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SEVEN YEAR STUDY OF THE EFFECTS OF PHOTOPERIODS ON THE CIRCANNUAL LOCOMOTOR RHYTHM OF THE GERBIL

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The locomotor activity of groups of 6 male Mongolian gerbils was maintained under four photoperiods, LL, 12L:12D, 18L:6D and 6L:18D. The consecutive experiments lasted from 18 to 30 months with different animals used for each study. The light intensity was 2.2 lux; ambient chamber temperature was 20°± 2°C with no seasonal correlation. Maintenance was kept at a minimum, and food/water were provided *ad libitum*. The composite curves for each experiment had High(s) statistically significant from the Low(s) ($P << 0.001$, Student's t test). The frequencies for each circannual activity rhythm varied with the different photoperiods. Thus, the light/dark cycle acts as a Zeitgeber for the circannual rhythm of the gerbil. Circannual rhythms are known to exist in humans with the LD cycle acting as a potential Zeitgeber, also.

ABSTRACT

The animal and plant kingdoms have various physiological and behavioral traits that increase the survivability of the organism. One such trait is the circannual or seasonal rhythm. The geophysical correlate of the seasonal rhythm is based upon the rotation of the earth around the sun lasting approximately 365 days or 12 months. Repetitive events recurring within a period of 12 months include migration, flowering, moulting, growth, hibernation, fattening, etc. Circannual rhythms are endogenous cycles that repeat at intervals approximating one year under the constant environmental conditions of the laboratory. Such rhythms appear to be under the control of a biological clock, whose mechanism is quite mysterious to present researchers. Even the name has changed over the past decades, from "circennial" (Farner, 1970) to "circannian" (Pengelley, 1967) to the present term "circannual".

Recently there has been an increase in the number of published, scientific papers on these long-term or ultradian rhythms. Circannual rhythms persisting under seasonally consistent environmental conditions of the laboratory have been observed in a variety of animals. Bats, for example, have circannual rhythms in body weight (Beasley et al., 1984). Ground squirrels also show similar rhythms in activity onset and termination (Lee et al., 1986), blood concentrations of luteinizing and testosterone hormones (Barnes, 1986), body temperature (Hengst and Wiebers, 1984), and body weight (Melnyk, 1983). Matsubayashi and Enomoto (1983) reported circannual, testosterone rhythms in primates. A cnidarian is known to have a circannual rhythm in growth (Brock, 1975). The antler replacement cycle in deer is stated to have a period length approximating one year (Coss, 1984). Natalini (1978, 1982) described circannual activity rhythms of the Mongolian gerbil under continuous dim illumination (LL) and a long-day photoperiod (18L:6D).

These cycles of activity occur spontaneously and may persist under experimentally constant environmental conditions. For activity rhythms timed by a circadian (24-hour) clock, either under natural or laboratory conditions, it is known that the photoperiod is the strongest Zeitgeber or synchronizer (Pittendrigh, 1981). Little is known about the Zeitgeber for circannual clocks. The present studies were conducted to determine and to compare the circannual patterns of gerbil locomotor activity held under various but consistent photoperiods. Therefore, the effects of a lighting regime similar to a daily photoperiod found in summer (18L:6D), the effects of a transitional lighting cycle (12L:12D), and the effects of a lighting regime imitating winter conditions (6L:18D) upon the activity rhythm of the gerbil were studied. Experimental activity cycles were compared to the cycles of animals housed under continuous dim illumination (LL). Results of some of these investigations were reported previously (Natalini, 1978; Natalini, 1982).

Since any consistent laboratory photoperiod would allow the expression of the activity profile of the corresponding circannual period, the existence of different activity patterns indicate that the light/dark cycle is an entraining agent. Circannual rhythms provide the organism with the adaptive advantage of anticipating seasonal situations and of helping to synchronize various complex physiological and behavioral traits. When the light/dark cycle not only entrains a rhythm but also induces an event such as found in various photoperiodic phenomena, such phenomena may also have annual periodicities. It is now known that even man possesses yearly cycles. Further considerations and the significance of such rhythms are deferred to the Discussion portion of this report.

