J. comp. Physiol. 90, 33—45 (1974)

The Effects of Constant Light and Light Pulses on the Circadian Rhythm in the Eye of *Aplysia*

Jon W. Jacklet

Department of Biological Sciences and Neurobiology, Research Center, State University of New York at Albany, Albany, New York

Received December 6, 1973

Summary. 1. A circadian rhythm in the frequency and amplitude of compound action potential (CAP) from the isolated eye of *Aplysia* persists for a week or more *in vitro* in constant darkness. This rhythm has a period of about 26 hours (Figs. 1, 2) in a specific culture medium and may express several periodic amplitude components (Fig. 3).

2. Constant light (LL) of low intensity shortens the period (Fig. 4) and reduces the range of oscillations. Higher intensity LL results in a further reduction in range, a greater variability of CAP frequency from hour to hour, alterations in the period, and possibly rhythm splits (Fig. 5).

3. Pulses of light given at specific points in the circadian cycle shift the phase of the rhythm (Fig. 7). The resulting phase response curve (Fig. 8) is similar to response curves for the activity of diurnal animals and potassium pulses on the eye rhythm.

Introduction

The brain has been shown to be important in the entrainment of some circadian rhythms to light-dark cycles (Menaker, 1968; Adler, 1970) and in some instances the brain is clearly shown to be the receptor for the zeitgeber stimulus and the site of the circadian clock that controls rhythmic functions (Truman and Riddiford, 1970; Truman and Sokolove, 1972). Another brain structure, the eye of *Aplysia*, has an inherent circadian rhythmicity in spontaneous dark activity (Jacklet, 1969a). The eye rhythm can be phase shifted by light and entrained by light-dark cycles *in vitro* (Jacklet, 1971; Eskin, 1971). Thus, under normal circumstances this eye serves the animal as a very sensitive detector of light, as a receptor for the zeitgeber stimulus and as a source of circadian timing information. The further characterization of the responses of the eye rhythm to light is of interest for several reasons: (1) the responses are important in determining if this rhythm *in vitro* is comparable to other rhythms studied in whole animals, (2) the responses might indicate something about the mechanism of the rhythm, (3) a phase response

curve for light pulses is needed to compare to the phase response curve for potassium pulses (Eskin, 1972) to investigate the idea that depolarization of the membrane is a natural mechanism for phase shifting the rhythm. Accordingly, the responses of the eye rhythm *in vitro* to constant light and constant darkness interrupted by light pulses were studied.

Methods

Aplysia californica were obtained from Pacific Bio-Marine (Venice, Ca.) and kept in LD 12:12 in Instant Ocean tanks (15° C) prior to experimentation. A preparation of the eye and attached optic nerve was dissected free of the animal and placed in 100 ml of culture medium regulated at 15° C for long term continuous recording of the optic nerve activity. The optic nerve was drawn into a tubing electrode (PE 10 or 20) in the recording chamber and the electrical activity was led off via a silver chloride/silver wire, amplified with a Tektronix 122 and recorded on a Grass polygraph. The recording chamber was housed in a light-tight box so that the eyes could be maintained at constant temperature in constant darkness or constant light from an incandescent illuminator. Phase response curves were generated by interrupting constant darkness with 1 hour pulses of light at appropriate phases of the rhythms. The culture medium consisted of 80% artifical sea water (ASW), 10% *Aplysia* blood and 10% nutrient mixture. The ASW composition in millimoles/liter was: NaCl, 425; KCl, 10; $CaCl_2$ 10; $MgCl_2$, 22; $MgSO_4$, 26; $NaHCO_3$, 2.5. The *Aplysia* blood has a similar ionic composition to sea water (Hayes and Pelluet, 1947) and was filtered (0.22 μ millipore) before it was added to the medium. Twenty-five ml of nutrient mixture contained: 2.5 ml of MEM vitamin solution (100 ×, Gibco), 2.5 g dextrose (Baker), 5 ml MEM amino acids (50 ×, Gibco), 2.5 ml non-essential amino acids (100 ×, Gibco), 2.5 ml L-glutamine (200 mM, Gibco), 2.5 ml penicillin-streptomycin solution (10000 units, Gibco), 6.7 ml 7.5% $NaHCO_3$, 2.7 ml 0.5 N NaOH and ASW. The final culture medium was filtered (0.22 μ, millipore) and the pH was adjusted to 7.8. This culture medium is modified from the medium of Strumwasser and Bahr (1966).

