to maintain the detection behavior.

Our experiments exploit brain-stimulation reinforcement as differential feedback for correct detections and rejections of the visual signal. Equivalent results can be obtained by standard food-pellet reinforcement in short sessions,²⁸ but then circadian variation is not easily measured. In contrast to food reinforcement, or pure informational feedback, direct brain stimulation can maintain thousands of trials per day, which is perhaps unique in animal or human psychophysics. Large samples are needed to establish the reliability of the sensitivity estimate, d' , which reflects a stochastic photoreception process. The experiment benefits further by use of the rat, who sleeps in short, sporadic episodes and is thus available to engage in continual detection behavior during both active and quiet phases of the circadian cycle. Upon initial access to brain-stimulation, the animal may self-stimulate ceaselessly for several days, to the exclusion of eating and sleeping. With further experience, a circadian self-stimulation rhythm emerges,²⁹ with lower total output and the opportunity to engage concurrently in normal daily activities.

Nevertheless, we find two types of rhythmic self-stimulator: the majority of animals respond faithfully throughout the subjective day (the rat's quiet phase), though at lower rates than at night; others, which we term the on/off type, abstain for up to several hours during the day, as if undershooting a reinforcement threshold. By raising stimulation current level, some on/off animals show increased daytime detection behavior, but others are recalcitrant. Across two weeks of testing, however, we can usually accumulate a sufficient number of detection trials at all circadian phases to estimate d' with high reliability.

Sensitivity Rhythm Across Signal Intensity

The validity of d' as a measure of a circadian visual sensitivity process is underscored by the parametric effect of signal intensity, as illustrated in FIGURE 1 (left panel). The animal was shown a 3-mm diameter spot of monochromatic light, centered at 500 nm, with the intensity varied in six steps throughout the scotopic threshold range. The levels were presented in random order for three weeks each, and detection data were pooled in four-hour blocks of circadian time (to correct for the free-running period in DD) across the final two weeks of each sample. At the highest intensity, the signal was clearly visible throughout the day, producing nearly errorless performance, with $d' > 4.0$. (Approximate correspondences of unbiased percent correct detections with d' : 50%, 0; 60%, 0.5; 70%, 1.1; 80%, 1.7; 90%, 2.6; 98%, 4.0.) There were ordered reductions in sensitivity with attenuation of the signal across 6.6 log units; a circadian pattern emerged within this error-prone range. Peak sensitivity at -5.4 log units attenuation, for example, approximately matched trough sensitivity at -4.8 log units. At -6.6 log units, the signal proved invisible ($d' \approx 0$) for half the day, with a relatively

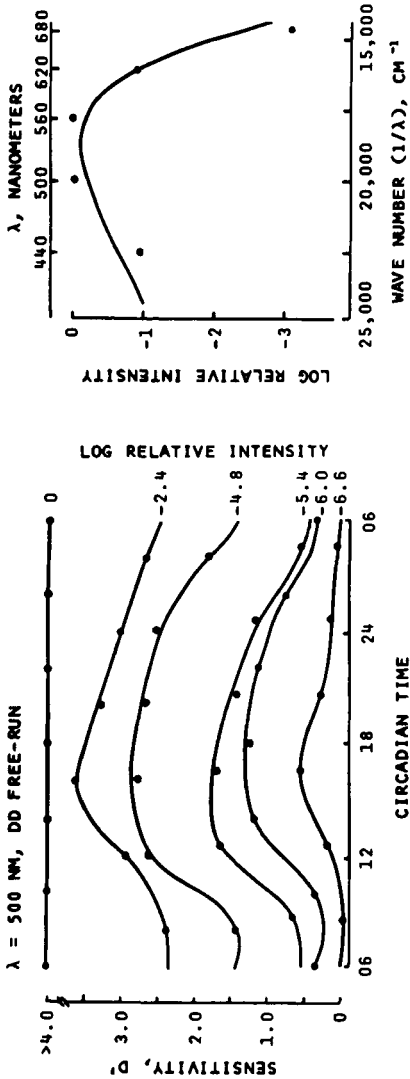


FIGURE 1. Left panel: Parametric manipulation of signal intensity for a rat tested in continuous darkness (DD, except for the 500 nm signals). Detection data for each intensity are pooled in four-hour circadian time blocks across two-week samples, yielding the sensitivity waveforms in d' , fitted by eye. At 0.069 cd/m^2 (0 log units attenuation) performance was nearly errorless at all circadian phases, with $d' > 4.0$. Reductions in signal intensity bared the circadian sensitivity pattern, with mean daily level ordered according to relative intensity. The curves are positioned on the abscissa so that the phases of highest sensitivity, corresponding to subjective night, fall between CT 12 and 24. Right panel: Parametric manipulation of wavelength for the same rat tested in discrete daily sessions at 1 P.M., after six hours of exposure to colony room light under LD 12:12, and one hour of dark adaptation. Signal intensity was adjusted to maintain 80% ($\pm 2\%$) correct detections across 100 trial samples. The data are superimposed on a Stiles π_4 relative field sensitivity curve corresponding to the spectral absorption of rhodopsin. These data are from Stephen Menitch's master's degree project in our laboratory.

narrow sensitivity peak ($d' = 0.6$) at circadian time (CT) 16, shortly before the midpoint of subjective night. Further attenuation undoubtedly would have yielded chance-level performance across the entire circadian cycle. By scanning d' across intensity levels at peak and trough phases, we estimate that the overall dynamic range of circadian visual sensitivity equals approximately 1.0 log unit (see also reference 30).

