abichi indicate that the convex ventral valve was downward, implying that this was the living position—not merely the final resting position of a disturbed shell.

7) The edges of the two valves are coincident; the edge of the recessed dorsal valve extends to the edge of the trail of the ventral valve, providing no space for gape in the inverted position.

8) The side view (Fig. 1) shows the anchoring spines curving to a common level that would have provided stability in the inferred living position, but neither stability nor mutual strengthening in the inverted position.

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References and Notes

1. W. Waagen, *Geol. Surv. India Mem., Palaeontol. Indica Ser. 13* **1**, pt. 4, pp. 611–728, pl. 58–81 (1884).
2. H. Muir-Wood and G. A. Cooper, *Geol. Soc. Amer. Mem. 81* (1960).
3. F. Noetling, *Centralblatt Mineral Geol. Palaeontol.* No. 17, p. 529 (1903).
4. P. J. Coleman, *Australia Bur. Mineral Resources Geol. Geophys. Bull. 40* (1957).
5. Survey of Pakistan sheet No. 38 P/4.
6. R. E. Grant, *J. Paleontol.* **37**, 134, pl. 19 (1963).
7. J. H. Johnson, *Colo. School Mines Quart.* **58**, No. 3 (1963).
8. R. E. Grant, *J. Paleontol.*, in press.
9. H. M. Muir-Wood, *Gt. Brit. Geol. Surv. Mem. 13* (1928), pt. 1.
10. R. R. Shrock, *Sequence in Layered Rocks* (McGraw-Hill, New York, 1948).
11. The expedition to Pakistan was made possible by the Smithsonian Institution through G. A. Cooper; A. N. Fatmi, Geological Survey of Pakistan, helped collect the specimens; Fig. 2 is by L. B. Isham, U.S. National Museum; the typescript was reviewed by J. T. Dutro, Jr., U.S. Geological Survey; publication is authorized by the director, U.S. Geological Survey.

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Circadian Rhythms in Rats: Effects of Random Lighting

Abstract. *Increase in body weight, spontaneous running activity, and adrenal cortical function have been studied in rats exposed to a random lighting schedule. In two separate experiments, grouped control animals were given 12 or 14 hours of light alternating with 12 or 10 hours of darkness, respectively, while corresponding grouped experimental animals were given the same total amounts of light and darkness per 24 hour period in a randomized pattern. Random light for periods of 17 to 40 days exerted no influence on growth rate, on weights of endocrine organs, or on adrenal response to adrenocorticotrophic hormone. However, the physiological fluctuation of group running activity and adrenal steroid secretion was abolished. Group desynchronization and the development of circadian rhythms having periods both shorter and longer than 24 hours appear to have replaced the synchronized group rhythmicity.*

Although numerous studies have appeared over the last 30 years on the ubiquity of circadian rhythms in plant and animal physiology and the effects of variations in environmental parameters on these rhythms, investigators generally have given little attention to the question of whether maintenance of these rhythms is essential in the overall homeostasis of the animal. Tentative evidence for such a requirement has come from studies of plant growth (*1*) and experiments with insectivora (*2*). The importance of these rhythms in mammals, however, has not been studied extensively.

In the past, many studies of circadian rhythms have involved the observation and analysis of physiological rhythms under constant environmental conditions. It is known that, with photoperiods of 12 hours of light alternating with 12 hours of darkness, for example, physiological rhythms in mammals tend to display a precise synchrony with the environmental lighting schedule; that under continuous light, period lengths will become shorter in diurnal, and longer in nocturnal, mammals; and that under continuous darkness the opposite will occur (*3*). However, constancy of the environment is only one of the possible modifications that can be used to study circadian rhythms. Another method, and one which to our knowledge had not been investigated, is a randomization of lighting conditions in which periods of light and darkness follow each other at irregular intervals. This experimental design provides control and experimental animals with the identical amount of light per day (that is, an equivalent net photoperiod), and yet prevents the acquisition of rhythmic light information by the experimental group. We now describe a study of locomotor activity, pituitary-adrenal function, and body growth in rats maintained in such a randomized lighting environment.

