Cold Spring Harbor Symposia on Quantitative Biology 1960;29:49-55

Phase Control of Activity in a Rodent

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Two main approaches to the problems of circadian rhythms have become apparent at this symposium, that of determining the underlying physiological mechanism or cause of the rhythms, and secondly that of determining their adaptive features. In relation to this second problem it has been noted that for a biological clock to be useful it must be maintained in phase with the environment. The mechanism of phase control resulting in synchronization of an animal's biological clock with its environment will be the subject of this paper.

The activity rhythms of many species show definite phase relationships with the day-night cycle. Nocturnal species such as *Peromyscus* [1], the poor-will [2], or bats [3, 4] start activity at a particular light intensity near sunset time and become inactive at dawn, while many day-active species of birds [5, 6] start activity at a certain level of dawn light intensity and cease activity about dusk. In my laboratory a study of the factors important in such phase control of activity has been carried out with nocturnal rodents; the results will be reported here for the flying squirrel, *Glaucomys volans*.

It has been possible to study the activity patterns of this species under natural daylight conditions both with captive and wild populations. In their natural habitat the flying squirrels are gregarious during the fall and winter months, and in this period the observation of the times when the squirrels left and returned to their den tree indicated the time of activity. A close correlation was seen between the time of departure from the den tree and the time of sunset. Similarly, with squirrels in a large outdoor enclosure, or in individual recording cages, the time that the squirrels became active could be accurately determined and the weather factors measured (Fig. 1). In these cases, also, a close parallel between the onset of activity and the time of sunset was noted. An even stronger correlation was seen with a narrow range of light intensities at dusk [7].

Such a correlation between activity onset and a particular light intensity clearly suggested a regulatory effect of the environment upon activity. It has been pointed out, however, by numerous speakers at this symposium and in the published work particularly of Aschoff [8, 9], Pittendrigh [11, 12, 13], and Rawson [14, 15] that activity rhythms characteristically drift out of phase with the outside world when light and temperature clues are removed. Activity rhythms in most species are not purely an exogenous response to favorable environmental conditions but are responses to both exogenous and endogenous factors.

In order to study the free running rhythms and their role in regulating activity, a number of flying squirrels were housed individually in recording wheel cages at 20°C. in darkness for various periods of time. Revolutions of the wheel for each animal were recorded on an Esterline-Angus Recorder. The daily records of an individual were subsequently mounted in a vertical series to give a concise graph of activity for extended periods of time. Several features are apparent in these records. The rhythm of locomotor activity continued to exist in the absence of rhythmic light and temperature clues; a period of strong running in the wheel alternated with a period of almost complete inactivity approximately every 24 hours (Fig. 2). The activity period drifted out of phase with normal day-night changes. Using the abrupt "onset" of activity as a measuring point, it was possible to calculate the average frequency of the free running rhythm for the period of darkness, and thus to measure the rate and direction of drift. Calculations of the average frequencies for 48 such test periods of 18 individuals in periods of darkness from 10 to 123 days showed a range from 22:58–24:21 (Fig. 3). The animals differed strikingly from one another in average frequency, but the variation from day to day for an individual during one test period was only several minutes. Thus the outstanding characteristics of the activity rhythms in darkness appeared to be (a) their persistent and apparently endogenous nature, (b) the stable frequency for one individual during one test period, (c) the restriction of the free running frequency to a narrow range close to and usually less than 24 hours with a resultant drift of the activity period, and (d) the very high accuracy of the rhythm of an individual.

What are the phase controlling agents in the natural environment, and what is their mechanism of action? Aschoff [9] has pointed out that within a limited band of frequencies repeated cycles of light or other agents may be effective "Zeitgeber."

