7. R. G. D. Steel and H. J. Torrie, *Principles and Procedures of Statistics* (McGraw-Hill, New York, 1960).
8. A. Arimura, J. A. Vilchez-Martinez, D. H. Coy, E. J. Coy, Y. Hirotsu, A. V. Schally, *Endocrinology* **95**, 1174 (1974).
9. G. Zeballos and S. M. McCann, *ibid.* **96**, 1377 (1975).
10. N. B. Schwartz, S. B. Cobbs, W. L. Talley, C. A. Ely, *ibid.*, p. 1171.
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Nucleus Suprachiasmaticus: The Biological Clock in the Hamster?

Abstract. *Destruction of the suprachiasmatic nuclei in the golden hamster by bilateral radiofrequency lesions abolishes three well-documented circadian rhythms—locomotor activity, estrous cyclicity, and photoperiodic photosensitivity. Entrainment of these rhythms by light cycles fails in lesioned hamsters; females become persistently estrous; in both sexes locomotor activity becomes sporadic, confined primarily to the light instead of darkness, and is totally arrhythmic when lesioned animals are exposed to continuous darkness; the photoperiodic gonadal response (gonadal regression induced by short day lengths) is abolished; lesioned animals remain reproductively mature irrespective of photoperiodic treatment.*

Several papers have described the effects in rats of ablation of the suprachiasmatic nuclei on rhythms of locomotor activity, drinking, pineal *N*-acetyltransferase activity, and adrenal corticosterone content (*1, 2*). Pittendrigh (*3*) suggested that the suprachiasmatic nuclei comprise a central pacemaker (biological clock) regulating circadian oscillations in various target organs. To investigate this possibility in the golden hamster we monitored three circadian rhythms—locomotor activity (wheel running), estrous cyclicity, and photosensitivity. Numerous studies have documented the precision and stability of the rhythm of locomotor activity (*4*), which, like others, is free-running in the absence of any entraining agents or zeitgebers; for this rhythm, the only documented entraining agent is the photoperiod. Thus, in constant conditions such as total darkness (DD) or constant, but dim, light (LL) the free-running activity rhythm has a period length of approximately 24 hours, thought to represent the periodicity of the endogenous oscillator driving the rhythm. In the presence of a light cycle of, for example, 14 hours of light (L) in 24 hours (LD 14:10), the oscillator and the rhythm that it drives assume the period length (24 hours) of the entraining agent. In this entrained state the hamster's activity is nearly entirely confined to the hours of darkness (Fig. 1, A and B). Interruption of the entraining signal from its receptor, which in this case is the retina (*5*), allows the oscillator to express its own endogenous periodicity and the rhythms it drives are accordingly free-running. If the oscillator could somehow be turned off, by electrocoagulation, for example, then the expresion of its driven rhythms, in the absence of a cyclic "driver" may be aperiodic.

The circadian rhythm of locomotor activity can serve as a marker for the less easily assayable rhythm of photosensitivity, which is the basis of the hamster's photoperiodic reproductive response (*6*). The two discrete phases of this rhythm, the photosensitive and the photoinsensitive, are each approximately 12 hours in duration (*6*). Light perceived during the photosensitive phase is interpreted as a long day and maintains gonadal function, whereas light perceived only during the insensitive phase is interpreted as a short day and promotes gonadal regression. The two rhythms of locomotor activity and photoperiodic photosensitivity appear to bear a fixed phase relationship such that the onset of activity corresponds closely to the onset of photosensitivity (*6*). If the hamster's clock entrains to a light cycle (for example, a short day of LD 6:18) such that light is presented during the photoinsensitive phase of the rhythm only, the reproductive system ceases to function; testes and sex accessory structures regress and estrous cyclicity ceases (*7*)—females enter a continuous diestrous condition. However, if the clock entrains so that light is present during at least a portion [as little as 1 hour is sufficient (*8*)] of the photosensitive phase of the rhythm, the reproductive system remains functional.