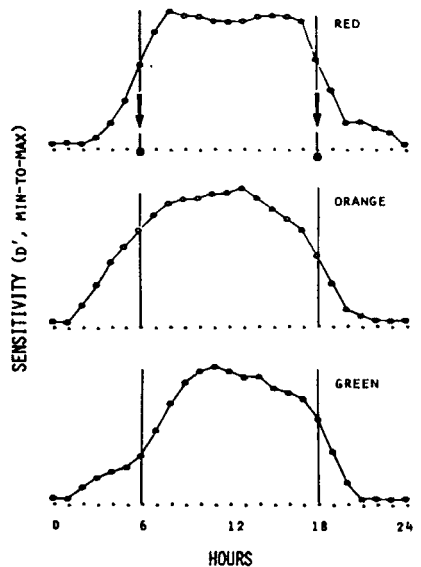


FIGURE 2. Relative sensitivity waveforms for a rat given dim red, orange, or green LED signals under skeleton photoperiod 12:12 entrainment. Hourly data are pooled across two-week samples at each color. Vertical lines indicate the times of 15-minute bright-light pulse presentations. Top panel, filled circles below arrows show the transient suppressive effect of light exposure on sensitivity—to less than zero on the relative scale—within the 45-minute interval following pulse presentations. Under a resonance design, occasional entrainment pulses (“morning” or “evening”) were omitted, permitting uncontaminated assessment of underlying dark-adapted sensitivity (top panel, main function at vertical lines) within pulse hours. These values are closely matched by interpolation across pre- and postpulse hours, and this method is applied to orange and green waveforms, as well as to subsequent entrainment data presented in FIGURES 3 and 6. Sensitivity begins to rise several hours before the evening pulse, and peaks within subjective night. Similarly, sensitivity falls gradually in the hours surrounding the morning pulse, and reaches its daily nadir within the first three to six hours of the subjective day.

Sensitivity Across the Visual Spectrum

The wavelength manipulation provides a second criterion for establishing the psychophysical performance as a valid index of visual sensory function, because the spectral sensitivity function in the rod-dominant eye is expected to peak in the region of maximum rhodopsin absorption. We sought this effect both in discrete daily sessions during which intensity was titrated every 100 trials in order to attain a constant-

accuracy level across wavelengths, and in continuous long-term sessions at fixed wavelength and intensity. The probe tests (FIGURE 1, right panel) yielded a fair match to the Stiles π_4 scotopic mechanism, reflecting the action of rhodopsin.³¹ In continuous testing at the various wavelengths (not shown here), the same relative intensities were successful in maintaining circadian sensitivity oscillations of equivalent amplitude, mean daily level, period, and phase relation to feeding.

A similar story holds for the sensitivity rhythm under the skeleton photoperiod (FIGURE 2). This animal was given two-week tests with red, orange, and green LED signals, intensity individually adjusted to maintain circadian oscillations at a moderate mean sensitivity level of $d' \approx 2.0$. In order to test the potential disruptive effect of the fluorescent entrainment pulses on the dark-adapted circadian sensitivity waveform, a resonance design was used during the red LED tests. Every few days one of the daily pulses (hour 6 = evening; hour 18 = morning) was omitted, permitting assessment of sensitivity at those times without bright light interference. These data were compared with sensitivity estimates taken during the 45-minute dark readaptation interval that followed each 15-minute entrainment pulse. The immediate aftereffect of bright light exposure was a suppression of sensitivity below the daily dark-adapted d' minimum within the animal's subjective day (hours 0 to 3). By the next hour, however, the animal completely recovered from the disruption: the rising sensitivity trend continued after the evening pulse, and the falling trend continued after the morning pulse. The pulses did not induce the cyclic reversals in daily sensitivity, but acted as a classical entraining agent. The evening rise anticipated the evening pulse by several hours, and the morning fall began at hour 18 even when the morning pulse was omitted. Periodogram analysis³² verified 24.00-hour periodicity.

For testing with the orange and green LED signals, the entrainment pulses were presented every day without exception. Pulse-hour sensitivity was estimated by interpolation across adjacent hours, which gives a close match to pulse-hour-omission data under the resonance procedure. The two waveforms show gradual anticipation of both evening and morning pulses; pulse-hour sensitivity always lies in the middle range of scaled sensitivity on ascending and descending limbs. The suggestive contrasts between the red, orange, and green waveforms are not systematic and should not be attributed to signal color: replications of a waveform using a single LED color, as under entrainment phase shifts (see FIGURE 6), give a similar range of variation.