MATERIALS AND METHODS

This report summarizes the results of past experiments and presents new data on two of four research projects. The photoperiod for each experiment changed. Experiment I, lasting from October, 1975, to March, 1977, was held under LL. Experiment II, lasting from September, 1977, to March, 1979, maintained a 12L:12D photoperiod. Project III, lasting from September, 1980, to March, 1982, had the long-day schedule of 18L:6D; and the final experiment with the short-day of 6L:18D started in October, 1983, and ended in March, 1986.

In each study six, 6-week-old male Mongolian gerbils, *Meriones ungiculatus*, served as the experimental animals. It should be emphasized that separate gerbils were used for each experiment. Lights on for the rectangular photoperiods were at 06:00 hr., Central Standard Time; lights off occurred at the appropriate time. The intensity of the incandescent white light at the center of the cage was 2.2 lux. Feedings consisted of Purina rat pellets and tap water *ad libitum*. The ambient chamber temperature was continuously monitored at 20° ± 2°C and was theromostatically controlled. Humidity was not controlled. Since there was no seasonal correlation with the environmental temperature change, since temperature is a poor entraining agent for homeotherms, and since biological clocks possess temperature compensation so that the clock runs independently of the temperature, it is unlikely that there were any temperature influences (also see: Tokura and Aschoff, 1983; Gibbs, 1983).

For the first two studies, the experimental animals were housed in tipping cage actographs, while the last two were conducted in running wheels. The tipping cage actographs pivot on a knife blade in such a way that any movement causes a deflection of microswitches (Boyer and Truchan, 1969). The Wahmann running-wheel cages have a wire mesh (9.0 mm) revolving wheel (diameter 35 cm) with access to a stationary living compartment (25.5L × 15.5W × 12.5H cm). Cleaning of the trays under the cages occurred once a month, and all maintenance was kept to a minimum. Data from the two kinds of cages might differ in amplitude, but not in period length (Natalini, unpublished).

Locomotor activity was recorded separately for each gerbil on an Esterline Angus event recorder in continuous operation with a chart speed of 45.5 cm/24 hr. Each microswitch activation produced a single pen deflection. Quantification of the data and statistical analysis have been described (Natalini, 1978). Briefly, each gerbil-hour of activity is assigned a value of 0 to 10 (0% to 100%) based on the amount of hourly activity. A Mean ± Standard Error of the Mean was calculated for the High (Peak) and Low (Trough) months of activity. Differences between the Peak and Trough were determined by a student's t test. Thus, each animal can act as its own "control" by comparing the High with the Low point. Results from the LL experiment act as a "control" also. The location of the experimental animals enabled olfactory and visual cues from each other, but I noted no mutual entrainment as indicated by the individual activity patterns.

RESULTS

Figure 1 illustrates the composite curves, each representing the mean, monthly activity of six gerbils under the various photoperiods. Under continuous dim illumination (LL), there is a High (Peak) in April and a Low (Trough) in February (Natalini, 1978). Under the equal light and dark photoregime (12L:12D), there is a High in October of year 1 and Highs in May and November of year 2. The Lows are located in December of year 1 and January of year 2. The six gerbils producing the composite curve of the long-day photoperiod (18L:6D) have Highs in November (year 1) and April (year 2) with a Low in January, beginning of year 3 (Natalini, 1982). Conditions of the short-day photoperiod (6L:18D) result in a Peak in May and a Trough 14 months later.

Figure 2 is another way by which the Highs and Lows may be demonstrated. Indicated in the figure are the Peaks and Valleys only for the composite curves. The error bars are plus/minus one standard error of the mean. Results from a student's t test indicated that the selected points are statistically significant with a P value much less than 0.001. Within the individual experiments, at least four of the six gerbils had graphs with Peaks/Troughs significant to the above level. The composite curves of Figures 1 and 2 still represent all 6 gerbils, no data was eliminated. Figure 2 also indicates the genetic individuality between animals by noting the differences in amplitude between the selected points.