Two chief methods have been used to distinguish the role of these regulatory agents. The first has been to change an animal from constant environmental conditions to an environment with one rhythmically fluctuating factor out of phase with the animal's free running rhythm, and finally to return the animal to constant conditions. This method was used in about 30 experiments, with various phase relationships between

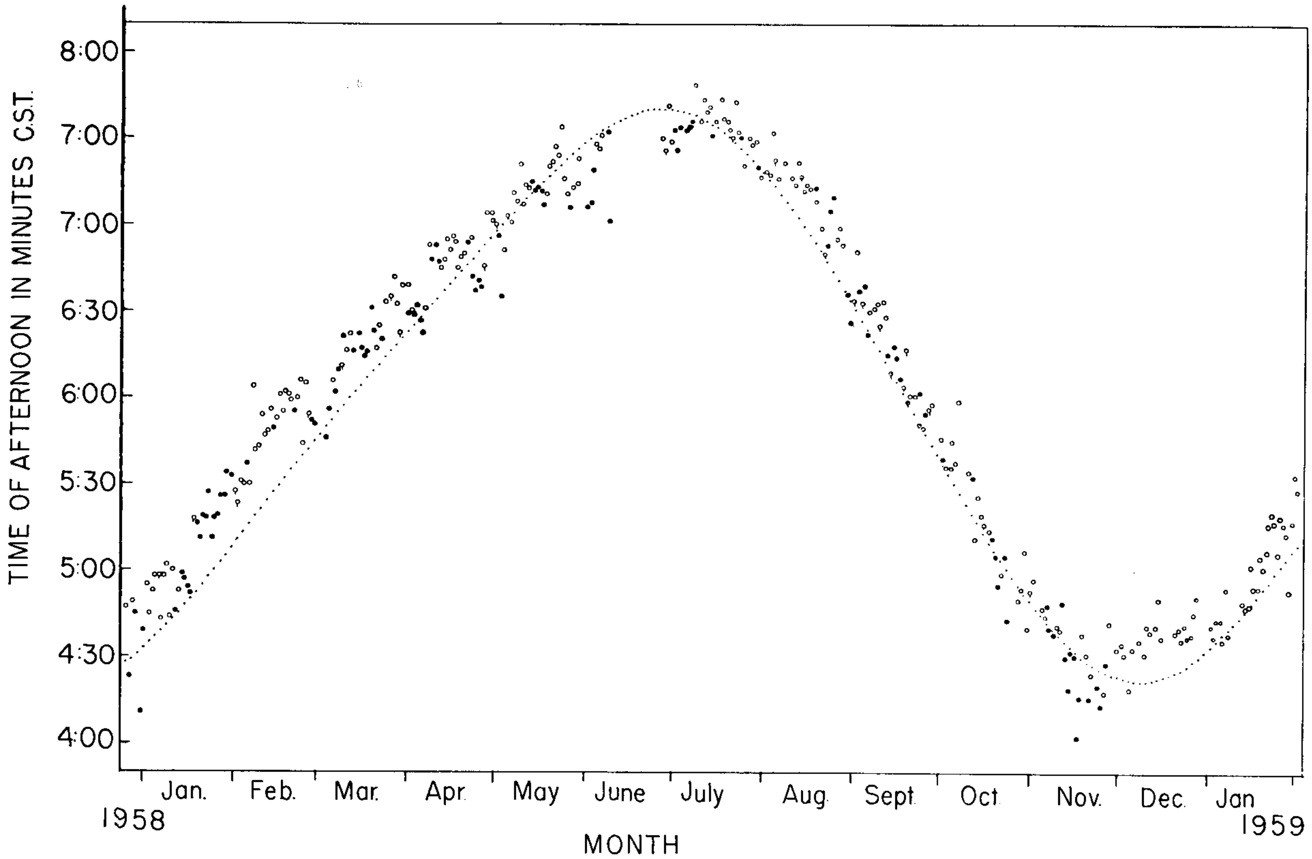

FIGURE 1. Onset of running wheel activity for one flying squirrel in natural daylight conditions throughout the year. Dots indicate the time of local sunset; circles indicate onset time: ○ with less than .7 of sky cloudy, Q with .7–.8 cloudy, ● with more than .8 sky cloudy.

the animal's endogenous rhythm and a 24 hour light-dark schedule. The records of activity indicated that light was a very effective synchronizing agent (Fig. 4). The use of 24 hour light-dark cycles, and the distinction of animals with free running rhythms in darkness of less than 24 hours from those with rhythms longer than 24 hours, is considered pertinent because this species in its natural environment is exposed to cyclic factors having a frequency of almost exactly 24 hours.

