RELATIONSHIP OF LIGHT INTENSITY AND
PHOTOPERIOD TO CIRCUANNAL RHYTHMICITY
IN THE HIBERNATING GROUND SQUIRREL,
CITELLUS LATERALIS

E. T. PENGELLEY, SALLY J. ASMUNDSON, BRIAN BARNES AND ROLAND C. ALOIA
Department of Biology, University of California, Riverside, CA 92502, U.S.A.

(Received 15 July 1975)

Abstract—1. Free-running circannual rhythms of the hibernator, Citellus lateralis, have been studied for nearly 4 yr in relation to light intensity and photoperiod. Animals were kept under constant temperature of 3°C but under a variety of lighting conditions ranging from constant light at 500 lx to total darkness.

2. It was found that the circannual period is virtually always less than 365 days (mean about 300 days), but that light did not affect the free-running period and did not appear to act as a zeitgeber. The significance of this is discussed.

3. Variations of the normal physiological behavior are also discussed.

INTRODUCTION

Since the discovery of endogenous circannual rhythms about 15 years ago, one of the principal lines of investigation has been to try to determine whether the mechanisms by which they are entrained are essentially the same as those for circadian rhythms (Pengelley, 1967; Pengelley & Asmundson, 1972, 1974; Pengelley et al., 1972). Endogenous circannual rhythms have now been demonstrated in a wide variety of animal species including invertebrates such as the cave crayfish (Jegla & Poulsom, 1970) and a marine cnidarian (Brock, 1974), a variety of birds (Gwinner, 1967, 1968a, b, 1969; Berthold, 1974; Klein, 1974; Rutledge, 1974), the insectivorous hedgehog (Kristofferson & Soivio, 1964; Johansson & Senturia, 1972), many species of hibernating rodents (Pengelley & Asmundson, 1974; Davis, 1967a, b; South et al., 1972), the sika deer (Goss et al., 1974) and even possibly man (McKerrow & Rosssiter, 1968; Weinberg, 1974; Senturia & Johansson, 1974). A recent symposium (Pengelley, 1974) has emphasized the fact that it is only after many years of research to establish these circannual rhythms that precise and meaningful experiments can be performed using various external zeitgebers as potential entraining agents.

Following the discovery of a circannual rhythm in the golden-mantled ground squirrel, Citellus lateralis, by Pengelley & Fisher (1957), subsequent experiments on entrainment have apparently implicated ambient temperature (Pengelley & Fisher, 1963), locomotor activity (Pengelley & Fisher, 1966), light (Pengelley & Asmundson, 1970) and more recently the effects of mating on female animals in the form of gestation and lactation (Pengelley & Asmundson, 1975). Since it has been known for many years that both the intensity of light and the photoperiod have profound effects on the behavior and physiology of a variety of animals (Rowan, 1925), including mammals (Bissonette, 1935), and since light in one form or another is almost invariably a zeitgeber for circadian rhythms (Cold Spring Harbor Symposia, 1960; Aschoff, 1965), it seemed a worthwhile approach to explore in greater depth the relationship of light to circannual rhythmicity in the golden-mantled ground squirrel. The animals involved in this experiment were born under controlled laboratory conditions to rule out any possible effect of previous natural environmental entrainment (Pengelley, 1966).

Due to the long term nature of this experiment (4 yr) a variety of individual behavioral differences, which many investigators consider to be important for a full understanding of circannual rhythmicity (Pengelley et al., 1972) in hibernating mammals, were also investigated. These included variations in the free-running period—whether it is greater or less than the geophysical period of a year, variations in the length of the homothermic period relative to the heterothermic period, variations in the relationship of the body weight rhythm to the homothermic heterothermic rhythm, and others. The results of these observations will also be reported here.

MATERIALS AND METHODS

During the first half of May 1970, pregnant females of the golden-mantled ground squirrel, Citellus lateralis, were trapped at an altitude of about 7500 ft, near Big Bear Lake in the San Bernardino Mountains of California. They were brought to the laboratory, housed in individual cages in a room with a photoperiod of 12 hr and an ambient temperature of 23 ± 1°C. Purina lab chow, water and bedding material were supplied ad lib., and this was supplemented with fresh carrots 3-times a week. Under these conditions (Pengelley, 1966) young were born between the middle of May and the first week in June. They were weaned at about 35 days after birth, and kept together until 31 July when each animal was placed in a separate cage. Subsequently they were divided into five groups of about 17 animals each, which were matched as far as possible for sex and body weight. Two of these groups were then bilaterally enucleated (Pengelley & Asmundson, 1970). On 31 August the experiment was started with each group
Fig. 1. Graphical representation of heterothermic (black bars), homothermic (open spaces) and free-running (onset to onset of black bars) periods for five groups of C. lateralis over nearly 4 yr. All groups at 3°C ambient temperature. Group 1, bilaterally enucleated (E), 24 hr darkness. Group 2, normal, 24 hr light, 500 lx. Group 3, bilaterally enucleated (E), 24 hr light, 500 lx. Group 4, normal, 24 hr light, 20 lx. Group 5, normal, 12L/12D, 200 lx. X = death, O = termination of animal in the experiment.