The least investigated circadian rhythm that we examined is estrous cyclicity. The periodic release of luteinizing hormone in both rats and hamsters is regulated by a circadian oscillator and depends on a differential sensitivity to estrogens (*9*). High concentrations of plasma estrogen (during the afternoon of proestrus) appear to sensitize the hypophysiotropic region of the hypothalamus to a signal from the clock and result in the release of sufficient luteinizing hormone to cause ovulation. Therefore, in females with estrogen implants that assure continuous high levels of plasma estrogen, there is a "preovulatory" release of luteinizing hormone at the same time every day (*9*). In the entrained state the hamster's estrous cycle repeats once every 4 days (96 hours), whereas in continuous dim light the period length of free-running estrous cycles is significantly greater than 96 hours (*10*). We have reproduced these results in a group of hamsters whose locomotor activity we also monitored. Our data demonstrate: (i) the free-running period length of estrus is a multiple of four of the free-running period length of locomotor activity; that is, estrus occurs once every 4 "days," as in the entrained state, but the length of the hamsters' "day" differs from 24 hours; (ii) arrhythmicity arises coincidentally in both rhythms after long-term subjection to continuous light—locomotor rhythmicity breaks down at the same time as the animals become persistently estrous (assessed by continuous vaginal cornification and continuous lordosis in the presence of a male). We suggest that estrous cyclicity and locomotor rhythmicity may be related in the same way as the photosensitivity and locomotor rhythms; we also suggest that all three rhythms may be driven by the same circadian clock.

We therefore attempted to locate and identify this clock by making radiofrequency lesions in the brains of adult, reproductively mature, male and female hamsters (*11*). If we could destroy the clock, (i) locomotor activity would be random with no indication of entrainment; (ii) estrous cyclicity would be replaced by persistent estrus; and (iii) animals without this clock would maintain gonadal function irrespective of photoperiod.

Histological studies were made in every hamster with a lesion (*12*), and the animals were placed into one of five groups (Table 1) according to the location and extent of the lesions. Those hamsters (groups 3 and 5) in which the suprachiasmatic nuclei were severely damaged or entirely destroyed failed to entrain to the ambient light cycle (LD 14:10); after 1 to 10 days of near total inactivity following the operation, such hamsters displayed patterns of running in the wheel that varied from sporadic bursts of activity throughout each 24-hour period (Fig. 1A) to more concentrated activity during the daylight hours, with little activity during darkness (Fig. 1B). When exposed to continuous darkness all animals were arrhythmic (Fig. 1, A and B). In addition, females with extensive lesions (group 5) quickly became persistently estrous, as judged by constant vaginal cornification, and these animals

also gave the lordosis response whenever they were placed with a male. In the male hamsters with extensive lesions, gonadal regression did not occur during the periods of constant darkness. The control animals (group 1) demonstrated normal entrainment to LD 14:10, and in DD the locomotor activity rhythm became free-running with a period length different from 24 hours. Reproductively these males responded to DD with testicular regression.

Our data show that the suprachiasmatic nuclei are required for the normal expression of three different circadian rhythms. The data are interpretable in several ways.

1) The suprachiasmatic nuclei may be an area of convergence of fibers from the clock, and the suprachiasmatic lesions may "unplug" these rhythms from the clock. Several points argue against this. First, lesions anterior, posterior, and dorsal to the suprachiasmatic nuclei fail to affect all three rhythms simultaneously. In fact, initial attempts to dissociate the three rhythms have succeeded only in the case of photoperiodic photosensitivity (groups 2 and 4) where there is normal or nearly normal locomotor entrainment to LD 14:10, free-running locomotor activity in DD, but failure of the reproductive system to regress with short day exposure; in males (group 2) testes and sex accessories remain large and functional, and females (group 4) continue to display 4-day estrous cycles throughout the period of DD treatment. Because the hamster's photoperiodic response relies on the integrity of both a circadian oscillator (driving the rhythm of photoperiodic photosensitivity) and the pineal gland and because the gonadal responses of the animals of groups 2 and 4 are identical to those of pinealectomized hamsters (*5, 7*), the lesions may have "unplugged" the pineal from the clock. We have not yet been able to abolish locomotor activity without affecting the remaining rhythms. In every case in which locomotor arrhythmicity is induced by lesions, the other two rhythms also become arrhythmic. Second, two reports (*13*) demonstrate that deafferentation of the preoptic region (including the suprachiasmatic nuclei) of female rats, leaving only the neural connections with the ventromedial hypothalamus and the eyes intact, allowed estrous cycles to persist; although the cycles were irregular, every female ovulated, which suggests that some structure located in this isolated area of the hypothalamus functions as a neural pacemaker in timing estrous cyclicity. Finally, since the only known zeitgeber of the hamster's clock is light, a neural pathway must exist from the retina to the clock. Eichler and Moore have reported the existence of a direct neural pathway from the retina to the suprachiasmatic nuclei (bilateral projection from each retina) of hamsters (*14*), similar to that reported in rats (*15*); in neither species were any other visual projections to the hypothalamus found.