A common analytical method of students of biological rhythms is to allow each animal to act as its own "control" so that within the longitudinal study one part (High) of a curve is compared to another part (Low). This is also true for the composite curves. LL can be considered a "control" since it lacks a photoperiod, but the graph would be expected to change in appearance if the light intensity were increased or continuous dark (DD) were used. In addition, to demonstrate a true endogenous, circannual period, one should have multiple Peaks or Valleys as noted in the 12L:12D light regime. At least two animals within each experiment indicated multiple Peaks (or Valleys); thus, I believe these results indicate true circannual periods.

DISCUSSION

With my series of experiments, I tested the hypothesis that the LD cycle could affect the appearance of the circannual activity period of the Mongolian gerbil. The data from the LL experiment indicates that the gerbils possess a circannual rhythm of locomotor activity. Figure 1 demonstrates the changes in the frequency of the activity rhythm under the different photoperiods, thus indicating that the endogenous circannual rhythm of activity is entrained by the photoperiodic Zeitgeber. While performing the analysis of the data, I clearly noted the daily (circadian) activity was entrained by the LD cycle, but the Highs and Lows of the seasonal rhythm were not entrained instead, the rhythms freeran with a period shorter or longer than 12 months.

There is a paucity of data demonstrating that the photoperiod can entrain the circannual period. Blake (1963) reported that the LD cycle can entrain the rhythm of diapause in an insect. Reproductive readiness in birds (Gwinner, 1977) and antler development in deer (Goss, 1977) are synchronized by the LD cycle. To consider the results valid, the experimenter should study the effects of the various photoperiods over many months and should not observe animals removed from the laboratory or natural environment to study for a few months. The effects of the previous lighting regime could have lasting influences. The constant conditions of food/water availability, temperature, social cues, and the photoperiod will manifest the circannual rhythm (Joy and Mrosovsky, 1985).

The LD cycle is pivotal for the process of photoperiodism where the length of the light (or dark), upon reaching a critical duration institutes some physiological or behavioral change in the organism. Thus, many photoperiodic phenomena such as moulting, fattening, reproductive readiness, migration, etc., have annual cycles. Nevertheless, the above annual rhythms need not be timed by a circannual bioclock. Models for the photoperiodic phenomena are based upon "external" or "internal" coincidence. (For a recent review see: Meier and Russo, 1984.) In any case models are simply "formulas" used by the students of biorhythms to organize their thoughts and research. Neither of the above two models has been proven or disproven.

Activity rhythms are not considered to be photoperiodic. Their circannual nature becomes apparent under the constant condition of LL, DD or LD, but LD conditions may not be totally information free (Chandola et al., 1983). It might be possible that the gerbil possesses some other physiologic parameter not observed that is photoperiodic and could provide timing cues to the locomotor rhythm. Thus, their circannual nature may not be due to an endogenous, self-sustained pacemaker.

Quantitatively the length of the circannual period should vary around 12 months (Gwinner, 1981). The ones that I have described vary a great deal more or less than 12 months since for some only half-cycles are indicated. These cycles are indistinguishable from the circannual rhythms. It is quite possible that there is a wide variety of interspecific variation of circannual periodicities. The pacemaker generating such periodicities would require environmental cues such as the LD cycle to synchronize the physiological rhythms with the environmental ones. It is not known to me why such a large deviation would exist between the environmental cues and the bioclock.

There are additional models which attempt to explain circannual rhythms. The organism could somehow count the circadian cycles (Meier and Russo, 1984). Joy and Mrosovsdky (1985) proposed an evolutionary sequences of stages which added together produce yearly cycles. The stages might have consisted of periods of fattening, reproductive readiness, reproduction, quiescence, etc., each stage lasting a few months, but when added sequentially a year is obtained. Another commonly discussed model is frequency demultiplication. The interaction of two circadian rhythms could be transduced into the longer period of a year (Dark et al., 1985). My future studies will relate to a search for an annual sensitivity to light, i.e., an annual phase response curve to light. A annual phase response curve must exist so that the LD cycle can entrain the yearly cycle. (For the classical study in this area see: DeCoursey, 1960).