Comment on Waveform Education

Given the unified cyclic trend across sensitivity levels within the threshold range (FIGURE 1, left panel) we have found it simplest to compare waveforms that differ in level and amplitude due to extraneous factors by stretching them linearly between the daily minimum and maximum, thereby creating a relative sensitivity scale (FIGURES 2, 3, 5, 6). The underlying data are based on the pool of detection trials from corresponding one-hour blocks across successive days in the sample. The waveform is clarified by filtering out random variation by a running median:^{33,34} the hourly raw data are scanned by a three-hour window shifted in one-hour steps, and the median d' is selected as the best estimate of sensitivity centered on that interval, thereby rejecting outliers. The process is iterated on the filtered data until the wave is stationary. Unlike a running mean, which assimilates outliers at the risk of distorting the result, this technique preserves original data points that best describe the circadian oscillation,

providing an accurate and unbiased curve fit. Systematic discontinuities, if present, are not averaged out. Note, however, that any systematic trend smaller than the window size (here, 3 hours) is obliterated.

The Circadian Repertoire: Free-Runs and Entrainment

The circadian repertoire is multifarious, and it is instructive to consider the covariation of visual sensitivity with other endpoints. Thus far, we have measured three behavioral functions concurrently with sensitivity: trial output in the signal detection task, drinking (water licks), and feeding (pellets delivered). The trial output measure is akin to the self-stimulation rate in the free-operant situation, which has been measured previously under various schedules of reinforcement, as well as under continuous darkness and illumination, and photic entrainment.^{24,35} The rat's ingestive behavior provides standard circadian reference data,³⁶ and has been successfully entrained to skeleton photoperiods.³⁷

FIGURE 3 compares the concurrent measures for a rat under free-running and

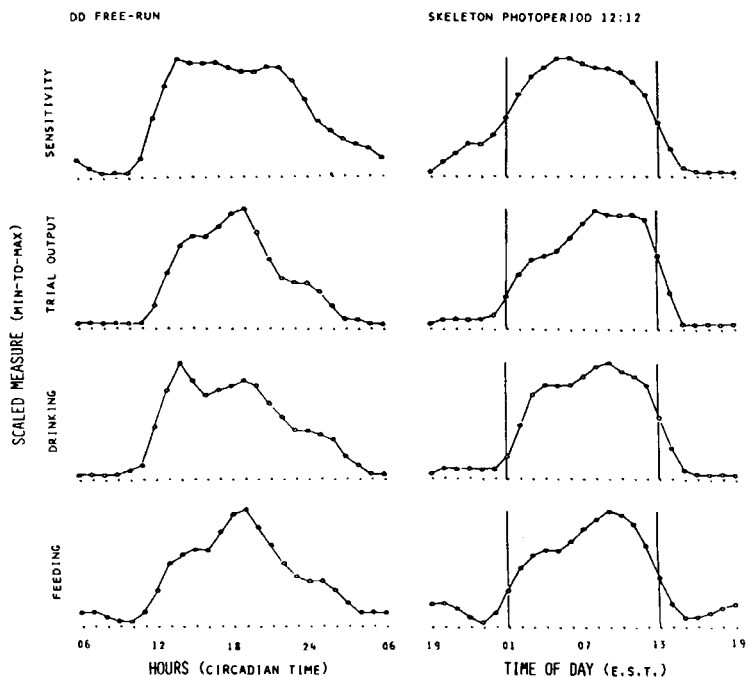


FIGURE 3. Comparison of free-running (DD) and entrained (skeleton photoperiod 12:12) waveforms for visual sensitivity, trial output in the signal detection task, drinking, and feeding, for a rat with an intact SCN. Data are pooled across two-week samples, with a circadian-time correction under DD ($\tau = 24.05$ hours). Under both lighting conditions, the region of high daily sensitivity encompasses that of the behavioral activity measures. Trial output peaks later in the day than does sensitivity. The skeleton photoperiod appears to compress the subjective night duration relative to DD.

