

## LIGHT AND HIGH POTASSIUM CAUSE SIMILAR PHASE SHIFTS OF THE *APLYSIA* EYE CIRCADIAN RHYTHM.

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The eye of *Aplysia*, with its circadian rhythm of spontaneous optic nerve impulses (Jacklet, 1969*a*), is a model system for studying the cellular mechanisms of circadian rhythms generated by nervous tissue (Jacklet, 1981). An approach to determine the mechanisms of rhythms is to apply pulses of experimental agents and then observe the shifts in phase of subsequent cycles of the rhythm. If the size and direction of the phase-shifts are dependent upon the phase of the cycle when the pulse was given (phase-dependent phase shifts), it is evidence that the rhythm timing mechanism has been perturbed. The specific cellular process acted on is then believed to be important in the cellular clock mechanism. Some agents cause phase-dependent phase shifts and the shifts are plotted in phase response curves (PRCs). It is useful to compare the PRCs produced by different agents in order to identify similar or dissimilar actions on the cellular clock (Jacklet, 1978).

Previous work has shown that there are at least two fundamental PRCs for the eye rhythm (Jacklet, 1978). One is for protein synthesis inhibitors (Rothman & Strumwasser, 1976; Jacklet, 1977), metabolic inhibitors (Eskin & Corrent, 1977), low temperature (Jacklet, 1978) and possibly serotonin (Corrent *et al.* 1978). The other is for light, a natural synchronizer, (Jacklet, 1974; Benson & Jacklet, 1977), and high potassium ( $K^+$ ) (Eskin, 1972). From the published results it is uncertain if the PRCs for light and high  $K^+$  are similar, since the experiments were carried out under different conditions. Eskin (1972) used sea water and estimated phase from the previous light-dark cycle. Jacklet (1974) used a nutrient culture medium and estimated phase from the measured rhythm. In the present paper experiments were carried out under uniform conditions with light and high  $K^+$  to investigate the relationship between the PRCs and to test for any effects of light or high  $K^+$  on protein synthesis.

Eyes were dissected from *Aplysia californica* kept in Instant Ocean at 16 °C in L.D. 12:12. They were placed in 50 ml chambers of culture medium (90% artificial sea water (ASW), 10% nutrient medium, Jacklet, 1974) and the optic nerve activity was recorded with a tubing electrode in complete darkness at 16 °C. Pulses (4 or 6 H) of high  $K^+$  (50, 90, or 109 mM) ASW (with reduced  $Na^+$  to maintain osmotic equivalence) were given at specific phases of the rhythm and chambers were rinsed with ASW three times before returning to normal culture medium. In separate experiments,

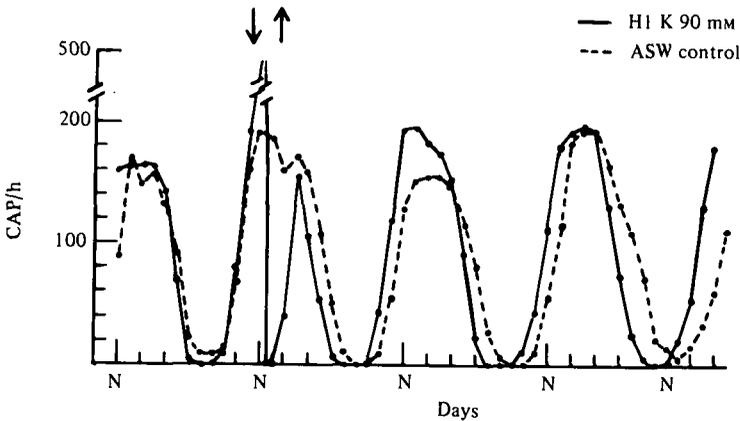


Fig. 1. Effects of high  $K^+$  on the circadian rhythm of eye CAP frequency. Each eye was pulsed for 4 h (arrow start, finish), one with ASW and the other with 90 mM- $K^+$  ASW. The latter was phase advanced 3 h. Time scale in successive noons (N).