Recently Czeisler et al. (1986) reported that light can act as a Zeitgeber for human circadian rhythms, also indicating the presence of a phase response curve in us. Light may be used to phase advance or delay human rhythms without the intervention of a chemical agent. Obviously, it would be impossible to maintain people under unvarying conditions for many months, but we are known to have seasonal rhythms in body temperature and length of sleep when maintained under constant conditions for short periods of time during the various seasons (Wirz-Justice et al., 1984). It is also obvious that annual clocks are required to synchronize the many reproductive and behavioral processes in animals. In the study of annual clocks, we still are researching at very preliminary quantitative levels.

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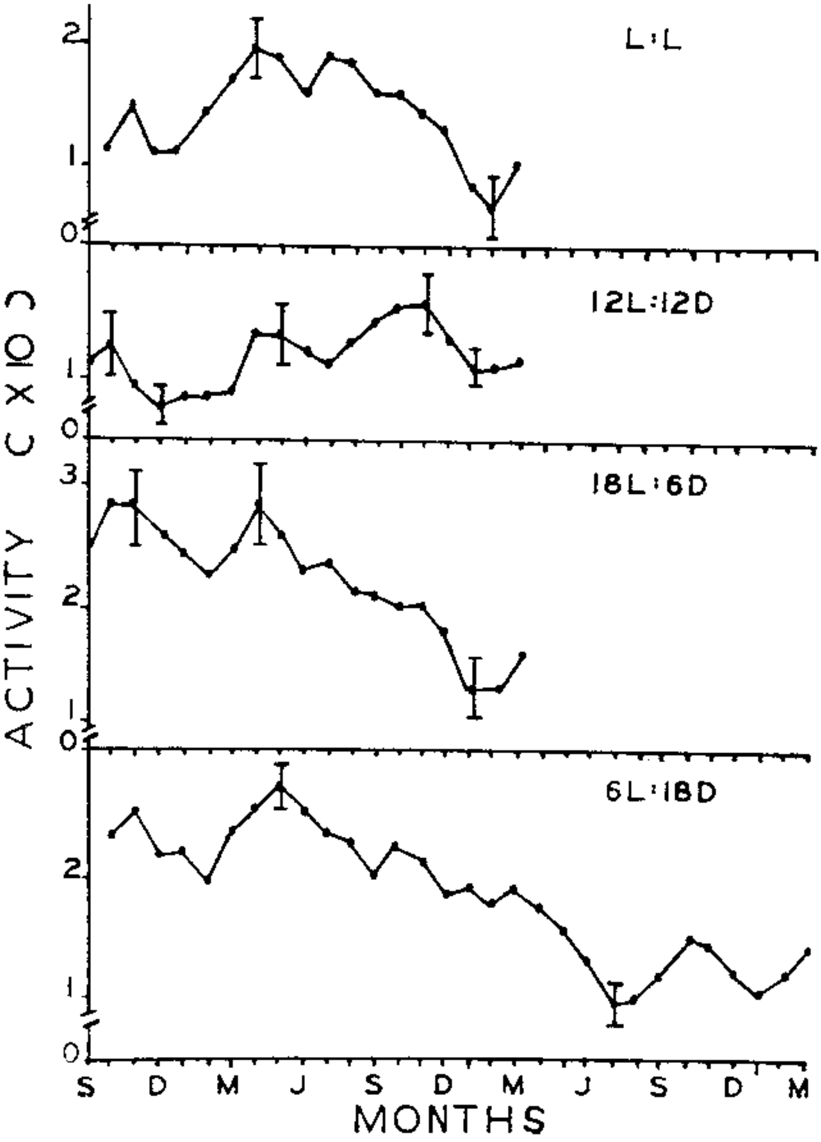

Figure 1. Summated curves for six gerbils under each of four experimental conditions: LL (Natalini, 1978); 12L:12D; 18L:6D (Natalini, 1982); and 6L:18D. Each value is the monthly mean ± SEM. Note the discontinuities in the ordinate axis.

