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SEASONAL RHYTHMS OF THE MONGOLIAN GERBIL UNDER A LONG-DAY PHOTOPERIOD

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ABSTRACT

The effect of a long-day photoperiod (18L:6D) was observed on the locomotor rhythms of six, male Mongolian gerbils, *Meriones unguiculatus*. Animals were housed individually in running wheel activity cages coupled to an Esterline Angus Event Recorder. Light intensity measured to the center of the housing compartment was 2.2 lux; temperature of the room was thermostatically controlled at 20 ± 2°C with no seasonal correlation. Hourly activity was quantified into monthly means for each gerbil. A composite curve for all experimental animals indicated peaks of activity in November (1980) and April (1981) statistically significant (P much less than 0.001) from a trough in January (1982). The light/dark (LD) cycle is a universal zeitgeber for circadian rhythms. In addition, it is essentially involved in the phenomenon of photoperiodism.

INTRODUCTION

The scientific literature dealing with the life sciences is replete with articles relating to periodic phenomena. Biological rhythms are any behavioral or morphological process recurring at somewhat predictable intervals. Many of them have geophysical correlates with frequencies similar to the solar, tidal, lunar, and annual oscillations. In the absence of any obvious entraining agents, these rhythms assume periods close to but not exactly the same as their geophysical correlates and are called: circadian, circatidal, circalunar, and circannual. Rhythms within range of those previously listed may be assigned a prefix indicating ultra- (less than) and infra- (more than). The exact clock or pacemaker is still unknown (but we may be getting closer to its discovery), and biologists remain forced to study the overt, observable expression of the pacemaker, or the proverbial "hands-of-the-clock" during their scientific investigations. Saunders (1977) has written a good, basic introduction to the field.

The Zeitgebers or entertaining agents, those which force the pacemaker and/or overt rhythm to maintain a particular period, have been found to be the cycles of day-night, temperature, barometric pressure, humidity, weather, sound, olfaction, and social cues. Nevertheless the most constant and noise-free environmental and laboratory Zeitgeber is the light/dark cycle (Englemann, 1981; Pittendrigh, 1981). The effects of LD cycles on circadian rhythms have been studied extensively, but the effects on long term rhythms are just beginning to be elucidated. The most prevalent, long term rhythm is the circannual period. Circannual rhythms are those processes repeating at intervals approximating a year in the absence of any varying, environmental Zeitgeber. Within the laboratory, experiments have to last for years. They have been found in the hibernation of goldenmantled ground squirrel (Mrosovsky et al., 1976; Pengelley and Asmundson, 1974), menstrual cycles of monkeys (Dailey and Neill, 1981; Michael and Zumpe, 1981), body mass of chipmunks and marmots (Ward and Armitage, 1981; Richter, 1978; Davis, 1976), breeding of crayfish (Jegla and Poulson, 1970), body mass of birds (Chandol et al., 1980) and growth of a dinoflagellate (Yentsch and Mague, 1980).

In addition to circannual rhythms, the literature lists examples of numerous types of long term periods. Many experimental designs (usually less than a year) exist for the observation of such periods. Organisms may be observed within their natural environment or captured from the natural state and immediately studied (Scelza and Knoll, 1982; Rosenblatt et al., 1982; Eales and Fletcher, 1982; Francezon et al., 1981; and Dziubek et al., 1981). Organisms may be observed within the laboratory but under natural photoperiods (Dang and Meusy-Dessolle, 1981; Pohl and West, 1976). Finally organisms may be removed from "natural" conditions and placed under constant conditions usually for a period of a few months (Kavaliers, 1981; Kenagy and Bartholomew, 1979; Pohl, 1977; Gwinner, 1975). In these various studies, conditions before the actual experimentation could have lasting "after-effects" on the organism and thus could have affected the results.

Not only is the light/dark cycle a necessary part of entrainment, it is also essential for the phenomenon of photoperiodism (not to be confused with photoperiod or LD cycle). Photoperiodism involves the induction of some seasonal event such as flowering, reproductive readiness, diapause, hibernation, moulting, migration, or pelt growth (Follett and Follett, 1981). Also, certain photoperiodic processes are known to have circannual periods.

In the present study, I continue to observe the effects of the light/dark regime on the locomotor activity of the Mongolian gerbil. Circannual rhythms were reported under continuous dim illumination (Natalini, 1978) and observed under 12L:12D conditions (Natalini, unpublished). With the significance of the light/dark cycle relating to entrainment of biological rhythms and to involvement in photoperiodism, I wanted to determine the effects of a long-day lighting regime on the rhythm of gerbil activity. By abolishing the natural light/dark cycle, the gerbils should be able to free-run under the experimental conditions producing no "exact" seasonal cycle. The aim of this and future experiments is to help obtain a better picture of circannual rhythms of the gerbil. Further considerations of the significance of such rhythms and possible mechanisms are deferred until the Discussion.