being placed under the following environmental conditions. Group 1, bilaterally enucleated, 24 hr darkness and 3 ± 1°C ambient temperature. Group 2, normal, 24 hr light at 500 lx and 3 ± 1°C ambient temperature. Group 3, bilaterally enucleated, 24 hr light at 500 lx and 3 ± 1°C ambient temperature. Group 4, normal, 24 hr light at 20 lx and 3 ± 1°C ambient temperature. Group 5, normal, 12L/12D at 200 lx and 3 ± 1°C ambient temperature. With only minor interruptions, due to equipment failure, these environmental conditions were maintained for nearly 4 yr. Maintenance and observations were carried out every day, while food and water were always ad lib.

Ground squirrels have distinct periods (months) of hibernation during which they are heterothermic and these alternate with periods of activity during which they are homothermic. While in the heterothermic state an animal has a body temperature close to the ambient (3°C in these experiments), but within these long periods of heterothermy there are short arousals during which the animal becomes fully active with a homothermic temperature of 37°C, maintains this for a few hours and then enters heterothermy again (Pengelley & Fisher, 1961). These short “periodic arousals” are not considered in this experiment, and reference is only made to the entire heterothermic and homothermic periods. Accurate weight records of each animal were kept by methods which do not disturb their hibernating behavior (Pengelley & Fisher, 1963).

RESULTS

The data from this experiment are summarized and expressed graphically in Fig. 1. It must be emphasized that the horizontal time scale is nearly 4 yr. The environmental conditions under which the various groups were kept (vertical axis) were held constant throughout the experiment. Consequently the only variable for each group was the intensity of light and/or the photoperiod. Thus any significant difference in the time of onset of the first and subsequent hibernation periods, the length of each heterothermic and homothermic period and the length of the free-running periods (i.e. from onset to onset of each heterothermic period) can justifiably be ascribed to the influence of light in one form or another. Despite the fact that light was expected to be a zeitgeber (Pengelley & Asmundson, 1970), the results do not support this thesis. A two-tailed t-test between each group for the length of the heterothermic, homothermic and free-running periods reveals no significant difference. Even with a more complex and sensitive test i.e. analysis of variance for one-way classification with unequal replication with subsamples (Snedecor & Cochran, 1967), no significant differences are revealed. There is also no significant difference between the groups, in the time of onset of the first hibernation period.

In Fig. 2, data from a representative animal from each group are plotted to show various features of the free-running circannual rhythm. Every animal in this figure completed four free-running periods before the experiment was terminated. It is obvious that with the beginning of each successive free-running period the animals are starting their heterothermic period earlier in relation to the presumptive seasons in the natural geophysical time scale of a year. The average free-running period is about 300 days so that by the onset of the third free-running period the animal is approx 180° out of phase with the presumptive seasons, and by the beginning of the fifth period the animal is back in phase again. This is true for all five groups. It may be pointed out here that for the
60 animals in this experiment from which meaningful data could be gained, only two exhibited a single free-running period of over a year. Figure 2 also shows the usual situation where the first free-running period is almost always longer than the subsequent ones. In addition it is also to be noted (Fig. 2) that typically the heterothermic period is longer than the homothermic one.

Figure 3 shows the body weight cycle and the heterothermic-homothermic cycle plotted on the same graph. Animals were chosen to show a specific behavioral pattern, hence there are two animals from group 1 and none from group 4. It is easily seen from the animals represented here, that the body weight and heterothermic-homothermic rhythms are normally phase locked in such a way that the weight peak is reached near the onset of the heterothermic period, and the minimum at or shortly after the end—see example of animal from group 5. However the first animal in group 1 shows the rather atypical situation in which there is considerable gain in weight long before the end of each heterothermic period. This is due to the fact that the animal started to eat enough food during its periodic arousals to gain some weight. Normally the animals do not eat during these arousals. However it may be noted that despite this variation, neither free-running rhythm is changed. The second animal in group 1 had one of its legs injured shortly after the beginning of the experiment, and it received periodic injections of an antibiotic for about a month until all danger of infection was removed. In view of this it is very interesting to note that while this considerably delayed the onset of the first heterothermic period, and damped the body weight rise, nevertheless the onset of the second heterothermic period was what would have been expected if it had had a normal first period. This clearly indicates that while the injury and treatment did affect the overt appearance of the first heterothermic period, it did not alter the basic circannual rhythm. The animal from group 3 is unusual in that the homothermic periods are longer than the heterothermic ones—see the more typical situation in the animals from groups 2 and 5, where the homothermic periods are shorter than the heterothermic ones. However, this does not seem to alter the free-running period. The animal in group 2 shows the unusual phenomenon of a general rise in the weight peak of each body weight rhythm, but again it remains phase locked with the heterothermic-homothermic rhythm.