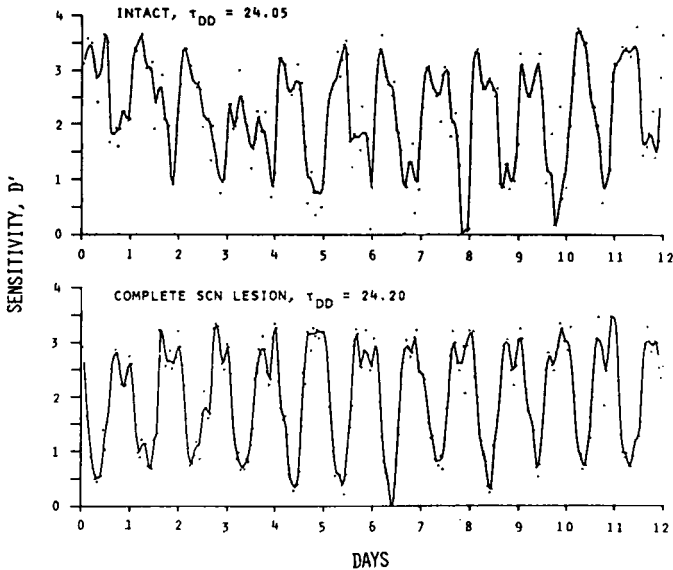


FIGURE 4. Daily sensitivity functions for two rats (intact SCN, and complete SCN lesion) given a dim green signal under DD. *Dots* indicate hourly d' estimates (three-hour pools of detection trials shifted in one-hour steps); *curve* clarifies the waveform by a running median. Both animals show regular circadian free-running oscillations in visual sensitivity; their patterns differ only in detail.

entrained conditions. Under entrainment, the cyclic rise in sensitivity begins earlier in the day, and peaks earlier, than any of the output functions; the daily descents are closely phase-locked. The same pattern holds in the free-run, though in this particular case increased drinking at the start of the subjective night closely matches the sensitivity function, while trial output and feeding lag. The skeleton photoperiod compresses the duration of subjective night by approximately two hours, as compared to DD, an effect we have seen repeatedly (*e.g.*, FIGURE 6). The sensitivity curves, which remain relatively high throughout the subjective night under both lighting regimens, encompass the high-activity phases of the output functions. Notably, trial output increases relatively gradually, reaching its maximum in the latter half of the subjective night: we have never seen it locked to the sensitivity function and so it is clear that the rate of psychophysical performance does not directly control sensitivity. These data, in themselves, would not raise the suspicion that the visual function needs a pacemaker separate from the others, because the circadian period of all measures is equivalent, and the phase relations are stable.

The Effect of the Suprachiasmatic Lesion on the Sensitivity Rhythm

Visual sensitivity and behavioral output respond differently when the SCN pacemaker is removed, however. SCN lesions abolish the circadian pattern of activity, drinking, and feeding, as well as self-stimulation.^{2,38} FIGURE 4 compares sensitivity patterns under DD for an intact animal and one bearing a complete SCN lesion.

Continuous 12-day samples are shown in order to emphasize the reliability of circadian oscillations. Though the intact record is somewhat more variable, sensitivity for both animals tends to peak around $d' = 3$, and decline to $d' < 1$, spanning the steeply-sloped threshold range of the psychometric function.³⁰ These curve positions were judiciously set by adjustments in LED intensity before the samples were taken. As illustrated in FIGURE 1 (left panel), a choice of lower or higher signal intensity would throw the entire rhythmic function toward floor or ceiling.

Waveform summaries for eight completely lesioned animals are shown in FIGURE 5. The range of free-running periods for the group, $\tau_{DD} = 23.85$ to 24.65 hours, is similar to that found for behavioral activity in intact rats,³⁵ though the SCN pacemaker presumed to generate such rhythmicity is absent. (Disruption of the

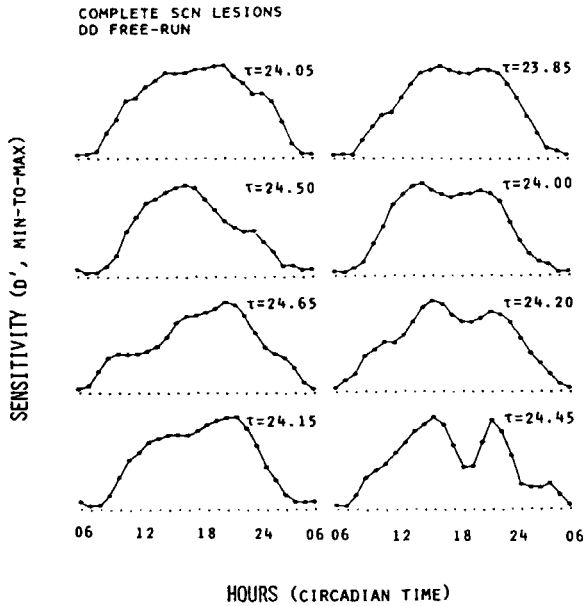


FIGURE 5. Relative sensitivity waveforms for eight rats bearing complete SCN lesions (dim green signal under DD). The data are pooled in circadian hours across two-week samples, with τ_{DD} determined by periodogram.

concurrent behavioral-output functions by the lesion will be described in a separate paper—in preparation.) The sensitivity curves are ordered by a trend toward bimodality, with distinguishable peaks early and late in subjective night, though these are not seen on every cycle even for animals with strongly bimodal pooled waveforms (compare, for example, the summary curve at $\tau = 24.20$ hours with the corresponding daily record in FIGURE 4, bottom panel.) It is possible that such data reflect morning and evening sensitivity oscillators that are normally tightly coupled,³⁹ as has been suggested for ocular rhythmicity in insects (W. Köhler, personal communication). Clearer evidence, however, is needed. The consistency of the main effect does, however, argue for a specialized pacemaker for circadian sensitivity, possibly within the eye, elsewhere in the visual system (excepting the SCN), or in other structures acting on the

visual system. In this respect our whole organism preparation is a liability, because it would be difficult to maintain the psychophysical performance if several of the likely pacemaker structures (*e.g.*, retina or ventral lateral geniculate nucleus) were selectively removed.