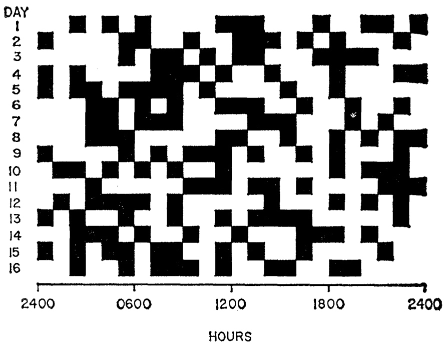

Fig. 1. Daily lighting schedule for experimental rats. Daily totals are 14 hours of light and 10 hours of darkness. Shaded areas represent periods of darkness.

A large wooden cabinet was constructed having two cages (40 by 70 by 60 cm) on each side; the cages were separated in the middle by an insulated compartment of 30 cm containing an exhaust fan to provide ventilation through each compartment and to mask sound transmission between cages. Two groups of ten male rats (Sprague-Dawley), selected at random from a group of 20 rats of equal age and weight, were separated into the two cages, with food and water being supplied to the animals as desired.

In the first experiment, the control group was subjected to 12 hours of light from 0600 to 1800 hours and 12 hours of darkness from 1800 to 0600 hours. The experimental group also received 12 hours of light and 12 hours of darkness, but in a randomized pattern throughout the 24-hour period. A random pattern of light and darkness, similar to that shown in Fig. 1, was prepared from random number tables and used throughout the experiment. The animals were weighed twice during the first week and weekly thereafter. Locomotor activity was recorded continuously for each group by means of a running wheel (40 cm in diameter) in each cage as well as by transduced measurements of floor screen movements. On the 41st day of the experiment, the rats were decapitated, and the thyroids, pituitaries, adrenals, and testes were recovered and weighed.

In a second experiment, 100 male rats of the same strain, age, and weight as in the first experiment were divided at random into two groups, housed two per cage, and given food and water as desired. The control group was subjected to 14 hours of light, from 0600 to 2000 hours, and 10 hours of darkness, from 2000 to 0600. The experimental group, kept in a similar room next to that of the control group, received an equivalent 14 hours of light, but divided into periods selected at random throughout the day, as shown in Fig. 1. Beginning at 0500 on the 17th day of the experiment and at 6-hour intervals thereafter through 0500 the following day, seven animals from each group, taken in alternating sequence, were decapitated. Trunk blood and both adrenals were collected individually for corticosterone determination by the fluorometric method (*4*). In addition, seven rats from each group were selected at 0500 and 1700, hypophysectomized, and tested 2 hours later for adrenal response to adrenocorticotrophic hormone (ACTH). A solution of ACTH (USP, XVI reference standard) was injected intravenously at a dose of 1.0 milliunits, the corticosteroid increase in the peripheral blood at 15 minutes being used as a parameter of the adrenal response to ACTH (*5*).

The mean body weights for the two groups measured in the first experiment show close agreement throughout the study, differences between the means being only a fraction of the standard deviations in all cases. These results suggest that rhythmic fluctuations in light may not be as essential to normal mammalian ponderal growth as they appear to be for plants and insectivora.

The weights of the endocrine organs provide further support for the similarity of the two groups. Differences in means between groups for anterior and posterior pituitaries, thyroid, adrenals, and testes are only fractions of the standard deviation in all cases. Further, adrenal responses to ACTH show no significant differences between the control and experimental groups or between values obtained for morning and afternoon. The concentrations of corticosterone in the peripheral plasma 15 minutes after injection were 16.4 ± 0.8 and 17.4 ± 1.8 μg/100 ml for control and experimental animals, respectively, at 0500; at 1700 the values were 17.9 ± 1.0 and 15.7 ± 0.8 μg/100 ml. Therefore, we conclude that random lighting for periods up to 40 days in the case of growth rate and organ weights and up to 17 days in the case of adrenal response to ACTH failed to produce significant alterations in these parameters.