DISCUSSION

As pointed out previously, light in one form or another, is a typical zeitgeber for circadian rhythms. Furthermore it has been demonstrated that in both birds (Menaker, 1968) and lizards (Hoffman, 1969) there are extraretinal light receptors, and that light can entrain circadian rhythms in these animals when bilaterally enucleated. However the evidence from mammals indicated that there are no extraretinal light receptors (Halberg & Visscher, 1954). Nevertheless we felt that a long term experiment using two groups of young bilaterally enucleated animals, one in total darkness (group 1) and the other in 24 hr light at 500 lx (group 3) would not only confirm whether these hibernating rodents had extraretinal light receptors but also whether there was any difference in their response to light in comparison to normal controls (groups 2, 4 and 5). On both counts the answer seems to be no. It is remarkable also that the intensity of light seems to have no effect (groups 1 and 2), for
it is known from Aschoff's rule (Hoffman, 1965) that the intensity of light is a factor in the control of circadian rhythms, having different effects on different species depending on their basic physiology. It should be noted that had our animals had access to activity wheels the results might have been different (Pengelley & Fisher, 1966). It is pertinent to note also that since the experiments reported here began, Goss et al. (1974) have shown that in the sika deer the circannual growth rhythm of antlers is affected by light.

It has been demonstrated by Heller & Poulsin (1970) that in C. lateralis the circannual rhythms of body weight and homothermy–heterothermy are apparently phase locked to the breeding cycle. While there are virtually no data on the effects of light on the breeding cycle of hibernating ground squirrels, which apparently breed only once a year, there is evidence that in both male and female hamsters (a hibernator) short photoperiods or total darkness cause atrophy of the gonads (Hoffman & Reiter, 1965a,b). On the other hand, in rats total darkness has little effect on the gonads. Constant light however, has little effect on hamsters but induces constant estrus in rats (Greenwald, 1963). Thus, the evidence seems quite clear that the effect of light, or the lack of it, on the reproductive physiology of various mammals is species specific. Furthermore, it has also been demonstrated that the effect of light is mediated through the pineal gland with subsequent profound effects on the reproductive hormones and probably others as well (Hoffman & Reiter, 1965b). Since in C. lateralis the sexual cycle is apparently phase locked to the homothermic–heterothermic and body weight rhythms, and since there is strong evidence that reproductive competence inhibits hibernation (Heller & Poulsin, 1970), it could justifiably have been suspected that, constant strong light or constant darkness would have affected the reproductive system in such a way that it would have altered, if not inhibited, the period of hibernation. The fact that this was not the case is very significant, not only for an understanding of the entrainment mechanisms of the circannual rhythms involved, but for the sexual physiology as well.

A circannual rhythm of fat deposition has been well demonstrated in another hibernator, the woodchuck, Marmota monax, by Davis (1967a,b), who has recently performed an important experiment concerning entrainment of the circannual rhythm. Laboratory held woodchucks in the U.S.A. exhibited a circannual weight rhythm with a 10-month free-running period; normal weight maximum in September and minimum in April. Yearling animals were captured in April kept under constant conditions until the following January, when they were flown to Sydney, Australia. In Australia they were exposed to the natural environment, and within 2yr they had shifted their weight maximum to April and maintained it there for an additional 2yr. Davis speculates that the length of the day was the zeitgeber, but controlled experimental evidence is lacking, and in the face of our results reported here one must be very skeptical. Indeed as Menaker (1974) has recently pointed out the analogies between circadian and circannual rhythms may have been overdrawn, and it appears unlikely that the physiological mechanisms by which organisms measure annual time will bear more than a superficial resemblance to those by means of which they measure circadian time. In the light of the results reported here, this hypothesis seems highly significant. Nevertheless it may be noted that if an animal does not respond to light on an annual basis, then it may be argued that a circannual clock is an even more important survival mechanism.

In conclusion we would like to point out that although our results may seem disappointing to some, we do not share his view. The influence of light on organisms has a vast array of effects, some of which are very subtle and there are no doubt many more of which we know nothing. Thus it is vital that experiments of this nature be continued and refined to elucidate the complex effects of light not only on most animals, but as Wurtman (1975) has also indicated on humans as well.

Acknowledgements—This study was supported by Grant No. GB-40827 from the National Science Foundation, and secondarily by an Intramural Grant from the University of California. We wish to thank Mrs. Dorothy Whitson for her dedicated technical assistance.

REFERENCES