Entrainability of the Visual Sensitivity Pacemaker

A fully elaborated circadian pacemaker should show not only the capability to free-run, but also vulnerability to entrainment by daily photic input. Entrainment is demonstrated when the free-running period is captured by that of the external time cue; when phase shifts of the external time cue guide the rhythm into newly appropriate phases; and when, upon release into constant conditions, the free-running phase originates from that set by the preceding entrainment regimen. FIGURE 6 (left panel) demonstrates these manipulations in an intact animal. The sensitivity rhythm is first entrained to 24-hour periodicity by a skeleton photoperiod with pulses at 08 and 20 hours. After stabilization, the animal is released into DD and shows a free-running rhythm whose peak phase originates from the former subjective nighttime interval. Two weeks later, a new skeleton photoperiod is imposed with pulses at 14 and 02 hours, and the rhythm is reentrained (this time with strong nocturnal bimodality). A subsequent three-hour advance of the pulse schedule produces another phase adjustment by means of an advancing transient.

The middle panel of FIGURE 6 shows a set of similar challenges for an animal who, we learned later, retained some SCN tissue after lesioning. The animal's ingestive patterns, however, showed no circadian component in the periodogram. Still, the sensitivity rhythm was robust. First, we captured the free-run at its momentary phase by placing the morning pulse just before the daily sensitivity minimum at 22 hours, and the evening pulse at 10 hours. Across several weeks of testing, sensitivity showed a daily rise and fall unusually phase-advanced relative to the pulses, with a waveform closely similar to that preceding in DD. We then presented a six-hour phase shift of the skeleton photoperiod, which is directionally ambiguous, and the animal adjusted by a six-hour phase delay in sensitivity, accompanied by great sharpening of the morning decrement. A further four-hour delay-shift of the schedule reentrained the rhythm accordingly, though the sensitivity peak remained stationary while the main body of the waveform repositioned itself symmetrically between the pulses at 20 and 08 hours. As a result, the daily anticipatory rise in sensitivity, before the evening pulse, was eliminated. A subsequent four-hour advance of the schedule led to an eight-hour delay adjustment, and reappearance of the extended evening-anticipation trend in sensitivity. Finally, the DD-release originated from the preceding entrainment phase.

The completely lesioned animal (FIGURE 6, right panel) was also successfully entrained. In this case, a four-hour advance of the schedule did induce an advancing transient and shift to earlier external clock time. A four-hour delay recaptured the earlier baseline pattern, and the DD release originated from the preceding entrainment phase. While there were contrasts in the mode of adjustment across intact, partially, and completely lesioned animals, all fulfilled the criteria for circadian entrainment.

We conclude that the SCN is not necessary for entrainment of the visual sensitivity rhythm. Indeed, although direct electrical and neurochemical stimulation of the SCN can induce phase responses without photic stimulation,⁴⁰⁻⁴² our data open the possibility that the SCN normally tracks peripheral ocular phase responses, thereby determining the daily adjustments required for entrainment of SCN-driven rhythms such as behavioral activity, ingestion, and pineal melatonin secretion.