The pattern of synchronization depended upon the length of the free running rhythm and upon the relation of light to the animal's activity cycle. When the free running rhythm in darkness was less than 24 hours, and the rephasing light fell during the subjective night period of the animal, synchronization was accomplished by the characteristic stepwise delay noted in many previous experiments [9, 14, 15, 16]. The activity onset stabilized shortly after the light-dark change. Subsequently, when the animal was returned to constant darkness, the onset resumed its forward drift. A summary of the chief types of synchronization found in these experiments is shown diagrammatically in Fig. 5. Animal #1 corresponds to the case discussed above. Figure 5—#2 represents animals with a free running rhythm of less than 24 hours, where the rephasing light did not fall during their subjective night, but only during their subjective day. Here the free running rhythm continued unchanged until the onset drifted up against the "dusk" light change and was then prevented from drifting forward by the delaying action of light. With a free running rhythm longer than 24 hours and the rephasing light occurring only during their subjective day (Fig. 5—#3), the animals would drift later each day until their activity onset fell 10–14 hours before the "dawn" light change. At this point they would stabilize. It appeared that light at the subjective dawn was shortening the activity rhythm, and thus synchronization was achieved by preventing the activity period from drifting back into the light period. In animals with rhythms in darkness longer than 24 hours and with light falling during the subjective night (Fig. 5—#4), the onset would be delayed in stepwise fashion through the light period and then would continue to drift later each day until it stablized with respect to the dawn light as described for #3. It should be pointed out that these longer than 24 hour rhythms occurred only infrequently (Fig. 3), and appeared relatively unstable. In controlled laboratory conditions they persisted only relatively short periods of time; for reasons too lengthy to discuss here it seems likely that such a method of synchronization with respect to "dawn" rarely occurs in free living populations of flying squirrels.

Similar types of synchronization have been observed

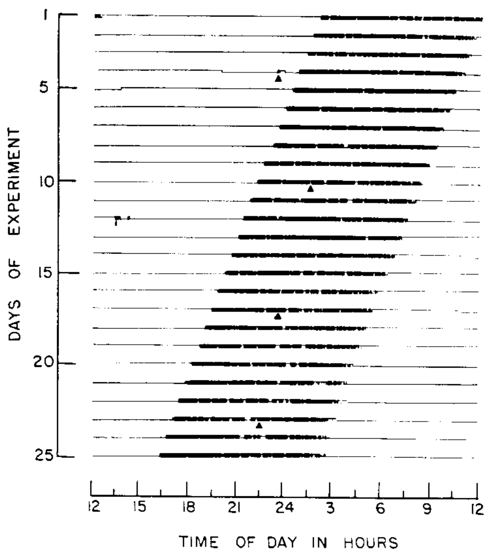

FIGURE 2. Activity graph of one flying squirrel in a recording wheel cage in continuous darkness at 20°C. from June 14–July 8, 1959. Solid triangles indicate the time of feeding in darkness; Σ the time of dim light during an equipment repair.