Results

Circadian Rhythm in DD

The eye is spontaneously active in constant darkness (DD). This activity is recorded in the optic nerve as a triphasic compound action potential (CAP) that is naturally synchronized by mechanism (electrotonic coupling) inherent to the eye (Jacklet, 1973). The CAP frequency varies with a circadian rhythm that is precise. The period of the rhythm varies (Jacklet, 1971) according to the medium composition, it is 22–24 hours in ASW alone and 26–27 hours in the culture medium used in these experiments. The rhythm persists quite clearly in culture for a week or longer as shown in Fig. 1. The temporal characteristics of the periodic frequency changes are: a rapid increase at onset, a sustained rate for about 10 hours and then a gradual decrease. During the week several changes occur in the rhythm: the period usually lengthens by 5% or so, the duration of the active phase lengthens with a corresponding decrease

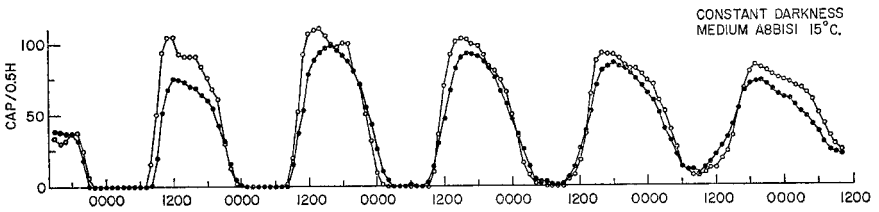

Fig. 1. The circadian rhythm in CAP frequency in DD of two eyes from the same animal. The first onset of CAP frequency in culture corresponds approximately with the last D-L transition at 0800 of the previous day seen by the whole animal. Medium A8B1S1: 80% ASW, 10% blood, 10% nutrient

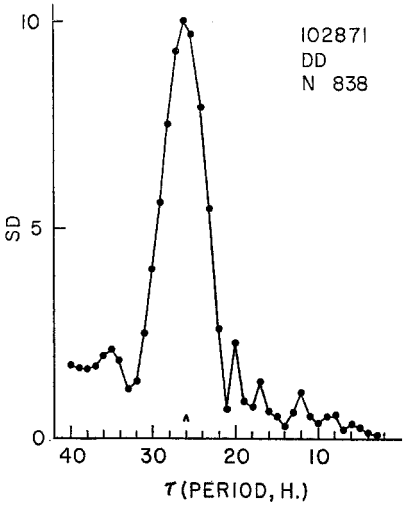

Fig. 2. Periodogram analysis of the CAP frequency from one eye of Fig. 1. Ten minute steps were used for 838 points over the six days of activity. The peak at 26 hours is flanked by minor peaks at 20 and 35 hours

in the inactive phase, and often the range of the oscillation decreases after a week in culture. The activity from both eyes from the same animal are plotted in Fig. 1 showing that the rhythm from one eye can be nearly identical to the other. However, significant phase differences can be produced and will be maintained in culture, indicating that any humoral influences from isolated eye to isolated eye in the same culture medium are slight.

Periodogram analysis (Fig. 2) of the rhythm shown in Fig. 1 shows that the eye has a single strong frequency mode with a peak at 26 hours