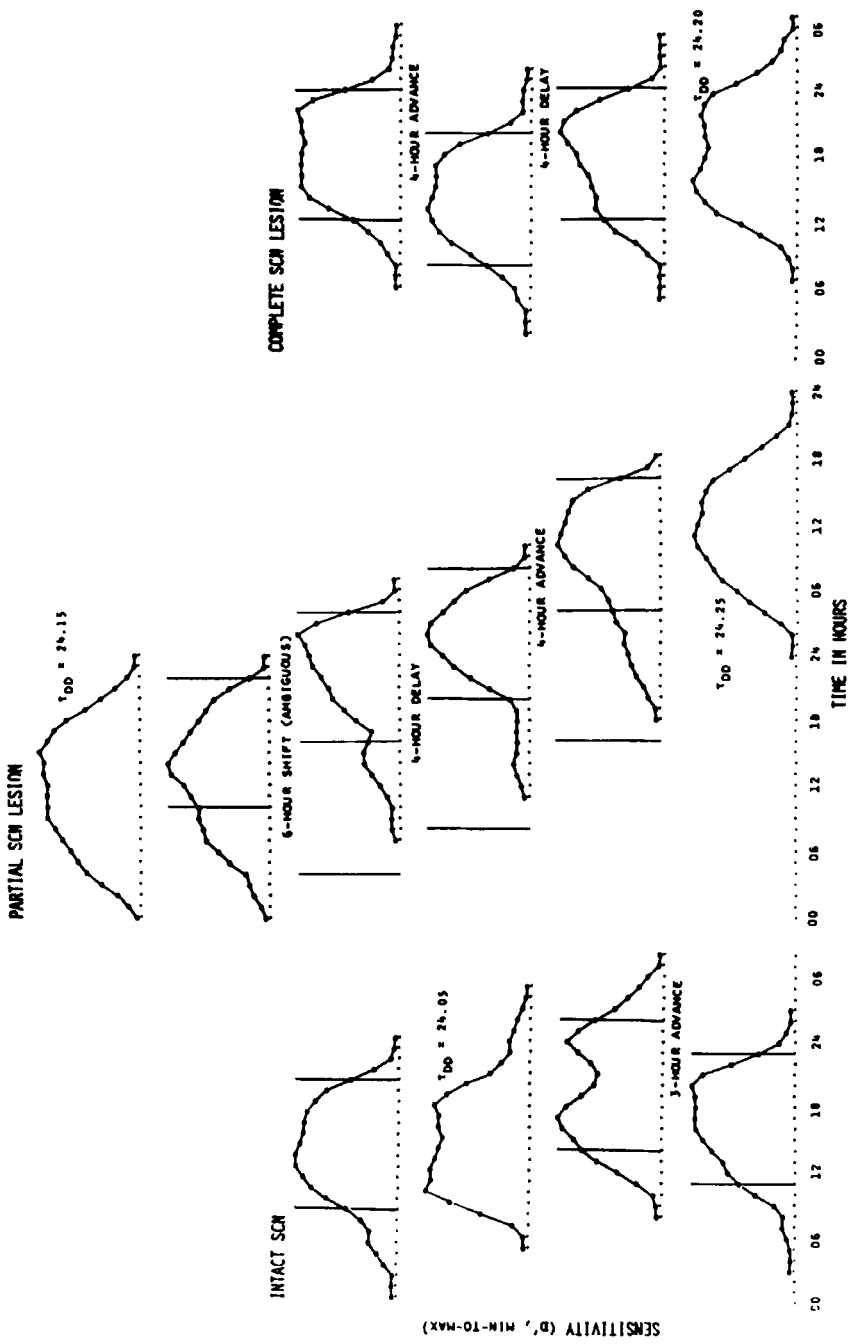


FIGURE 6. Succession of entrainment and DD regimens for three rats with varying damage to the SCN: intact, partial lesion (but arrhythmic ingestive patterns), and complete lesion. Each curve represents a steady-state two-week sample, and omits transient adjustments induced by skeleton photoperiod phase shifts. DD curves, plotted on the circadian time base are phase-anchored to the day of release from entrainment (except for the *top curve, center panel*, which is anchored at the end of the free run). All three animals show appropriate phase shifts to delays and advances of the skeleton photoperiod, as well as appropriate DD-release phases after entrainment. It follows that a visual system pacemaker, exclusive of the SCN, mediates entrainment of the sensitivity rhythm.

CONCLUSION

Whereas the mammalian circadian system is generally acknowledged to be organized in a multioscillatory hierarchy,⁴³ there have been few clearcut demonstrations of persistent rhythmicity in the absence of the SCN and light-dark cycles. Two such cases are the rat's anticipatory responding to periodic meal availability³⁸ and the squirrel monkey's body temperature rhythm.⁴⁴ The squirrel monkey's drinking rhythm persists after the lesion, too, but shows disruption and dampening within four to six weeks, and eventual arrhythmicity.⁴⁵ By contrast, the visual sensitivity rhythm persists in tests up to eight months after lesioning.

Our data do not localize the visual clock to the eye, although several lines of evidence point to ocular circadian pacemakers that may subserve rhythmic sensitivity to light. Potential correlates include rod outer disk shedding,¹⁵ autophagy within rod inner segments,⁴⁶ and dopamine concentration and synthetic rate within amacrine cells.⁴⁷ It is tempting to envision a functional connection with localized retinal melatonin level, which may show persistent daily rhythmicity even after pinealectomy.^{48,49}

The induction of circadian phase responses requires bright light exposure, and is thus distinct from signal detection.²⁷ It is possible, however, that phase advances and delays—essential for entrainment—occur when bright light strikes the eye at particular states of visual sensitivity, as indexed by the level and slope of the *d'* waveform. The instigated process may first induce an ocular phase response that in turn precipitates the phase shift of the SCN pacemaker.

SUMMARY

Visual signal detectability oscillates as a circadian rhythm, capable of free-running in constant conditions, and entrainment by external lighting schedules. These functions persist after lesioning of the suprachiasmatic nucleus (SCN), implicating a separate pacemaker for visual sensitivity. Its locus, physiology, and mode of interaction with the SCN have yet to be established.