In contrast to the foregoing findings from physiological parameters which normally show no rhythmicity, studies of locomotor activity and plasma and adrenal corticosterone show a disruption of the usual temporal patterns. Comparison of Figs. 2 and 3 reveals a clear diurnal variation of group running activity for the control animals, whereas the experimental group in random light shows a gradual but progressive loss of this pattern. The distinction between group activity and inactivity remains fairly clear for the first 4 or 5 days but then fades as running activity begins to appear both earlier and later than usual, ultimately occurring almost uniformly throughout the

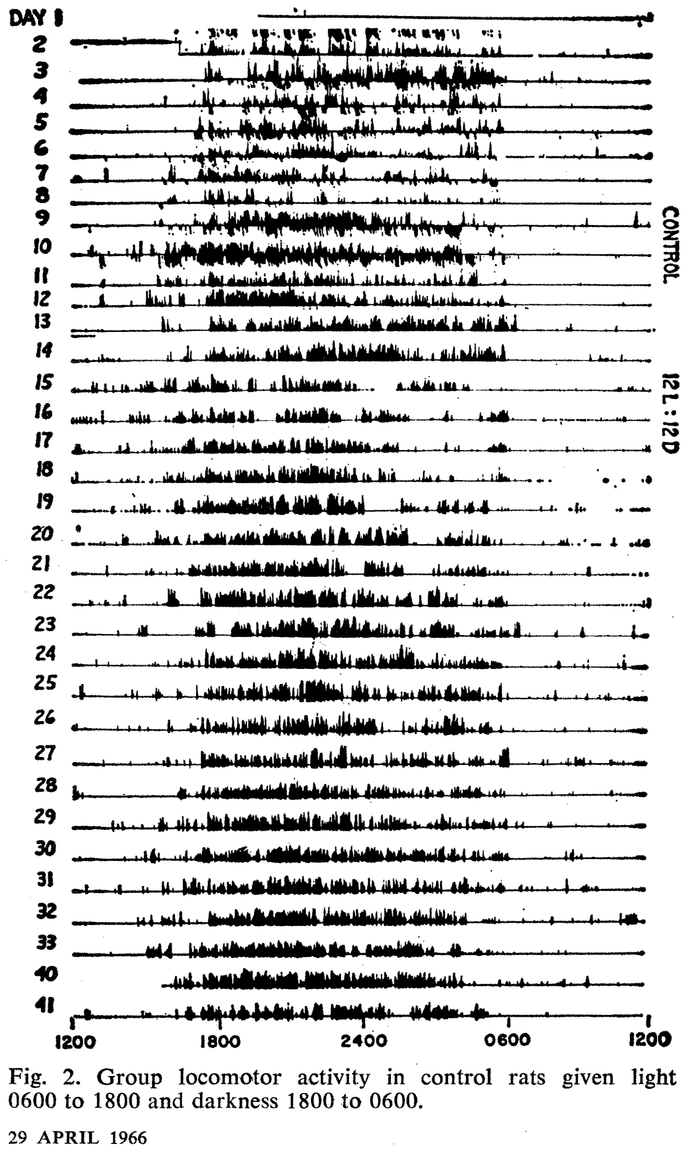

Fig. 2. Group locomotor activity in control rats given light 0600 to 1800 and darkness 1800 to 0600.