MATERIALS AND METHODS

Six-week-old male Mongolian gerbils, *Meriones ungiculatus*, were purchased from Tumblebrook Farms, West Brookfield, MA. Upon arrival, the animals were observed for one week under a 12L:12D photoperiod (light on -0600 hours CST) in order to ascertain any immediate problems. On 1 September, 1980 the gerbils were placed in a rectangular photoperiod of 18L:6D (light on -0600 hours CST). Data collection ceased on 31 March, 1982. Two out of the six experimental animals died within the last two months of the experiment.

Animals were housed individually in Wahmann activity cages (model LC14) having a wire mesh (9.0 mm) revolving wheel (diameter 35 cm) with access through a partition to a living compartment (25.5 L x 15.5 W x 12.5 H cm) made of wire mesh cloth. In order to prevent self-selected lighting regimes (see: Aschoff, 1981a), no bedding was provided in the living compartment. The activity recorders were placed concentrically around four, six-watt incandescent light bulbs with a light intensity of 2.2 lux measured at the center of the housing cage (Weston light meter Model No. 703-60). The same intensity used in my previous experiments. Frequent repacement of the lamp bulbs prevented and accidental failures or diminution of the light intensity. The gerbils had free access to water and Purina rat pellets. Necessary feeding and maintenance were conducted at random times on 10- to 15-day intervals. A Taylor Thermograph Recorder (Model No. 2350) continuously monitored the room temperature, thermostatically controlled at 20 ± 2°C. Over the seasons, there was no systematic change in temperature, and in any case temperature has little entrainment capabilities for homeotherms (Bahnak and Kramm, 1979; Stewart and Reeder, 1968).

Attached to the running wheel of each cage was an eccentric cam which activated a microswitch coupled to an Esterline Angus recorder which continuously registered the locomotor activity of the gerbils. Analysis of the 19 months of data was previously described (Natalini, 1972). Briefly, each hour of the day was assigned a value from 0 to 10 indicating no activity (0%) to complete activity (100%). Via this method, daily and monthly means of activity were calculated for each gerbil. The experimental animals were within hearing and/or olfactory distances of each other, but I noted no such mutual entrainment as demonstrated by comparing individual graphs of activity.

RESULTS

Figure 1 demonstrates the seasonal, composite rhythm for the mean monthly activity for the six gerbils. There are distinct peaks (H_1 and H_2) in November (1980) and April (1981) respectively with a trough (L_1) in January (1982). Figure 2 is a graphic representation of the maxima and minima for the experimental animals and the composite (c) for all six animals. A student's t test was used to measure the selective points of interest for statistical significance. The maxima were significantly (P much less than 0.001) greater than the minima for all gerbils except animal 4. (The error bars are $\bar{X} \pm$ SEM.) Since the highs and low of Animal 4 are only significant to the 0.01 level, I reject the animal as having a true seasonal rhythm seeing that the remaining animals had a much greater significance between points. Every organism has its own genetic history. This can be demonstrated in the range of activity from the maximum to the minimum for each gerbil which varied from 29% to 74% of the maximum. Thus, a common analytical method in periodic studies is for an experimental animal to act as its own control. Figure 2 illustrates statistical significance in the comparison of the high(s) and low(s) for each animal except animal 4. Also, Figure 2 illustrates genetic individually between animals by noting the difference in magnitude of the peaks and troughs among the experimental animals.

The null hypothesis would assert that there is no difference between any monthly mean of activity. Values falling between the maxima and minima were not statistically significant with each other nor the selected point(s) of interest. Only the highs and lows were significant to the 0.001 level. The statistical description reinforces the strong visual impression of Figure 1.

DISCUSSION

Locomotor activity experiments are used widely to study one of the "hands-of-the-clock". Wheel running results possibly exclude less periodic events such as grooming and/or drinking. They have proven to be clear in measurement, accurate in results, demonstrable in pacemaker properties, and continuous in duration which contrasts to once in a life time events like hatching or pupation studies. Such activity chambers can be supplied easily with food and water so that a minimum of disturbances is necessary which affect phasing properties of the overt rhythm.

Using such wheel running activity chambers, I have demonstrated distinct, free-running, seasonal cycles which are obvious through visual inspection alone. Statistically significant peaks were recorded in November (1980) and April (1981) with a trough approximately a year later in January (1982). Because the experiment did not continue to allow completion of the second half of the cycle, the indicated rhythms can not be called true circannual periods (Gwinner, 1981a). Since true circannual periods were observed from my previous studies using the same species (Natalini, 1978), I have no doubt that they represent the result of a circannual pacemaker.