REFERENCES

1. STEPHAN, F. S. & I. ZUCKER. 1972. Rat drinking rhythm: central visual pathways and endocrine factors mediating responsiveness to environmental illumination. *Physiol. Behav.* **8**: 315–326.
2. STEPHAN, F. S. & I. ZUCKER. 1972. Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. *Proc. Natl. Acad. Sci. USA* **69**: 1583–1586.
3. ZUCKER, I., B. RUSAK & R. G. KING, JR. 1976. Neural bases for circadian rhythms in rodent behavior. *In Advances in Psychobiology*. A. H. Riesen & R. F. Thompson, Eds. Vol. 3: 35–74. Wiley, New York.
4. MOORE, R. Y. & N. J. LENN. 1972. A retinohypothalamic projection in the rat. *J. Comp. Neurol.* **146**: 1–14.
5. ANDERSON, K. V. & W. K. O'STEEN. 1972. Black-white and pattern discrimination in rats without photoreceptors. *Exp. Neurol.* **34**: 633–637.
6. LAVAIL, M. M., M. SIDMAN, R. RAUZIN & R. L. SIDMAN. 1974. Discrimination of light intensity by rats with inherited retinal degeneration: a behavioral and cytological study. *Vision Res.* **14**: 693–702.
7. DUNN J., R. DYER & M. BENNETT. 1972. Diurnal variation in plasma corticosterone following long-term exposure to continuous illumination. *Endocrinology* **90**: 1660–1663.

8. EBIHARA, S. & T. KEIICHIRO. 1980. Entrainment of the circadian activity rhythm to the light cycle: effective light intensity for a zeitgeber in the retinal degenerate C3H mouse and the normal C57BL mouse. *Physiol. Behav.* **24**: 523-527.
9. HUDSON, D. J. & M. MENAKER. 1983. Circadian photoreception in rodless (*rd*) mice. *Soc. Neurosci. (Abstract)* **9**: 1073.
10. RICHTER, C. P. 1965. *Biological clocks in medicine and psychiatry*. C.C. Thomas. Springfield, Ill.
11. KAITZ, M. 1976. The effect of light on brightness perception in rats with retinal dystrophy. *Vision Res.* **16**: 141-148.
12. MCGUIRE, R. A., W. M. RAND & R. J. WURTMAN. 1973. Entrainment of the temperature rhythm in rats: effect of color and intensity of environmental light. *Science* **181**: 956-957.
13. RUSAK, B. 1977. Involvement of the primary optic tracts in mediation of light effects on hamster circadian rhythm. *J. Comp. Physiol.* **118**: 165-172.
14. PICKARD, G. E. & M. R. RALPH. 1984. Ablation of the ventral lateral geniculate nucleus (vLGN) alters the phase shifting effects of light on the mammalian circadian system. *Soc. Neurosci. (Abstract)* **10**: 294.
15. LAVAIL, M. M. 1976. Rod outer segment disk shedding in rat retina: relationship to cyclic lighting. *Science* **194**: 1071-1074.
16. GOLDMAN, A. I., P. S. TEIRSTEIN & P. J. O'BRIEN. 1980. The role of ambient lighting in circadian disc shedding in rod outer segment of the rat retina. *Invest. Ophthalmol. Visual Sci.* **19**: 1257-1267.
17. TEIRSTEIN, P. S., A. I. GOLDMAN & P. J. O'BRIEN. 1980. Evidence for both local and central regulation of rat outer segment disc shedding. *Invest. Ophthalmol. Visual Sci.* **19**: 1268-1273.
18. LAVAIL, M. M. & P. A. WARD. 1978. Studies in the hormonal control of circadian outer segment disc shedding in the rat retina. *Invest. Ophthalmol. Visual Sci.* **17**: 1189-1193.
19. JACKLET, J. W. 1969. Circadian rhythm of optic nerve impulses recorded in darkness from isolated eye of *Aplysia*. *Science* **164**: 562-563.
20. BARLOW, R. B. JR. & S. C. CHAMBERLAIN. 1973. Light and a circadian clock modulate structure and function in limulus photoreceptors. *In The Effects of Constant Light on Visual Processes*. T. P. Williams & B. N. Baker, Eds. 247-269. Plenum. New York.
21. BLOCK, G. D. & S. F. WALLACE. 1982. Localization of a circadian pacemaker in the eye of a mollusc, *Bulla*. *Science* **217**: 155-157.
22. TERMAN, M. & M. RAIBERT. 1974. Circadian rhythm of luminance detectability in the rat: operant-psychophysical analysis. Psychonomic Society meetings. (Abstract) Boston, Mass.
23. ROSENWASSER, A. M., M. RAIBERT, J. TERMAN & M. TERMAN. 1979. Circadian rhythm of luminance detectability in the rat. *Physiol. Behav.* **23**: 17-21.
24. TERMAN, M. 1983. Behavior analysis and circadian rhythms. *In Advances in Analysis of Behaviour*. M.D. Zeiler & P. Harzem, Eds. Vol. 3: 103-141. Wiley. Chichester, England.
25. GREEN, D. M. & J. A. SWETS. 1966. *Signal Detection and Psychophysics*. Wiley. New York.
26. PITTENDRIGH, C. S. & S. DAAN. 1976. A functional analysis of circadian pacemakers in nocturnal rodents. IV. Entrainment: pacemaker as clock. *J. Comp. Physiol.* **106**: 291-331.
27. TAKAHASHI, J. S., P. J. DECOURSEY, L. BAUMAN & M. MENAKER. 1984. Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. *Nature (London)* **308**: 186-188.
28. TERMAN, M. & J. W. KLING. 1968. Discrimination of brightness differences by rats with food or brain-stimulation reinforcement. *J. Exp. Anal. Behav.* **11**: 29-37.
29. TERMAN, M. & J. Terman. 1970. Circadian rhythm of brain self-stimulation behavior. *Science* **168**: 1242-1244.
30. TERMAN, M. 1981. Behavioral dynamics of the psychometric function. *In Quantitative Analyses of Behavior*. M. L. Commons & J. A. Nevin, Eds. **1**: 321-344. Ballinger. Cambridge, Mass.