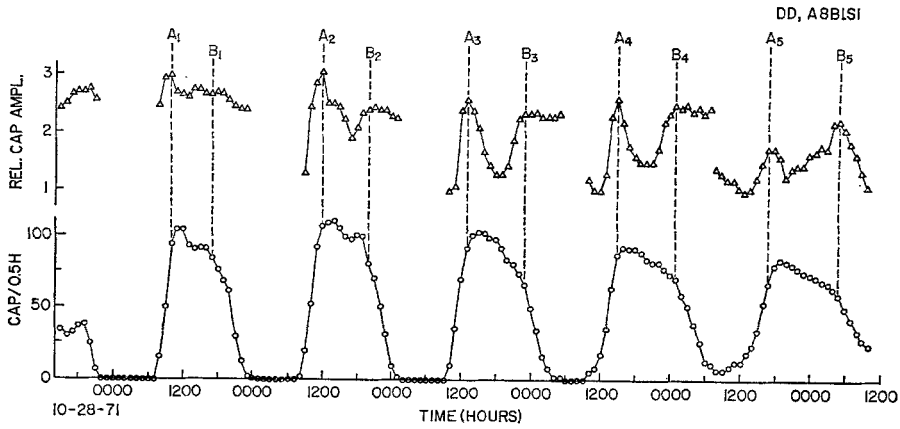

Fig. 3. The rhythms in CAP amplitude and CAP frequency have a definite phase relationship in DD. The first peak in CAP amplitude A, A_2, etc. and the second peak in CAP amplitude B_1, B_2, etc. correspond to changes in the CAP frequency. The interval between Y and B increases with time in DD

flanked by persistent but weaker peaks at 35 hours and 20 hours. The broadness of the major mode is partially due to the variation in period over the week and the increase in duration of the active part of each cycle.

In addition to the rhythm in CAP frequency there is also a rhythm in CAP amplitude (Jacklet and Geronimo, 1971; Jacklet, 1973). Fig. 3 shows the rhythm in amplitude and frequency from one of the eyes (open circles) in Fig. 1. The CAP amplitude rhythm has 2 peaks for each cycle of CAP frequency. In the first day after isolation in DD the second peak is not clear, but with increasing time in DD it develops. The first peak in CAP amplitude has a persistent phase relationship with the CAP frequency rhythm as shown by the dotted lines (A_1, A_2, A_3, etc.) in Fig. 3. This peak always occurs at the point where the CAP frequency changes from a rapid increase to a sustained rate. The second peak of CAP amplitude is more variable than the first peak, but it usually occurs in phase with the point where the CAP frequency changes from a sustained rate to a rapid decrease (B_1, B_2, B_3, etc. of Fig. 3). The CAP amplitude is indicative of the number of individual cells that contribute voltages to the CAP, if one makes the assumption that each individual cell contributes equal voltage to the CAP. Thus this data suggests that these are 2 temporally distinct subpopulations of cells that contribute to the rhythm. An interesting observation on the 2 peaks in CAP amplitude shown in Fig. 3 is that the periods of the 2 peaks are not identical. The A peak has periods of about 26, 25, 26, and 26 hours and the B peak has periods of

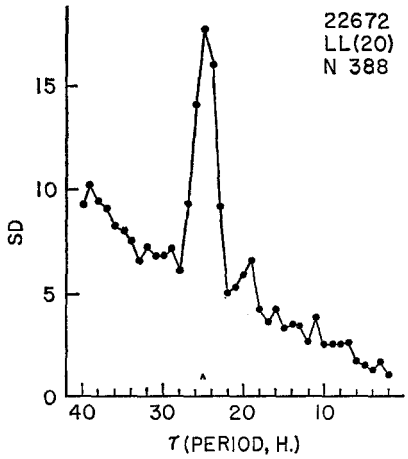

Fig. 4. Periodogram analysis of CAP frequency from an eye in LL (20). Thirty minute steps were used for 388 data points over the eight days of activity. The peak at 25 hours is flanked by the persistent minor peak at 19–20 hours

about 27, 27, 26 and 28 hours. The interval between the A peak and the B peak increases (7, 8, 10, 10, 12) with the lenght of the run in DD. The numerical value of these periods and intervals are somewhat subjective but the trend can be observed in Fig. 3. Thus one component of the eye rhythm appears to be running at a different period from the other component but still maintaining relative coordination. The first peak of CAP amplitude is typical of the eye rhythms studied but in some eye rhythms the second (B) peak may be fractionated into several subpeaks.