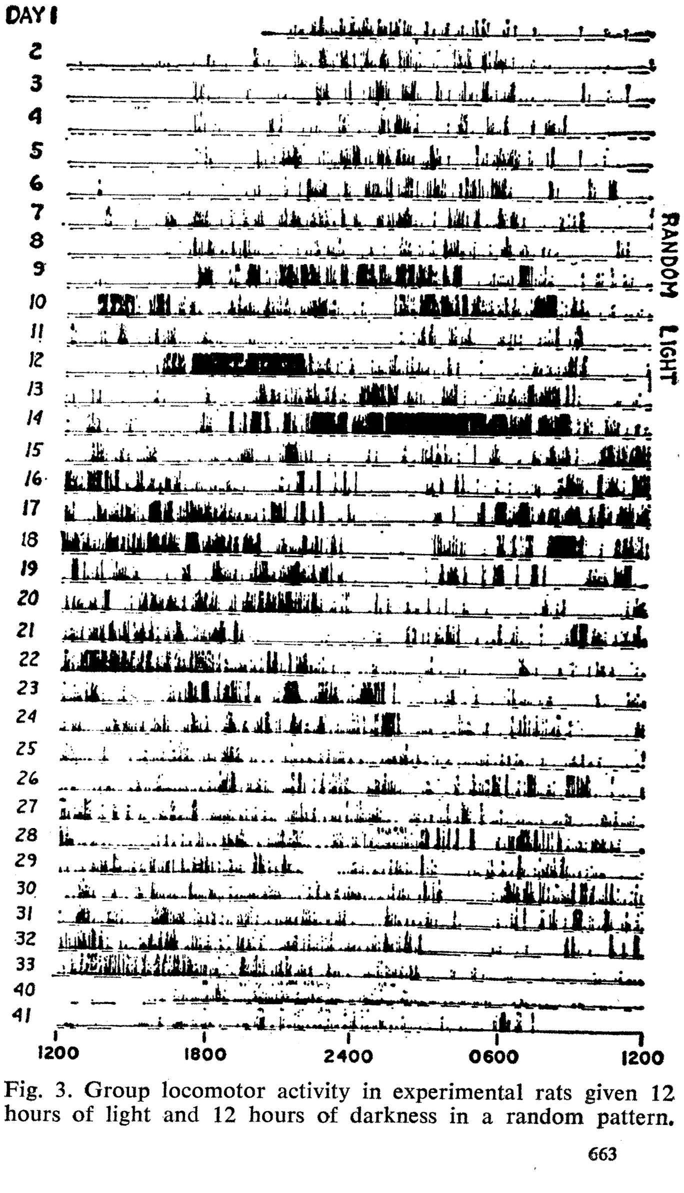

Fig. 3. Group locomotor activity in experimental rats given 12 hours of light and 12 hours of darkness in a random pattern.

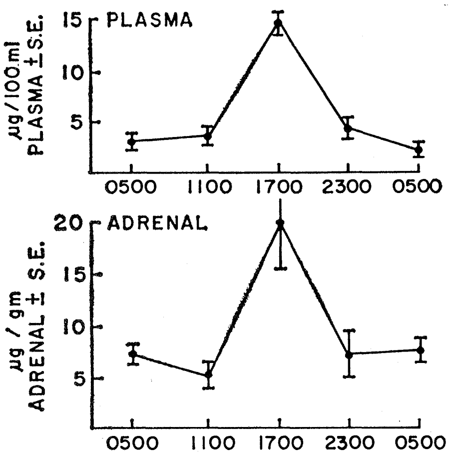

Fig. 4. Diurnal variation of plasma and adrenal corticosterone in control rats given light 0600 to 2000 and darkness 2000 to 0600.

day in a random relation to light and darkness. Although this pattern of disruption could represent a complete loss of physiological rhythms, it seems equally reasonable to view this as a desynchronization of ten separate but continuing activity rhythms which show no clear rhythmicity when summed as a group.

The concentrations of plasma and adrenal corticosterone throughout the final 24-hour day in the second experiment are shown in Figs. 4 and 5. The graph of the corticosterone values for the control animals demonstrates the usual group diurnal cycles found in normal rats (*6*), with a distinct peak just prior to the onset of darkness in the late afternoon and lowest values at the onset of light in the early morning. The peak value at 1700 is significantly different from all other values.

Although the graph of adrenal and plasma steroids for the experimental animals bears some resemblance to that of the controls, the group diurnal cycle is attenuated, and the standard errors for these values, except at 0500, are so large that none of the means is statistically distinct.

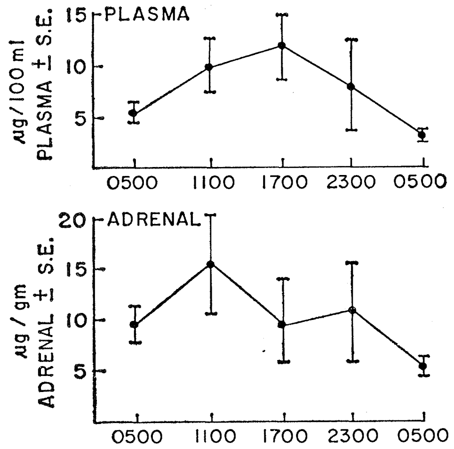

Fig. 5. Diurnal variation of plasma and adrenal corticosterone in experimental rats given 14 hours of light and 10 hours of darkness in a random pattern.

