CHANGING THE PERIOD OF THE CLOCK OF THE KANGAROO RAT, *DIPODOMYS MERRIAMI*, BY LIGHT PERTURBATIONS

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ABSTRACT.—Eleven Kangaroo Rats were maintained under continuous dim illumination and nearly constant temperature, 20°C±1°C, for eight months. Their persistent locomotor activity was monitored by an event recorder. Shifts in time of activity onset in response to hour-long light perturbations given at 4- to 7-day intervals randomly distributed over a 24-hour period were investigated. It was noted that at certain critical times during the circadian cycle, the period of the free-running activity rhythm was altered concurrently with the change in phase induced by the light perturbation. In most instances, phase shifts were transient, but the period changes commonly persisted unless caused to be altered later by another light pulse occurring within a critical period. These critical periods were located over the times of maximum phase-responsiveness to the light, both for phase advance or phase delay in the phase-response curve.

For years, investigators of biological clocks sought means to change the period or in some way stop the clock. During the studies it was assumed that in altering either the period or phase of an observed rhythm the clock was being correspondingly changed. Despite all such attempts, little or no knowledge of the biochemical or biophysical nature of the basic oscillator was gained (see editorial in *Nature New Biology*, 1971).

The clock seemed to be uninfluenced by almost all pharmacological agents that were tested. This list of agents included many dozens of compounds, ranging from metabolic inhibitors of protein synthesis to tranquilizers (Hastings, 1960; Hastings and Bode, 1962; Wahlström and Widerlöv, 1968). However, ethanol Bünning and Baltes, 1963) seemed to alter slightly the period.

Deuterium Oxide (D_2O) has been shown to affect the steady-state period of the circadian rhythm. Suter and Rawson (1968) demonstrated that heavy-water modified the free-running period of the Deer Mouse, *Peromyscus leucopus noveboracensis*. The period of the activity rhythm in constant darkness (DD) increased directly with the concentration of heavy-water added to the animal's drinking water. The response was reversible. This compound has been shown to affect the circadian rhythm of other organisms: the phototactic response of *Euglena* (Bruce and Pittendrigh, 1960), the leaf rhythm in *Phaseolus* (Bünning and Baltes, 1963), and locomotor rhythms of mice (Palmer and Dowse, 1969), pigeons (Snyder, 1969), and blinded rats (Richter, 1970), and the tidal rhythms of isopods (Enright, 1971). Cycloheximide, an inhibitor of protein synthesis, has also been reported to alter the free-running circadian period (Feldman, 1967).

The literature describes many instances of period changes of free-running rhythms. Often an organism being studied in continuous light or darkness is subjected to some experimental procedure after which it continues to be observed under the previous environmental conditions. Frequently, it is noted that the free-running period has been altered. Since this free-running period is postulated to be a direct expression of the clock period it is assumed that the basic timer has been influenced by the procedure. "Spontaneous"

frequency changes are also commonly observed under continuous light (LL) or continuous (DD). These may be abrupt changes or gradual ones.

Harker (1964) reported a spontaneous frequency change in the free-running rhythm of the cockroach *Blaberus*. The activity of the lizard, *Lacerta sicula* (Hoffmann, 1959) showed a period change under constant conditions. DeCoursey and DeCoursey (1964) described sudden frequency changes in the daily rhythm of activity for bats, *Rhinolophus ferrumequinum*, under DD. Such changes are also common for mice (Johnson, 1939; Terracini and Brown, 1962; Brown, 1965), flying squirrels (DeCoursey, 1961; 1964), and arctic rodents (Swade and Pittendrigh, 1967). Palmer (1964) noted them for bird activity rhythms. Among proposed "explanations" have been abrupt physiological changes within the organism, dissociation of rhythms within the animal, and seasonal changes.

Period fluctuations have also been reported under conditions in which they may have been induced in as much in these instances a known stimulus immediately preceded the observed period change. For example, the free-running activity rhythm in the cockroach *Leucophaea* was recorded in DD for 17 days, then in an altered temperature regime, and afterwards in the former DD; a change in frequency resulted (Roberts, 1962). Comparable frequency changes also probably brought about by temperature changes were reported by Enright (1966) for the house finch, Rawson (1960) for bats, and Hoffman (1969) for wild mice.

Experiments involving alterations in photoperiod and in electrostatic field have also been reported to be associated with period changes. Aschoff and Wever (1966) investigating photoperiodic effects on the free-running period of Chaffinches reported this. Dowse and Palmer (1969) described a frequency change following entrainment of mice activity rhythms to electrostatic fields.