Circannual rhythms, which persist in the constancy of the laboratory environment, have properties similar to circadian rhythms. Some of these properties include periods close to 12 months, temperature compensation, synchronization by the photoperiod, genetic individuality, ranges of entrainment, and the appearance of transients before reaching steady-state conditions (Gwinner, 1981a; Gwinner, 1981b). Very few reports exist for circannual rhythms which clearly indicate the photoperiod to be an effective environmental Zeitgeber. Synchronization by the LD cycle has been demonstrated for reproductive readiness in birds (Gwinner, 1977), antler development in deer (Goss, 1977), and diapause in insects (Blake, 1963).

The light/dark cycle (photoperiod) is essential for the process of photoperiodism such that organisms measure the length of the light (or dark) in order to make some physiological modification in their life cycle. Various hypotheses exist which attempt to explain the mechanism(s) for the photoperiodic phenomenon. An original model was proposed by Buning (1969) which entails the LD cycle in entraining a circadian rhythm; and if the length of the light portion exceeds a particular amount, light will fall on a photosensitive phase inducing some seasonal event. Later, the Bunning hypothesis was called the "external coincidence" model by Pittendrigh (1972). Also, Pittendrigh (1972) introduced a second model entitled "internal coincidence" where the LD cycle entrains various oscillators such that the phase relationship between them brings about the seasonal response. The above theories involve circadian pacemakers. One which does not involve circadian pacemakers has been proposed by Lees (1966) described as an "hour-glass" model where the actual length of the light (or dark) portion allows the synthesis of some product so that if enough product is accumulated the photoperiodic response occurs; thus, no circadian rhythm is involved. Aschoff (1981b) and Follett and Follett (1981) have edited recent reviews encompassing photoperiodism.

Confusion may exist between true circannual rhythms and photoperiodic phenomena since many photoperiodic phenomena occur annually. Only a few photoperiodic processes have been reported to occur with a true circannual frequency in the consistency of the laboratory conditions. Circannual rhythms may be expressed indepdently of the LD cycle. Photoperiodism indicates that there is a critical length of the light (or dark) span which can induce the photoperiodic response at any time of the year if proper laboratory conditions exist.

The location of the circadian and especially the circannual pacemaker is still uncertain. The supra-oesophageal ganglion (Page, 1981) or the optic lobes (Page, 1982) in insects, the eyes of molluscs (Block and Wallace, 1982), the pineal organ in birds (Menaker et al., 1981), and the suprachiasmatic nuclei in mammals (Menaker and Binkley, 1981; Turek, 1981) appear to be excellent prospects for the location of the circadian pacemaker. In addition Gwinner (1981a; 1981b) has proposed that the interaction of multiple circadian pacemakers can result in circannual rhythms by the process of frequency demultiplication or via a process of external/internal coincidence similar to photoperiodism, thus negating the existence of circannual pacemakers. For alternate models of biological clocks see Brown (1972, 1978).

Circannual and other long term rhythms have been reported for the various behaviors of man (Rubin, 1979; Herbert, 1982). Their significance in animals for the preparation of breeding readiness and adverse environmental conditions is commonly known. Today, LD cycles are being utilized to increase the efficiency of milk, eggs and meat production of many domesticated animals (Tucker and Ringer, 1982). Further research is required to determine additional relationships between LD cycles and circannual or photoperiodic phenomena. The present study is a continuation of investigations of the effect of LD cycles on long term activity rhythms of the Mongolian gerbil in search of the Zeitgeber of such rhythms. Finally, I would like to point out that evidence clearly indicates the existence of long term cycles; and thus, since the physiology of animals varies from month to month, experiments performed without regard to this fact are likely to be questionable.