Circadian Rhythm in LL

In constant dim light the rhythm persists and the period is shortened. For example, in 20 lux the period is shortened to a peak at 25 hours and the minor mode at 19–20 hours persists (Fig. 4). When the intensity of constant light (LL) is increased to 100 lux, significant changes occur in the rhythm (Fig. 5). The frequency does not decrease to zero in each cycle, as it had in DD, and so the range of the CAP frequency (maximum-minimum) decreases and the shape of the oscillation is flattened. As the range of the oscillation decrease, greater variability in the CAP frequency from hour to hour occurs and the smooth profile of CAP frequency becomes more ragged. Additionally, it appears that the two rhythms which were initially in phase are driven out of phase even though they are in the same medium exposed to the same light. Examination of the record

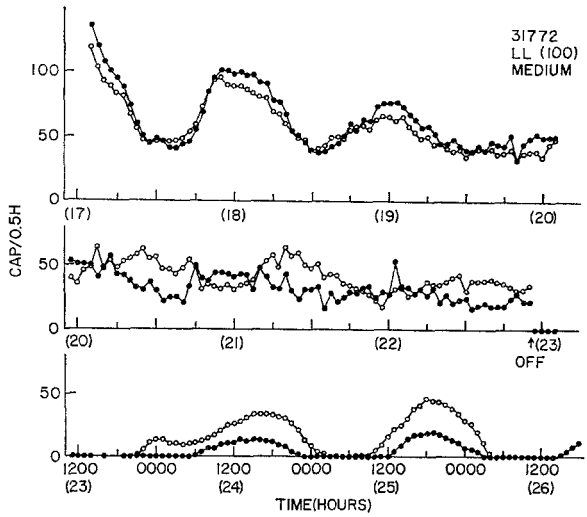

Fig. 5. Circadian rhythm in CAP frequency for days in LL (100) followed by three days of DD (at off) for two eyes from the same animal. On the 20th a change of period occurred (open circles) and produced a shift in phase between the eyes as a result of LL. At the same time the solid periodic components appear to fragment into multiple components. In DD one rhythm is split in the first cycle before it reverts to a single solid component

in Fig. 5 shows that one eye (open circles) changed to a longer period on March 20. This observations was supported by a periodogram analysis of the activities which shows that one eye (open circles) has 2 strong peaks of activity at 26 and 20 hours for the six days in LL. In contrast, the other eye shows one peak at 24 hours and a pronounced shoulder at 20 hours. However the periodogram analysis is of limited value for situations where the period of the oscillation is changing during the run and the run is short. Fig. 5 indicates that an exact measurement of the period after six days in LL (100) is extremely difficult and the record is not long enough to make a confident estimate of the persistence of the periodic components. However, prolonged constant light does produce multiple peaks in CAP frequency from what was formally a single periodic component. When the eyes were exposed to DD on the 23rd (Fig. 5), several changes occurred in the rhythm. The CAP frequency reverted back to the smooth transitions from hour to hour and the pronounced variability in CAP frequency disappeared. The eye rhythm (open circles) that had shown two apparent periodic components (26 and 20 hours) in LL clearly shows two components in the first cycle in DD.

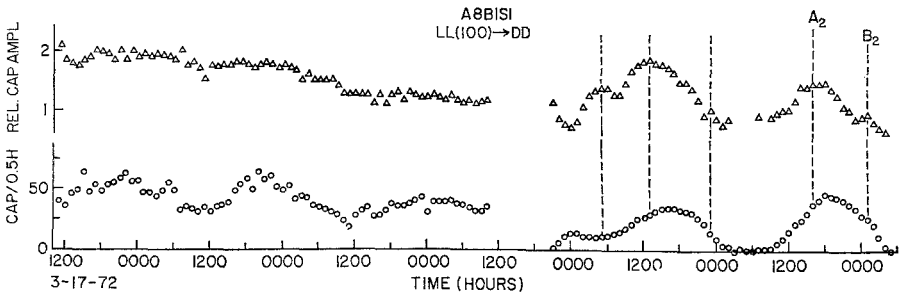

Fig. 6. The rhythm in CAP amplitude and CAP frequency for the last three days in LL followed by DD for one eye (open circles) shown in Fig. 5. In DD the phase relationship between the peaks (A and B) in CAP amplitude and CAP frequency are re-established

These components apparently rejoined in the second cycle in DD. This is evidence that a split did occur in LL. On the other hand, that eye which had a single periodic component in LL maintained a single period component in DD.