The purpose of this paper is to report the induction of frequency changes in the free-running period of Kangaroo Rat in response to brief light perturbations. The Kangaroo Rats were chosen for these experiments because Lowe et al. (1967) reported that the activity patterns of these animals are distinct and have a degree of precision exceeded by few other animals.

Materials and Methods

The test animals were male and female Kangaroo Rats, *Dipodomys merriami*. Eleven subjects were maintained in two types of actographs, running-wheels and tipping-cages (Natalini, 1972). The running wheel actographs had a cage of 1-cm. mesh hardware cloth, 15x25x13 centimeters, with a 35-cm. running wheel. The tipping actographs were made of 1-cm. hardware cloth 13x30x15 centimeters, including a horizontal circular running area and a central nesting area (Boyer and Truchan, 1969). These latter cages were supported near their center on a knife-blade pivot; movement by the rat produced a small amplitude rocking of the cage. Food and water were freely available.

Each revolution of the running wheel, or tilt of the tipping cage, closed a microswitch which caused a pen deflection on an Esterline Angus Event Recorder. This instrument was situated in an adjoining room so its operation and the daily removal of the records would not disturb the Kangaroo Rats.

All the experiments were conducted in a fairly sound-proof photographic dark room. During the experiment, the temperature was 20°C±1°C. The

behavior was recorded from 15 January to 31 August 1970. During this study 66,240 rat-hours of activity were recorded.

Each test organism in its individual actograph was housed in a separate compartment of a large cabinet. There were two six-watt incandescent lamps above each Kangaroo Rat. One provided continuous illumination of an average intensity of 4 lux. The other provided the hour-long light perturbations of approximately 35 lux which were given once every 4 to 7 days at random times within a solar day. The illumination levels were measured with a Weston Illuminator meter (#756) at the usual level of the animal which was approximately three inches from the surface of the shelf.

The Esterline Angus recordings were retraced with consecutive days of data for each individual rat on a single long sheet of tracing paper. The free-running period was estimated by drawing a line through successive onsets of activity. The effect of a light perturbation was determined by comparing the first onset following the light flash with the value expected on the basis of the extrapolation of the apparent preceding period. Each Kangaroo Rat thus acted as its own control, to compensate for the considerable individual variability in response patterns between rats.

Results

The phase-response curves for the pooled data for those eight animals with free-running periods greater than 24 hours and for three animals with periods less than 24 hours have been previously reported (Natalini, 1972).

It became obvious even during the initial stages of this phase-response study that the period as well as the phase often changed suddenly. Observing this fact more closely, it was noted that these changes most commonly occurred near the time of a disturbance such as the experimental light perturbations, feeding interruptions, or cage servicings and especially when such disturbances occurred at, or close to, the times of maximum phase-response. delay or advance, to the light perturbations. These will be referred to as "critical" times.

For a closer observation of this phenomenon, the data were reexamined for the effects on the free-running period of light perturbations or any other recorded disturbances that occurred during a three-hour interval

Table 1.—Disturbances causing Phase Shifts.

Animal	Permanent Phase Shifts With Period Change	Permanent Phase Shifts Without Period Change	Non permanent Phase Shifts	Total Number of Disturbances	Percent Permanent Phase Shifts
1	4	6	6	16	62.5
2	2	1	6	9	33.3
3	5	1	7	13	46.2
4	1	1	6	8	25.0
5	1	0	4	5	20.0
6	1	5	10	16	37.5
7	1	10	9	20	55.0
8	1	2	5	8	37.5
9	1	4	6	11	45.5
10	0	5	7	12	41.7
11	0	3	8	11	27.3

over the onset of activity and a three-hour one over the end of the activity period. These were considered to be, in general, the critical periods for the Kangaroo Rats. Arbitrarily, no exceptions were made for those individual rats whose maximum phase-response times appeared to have occurred beyond the limits of defined times.

Table 1 gives the percentage of disturbances that occurred during the critical periods that caused permanent phase and/or period change. An abrupt phase-change may, or may not, be associated with a sudden change in period. It also must be emphasized that almost every activity alteration followed a light perturbation but not all perturbations caused a permanent alteration.

Some illustrations of these phase shifts with or without a change in period are presented. These figures are enlarged photographs of small portions of the total activity tracings. The black areas represent the activity near the onset; solid black areas show continuous activity; white areas signify inactivity. Each line is a separate day. For Figure 1, the light perturbation took place 3 hours after onset.