Thus, with regard to circadian rhythms, the biological organism appears to behave as an open, or environmentally sensitive, system in which light is the single most important sensory modality. Circadian rhythms differing in a predictable way from 24-hour periods have been described classically for animals in an environment of continuous light or darkness or during periods of phase shifts in the lighting periodicities (*3, 7*). However, for the animal in an environment of random light, in which the net balance between light and darkness is maintained in the absence of lighting rhythmicity, there is no previous evidence on which to predict the behavior of circadian rhythms or even their existence under such conditions. While there are undoubtedly several explanations for our data, we suggest one that seems reasonable and promising.

The alteration in activity rhythms, such that activity occurs both earlier and later in the day, would appear to be best explained as a development within the group of activity rhythms having periods both shorter and longer than 24 hours; this occurrence would produce the widening of the activity trace in both directions as observed. If, on the other hand, the group developed a set of varying rhythms having periods that were all longer or all shorter than 24 hours, the activity trace would still widen but would do so in only one direction, with the leading or trailing edge drifting (the so-called Aschoff effect) in the same direction as the widening.

The similarity of the low concentrations of corticosterone at 0500 in both experimental and control groups, contrasted with the wide range of values occurring at 1100, 1700, and 2300, suggests that some of the large peak values normally seen at 1700 have shifted in time both forward and backward sufficiently to increase the average values at 1100 and 2300 without yet affecting the 0500 values. The persistence of low levels in both 0500 samples would appear to rule out the existence of individual rhythms with periods all less than or all greater than 24 hours, since a movement of all peak values only forward or backward in time would leave either the 2300 or 1100 samples unaffected instead of those at 0500. One would expect in a longer experiment that even the 0500 values would show an increased average and standard error, such that no semblance of group rhythmicity would remain. Thus, it would appear that randomized lighting provides a unique environment in which light-dark fluctuations become useless as time-givers and in which circadian rhythms within a group become less constrained to follow a fixed pattern of alteration of period length. Under conditions of continuous light or darkness conversely, there would appear to be some but not total independence of period length from photoperiod.

In summary, it has been shown that whereas random environmental lighting schedules appear to have no effect on ponderal growth, endocrine organ weights, or adrenal response to ACTH, the periodic fluctuations of group activity and adrenal steroid secretion were abolished. Individual circadian rhythms, both longer and shorter than 24 hours in period, appear to have replaced the synchronized rhythmicity in the group. Simple removal of only the temporal aspect of the lighting environment, without alteration of the net photoperiod, would appear to be a unique method of modifying and in some cases nullifying the important effect of light on biological rhythms in mammals.

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References and Notes

1. H. R. Highkin, *Cold Spring Harbor Symp. Quant. Biol.* **25**, 231 (1960).
2. J. Harker, *The Physiology of Diurnal Rhythms* (Cambridge Univ. Press, London, 1964).
3. J. Aschoff, *Cold Spring Harbor Symp. Quant. Biol.* **25**, 11 (1960).
4. R. H. Silber, R. D. Bush, R. Oslapas, *Clin. Chem.* **4**, 278 (1958).
5. R. Guillemin, G. W. Clayton, J. D. Smith, H. S. Lipscomb, *Endocrinology* **63**, 349 (1958).
6. R. Guillemin, W. E. Dear, R. A. Liebelt, *Proc. Soc. Exp. Biol. Med.* **101**, 394 (1959); V. Critchlow, R. A. Liebelt, M. Bar-Sela, W. Mountcastle, H. S. Lipscomb, *Am. J. Physiol.* **205**, 807 (1963).
7. J. Aschoff, in *Circadian Clocks*, Proc. Feldafing Summer School 7–18 September 1964 (North-Holland, Amsterdam, 1964), p. 95; F. Halberg, R. E. Peterson, R. H. Silber, *Endocrinology* **64**, 222 (1959).
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