The rhythm in CAP amplitude is influenced by LL (100) in much the same way as the rhythm in CAP frequency. The variation in CAP amplitude is decreased and conspicuous peaks are not obvious (Fig. 6). Fig. 6 shows the CAP amplitude and CAP frequency for the last three days of LL (100) and the three subsequent days in DD for one of the eyes (open circles) in Fig. 5. Since the peaks in CAP amplitude are not conspicuous, the phase relationship between the CAP amplitude and frequency rhythms is uncertain. However, in DD the phases revert to the typical relationship for DD (Fig. 3) by the second cycle in DD. That is, the first peak (A) in CAP amplitude corresponds with the point of change from the rapid CAP frequency increase to the sustained rate and the second peak (B) corresponds with the point of change from sustained CAP frequency to rapid decrease. In the first cycle in DD after LL these relationships are somewhat distorted, perhaps because the rhythm is in a transient state and complicated by the apparent split in the frequency rhythm.

All the eyes (9) that were tested in constant light of 100 lux or greater showed the characteristic of increased average CAP frequency, decreased range of CAP frequency and CAP amplitude, increased variability in CAP frequency and shortened periods. The run *in vitro* is limited, presently, to a week or so but with further improvements in culture technique longer runs should be possible and the persistence of periodic components can be more accurately estimated.

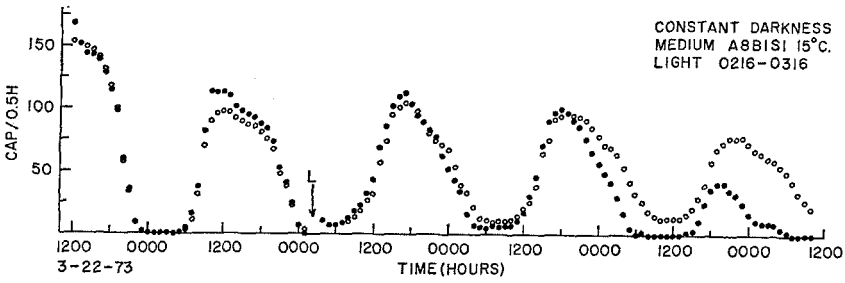

Fig. 7. Circadian rhythm of frequency interrupted by a one hour pulse of light at 18 hours circadian time (0216 EST) which produced a persistent delay in phase. The light evoked activity is not plotted but was several hundred CAP/0.5 hours. This is an example of the data used to plot the phase response curve in Fig. 8. Both eyes received the light pulse; phase shifts were determined by comparison to expected phase

Effects of Light Pulses on the Circadian Rhythm

The eye rhythm was previously shown to be phase shifted and entrained by light pulses *in vitro* (Jacklet, 1971; Eskin, 1971). In order to determine if this rhythm *in vitro* had the characteristics of other circadian rhythms, light pulses (1 hour duration, 600 lux) were given at various phases of the rhythm in DD to generate a phase response curve. An example of the data used to generate the phase response curve is shown in Fig. 7. This rhythm in DD was interrupted with a light pulse at 18 hours (0216 EST) after the first onset of the cycle in DD. This produced a phase delay that is maintained in subsequent cycles of the rhythm in DD. Note that the increased CAP frequency produced during the light has not been plotted, but it was several fold the maximum spontaneous DD frequency. In addition to producing a phase delay in the rhythm the light pulse temporarily changed the shape of the frequency cycle and also caused a temporary perturbation of the amplitude cycle (not shown). The peak in CAP frequency is sharper and the plateau is less obvious. The rhythm was always allowed to run for one or two cycles in DD prior to the light pulse in order to accurately access the phase point for presentation of the light. Zero hour circadian time was taken as the point at which the CAP frequency was going through its maximum increase. This point appears to be the least variable for this particular rhythm and usually coincides with the projected D-L transition on the first day *in vitro* in culture medium.