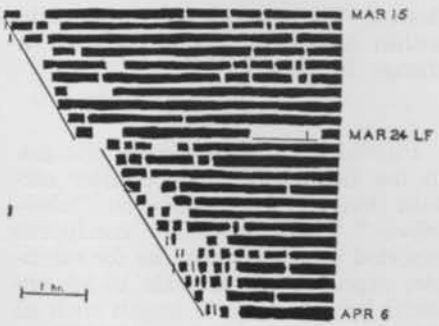

FIGURE 1.—Data of 15 March to 6 April of Animal 1, showing a phase change from a Light Flash (indicated by horizontal line) occurring 3 hours after onset on 24 March; period = 24.13 hours. LF = Light Flash, F = Feeding, N = Noise Disturbance.

The free-running period after the light disturbance remained the same as before, i.e., a phase-shift occurred without a period change.

FIGURE 2.—Data of 21 June to 12 July of Animal 7 showing a period change on 29 June from a Light Flash occurring 3 hours after onset (not shown on photograph) (24.05 hours to 24.10 hours) and on 6 July from a Light Flash and Feeding occurring 1 hour after onset (24.10 hours to 24.17 hours). See Figure 1 for abbreviations.

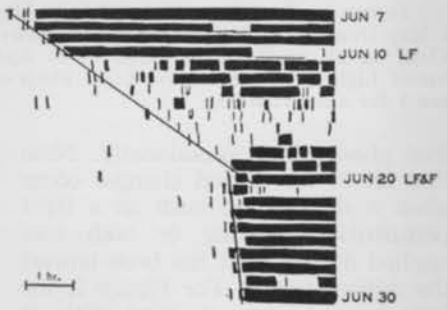

FIGURE 3.—Data of 7 June to 30 June of Animal 7 showing a period change on 20 June from a Light Flash and Feeding occurring at the end of the preceeding activity period (not shown in photograph) (24.33 hours to 24.05 hours); Light Flash on 10 June. See Figure 1 for abbreviations.

Figures 2, 3, and 4 depict a number of examples of period changes. These figures are also enlarged areas of activity records. Most of the animals in the experiment exhibited

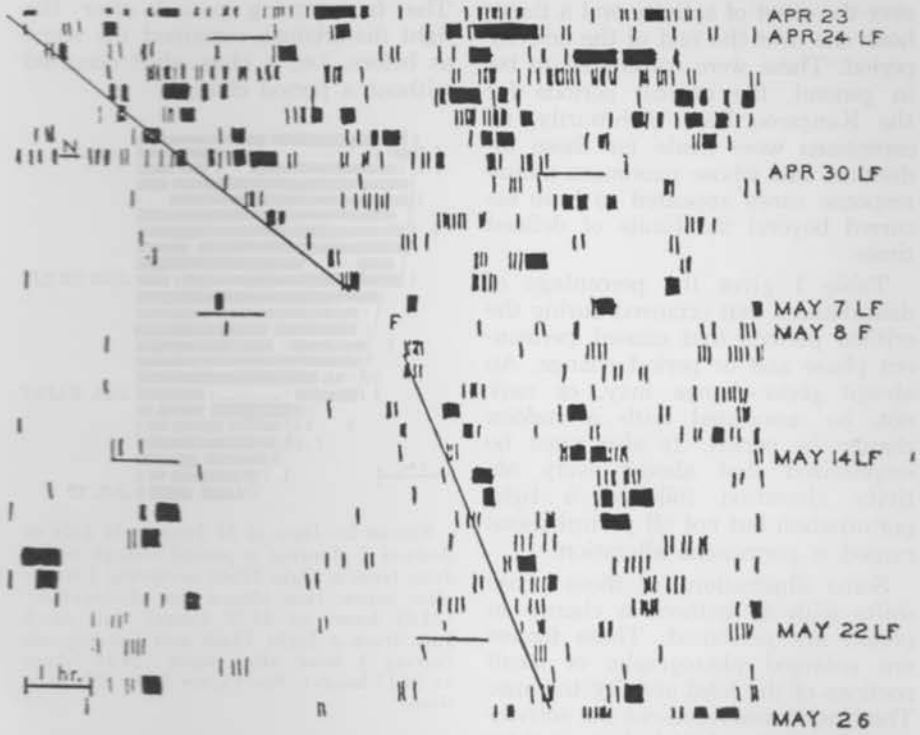

FIGURE 4.—Data from 23 April to 26 May of Animal 11 showing a period change on 8 May from a Feeding occurring at activity onset (24.33 hours to 24.08 hours); Light Flash on 24 April; Light Flash on 30 April; Noise Disturbance over 29 April to 30 April; Light Flash on 7 May; Light Flash on 14 May; Light Flash on 22 May. See Figure 1 for abbreviations.