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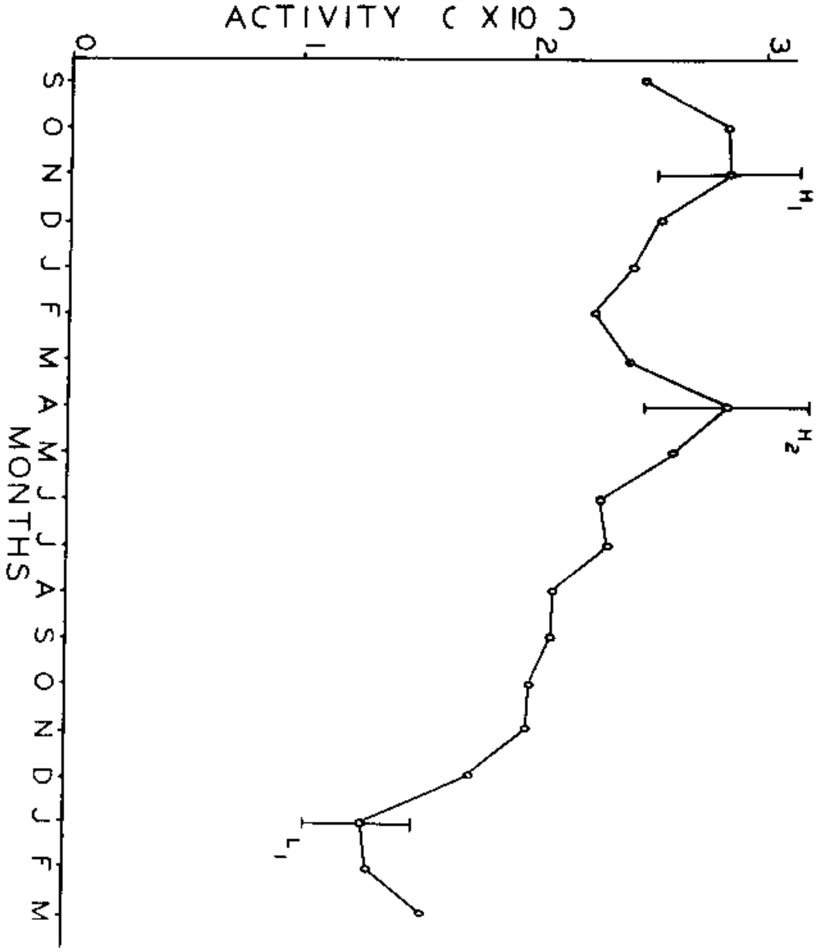

Figure 1. Summated curve for six gerbils. Each reference point is the monthly mean with $\bar{X} \pm$ SEM for High One (H_1), High Two (H_2), and Low One (L_1).

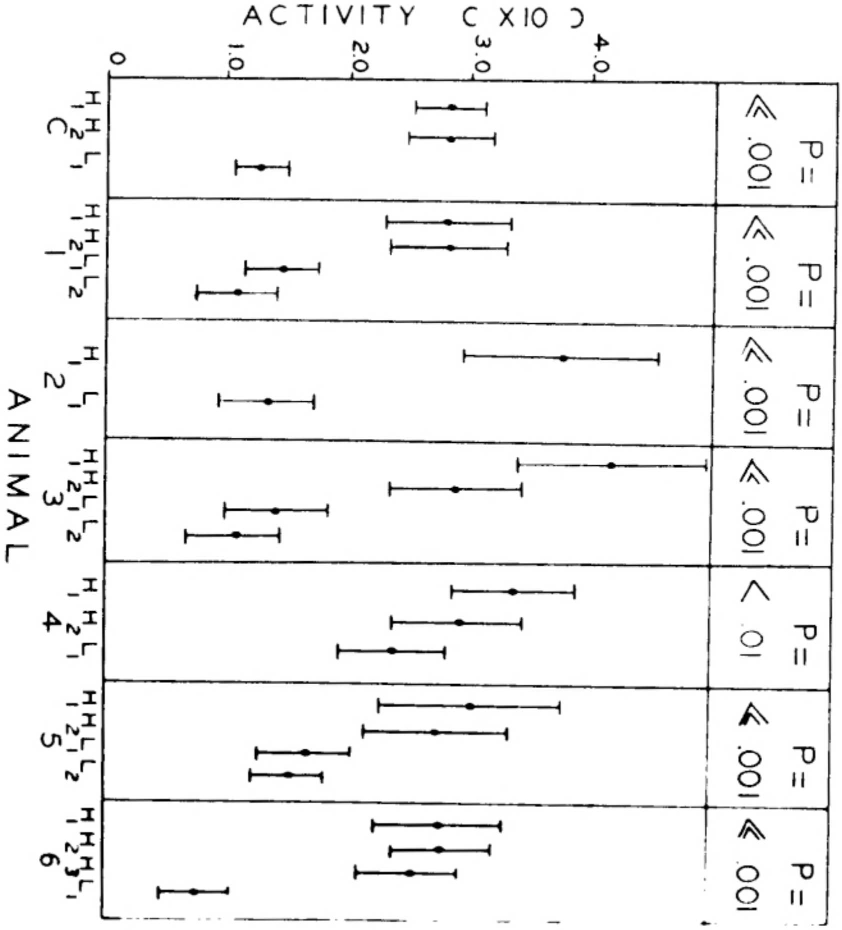

Figure 2. ± one standard error of the mean X̄ ± SEM for the Highs and Lows of each experimental animal (acting as its own control). Probability was determined by a student's t test indicating significance at the level much less than 0.0001. C = composite of all six experimental animals.