31. GRAHAM, C. H. 1965. Discriminations that depend on wavelength. *In* *Vision and Visual Perception*. C.H. Graham, Ed. 350–369. Wiley, New York.
32. SOKOLOVE, P. G. & W. N. BUSHNELL. 1978. The chi-square periodogram: its utility for analysis of circadian rhythms. *J. Theor. Biol.* **72**: 131–160.
33. TUKEY, J. W. 1977. *Exploratory Data Analysis*. Addison-Wesley, Reading, Mass.
34. TRYON, W. W. 1983. Digital filters in behavioral research. *J. Exp. Anal. Behav.* **39**: 185–190.
35. TERMAN, J. & M. TERMAN. 1980. Effects of illumination level on the rat's rhythmicity of brain self-stimulation behavior. *Behav. Brain Res.* **1**: 507–519.
36. ROSENWASSER, A. M., Z. BOULOS & M. TERMAN. 1981. Circadian organization of food intake and meal patterns in the rat. *Physiol. Behav.* **27**: 33–39.
37. ROSENWASSER, A. M., Z. BOULOS & M. TERMAN. 1983. Circadian feeding and drinking rhythms under complete and skeleton photoperiods. *Physiol. Behav.* **30**: 353–359.
38. BOULOS, Z., A. M. ROSENWASSER & M. TERMAN. 1980. Feeding schedules and the circadian organization of behavior in the rat. *Behav. Brain Res.* **1**: 39–65.
39. PITTENDRIGH, C. S. & S. DAAN. 1976. A functional analysis of circadian pacemakers in nocturnal rodents. V. Pacemaker structure: a clock for all seasons. *J. Comp. Physiol.* **106**: 333–355.
40. RUSAK, B. & G. A. GROOS. 1982. Suprachiasmatic stimulation phase shifts circadian rhythms. *Science* **215**: 1407–1409.
41. ZATZ, M. & M. J. BROWNSTEIN. 1979. Intraventricular carbachol mimics the effects of light on the circadian rhythm in the rat pineal gland. *Science* **203**: 358–361.
42. ALBERS, H. E., C. F. FERRIS, S. E. LEEMAN & B. D. GOLDMAN. 1984. Avian pancreatic polypeptide phase shifts hamster circadian rhythms when microinjected into the suprachiasmatic region. *Science* **223**: 833–835.
43. MOORE-EDE, M. C., F. M. SULZMAN & C. A. FULLER. 1982. *The Clocks that Time Us: Physiology of the Circadian Timing System*. Harvard Univ. Press, Cambridge, Mass.
44. FULLER, C. A., R. LYDIC, F. M. SULZMAN, H. E. ALBERS, B. TEPPER & M. C. MOORE-EDE. 1981. Circadian rhythm of body temperature persists after suprachiasmatic lesions in the squirrel monkey. *Am. J. Physiol.* **241**: R385–R391.
45. ALBERS, H. E., R. LYDIC, P. H. GANDER & M. C. MOORE-EDE. 1984. Role of the suprachiasmatic nuclei in the circadian timing system of the squirrel monkey. I. The generation of rhythmicity. *Brain Res.* **300**: 275–284.
46. REMÉ, C., A. WIRZ-JUSTICE & M. DAPRADA. 1983. Retinal rhythms in photoreceptors and dopamine synthetic rate: effect of a monoamine oxidase inhibitor. *Trans. Ophthalmol. Soc. U.K.* **103**: 405–410.
47. WIRZ-JUSTICE, A., M. DAPRADA & C. REMÉ. 1984. Circadian rhythm in rat retinal dopamine. *Neurosci. Lett.* **45**: 21–25.
48. YU, H. S., S. F. PANG & P. L. TANG. 1981. Increase in the level of retinal melatonin and persistence of its diurnal rhythm in rats after pinealectomy. *J. Endocrinol.* **91**: 477–481.
49. REITER, R. J., B. A. RICHARDSON, S. A. MATTHEWS, S. J. LANE & B. N. FERGUSON. 1983. Rhythms in immunoreactive melatonin in the retina and Harderian gland of rats: persistence after pinealectomy. *Life Sci.* **32**: 1229–1236.