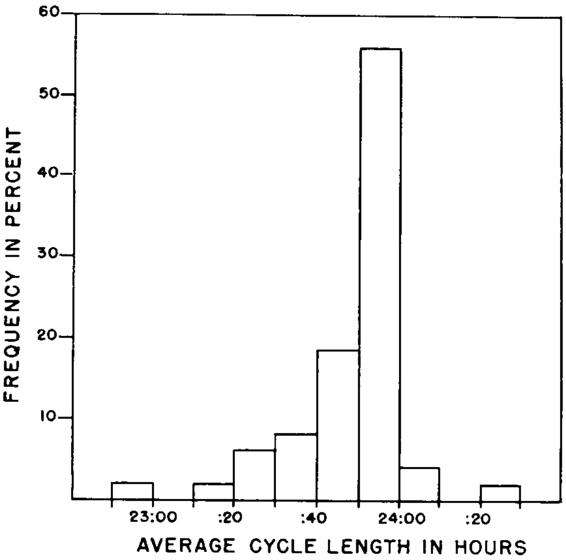

FIGURE 3. Histogram showing the distribution of the average activity rhythm frequency for each test period of continuous darkness. For further explanation see text.

with other nocturnal rodents, both in my own research (unpublished experiments) and in other laboratories [9, 10, 13, 14, 15]. They suggested that the direction of resetting depended upon the time that light had occurred (Fig. 5—lower part). It seemed feasible that synchronization to rhythmic time-givers by stepwise phase-resetting might be due, as postulated by Rawson [14], to a daily rhythm of response to light.

The above experiments could not pinpoint the effect of light, but led to a second method of studying the problem by using short, isolated, single exposures to light. Similar "light-shock" techniques have been used by Pittendrigh [11, 12, 13, 17, 18], Bünning [19], Rawson [14, 15], Hastings and Sweeney [20], and others to detect rhythms of response of organisms to light. An animal was kept in darkness for a period of several days or weeks in order to measure its free running rhythm in a steady state, and then at a specific time it was exposed to a standard light exposure (10 minutes at 0.5 ft. c. for most individuals). From the slope of the free running rhythm it was possible to calculate the expected time of activity onset. Any difference between the expected and actual onset was considered a phase

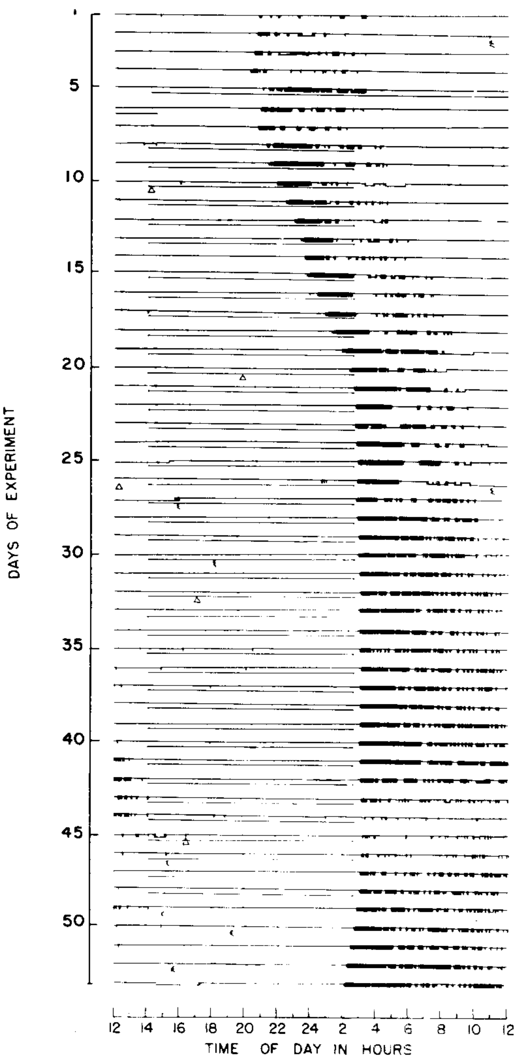

FIGURE 4. Synchronization of a flying squirrel to a 12L:12D cycle out of phase with its endogenous activity rhythm. Days 1–4 in DD, day 5 with 1.0 ft.c. of light for 24 hours (for an unrelated experiment) with light indicated by horizontal underlining; days 6–7 in DD; days 8–48 in 12L:12D with 1.0 ft.c. of light indicated by underlining; days 49–53 in DD. Symbols indicate the time of disturbances for feeding (triangles) or repair of equipment (Σ). For further explanation see text.