Table 1. Lesion effects on three circadian rhythms in adult hamsters. In group 1 (control males), the lesions completely missed the suprachiasmatic nuclei. In groups 2 (males) and 4 (females), the lesions were located posterior or dorsal to the suprachiasmatic nuclei. In groups 3 (males) and 5 (females), the suprachiasmatic nuclei were either severely damaged or destroyed. Females in presistent estrus demonstrated continuous vaginal cornification and, in the presence of a male, continuous lordosis. Abbreviations: Entr, entrained; FR, free-running; TR, testicular regression; Arr, arrhythmic.

Group	*N*	LD 14:10			DD		
		Activity (motor)	Estrous cycle	Testis function	Activity		Terminal testis weight (mg)*
					Motor	Gonadal	
1	5	Entr		Normal	FR	TR	789.1 ± 241.6
2	7	Entr		Normal	FR	No TR	3345 ± 300.9
3	6	Arr		Normal	Arr	No TR	3262.8 ± 287.6
4	9	Entr	Cyclic		FR	FR cycle	
5	4	Arr	Persistent estrus		Arr	Persistent estrus	

*Mean ± standard error after 9 to 15 weeks in constant darkness.

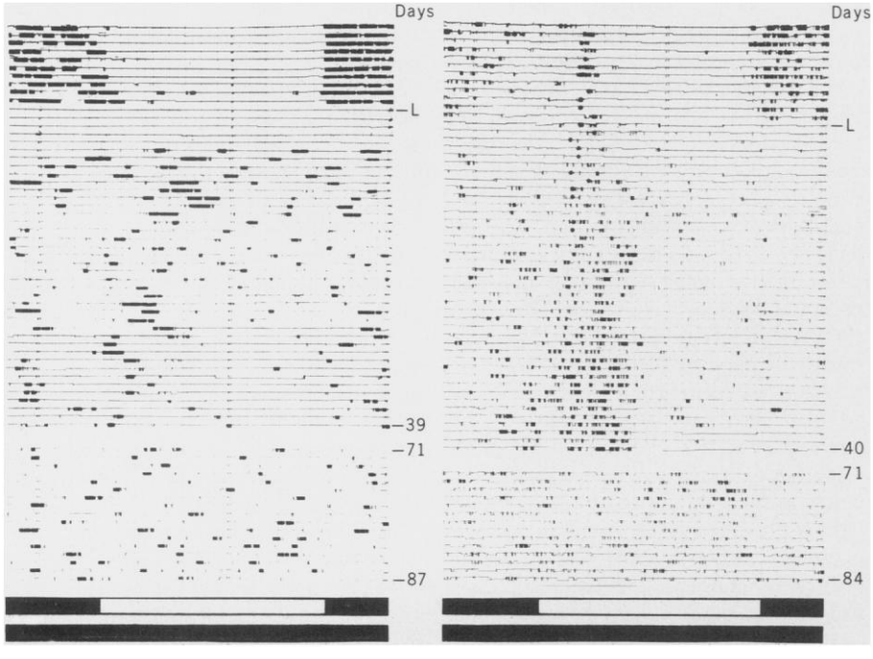

Fig. 1. Locomotor activity records of a male (A) and a female (B) hamster with lesions of the suprachiasmatic nuclei. Both animals were exposed to LD 14:10 before and for 10 weeks after the lesions were made. They were then transferred to DD for 10 to 15 weeks during which they were allowed access to a running-wheel for 2 to 3 weeks. The light cycle is diagramed beneath each record. The activity for each successive day appears beneath the record for the previous day. The numbers on the side of each record represent the number of days after the lesions (*L*) were made. Both records demonstrate entrainment of locomotor rhythm to LD 14:10 (lights 0600 to 2000) before the operation and loss of entrainment to this photoperiod afterward. In (A) activity becomes sporadic, occurring in bursts of approximately 0.1 to 3.0 hours in duration in both the dark and light phases of the photocycle. When the animal was exposed to continuous darkness (days 71 to 87), activity remained sporadic, with bursts of more uniform and briefer duration. The testes in this animal remained large and functional during LD 14:10 and DD treatment. In (B) activity after the operation was confined primarily to the early morning hours. This apparent entrainment results from our daily presence in the animal room at that time, when we disturbed every female daily by assaying her estrous cycle. This is apparent even in activity records made before the operation, where a daily burst of activity at approximately 0800 corresponds to our presence in the room. However, while in DD (days 71 to 84) the animal was not disturbed, and activity was fairly uniform throughout each 24-hour period. After the lesion was made, this animal was in estrus on days 5, 10, 11, 14, 15, and 17 through 70 (persistent estrus), throughout 12 weeks of DD treatment, and for 8 weeks on LD 14:10. Thus this animal remained in persistent estrus from day 17 until she was killed on day 210.