The delay or advance of phase as a function of the time of the light presentation in the cycle is shown in Fig. 8. The abscissa is circadian time with zero corresponding to the CAP frequency maximum increase. It is

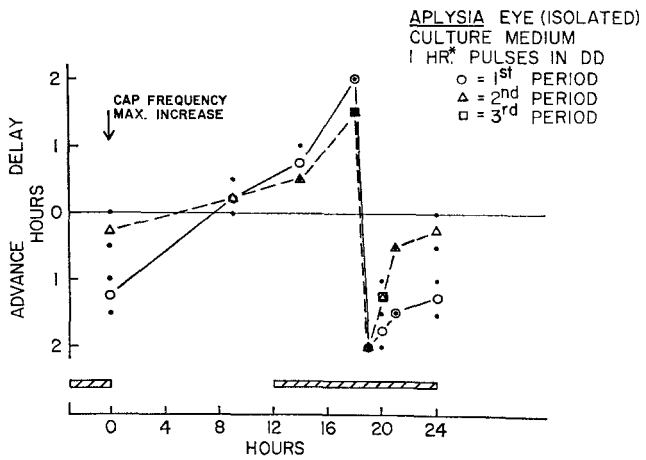

Fig. 8. Phase response curve for one hour pulses of light in DD. Zero circadian time is taken as the point maximum CAP frequency increase in the previous cycle. Each point represents the average of two eyes. The solid line shows the phase one cycle after the light pulse and the dashed line the phase at the second cycle. The squares show the phase at the third cycle after the light pulse. There is some compensation for the phase shift on the second day but the phase is stable by the third day

apparent that maximum delays occur early in the subjective night and maximum advances occur late in the subjective night. The curves also show that some compensation for both advances and delays, [usually delays are established immediately and advances show transients in other systems (Pittendrigh, 1960)], occurs in the second cycle after the light pulse but the third cycle is relatively stable and corresponds to the steady state phase shift. The maximum phase shifts are only two hours; perhaps this would have increased with more intense light or pulses of longer duration, but the eye rhythm shows strong resistance to large perturbation as do some rhythms in whole organism (DeCoursey, 1960).

Discussion

The response of the eye rhythms *in vitro* to light pulses is clearly a function of the phase of the rhythm as are the responses for rhythms in whole organisms (DeCoursey, 1960; Pittendrigh, 1960; Aschoff, 1965). Phase delays occur during the early subjective night, and phase advances occur during the late subjective night. Both of these shifts show 'decreasing transients' (Aschoff, 1965) toward the unperturbed state. The sharp transition between these delays and advances in subjective night is

characteristic of diurnally active species (like *Aplysia*, Jacklet, 1972). The response curve for the eye is also very similar to the response curve of the eye for potassium pulses (Eskin, 1972). Both curves show compensation on the second cycle toward a smaller phase difference than on the first cycle after the pulse. The phase reversal point in the middle of the subjective night for potassium pulses is later than that for light pulses. This difference could be related to several differences in experimental procedure. For example, Eskin used the peak of CAP frequency for measuring phase and I used the maximum increase in CAP frequency. The potassium pulses were tested in filtered sea water and the light pulses were tested in culture medium where the period of the rhythm is 26–27 hours instead of less than 24 hours in filtered sea water. Also the amount of phase shift is related to the duration of the potassium pulse (Eskin, 1972) or the light pulse. In general, the phase response curve for potassium is remarkably similar to the phase response curve for light (the natural zeitgeber) and lends support to Eskin's suggestion that the clock can be phase shifted by depolarization of the membrane. Perhaps the clock can be set by membrane depolarization regardless of the means of producing the depolarization, but the way that light influences the clock is not known with certainty. A reasonable possibility is that light is transduced by the receptor cells and produces a depolarizing receptor potential (Jacklet, 1969b). This receptor potential depolarizes, by electronic coupling, the secondary neurons (pacemakers which apparently are the site of the circadian rhythm) and sets the phase of the circadian rhythm.