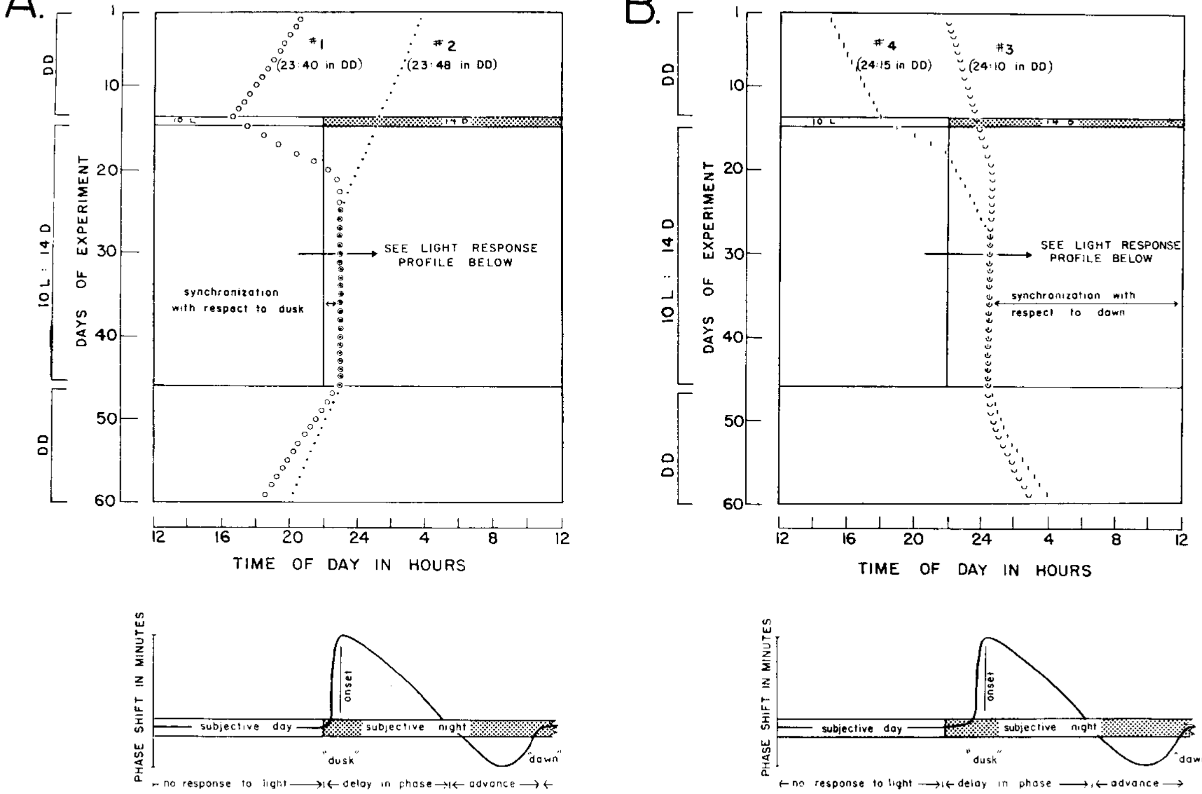

FIGURE 5 *A*,*B*. Diagrammatic representation of synchronization for (*A*) squirrels with DD activity rhythms less than 24 hours in length, and (*B*) for squirrels with DD rhythms greater than 24 hours. The onsets of activity for the days of the experiments are shown above; light response profiles are shown below. For further explanation see text.

shift due to light (method shown diagrammatically in Fig. 6).

In making these calculations it was apparent that several precautions were necessary. First of all, the squirrels were not perfect time pieces. The precision of animals was evaluated by calculating the standard deviation of the activity rhythm. For the test periods in darkness (Fig. 3) values ranging from ±2 to ±15 minutes for different animals were found. By selecting the highly "accurate" runners the expected error in phase shift calculations could be reduced to a few minutes. It also seemed possible that the response of an animal to a test light depended in part upon exposures to light or other stimuli in its previous history. Changes in frequency of free running rhythms, such as Dr. Pittendrigh pointed out in his lecture, have also been noted in *Glaucomys*. The use of a control period of darkness immediately before each light shock eliminated some of the difficulties. The response to a standard test point repeated after a variety of light schedules suggested that shifts in light responsiveness had not occurred.