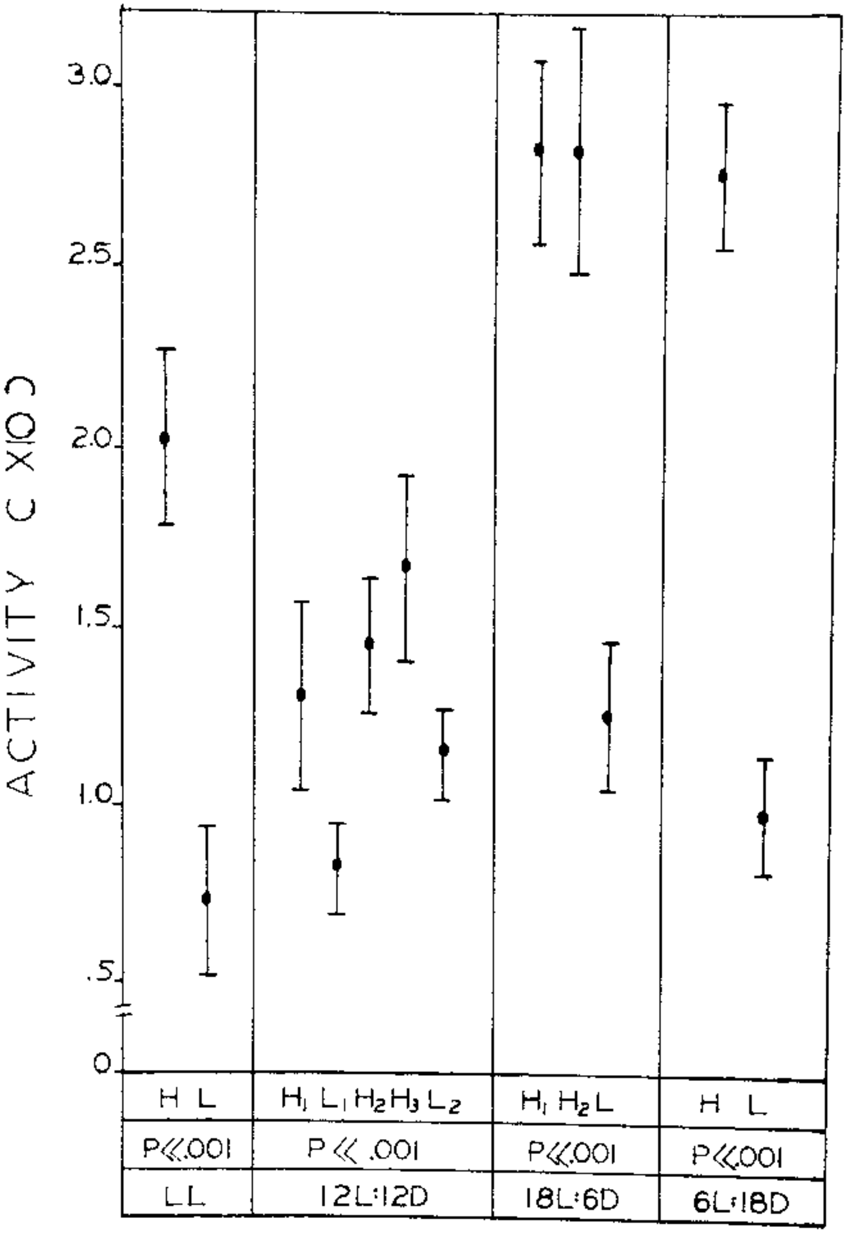

Figure 2. ± One standard error of the mean for the Highs and Lows of Figure 1. Each value represents 6 gerbils. Probability was calculated by a student's t test indicating a level of significance much less than 0.001. Notice discontinuity in the ordinate axis.