Constant light alters the circadian rhythm in the eye by decreasing the free running period. This effect of light is consistent with Aschoff's Rule (Pittendrigh, 1960) for diurnally active animals. The eye rhythm is consistent with other generalization on whole animal rhythms too. If the frequence of CAP can be compared to total activity of an animal and if activity (α) and rest (ϱ) can be compared to the high frequency and low frequency phases of the rhythm, then the eye rhythm also obeys the Circadian Rule (Aschoff, 1960). The Circadian Rule states that for light-active animals spontaneous frequency, the activity: rest ratio ($\alpha:\varrho$) and total activity increase with increasing light intensity. The locomotion circadian rhythm of *Aplysia* does obey the Circadian Rule (Jacklet, 1972).

Comparison of the periods of the *Aplysia* eye rhythm *in vitro* in culture medium and the locomotion rhythm of the intact animal (Jacklet, 1972) indicates that at approximately the same light intensities in LL both rhythms have periods of about 25 hours. Also periodogram analysis of each of these rhythms (eye and locomotion) shows that they have persistent minor modes at 19–20 hours and about 30 hours that flank the major mode of periodicity. A distribution of frequencies with a strong

central mode flanked by weaker minor modes was recognized by Wiener (1958) as characteristic of an ensemble of mutually entrained non-linear oscillators and suggested by Barlow (1960) as theoretical support for the concept of an ensemble of oscillators instead of a single central one.

It has been proposed (Jacklet and Geronimo, 1971) that the eye rhythm of *Aplysia* from produced by a population of interacting oscillators based on evidence from reducing the number of cells in the eye by ablation. The appearence of the rhythm in some of the greatly reduced eyes is similar in several ways to the appearance of the rhythm after several days in LL. The rhythm of CAP amplitude is flattened, the range of the CAP frequency oscillations is reduced and the changes in CAP frequency from hour to hour may be abrupt and erratic. This results in uncertainty about the phase and period of the, previously obvious, circadian oscillations. These similar results are apparently brought about by a reduction in the number of neurons to a critical level on the one hand and depolarization by constant light to a critical level on the other hand. A detailed presentation of the rhythm in reduced eyes under various light conditions is in preparation.

The influence of constant light on circadian rhythms is variable and apparent arrhythmia is not uncommon (Pittendrigh, 1966; Hoffmann, 1971). A phenomenon of considerable interest is rhythm splitting. At particular constant light intensities a single rhythm may split into several discrete periodicities that exhibit spontaneous changes in their phase relationships (Pittendrigh, 1960; Swade, 1971; Winfree, 1971; Hoffmann, 1971). Their occurrence has been viewed as evidence that circadian rhythms have a multioscillator basis. Rhythm splits have been observed after many days in constant light and a long run is usually needed to definitely established the phase relationship of the several components (Hoffmann, 1971). Unfortunately, the eye rhythm has not yet been maintained for months *in vitro*, but the short term results suggest that splits may occur in the rhythm. Splits in the eye rhythm are suggested by the appearance of 2 CAP frequency components after the transition from LL to DD (Fig. 5) and by the drifting apart of the peaks of CAP amplitude in DD (Fig. 3). The changes in CAP amplitude may be proportional to changes in the number of neurons constributing voltage to each CAP. This assumes that each neuron contributes an equivalent voltage to the CAP and that the duration of the CAP does not change. The peaks of CAP amplitude may represent subpopulations of neurons that have different periods and drift apart with time in DD. This interpretation is consistent with a model (Winfree, 1967) that shows rhythm splits (Winfree, 1971) but there is uncertainty about the temporal activity of individual neurons and how they contribute to the CAP and the rhythm. For example, a neuron may contribute to only one peak or

several peaks of CAP amplitude. Definite splits in the eye rhythm may be produced by prologed runs in DD to accentuate the apparent drifting apart of components or by prolonged LL runs followed by DD.

I thank Heb Soon Kim for technical assistance and Arthur Winfree for comments on a preliminary draft. Supported by NIH grant 08443.