These single light stimuli demonstrated that in *Glaucomys* the direction and amount of shift in phase depended upon the time during the animal's subjective cycle at which light occurred [21]. Light had a maximal delaying effect at the onset of activity. Delay responses gradually declined during the six or seven hours after onset, followed by a 3–5 hour period of advancing responses (Fig. 5, 6). Light during the animal's subjective day had no phase shifting effect until about an hour before the onset of running. Such a system of responses involving resetting in opposite directions seemed capable of giving conflicting instructions. However, a series of experiments in which a delaying and an advancing signal were given during one activity period indicated that only the delay effect occurred in case of conflict of signals.

The single isolated light stimuli clarified the light response rhythm first suggested by the synchronization experiments. They suggested even more convincingly that synchronization was a series of daily resetting or phase shifting phenomena, with the light response rhythm as the phase controlling agent.

A few clear demonstrations of phase controlling agents other than light have been pointed out in the papers and discussions at this symposium. Temperature and sound have been considered important secondary, latent Zeitgeber. With *Glaucomys* the effect of temperature and sound as phase controlling agents was tested using both rhythmically repeated 24 hour cycles and also using the single stimuli technique (unpublished experiments). Fluctuating temperature cycles (12 hours 25°C:12 hours 15°C.) were not effective in modifying the timing of the free running rhythm. In some cases an inhibition of activity during the warm period occurred, but when the animal was returned to constant temperature conditions it was apparent that

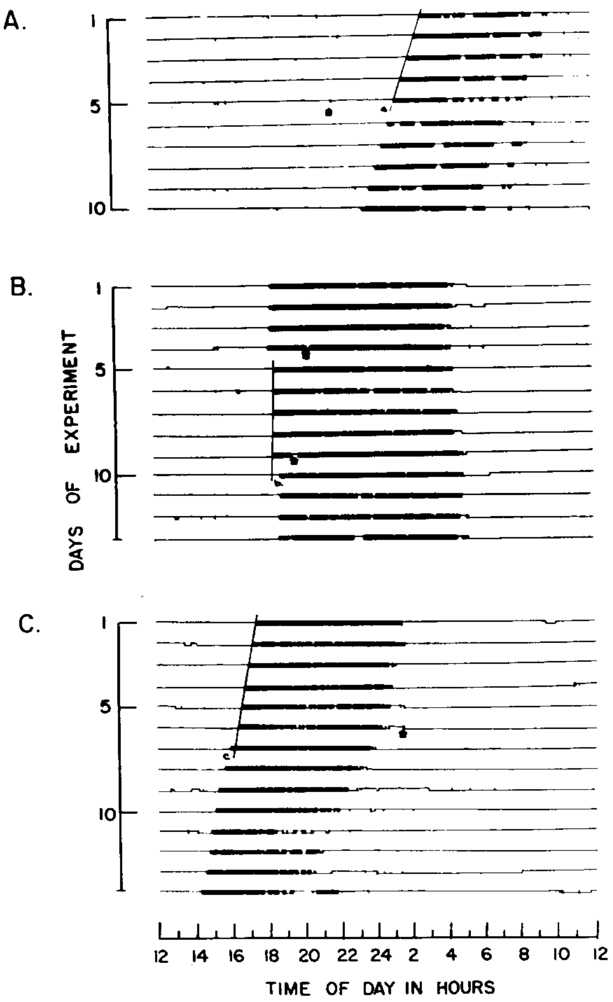

FIGURE 6 *A–C*. Light shock technique showing (*A*) light in subjective day, (*B*) light in subjective night, and (*C*) light at subjective dawn. Arrows indicate one light shock lasting 10 minutes with 0.5 ft.c. of light. For further explanation see text.

no true synchronization or phase shifting had occurred (Fig. 7). The rhythms continued to drift across the transition points in various kinds of noise cycles with no indication of synchronization. In marked contrast to the light signals, the single temperature or sound stimuli had no effect in modifying the activity rhythm frequency at any time during the animal's daily cycle.