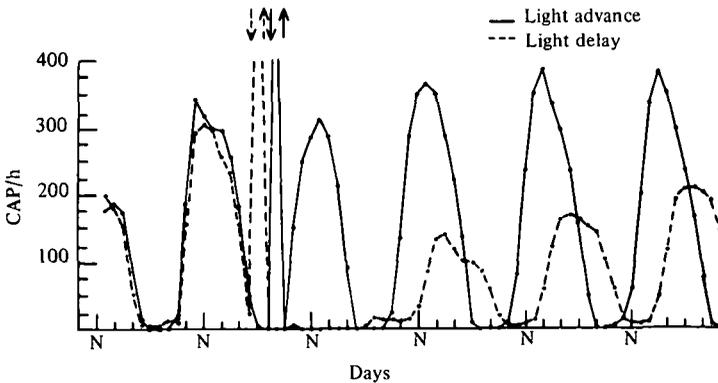


Fig. 2. Effect of light pulses on the circadian rhythm of eye CAP frequency. Each eye was pulsed with light for 3 h, one from CT 16-19 and the other from CT 19.5-22.5 (arrows). One rhythm was delayed 3 h and the other advanced 4 h. Time scale in successive noons (N). The amplitude of the delayed rhythm was restored to normal after a few cycles.

light pulses were given with white (tungsten) light of 10000 lx for 3 h. Temperature changes were prevented by the plexiglass chamber cover and circulated cooled water. The effect of 10000 lx light or 109 mM- $K^+$  on protein synthesis was determined by measuring the incorporation of [ $^3H$ ]leucine into TCA precipitable material (Jacklet, 1977). Eyes were treated with light or high  $K^+$  for 1 h at CT 20 and then pulsed for 1.5 h with [ $^3H$ ]leucine during the continued light or high  $K^+$  treatment.

The high  $K^+$  experiments confirmed the basic findings of Eskin (1972). Pulses given in late subjective night and early subjective day caused phase advances, as shown in Fig. 1, and pulses given during early subjective night caused phase delays. Weak (50 mM) pulses of high potassium produced much smaller shifts than stronger (90 or 109 mM) pulses. During the pulse treatment, the CAP activity was very high for 30 min or so and then the eye became silent. When the solution was changed back to

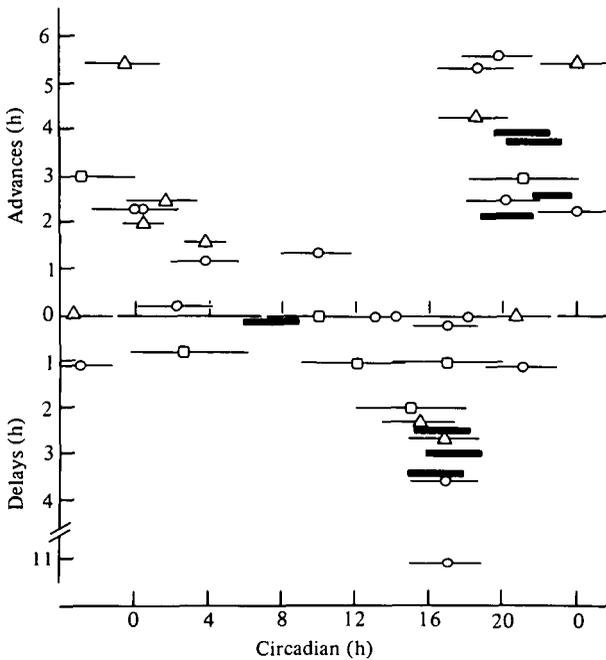


Fig. 3. PRC for high potassium or light pulses. Bars show the duration of the pulses, each symbol (one eye) shows the treatment. Squares are for 50 mM, circles 90 mM and triangles 109 mM- $K^+$ . Heavy bars are for light pulses. The steep transition from delays to advances occurs at CT 18 for both treatments.

normal, a rebound firing was observed. The low  $Na^+$  control did not show rebound firing or cause phase shifts; also found by Eskin (1972).