References

Adler, K.: The role of extraoptic photoreceptors in amphibian rhythms and orientation a review. J. Herpetol. **4**, 99–112 (1970)

Aschoff, J.: Exogenous and endogenous components in circadian rhythm. Cold Spr. Harb. Symp. quant. Biol. **25**, 11–28 (1960)

Aschoff, J.: Response curves in circadian periodicity. In: Circadian clocks (ed. J. Aschoff), p. 95–111. Amsterdam: North Holland Publishing Co. 1965

Barlow, J.: Cold Spr. Harb. Symp. quant. Biol. **25**, 54–55 (1960)

DeCoursey, P. J.: Phase control of activity in a rodent. Cold Spr. Harb. Symp. quant. Biol. **25**, 44–55 (1960)

Eskin, A.: Properties of the *Aplysia* visual system: *in vitro* entrainment of the circadian rhythm and centrifugal regulation of the eye. Z. vergl. Physiol. **74**, 353–371 (1971)

Eskin, A.: Phase shifting a circadian rhythm in the eye of *Aplysia* by high potassium pulses. J. comp. Physiol. **80**, 353–376 (1972)

Hayes, F. R., Pelluet, D.: Inorganic constituents of molluscs. J. Mar. Biol. Assoc. U. K. **26**, 580–589 (1947)

Hoffmann, K.: Splitting of the circadian rhythm as a function of light intensity. In: Biochronometry (ed. M. Menaker), p. 134–145. Washington, D. C.: National Academy Sciences 1971

Jacklet, J. W.: Circadian rhythm of optic nerve impulses recorded in darkness from isolated eye of *Aplysia*. Science **164**, 562–563 (1969a)

Jacklet, J. W.: Electrophysiological organization of the eye of *Aplysia*. J. Gen. Physiol. **53**, 21–42 (1969b)

Jacklet, J. W.: A circadian rhythm in optic nerve impulses from an isolated eye in darkness. In: Biochronometry, (ed. M. Menaker), p. 351–362. Washington, D. C.: National Academy Science 1971

Jacklet, J. W.: Circadian locomotor activity in *Aplysia*. J. comp. Physiol. **79**, 325–341 (1972)

Jacklet, J. W.: Neuronal population interactions in a circadian rhythm in *Aplysia*. Neurobiology of invertebrates, Tihany 1971. (ed. J. Salánki), Hungarian Academy Sciences, Budapest pp. 363–380, 1973

Jacklet, J. W., Geronimo, J.: Circadian rhythm: Population of interacting neurons. Science **174**, 299–302 (1971)

Menaker, M.: Extraretinal light perception in the sparrow. I. Entrainment of the biological clock. Proc. nat. Acad. Sci. (Wash.) **59**, 414–421 (1968)

Pittendrigh, C. S.: Circadian rhythms and the circadian organization of living systems. Cold Spr. Harb. Symp. quant. Biol. **25**, 159–184 (1960)

Pittendrigh, C. S.: The circadian oscillation in *Drosophila pseudoobscura* pupae: A model for the photoperiodic clock. Z. Pflanzenphysiol. **54**, 275–307 (1966)

Strumwasser, F., Bahr, R.: Prolonged *in vitro* culture and autoradiographic studies of neurons in *Aplysia*. Fed. Proc. **25**, 512 (1966)

Swade, R. H.: A split activity rhythm under fluctuating light cycles. In: Biochronometry (ed. M. Menaker), p. 148–149. Washington D. C.: National Academy Science 1971

Truman, J., Riddiford, L.: Neuroendocrine control of ecdysis in silkworms. Science **167**, 1624–1626 (1970)

Truman, J. W., Sokolove, P. G.: Silk Moth eclosion: Hormonal triggering of a centrally programmed pattern of behavior. Science **175**, 1491–1493 (1972)

Wiener, J.: Nonlinear problems in random theory, p. 69–70 Cambridge: MIT Press 1958

Winfree, A. T.: Biological rhythms and the behavior of populations of coupled oscillators. J. theor. Biol. **16**, 15–42 (1967)

Winfree, A. T.: Comments on Rhythm Splitting, p. 150–151. In: Biochronometry (ed. M. Menaker). Washington, D. C.: National Academy Sciences 1971

Dr. Jon W. Jacklet
Department of Biological Sciences
and Neurobiology, Research Center
State University of New York at Albany
Albany, New York 12222, USA