Thus in *Glaucomys* light appears to be a strong and very specific phase controlling agent. It has been pointed out by several of the symposium speakers and in the work of Tribukait [22] that a strong synchronizer such as light may be able to entrain a biological clock over a relatively wide range of frequencies, whereas latent, weaker ones may be effective only in a very narrow zone. While temperature and sound were not able to entrain the activity rhythm of *Glaucomys* under the conditions tested, it is possible that these stimuli are very weak synchronizers and could be effective in certain conditions.

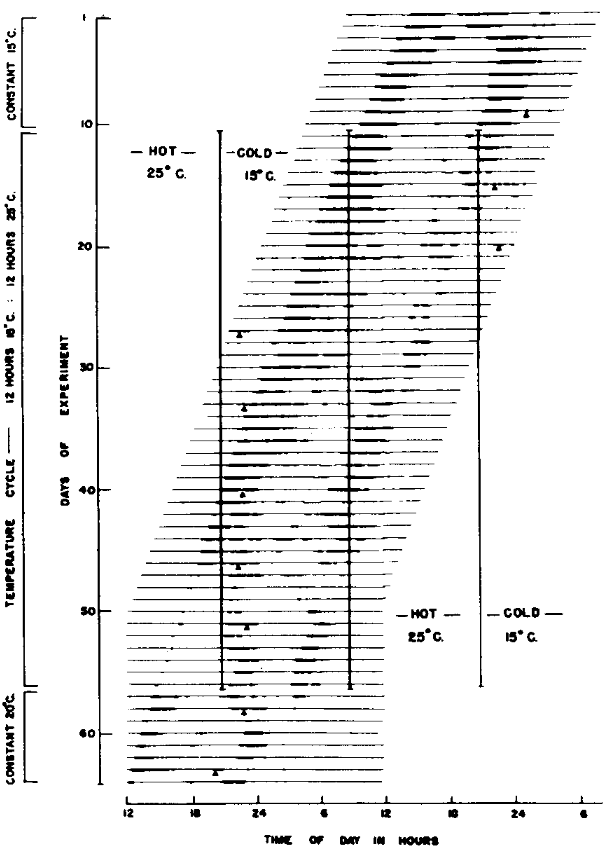

FIGURE 7. Temperature synchronization experiment for one flying squirrel. Days 1–10 in DD at 15°C.; days 11–56 in DD with 12 hours 25°C.: 12 hours 15°C.; days 57–64 in DD at 20°C. Solid triangles indicate feeding time. For further explanation see text.

In summary, it may be said that in *Glaucomys* the activity in natural conditions coincides with the period of darkness, and this relationship is maintained throughout the course of the year by the interaction of an endogenous activity rhythm and a related daily rhythm of responsiveness to light. Such a resetting rhythm could not be demonstrated to a variety of temperature or sound stimuli.

ACKNOWLEDGEMENTS

I wish to express my appreciation for the following invaluable assistance with this research. Dr. John T. Emlen gave many helpful suggestions. Mrs. Ardis Stewart provided technical aid. The conceptual contributions of Dr. Kenneth S. Rawson to the field of rhythms, and his generosity in providing equipment and assistance, have made these experiments in large measure possible. This research was supported by Fellowships from the American Association of University Women, and the National Science Foundation, and was also supported by the United States Air Force through the Air Force Office of Scientific Research of the Air Research and Development Command, under contract No. AF 49(638)-691.