Light pulses were given in separate experiments and largest phase shifts were obtained in the subjective night, circadian time (CT) 12–24. Both phase delays and phase advances were obtained (Fig. 2). Light caused rapid firing of CAP for the duration of the light pulse but no rebound firing, as previously noted (Jacklet, 1974). The phase shifts for light and the phase shifts for high  $K^+$  are shown in the PRCs in Fig. 3. The largest  $K^+$  phase delays were obtained near CT 16 and the largest phase advances were obtained about CT 20 but there was some overlays of times. The  $K^+$  PRC shows a steep transition from delays to advances centered at about CT 18. The light experiments gave advances and delays that were very similar to the high  $K^+$  results and the transition from delays to advances was also at CT 18. In previous studies with 1 h light pulses (Jacklet, 1974), the steep transition from phase delays to phase advances occurred at about CT 18 and 2 h light pulse experiments by Benson & Jacklet (1977) showed a steep transition at CT 18–19 (after corrections for the CT). Eskin (1977) found 3 h phase advances at CT 18–24. Those experimental results taken together with the present ones demonstrate that light and high  $K^+$  pulses cause similar phase shifts of the circadian oscillator in the *Aplysia* eye.

Eskin (1972) concluded that membrane depolarization was the mechanism of action of high potassium. Depolarization induced secretion was not likely since low  $Ca^{2+}$ -high  $Mg^{2+}$  ASW did not block the  $K^+$  effect. The necessary reduction in  $[Na]$  (to 326 mM)

did not cause phase shifts alone (Eskin, 1972). The high  $K^+$  treatments should depolarize the eye cells, assuming that the resting potential is determined largely by  $K^+$  conductance. Using an intracellular  $K^+$  activity of 142 mM and an activity coefficient of 0.7 for extracellular  $K^+$  (Kunze & Brown, 1971), shifts in  $E_K$  were calculated. For normal 10 mM- $K^+$   $E_K$  is -76 mV, for 50 mM- $K^+$   $E_K$  is -35 mV, for 90 mM it is -20 mV, and for 109 mM it is -16 mV. As 50 mM had only a slight phase-shifting effect, the threshold potential must be near -35 mV. It is known that light depolarizes the photoreceptors and secondary neurones of the eye (Jacklet, 1969*b*). The phase shifting effect of light is blocked only by treatment, 10  $Na^+$ -hi  $Mg^{2+}$ -lo  $Ca^{2+}$ , which reduces the ERG by 90% (Eskin, 1977). The common action of both light and high  $K^+$  in causing phase shifts appears to be depolarization of the retinal cells.

An effect of high  $K^+$  is inhibition of protein synthesis (Ram, 1974). His studies of the *Aplysia* abdominal ganglion showed that 90 mM- $K^+$  blocked total amino acid incorporation by 50%, while enhancing incorporation into certain MW proteins. In the present study 109 mM- $K^+$  reduced the incorporation of leucine into eye protein by  $68.3 \pm 7.4\%$  (S.D.)  $n = 6$ . Light, however, did not substantially reduce incorporation,  $7\% \pm 10$  (S.D.)  $n = 3$ . The inhibition of protein synthesis by high  $K^+$  is interesting because specific inhibitors of protein synthesis such as puromycin (Rothman & Strumwasser, 1976) and anisomycin (Jacklet, 1977) cause phase dependent phase shifts. The PRCs for puromycin and anisomycin are similar (Lotshaw & Jacklet, 1980) but they are not like the PRC for high  $K^+$ . For example, protein synthesis inhibitors induce phase delays during the part of the cycle when high  $K^+$  induces phase advances. Furthermore, anisomycin has been shown to inhibit [ $^3H$ ]leucine incorporation by up to 50% without causing phase shifts (Lotshaw & Jacklet, unpublished). Inhibition greater than 50% is needed. It seems possible that high  $K^+$  induced inhibition of protein synthesis is not involved in high  $K^+$  induced phase shifting or it is insignificant relative to the depolarizing effect. It may also be that the portion of protein synthesis blocked by high  $K^+$  is not the specific protein synthesis needed for the integrity of the rhythm, since high  $K^+$  does not block protein synthesis uniformly (Ram, 1974) and some eye proteins may be spared or enhanced.

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