2) Suprachiasmatic lesions do not affect the clock directly but instead, regardless of photoperiod, produce the perceptual illusion of constant high intensity illumination which may have effects on the three rhythms in question identical to those described in hamsters with lesions (*3, 16*). In many animals with such lesions, patterns of locomotor activity in LD 14:10 differ from those in DD (Fig. 1). If the lesions in these animals produced a perceptual illusion of continuous illumination, one would expect similar locomotor patterns regardless of the ambient photoperiod. We have seen animals in which the lesion did not abolish locomotor rhythmicity, but, rather, altered its phase relationship to the photoperiod. In every animal thus affected, the suprachiasmatic nuclei were only partially destroyed. This suggests that less than total damage to the clock may alter either tau (τ), the free-running period length of the clock, or the relationship of the clock to the entraining light cycle.

3) The suprachiasmatic nuclei may not be the primary clock but rather a coupled oscillator in a two- or multi-oscillator system. To negate this hypothesis, it would be necessary to isolate the suprachiasmatic region from all the neural input (except that from the retinas) leaving efferent connections intact, and then to view maintained entrainment of the rhythms in question. This monumental feat has not yet been accomplished in hamsters. However, these facts remain: (i) in hamsters the primary (self-sustained) oscillator in a multioscillator system must receive photic input, whereas other driven oscillators need not necessarily be so endowed, and (ii) the only known direct visual projection outside of the classical primary and accessory optic pathways in both the hamster and the rat is to the suprachiasmatic nuclei.

4) The suprachiasmatic nuclei may be a primary oscillator regulating a variety of rhythms. The strongest supporting evidence is (i) the visual pathway from the retina to the suprachiasmatic nuclei, and (ii) the fact that seemingly unrelated rhythms ranging from locomotor activity to adrenal corticosterone content (*1, 2*) are abolished when the suprachiasmatic nuclei are destroyed. We believe it unlikely, although by no means impossible, that a single driven oscillator would be involved in the expression of so many different rhythms. Although further research is needed, we tentatively conclude that the nucleus suprachiasmaticus is a biological clock in the hamster.