this phenomenon occasionally. Note that all of the period changes occur when a disturbance such as a light perturbation, feeding or both was applied during what has been termed the critical period. For Figure 2, the light perturbation of June 29th is not indicated on the figure. For Figure 3, the light shock and feeding on June 20th occurred at the end of the preceding activity period also not indicated on the photograph. Notice, the light shock on June 10th (Fig. 4) did not cause any clear period-change; it is outside the critical period. Figure 4 illustrates similar apparent absence of period-change on April 30th and May 22nd in response to light flashes. The feeding on May 8th, within a critical period, effected a change in period.

DISCUSSION

Pittendrigh (1960) termed changes in the free-running period after certain long pretreatments as "after-effects." There are a few conditions reported to cause them, as for example, exposing the animals to an unusual light-dark cycle length such as thirteen hours of light and thirteen hours of dark (13L:13D). When the organism was released into total darkness (DD), the period was found to depend upon the pretreatment. Also, the free-running period in DD ap-

pears related to the light intensity during a pretreatment period in continuous light (LL). Another condition inducing period change involves imposition of a long pulse of light (at least 12 hours duration) within other wise continuous darkness. A final condition inducing such postulated "after-effects" is pretreatment by a 24 hour photoperiod, 12L:12D, preceding release into continuous lighting conditions (LL).

There are only a few instances in the literature where it is reported that short light perturbations might have induced frequency changes. DeCoursey (1964) found that ten minute light flashes sometimes changed the free-running period of the hamster, *Mesocricetus auratus* Kramm (1972) also suggested that short pulses affected the period of activity of the Antelope Ground Squirrel, *Ammospermophilus leucurus leucurus*. A six-hour light pulse changed the frequency of the locomotor ryhthm of the House Sparrow, *Passer domesticus* (Eskin, 1971). Engelmann (1969), similarly, suggested this might occur in *Drosophila* in response to a short pulse (up to 15 minutes) of blue light. A new steady state appeared after the passage of a few transient or temporary period changes. He hypothesized that the light flash caused the new period. However, he recognized that he could not exclude the possibility that he was working with a population of *Drosophila* that may have undergone a genetic change. In all of the above reports, the disturbance was given at, or near the previously defined critical period of the phase-response curve. The present study supports the hypothesis that a brief pulse of light can cause an alteration of a free-running circadian rhythm.

Around 50 percent of the times that a light flash occurred within the critical period for Kangaroo Rats, a new steady-state period or phase was induced. These changes lasted for a minimum of about a week. In many cases the reason they lasted only one week was that a later pulse again altered the period or phase. The smallest period change that could be noted with reasonable confidence was about 10 minutes. Not all flashes within the critical period alter the frequencies. It has been shown that the response curve is associated with the animals' circadian or free-running period (Natalini, 1972), and therefore as the free-running period changed so would, presumably, the response-curve. A sensitivity peak within the critical period might even become relocated. An arbitrary interval of three hours was chosen as the duration of the critical period. This could, and probably does, vary among the individual animals. Also, if there were an annual variation in light sensitivity, an annual variation would also be expected in the phase-response curve, and this would be reflected in the amount of period change to any given phasing stimulus.

The circadian rule states that the free-running period varies with light intensity (see: Aschoff, 1960). It may be possible that following light pulses within the critical periods the free-running period does not return fully to its previous frequency but continues at a different circadian period like it would if the light intensity were changed. Since the organism is a physiochemical system and clearly a rhythmic one, the lengthening of one cycle of activity could quite conceivable effect an alteration which would persist for later cycles.

It also seems reasonable to postulate that the time of a light pulse within a circadian cycle would determine concurrently both the amount of phase shift within the cycle and amount of the persistent period change. Consistent with such an hy-

pothesis, Lohman (1967) using the beetle *Tenebrio,* reported that for shifts from one level of continuous light to a higher one (LL_1 to LL_2), the period in the LL_2 was a function of the phase within the phase-response rhythm during LL_1 when the transition occurred.

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