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DISCUSSION

Bünning: The rule that a short light shock may delay, advance, or be without any phase shifting influence, depending on the time of its presentation, holds for plants too. The first to observe this was Kleinhoonte in her experiments on leaf movements in *Canavalia.*

DeCoursey: I quite agree with Dr. Bünning that daily rhythms of response to light have been known for many years in both plants and animals. However, it was not recognized that such a daily rhythm of light responsiveness could account for phase control in organisms until Rawson's clear demonstration of this a few years ago in *Peromyscus.*

Barlow: With respect to "social entrainment," i.e., mutual entrainment of a population of organisms, the apparently predominating effect of light as a stimulus in many situations suggests that those forms be examined for entrainment for which emission of light as well as reception of light occur in normal behavior. One possibility is that of the firefly, a suggestion which Professor Norbert Wiener has made elsewhere. Dr. Hastings has pointed out to me that there are species of fireflies, in Southeast Asia at least, for which mutual entrainment, with respect to rhythmic flashing, appears to be well established. Quantitative studies of such phenomena are of some importance from the standpoint of understanding the basic nature of the rhythms, for, as has been indicated by Wiener, mutual entrainment of an ensemble of oscillators is only possible if the individual oscillators concerned are of a non-linear type. Dr. Klotter has already indicated in his paper at this Symposium that it is not possible to drive or to entrain a linear oscillator at a frequency different from its natural or free-running frequency.

An ensemble of mutually entrained non-linear oscillators, in fact, has a most interesting characteristic: the frequency resulting from mutual entrainment is more stable than the frequency of any of the individual oscillators in isolation. Consider an ensemble of identically constructed oscillators, for which in the un-

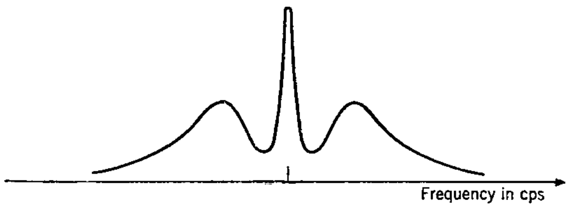

Figure 1. Spectrum of an ensemble of mutually coupled non-linear oscillators. The spectrum shows a peak, with dips on either side, instead of the normal (gaussian) distribution characteristic of such an ensemble in the absence of coupling.

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coupled state, the frequency spectrum of the ensemble is normal or gaussian. Now let the oscillators be mutually coupled; the spectrum of the ensemble is no longer gaussian, but has the shape shown in Fig. 1, exhibiting a peak with dips on either side. This shape results from the fact that, in the center part of the spectrum, the frequencies are "pulled together," and the two regions on either side of the peak, from which this attraction has taken place, are left as dips in the spectrum. The result has been to generate a narrow band of frequencies, from an initial relatively broad band. Now Fig. 1 represents a measurement over the *ensemble* of oscillators, but by the ergodic theorem, under appropriate conditions, a measurement over an ensemble is equivalent to a measurement over *time*, and with respect to the latter we can state that the effect of coupling has been to generate a more stable frequency (or actually, a narrow band of frequencies) in time.

From the above considerations, one might expect, then, that the rhythm of flashing of a large mutually entrained group of fireflies would be more stable in time under constant conditions (of temperature for example) than the rhythm of flashing of any one of the fireflies isolated from all the rest. More generally, with respect to other types of mutual entrainment, the above characteristic of an ensemble of mutually-entrained non-linear oscillators suggests itself as a property that might well be found in individual organisms, and from a purely theoretical standpoint, it would lend support to Dr. Pittendrigh's concept of "ensembles of oscillators" instead of a single central clock. It should perhaps be noted, however, that with respect to the problem of relative temperature insensitivity exhibited by many biological systems, the above considerations pertain only to generation of a more stable rhythm at a particular temperature, i.e., the Q_{10} of the ensemble would be the same as the Q_{10} of the individual oscillators, which we have assumed to be identical. Stability with respect to temperature as well as time could perhaps result if the Q_{10}'s of the individual oscillators were not indentical, but instead included values greater and less than one.