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References and Notes

1. F. K. Stephan and I. Zucker, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1583 (1972); *Physiol. Behav.* **8**, 315 (1972).
2. R. Y. Moore and V. B. Eichler, *Brain Res.* **42**, 201 (1972); R. Y. Moore, in *The Neurosciences: Third Study Program*, F. O. Schmitt and F. G. Worden, Eds. (MIT Press, Cambridge, Mass., 1974), pp. 537– → R. Y. Moore and D. C. Klein, *Brain Res.* **71**, 17 (1974).
3. C. S. Pittendrigh, in *The Neurosciences: Third Study Program*, F. O. Schmitt and F. G. Worden, Eds. (MIT Press, Cambridge, Mass. 1974), pp. 437–458.
4. J. Aschoff and J. Meyer-Lohman, *Z. Tierpsychol.* **11**, 476 (1954); J. Aschoff, J. Figala, E. Poppel, *J. Comp. Physiol. Psychol.* **85**, 20 (1973); P. J. DeCoursey, *J. Cell. Comp. Physiol.* **63**, 189 (1964); C. S. Pittendrigh, *Cold Spring Harbor Symp. Quant. Biol.* **25**, 159 (1960).
5. R. J. Reiter, *Anat. Rec.* **160**, 13 (1968); *Gen. Comp. Endocrinol.* **12**, 460 (1969).
6. J. A. Elliott, M. H. Stetson, M. Menaker, *Science* **171**, 1169 (1972); M. H. Stetson, J. A. Elliott, M. Menaker, *Biol. Reprod.* **13**, 329 (1975). The annual reproductive cycle of the hamster is regulated by seasonal changes in day length (photoperiod). Such regulation is referred to as photoperiodism. Normally, photoperiods of more than 12.5 hours per 24 promote gonadal activity, whereas shorter photoperiods promote gonadal quiescence [S. Gaston and M. Menaker, *Science* **158**, 925 (1967)]. These differing effects of photoperiod necessitate that the hamster be able to distinguish between long and short photoperiods. The hamster does so through a circadian rhythm of photosensitivity, the parameters of which are discussed in the text. If light is present during that portion of the hamster's day coincident with the sensitive phase of this rhythm it is interpreted as a long photoperiod and maintains gonadal function by an as yet unresolved photo-neuro-endocrine reflex. Conversely, light present during that portion of the hamster's day coincident with the insensitive phase of this rhythm is interpreted as a short photoperiod and does not maintain gonadal function. Thus the hamster's photoperiodic gonadal response depends not on the total amount of light to which the hamster is subjected, but rather on the temporal position of light with respect to the hamster's circadian system. One can maintain gonadal function in hamsters subjected to far less than the minimum 12.5 hours of light per day by presenting the light during the sensitive phase of the rhythm of photosensitivity.
7. S. Sorrentino, Jr., and R. J. Reiter, *Gen. Comp. Endocrinol.* **15**, 39 (1970).
8. J. A. Elliot, thesis, University of Texas (1974).
9. R. L. Norman and H. G. Spies, *Endocrinology* **95**, 1367 (1974); S. J. Legan, G. A. Coon, F. J. Karsch, *ibid.* **96**, 50 (1975); S. J. Legan and F. J. Karsch, *ibid.*, p. 57.
10. J. J. Alleva, M. V. Waleski, F. R. Alleva, *ibid.* **88**, 1368 (1971).
11. The hamsters were raised from stock purchased from Charles River Lakeview, Newfield, N.J. They were placed in the running-wheel cages for continuous recording of locomotor activity. The photoperiod was LD 14:10. Food and water were available. The animals were anesthetized with pentobarbital (10 mg in 0.5 ml) and placed in a Kopf stereotaxic apparatus. Radiofrequency lesions (100 khz, 50 volts, 20 ma; LM4 Lesion Maker, Grass Instruments, Quincy, Mass.) were made bilaterally with an electrode (size 0 insect pin insulated except for its tip with Insl-x (Insl-x Products Corp., Yonkers, N.Y.). The animals were held overnight in small plastic cages and returned to their running-wheel cages the following morning. All animals survived the operation. Activity was recorded for at least 70 days after the operation, during which the photoperiod remained LD 14:10. The animals were then transferred to continuous darkness (DD), and activity was again recorded for 14 to 21 days, after which the animals were removed from activity cages and housed individually in small plastic cages for the duration of DD treatment. When the experiment was completed, animals were anesthetized with pentobarbital, perfused via the left ventricle with saline followed by 10 percent formalin or Bouin's fluid. The gonads were weighed and preserved with the brain in 10 percent formalin.
12. Brains were washed with 70 percent ethanol, trimmed to give a uniform plane of section, dehydrated in ethanol, cleared in toluene and embedded in Paraplast. Serial 10-μm sections were mounted on glass slides and stained [H. Klüver and E. Barrera, *J. Neuropathol. Exp. Neurol.* **12**, 400 (1953)].
13. B. Halász, in *Frontiers in Neuroendocrinology*, W. F. Ganong and L. Martini, Eds. (Oxford Univ. Press, London, 1969), pp. 307–342; W. A. Kaasjager *et al.*, *Neuroendocrinology* **7**, 54 (1971).
14. V. B. Eichler and R. Y. Moore, *Acta Anat.* **89**, 359 (1974).
15. R. Y. Moore and N. J. Lenn, *J. Comp. Neurol.* **146**, 1 (19 → A. E. Hendrickson, N. Wagoner, W. M. Cowan, *Z. Zellforsch. Mikrosk. Anat.* **135**, 1 (1972).
16. G. C. Kent, P. M. Ridgeway, E. F. Strobel, *Endocrinology* **82**, 699 (1968).
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Selective Production of *cis*- and *trans*-Verbenol from (−)- and (+)-α-Pinene by a Bark Beetle

Abstract. *A unique biological system whereby optical isomers are selectively transformed to geometrical isomers is demonstrated in* Ips paraconfusus. *Exposure of adult male and female beetles to vapor of (−)-α-pinene resulted in the production of (+)-*cis*-verbenol, a pheromone of this species, whereas (+)-α-pinene was oxidized to (+)-*trans*-verbenol. It appears, therefore, that the ability of a bark beetle to produce its aggregation pheromone can be governed by the chirality of a precursor in the host tree.*

Bark beetles that attack coniferous trees are exposed to high concentrations of monoterpenes as they encounter resin exuding from the injured tissues of their hosts. Exposure of adult beetles to vapors of individual terpenes results in the formation of oxidation products, including pheromones, which are detected in the hindgut (*1*). The host monoterpene α-pinene has been converted to *cis*- and *trans*-verbenol and myrtenol by every species that we studied.

Since the verbenols are dissymmetric molecules and the biological activity of an insect pheromone may depend on its chirality (*2*), we were interested in determining whether bark beetles might preferentially oxidize (+)- or (−)-α-pinene and whether the optical rotation of the products would be dependent on the rotation of the precursor. The species chosen for this study was *Ips paraconfusus* Lanier, since *cis*-verbenol is a component of the pheromone system of this beetle (*3*).

Adult male and female beetles were exposed to the vapors of (+)-α-pinene